Partially differentiated polyfunctional T cells dominate the periphery after tumor-infiltrating lymphocytes therapy for cancer

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Adoptive Cellular Therapy

O1
IL-15 primes an mTOR-regulated gene-expression program to prolong anti-tumor capacity of human natural killer cells

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Background
NK cell–based immunotherapy is a potential therapeutic modality in patients with advanced cancers as transfer of haploidentical NK cells induces beneficial responses in patients with hematological malignancies; and leukemia clearance correlates with persistence and in vivo expansion of NK cells after infusion. Thus, sustained NK cell activity in vivo likely represents a therapy-performance-limiting factor.

Methods
We performed genome-wide analysis of cytosolic and polysome-associated mRNA from interleukin (IL)-2 and IL-15 activated NK cells. Furthermore, the ability of IL-2 and IL-15 to sustain human NK cell activity following cytokine withdrawal as well as their effect on NK cells to resist tumor-induced immunosuppression was compared.

Results
After cytokine withdrawal, IL-15-treated NK cells maintained a higher level of cytotoxicity (p < 0.05) and showed lower levels of apoptosis (p < 0.05) compared with cells treated with IL-2. IL-15 augmented mTOR signaling, which correlated with increased expression of genes related to cell metabolism and respiration. Consistently, mTOR inhibition abrogated IL-15–induced cell function advantages. Moreover, mTOR-independent STAT-5 signaling contributed to improved NK cell function during cytokine activation but not following cytokine withdrawal. Upon co-culture with tumor cells or exposure to tumor cell supernatant, IL-15 activated NK cell maintained a significantly higher level of proliferation and cytotoxic activity (p < 0.05). Mechanistically, tumor-derived prostaglandin-E2 suppressed IL-2 cultured NK cells while IL-15 cultured NK cells remained activated. The superior performance of IL-15 stimulated NK cells was also observed using a clinically applicable protocol for NK cell expansion in vitro and in vivo.

Conclusions
This study adds to our understanding about establishment and maintenance of tumor-reactive NK cells and supports clinical implementation of IL-15 for adoptive NK cell therapy. More broadly, our studies suggest that a large aspect of cytokine-mediated gene expression programs and downstream cellular functions, including anti-tumor capacity, are overlooked if post-activation conditions are omitted. This is likely not limited to NK cells and should hence be considered in similar studies of other immune cells.

Biomarkers and Immune Monitoring

O2
ImmuNoMap: a novel bioinformatics tool for analysis of T cell receptor repertoire data in model systems and clinical settings


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Background
There has been a dramatic increase in T cell receptor (TCR) sequencing spurred, in part, by the widespread adoption of this technology across academic medical centers and by the rapid commercialization of TCR sequencing. While the raw TCR sequencing data has increased, there has been little in the way of approaches to parse the data in a biologically meaningful fashion. The ability to parse this new type of ‘big data’ quickly and efficiently to understand the T cell repertoire in a structurally relevant manner has the potential to open the way to new discoveries about how the immune system is able to respond to insults such as cancer and infectious diseases.
Methods
Here we describe a novel method utilizing phylogenetic and sequencing analysis to visualize and quantify TCR repertoire diversity. To demonstrate the utility of the approach, we have applied it to understanding the shaping of the CD8 T Cell response to self (Kb-TRP2) and foreign (Kb-SIY) antigens in naive and tumor bearing B6 mice. Additionally, this method was applied to tumor infiltrating lymphocytes (TIL’s) from patients undergoing Nivolumab (anti-PD-1) therapy in a clinical trial for metastatic melanoma to understand TCR repertoire characteristics between responders and non-responders.

Results
Analysis of the naïve CD8 response to SIY showed a lower clonality yet more closely structurally related response whereas CD8 responses to TRP2 were highly clonal yet less structurally related. Presence of tumor exhibited interesting differential effects on SIY vs. TRP2. We believe that differences in TCR repertoire suggest effects from central and peripheral tolerance on self vs. foreign antigens. In clinical trial data, the phylogenetic analysis revealed unique TCR repertoire signatures that differentiated responders from non-responders to anti-PD-1 therapy, including some that could be detected prior to initiation of therapy. Additionally, this analysis revealed that patients whose CD8 response had a larger contribution from novel and unique structural clones responded better to therapy.

Conclusions
In summary, we have developed and demonstrated a novel method to meaningfully parse and interpret TCR repertoire data and have applied it to yield a novel understanding of CD8 T Cell responses to different types of antigens as well as key characteristics in those who respond to anti-PD-1 therapy.
Increased STAT3 signaling and decreased suppressive function of regulatory T cells are biomarkers of positive patient outcome to nivolumab therapy

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Methods
Pre and post-treatment Tregs were isolated from the peripheral blood of surgically resected stage III/IV metastatic melanoma patients treated with adjuvant nivolumab. Suppressive capacity was assessed in an allogeneic mixed lymphocyte reaction. Paired (pre vs. post-treatment) Tregs were assessed by flow cytometry for phosphorylated STAT3 (pSTAT3) expression. Finally, paired Treg samples were assessed for gene expression by RNA-sequencing.

Results
Tregs from non-relapsing patients demonstrated a significant decrease in suppressive capacity post-treatment (p < 0.05). However, suppressive capacity in relapsing patients did not decrease and their Tregs were significantly more suppressive post-treatment relative to non-relapsers (p < 0.01). Significantly increased levels of pSTAT3 post treatment were observed in non-relapsers (p < 0.05) but not in relapsers (p < 0.40). Significantly increased pSTAT3 was not seen in conventional T cells after nivolumab therapy. Culturing treatment-naive T cells with PD-1 blocking antibodies in vitro resulted in increased levels of pSTAT3 in Tregs compared to IgG controls (p < 0.01). In vitro PD-1 blockade also significantly increased the number of Tregs (p < 0.01), and significant increases were seen in paired patient samples (p < 0.05). Paired analysis of Treg RNA-seq data using Panther and GeneGo. Metacore showed several significantly increased pathways associated with proliferation in non-relapsers. Changes in these pathways were absent in relapsers. Gene Set Enrichment Analysis of non-relapsers Tregs showed significant overlap with known STAT3 target genes. Conversely, Enrichr analysis of relapsers showed significant upregulation of STAT1 and STAT2 target genes. No overlap of significantly changed gene expression or pathways in Tregs vs. conventional CD4+ T cells were observed.

Conclusions
These results highlight the potential importance of Tregs in mediating benefit with PD-1 blockade, demonstrating pSTAT3 induction and reduced suppressive capacity as biomarkers of clinical benefit. PD-1 blockade also increased the percentages of Tregs, consistent with the known roles of STAT3 in promoting cell survival and proliferation. RNA-seq data demonstrated increased STAT3 and proliferation associated gene expression. Intriguingly, Tregs from relapsing patients had increased expression of genes associated with STAT1/2 signaling, warranting further investigation of these pathways. In addition to highlighting STAT signaling as a biomarker of relapse, these results demonstrate distinct differences in the impact of PD-1 blockade in Treg vs. conventional T cells.

Analysis of pharmacodynamic biomarkers in the first in-human trial of GITR co-stimulation with the agonist antibody TRX-518 in advanced solid cancer patients

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Methods
GITR is a tumor necrosis factor receptor expressed at high levels on regulatory T cells (Tregs) and up-regulated on T cells upon activation. GITR stimulation abrogates Treg suppression and enhances T cell effector function. These observations suggest that GITR could be an attractive target for immunotherapy with agonist antibodies. GITR stimulation in tumor-bearing mice has shown therapeutic activity associated with both Treg reduction and modulation. Here we report results of pharmacodynamic analyses in the first in-human phase I trial with the fully humanized agonist anti-GITR antibody TRX518 as monotherapy in patients with advanced refractory solid tumors.

Results
Patients were accrued to 9 cohorts (up to 6 patients/ cohort) to receive a single dose of TRX518 (dose range: 0.0001-8 mg/kg). Pharmacodynamic analyses included flow cytometric evaluation of frequency and phenotype of circulating T cells and cytokine quantification in serum samples at different time points up to 12 weeks after treatment. Relevant changes observed with these analyses were monitored in pre- and post-treatment tumor biopsies by immuno-fluorescence staining.

Conclusions
Here we report results obtained in 37 patients treated with ≥0.005 mg/kg TRX518 (cohorts 3-9), including 6 melanoma, 7 non-small cell lung cancer (NSCLC) and 7 colorectal cancer (CRC) patients and 17 patients with 11 other solid tumors. Among the T cell parameters analyzed, we found frequent reduction in circulating Tregs after treatment with TRX518 across all cohorts, with some exceptions. Importantly, this effect could be maintained over the 12-week observation period. When the analysis was performed by disease type, it revealed a pronounced TRX518 dose-dependent down-regulation of peripheral Tregs in both melanoma and CRC patients. Interestingly, in NSCLC cancer patients, Tregs did not always decrease after treatment. In a subset of patients (n=6; 2 melanoma, 2 CRC, 2 lung), for whom we had pre- and post-treatment tumor biopsies in addition to PBMCs, we tested whether intra-tumor Tregs were consistently affected. In melanoma and CRC patients, intra-tumor Foxp3+ Tregs were significantly reduced after treatment, in agreement with the peripheral Treg down-modulation observed in the same patients. In lung cancer patients, lack of circulating Treg reduction was consistently associated with stable or increased intra-tumor Treg infiltration after TRX518.

Conclusions
Circulating Treg reduction is a potential pharmacodynamic biomarker of TRX518 biological activity. This parameter may allow predictive correlation with changes in intratumoral Treg infiltration. We plan to further investigate this effect and its relevance for the association with clinical responses in our recently opened TRX518 multi-dose study.
Clinical trials conducted at KCI.

The study was made possible due to the efforts of clinical nursing support staff. Funding for this study was provided by Helen Kay Trust and Philanthropy at KCI. We acknowledge the efforts of clinical trial teams.

**Bispecific Antibodies**

**O5**

Clinical responses in advanced pancreatic patients treated with bispecific antibody armed T cells (BATS)

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**Background**

Conventional chemotherapy (chemo) for locally advanced pancreatic cancer (LAPC) and metastatic pancreatic cancer (PC) is associated with dismal responses and poor survival rates. Arming T cells (ATC) with anti-CD3 x anti-EGFR bispecific antibody (EGFRBi) turns every ATC into a non-MHC restricted EGFR-specific cytotoxic T lymphocyte [1]. Engagement of CD3 on T cells and EGFR on Mia PACA-2 leads to cytokine secretion, proliferation, cytotoxicity by ATC and inhibition of tumor growth [2]. An earlier study using Infusions of anti-CD3 x anti-HER2 (HER2Bi) armed ATC in metastatic breast cancer provided encouraging survival (OS = 36 months) and evidence of anti-breast cancer immunity [3].

**Methods**

In this study, we used anti-CD3 x anti-EGFR bispecific antibody (EGFRBi)-armed T cells (EGFR BATs) to target EGFR in 5 metastatic PC patients and 6 colorectal cancer patients treated at Karmanos Cancer Institute on Protocol #2014-025 in a phase I dose escalation involving 3 weekly infusions of 10, 20, and 40 x 10^9 BATs/infusion followed by a booster infusion 3 months later.

**Results**

In the 5 PC patients, we report 1 patient was stable for 6.5 months and 2 patients in whom infusions of EGFR BATs may have “sensitized” the tumor to subsequent chemotherapy. The patient with stable disease had a near partial response. The median overall survival in 5 patients is 23.5 months with the median time to progression (TTP) of 7.0 months. Patient IT20102 received BATs and was responding to chemotherapy after progressing after EGFRBi-BATs therapy; and 2) two patients with slow progressive disease who survived beyond 400 days. Survival for the 5 patients was 13.6, 14.5, 23.3 (alive in CR), 24.9 (alive, stable), and 31.0 months after enrollment, respectively (as of 7-20-16).

**Conclusions**

Targeting PC with EGFR BATs resulted in improved survival and remarkable post-immunotherapy chemotherapy responses in a small series of patients. The series provides evidence for anti-tumor activity of EGFR BATs as well as evidence that BATs infusions can sensitize tumors to subsequent chemotherapy.

**Acknowledgements**

Funding for this study was provided by Helen Kay Trust and Philanthropy and Startup Funds at KCI. We acknowledge the efforts of clinical coordinating staff, the clinical trials office staff, GMP laboratory staff, and clinical nursing support staff to making this study possible. The study was conducted at KCI.

**References**


**Combinations: Immunotherapy/Immunotherapy**

**O6**

Reactivating the anti-tumor immune response by targeting innate and adaptive immunity in a phase I/II study of intratumoral IMO-2125 in combination with systemic ipilimumab in patients with anti-PD-1 refractory metastatic melanoma

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**Background**

While checkpoint inhibitor (CPI) therapy has transformed metastatic melanoma (MM) treatment, many patients remain refractory. We reasoned that combining CPI with an agent that activates antigen presenting cells and improves T cell priming may result in improved response. Our approach is to modulate the tumor microenvironment through intratumoral (i.t.) injection of the TLR9 agonist, IMO-2125, in combination with ipilimumab (ipi). We hypothesize that this will result in dendritic cell (DC) activation and induction of tumor-specific CD8+ T cells which will synergize with ipilimumab to overcome immune-escape. Based on this rationale we initiated a phase I/II clinical trial.

**Methods**

Adults with refractory MM despite up to 2 lines of CPI including PD-1 blockade therapy (with or without a BRAF inhibitor) are eligible. IMO-2125, in doses escalating from 4mg to 32mg, is administered i.t. once every 2 weeks in conjunction with ipilimumab (ipi) 3 mg/kg every 4 weeks. Based on prior trials of ipilimumab, the dose of anti-PD-1 is 3 mg/kg every 4 weeks in conjunction with IMO-2125. Patients are concurrently receiving ipilimumab and IMO-2125 until patients experience disease progression, unacceptable toxicity, or withdraw from the study. MTX, a purine antagonist, is administered to block proliferation of lymphocytes and myeloma cells.
given i.t. weeks 1, 2, 3, 5, 8, and 11 along with ipilimumab i.v. 3 mg/kg weeks 2, 5, 8, and 11. Dose-limiting toxicity (DLT) is evaluated using a modified Toxicity Probability Interval design. Primary endpoints are safety, tumor response, and PK. Blood and injected and distal tumor biopsies are obtained pre- and on-treatment. Immune analyses include DC subsets and their activation status as well as T cell activation, function and proliferation. T cell repertoire diversity will be evaluated by high throughput CD83 sequencing.

Results

As of August 2, 2016, 11 pts have been enrolled. DLT has not been observed. Grade 3 hypophysitis (2 subjects) is the only immune-related AE observed to date. No other drug-related grade 3-5 AEs were documented and only 1 subject experienced a grade 2 fever. Five patients are evaluable for response - 2 PR, 2SD, 1PD per investigator assessment. Fresh tumor biopsies show maturation (upregulation of HLA-DR) of the myeloid DC1 subset (CD1c+CD303-) in the IMO-2125 injected tumor lesion 24 hr post-treatment compared to pre-treatment biopsy. On-treatment biopsy results are consistent with a higher rate of proliferative (Ki67) effector CD4+ and CD8+ T cells in responders. Cytokine analysis shows a 2-3 fold increase in circulating IFNγ levels compared to pretreatment in responders.

Conclusions

Though preliminary, these results demonstrate that the combination of ipi and IMO-2125 is well tolerated with encouraging preliminary activity in a PD-1 refractory population. Dose escalation is ongoing and a phase II expansion will include IMO-2125 in combination with both ipi and anti-PD-1. Updated safety, antitumor activity, and biomarker data will be presented.

Trial Registration

ClinicalTrials.gov identifier NCT02644967.

O7

Clinical safety and efficacy assessment of the CD137 agonist urelumab alone and in combination with nivolumab in patients with hematologic and solid tumor malignancies


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Background

Urelumab is a fully human CD137 agonistic monoclonal antibody (mAb) that enhances T cell and natural killer (NK) cell antitumor activity in preclinical models. Nivolumab, a fully human programmed death-1 (PD-1) mAb that blocks the inhibitory function of the PD-1 receptor on T cells, has shown single-agent activity in many advanced malignancies. We hypothesized that the distinct, non-redundant mechanisms of these two mAbs could enhance antitumor activity without compromising safety. Here we report safety/tolerability, pharmacodynamics, and preliminary efficacy of urelumab and urelumab plus nivolumab combination therapy in patients with advanced malignancies.

Methods

The monotherapy study evaluated urelumab in patients with advanced malignancies (0.1 or 0.3 mg/kg Q3W) or advanced non-Hodgkin lymphoma (8 mg Q3W or Q6W). The combination study evaluated urelumab (3 or 8 mg Q4W) plus nivolumab (3 mg/kg or 240 mg Q2W) in patients with advanced solid tumors or B cell lymphoma (dose escalation) or patients with diffuse large B cell lymphoma (DLBCL), melanoma, non-small cell lung cancer (NSCLC), or squamous cell carcinoma of the head and neck (SCCHN; cohort expansion). Based on preliminary safety/tolerability/pharmacokinetic assessments of urelumab, cohort expansion focused on flat doses of 8 mg.

Results

Overall, patients who received urelumab monotherapy (N=123) experienced infrequent treatment-related serious AEs (7%) and treatment-related AEs (TRAEs) leading to discontinuation (5%; Table 2). In 104 patients treated with urelumab plus nivolumab (melanoma, n=40; NSCLC, n=20; SCCHN, n=22; DLBCL, n=22), the most frequent TRAE was fatigue (26%); grade 3/4 ALT/AST elevations (3%/3%) and TRAEs leading to discontinuation (7%) were infrequent. No treatment-related deaths were reported. Urelumab stimulated peripheral IFN-γ-induced cytokine production; induction was greater with urelumab plus nivolumab. In most melanoma tumors evaluated, a trend toward increased T and NK cell number and expression of IFN-γ and CXCL9 was observed upon treatment with the combination. Six patients with lymphoma treated with urelumab monotherapy had a partial (n=3) or complete (n=3) remission. Nine of 86 evaluable patients treated with the combination had partial responses (melanoma, n=8; SCCHN, n=1); no patients with NSCLC or DLBCL had confirmed responses at the interim analysis. Of 71 patients treated with urelumab plus nivolumab with RECIST/IWG assessments, 33 had reductions in tumor burden (Fig. 4).

Conclusions

Urelumab with or without nivolumab is safe/tolerable at flat and weight-based doses of 8 mg and 0.1 mg/kg. Although urelumab has demonstrated single-agent pharmacodynamic and antitumor activity in lymphoma, combination with nivolumab did not appear to provide significant additive/synergistic clinical benefit at the doses evaluated.

Trial Registration

ClinicalTrials.gov identifier NCT01471210 and NCT02253992.

Table 2 (Abstract O7). Treatment-related safety events

<table>
<thead>
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<th>Patients, n (%)</th>
<th>Urelumab monotherapy</th>
<th>Urelumab + nivolumab</th>
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</thead>
<tbody>
<tr>
<td>Treatment-related AEs</td>
<td>65 (53)</td>
<td>65 (53)</td>
</tr>
<tr>
<td>Most frequent treatment-related AEsa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>18 (15)</td>
<td>27 (26)</td>
</tr>
<tr>
<td>AST increased</td>
<td>16 (13)</td>
<td>9 (9)</td>
</tr>
<tr>
<td>ALT increased</td>
<td>12 (10)</td>
<td>13 (13)</td>
</tr>
<tr>
<td>Treatment-related grade ≥4 AST elevation</td>
<td>4 (3)</td>
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<tr>
<td>Treatment-related ≥4 ALT elevation</td>
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<tr>
<td>Treatment-related serious AEs</td>
<td>9 (7)</td>
<td>10 (10)</td>
</tr>
<tr>
<td>Treatment-related AEs leading to discontinuation</td>
<td>6 (5)</td>
<td>7 (7)</td>
</tr>
<tr>
<td>Treatment-related deaths</td>
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<td>0</td>
</tr>
</tbody>
</table>

AE, adverse event; AKT, alanine aminotransferase; AST, aspartate aminotransferase.
aTreatment-related AEs occurring in ≥10% of all patients
Beyond immune checkpoint: first-in-class antibody targeting soluble NKG2D ligand sMIC for cancer immunotherapy
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Background
In response to oncogenic insult, human cells were induced to express a family of MHC I-chain related molecules A and B (MICA and MICB, generally termed MIC) on the surface which serve as the ligands for the activating immune receptor NKG2D expressed by all human NK, CD8 T, NKT, and subsets of gamma-delta T cells. Theoretically, engagement of NKG2D by tumor cell surface MIC is thought to signal and provoke the immune system to eliminate transformed cells. Clinically, almost all advanced tumors in cancer patients produce soluble MIC through proteolytic shedding mediated by metalloproteases, or by release in exosomes derived from the cell membrane. Tumor-derived sMIC is known to be highly immune suppressive and profoundly insults the immune system by downregulating receptor NKG2D expression on effector NK and T cells, driving the expansion of tumor-favoring myeloid suppressor cells, skewing macrophages into alternatively activated phenotypes, and perturbing NK cell peripheral maintenance. High levels of serum sMIC significantly correlate with advanced diseases of many types of cancer. These observations clearly endorse sMIC to be a cancer immune therapeutic target. However, due to mice lacking homologues to human MIC, this concept was not proven until our recent studies.

Methods
Using a "humanized" MIC-transgenic spontaneous mouse model which recapitulates the NKG2D-mediated onco-immune dynamics of human cancer patients, we addressed whether sMIC is a cancer immunotherapeutic target and whether antibody targeting sMIC synergizes with immune checkpoint blockade or adoptive T or NK cell therapy.

Results
We show that therapy with a first-in-field non-blocking antibody B10 that does not block the interaction of MIC with NKG2D revamps endogenous innate and antigen-specific CD8+ T cell responses and remodels immune reactive tumor microenvironment, by restoring NK cell hemostatic maintenance and function, enhancing CD8+ T cell infiltration to tumors and TCR clonality/diversity, modulating CD8+ T cells metabolic preferences, eliminating MDSCs and TAMs. Anti-sMIC stand-alone therapy resulted in effective debulking of primary tumors and eliminated metastasis. Using multiple pre-clinical animal models, we further demonstrate that antibody B10 neutralizing sMIC synergizes with CTLA-4 and PD-1/PD-L1 checkpoint blockade therapy and adoptive cell based therapy with no observed toxicity.

Conclusions
Our study has launched a new avenue of cancer immunotherapy which can be readily translated into clinical trials.
Background
Recent work from our laboratory has shown that anti-tumor immunosuppression is suppressed in mice housed at standard temperatures (ST; 22°C) which could be reversed by housing mice at warmer, thermoneutral temperatures (TT; 30°C) [1]. However, the mechanisms causing this impairment at ST remain unclear. Cold stress is mediated specifically by activation of the sympathetic nervous system and the release of norepinephrine (NE), which is highly suppressive when signaling through β-adrenergic receptors (β-ARs) on immune cells. We found that NE levels are significantly elevated in tumor-bearing mice housed at ST compared to TT, which led us to hypothesize that chronic stress induced by cool housing temperatures increases β-AR signaling that dampens the anti-tumor immune response and the efficacy of immune modulating therapies.

Methods
We used both physiologic (housing temperature; ST and TT) and pharmacologic blockade (β-blockers) to modulate β-AR signaling levels in immune-competent and SCID mice bearing 4T1 or B16-OVA tumors. Flow cytometry was used for immune cell analysis. Anti-PD-1 checkpoint blockade was given in 6, 200μg doses (Days 0, 2, 4, 6, 9, and 12) starting the day after tumors became detectable.

Results
We found that the addition of β-blockade significantly delayed 4T1 and B16-OVA tumor growth in mice housed at ST, recapitulating the slower tumor growth observed in mice housed at TT. However, β-blockade had no impact on tumor growth in SCID mice at ST or TT indicating dependence on the adaptive immune system. Analysis of 4T1 and B16-OVA tumors from immune-competent mice showed increased IFN-γ expression in both CD4+ and CD8+ T cells in mice treated with β-blockade indicating a more robust anti-tumor immune response. Lastly, we investigated the impact of β-AR signaling on anti-PD-1 checkpoint blockade efficacy and found that reducing β-AR signaling by both physiologic (TT) and pharmacologic (β-blockade) strategies improved responses in both tumor models. Further analysis of 4T1 tumors from mice treated with β-blockade and anti-PD-1 showed an increase in IFN-γ, producing CD8+ T cells compared to either β-blockade or anti-PD-1 alone.

Conclusions
Taken together, these data indicate that elevated β-AR stress signaling caused by cool housing temperatures impairs anti-tumor immunity and the response of tumors to anti-PD-1 checkpoint blockade.

Acknowledgements

Reference

Immune Metabolism

O10
NAD-Sirt1 axis is central to the unique immuno-metabolic phenotype of Th1/17 hybrid cells in regulating its enhanced anti-tumor potential
Shilpak Chatterjee, Anusara Darendhasanam, Paramita Chakraborty, Kyle Toth, Megan Meek, Elizabeth Garrett-Mayer, Michael Nishimura, Cristylaulos, Craig Beeson, Xuezhong Yu, Shikhar Mehrotra
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Background
Th17 cells hold promise for immunotherapy of cancer [1]. While the anti-tumor potential of Th17 cells primarily depends upon IFN-γ secretion and persistence [1], a long-term tumor control has still remained elusive. Given that both the “effector” and “stemness like” features are prerequisites for T cells to mount durable anti-tumor responses, we hypothesized that combining the culture conditions of Th1 (effector) and Th17 (stemness like) cells could generate hybrid Th1/17 cells with improved anti-tumor properties.
Methods
Melanoma epitope tyrosinase reactive CD4+ T cells obtained from h3T TCR transgenic mice were differentiated ex vivo to Th1, Th17, and Th1/17 cells before adoptive transfer (0.25x10^6 cells/animal i.v.) to C57BL/6 recipient animals with subcutaneously established B16 melanoma. Quantitative PCR (q-PCR), flow cytometry, and metabolomic analyses were used to evaluate the expression of various metabolism and stemness associated genes as well as protein expression in the T cells. To compare the metabolic commitment between different subsets (Th1, Th17 and Th1/17), real time metabolic flux analyzer (Seahorse Biosciences, USA) and radioactive tracer studies were used.

Results
The combined culture conditions of Th1 and Th17 generates hybrid Th1/17 cells with a IFNyhi, IL17hi, GM-CSFhi, 4D107a2hi, T-bethi, Granzyme Bhi, IL23Rhi, IL22hi, Bcl6hi, Tcf7hi signature. These hybrid Th1/17 cells exhibit enhanced tumor control in subcutaneous and lung metastasis models of murine melanoma. A hypothesis generating transcriptional, metabolic, and proteomic profiling, followed by confirmatory experiments established that the enhanced anti-tumor properties were attributed to increased NAD+ mediated activity of histone deacetylase Sirt1 in hybrid Th1/17 cells. Inhibition of NAD+ and Sirt1 activity either pharmacologically or by genetic ablation (Sirt1-KO T cells) led to loss of stable anti-tumor control. Importantly, anti-tumor T cells or tumor infiltrating lymphocytes programmed in the presence of exogenous NAD+ also led to the similar metabolic phenotype and improved anti-tumor control.

Conclusions
The present study discloses that metabolic status plays an important role in dictating the anti-tumor response of the T cells. Combining the culture conditions of Th1 and Th17 cells renders hybrid Th1/17 cells with a unique immune-metabolic feature that enables them to orchestrate distinct transcriptional programs leading to highly effector and stem-like T cells.

Reference

O11
The Wnts5a-beta-catenin pathway triggers a metabolic switch that drives indoleamine 2,3-dioxygenase activity and dendritic cell tolerization in the melanoma microenvironment: optimizing checkpoint inhibitor immunotherapy
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Background
Despite recent advances, many cancers remain refractory to available immunotherapies by developing various strategies to evade the immune system. Emerging evidence indicates that the tolerization of local dendritic cells (DCs) within the tumor microenvironment plays a critical role in immune evasion. The role of metabolic re-programming in DC tolerization remains poorly characterized and the mechanisms by which cancers may utilize these pathways to promote the establishment of an immunotolerant microenvironment have not been described.

Methods
We investigated the role of the Wnt-beta-catenin pathway in the metabolic reprogramming of melanoma-derived DCs using real-time metabolic flux analysis. The impact of DC metabolic re-programming on the enzymatic activity of indoleamine 2,3-dioxygenase (IDO) was analyzed by HPLC while protoporphyrin IX(PpIX) levels were quantified by flow cytometry. The role of DC fatty acid oxidation (FAO) on regulatory T cell (Treg) generation was investigated using pharmacologic and genetic approaches. The impact of FAO inhibition on anti-tumor immune responses to anti-PD-1 antibody therapy were investigated in a transgenic melanoma model.

Results
We show that the Wnts5a-beta-catenin-PPARg pathway shifts DCs from glycolysis to FAO in the melanoma microenvironment in a manner dependent upon induction of the mitochondrial fatty acid transporter, CPT1A (Fig. 9). This metabolic shift promotes DC tolerization by 1) elevating DC levels of the PpIX prosthetic group of IDO, resulting in the enhanced activity of this enzyme (Fig. 10) and 2) potently suppressing DC-expression of IL-6 and IL-12, both culminating in the generation of Tregs both in vitro and in vivo (Fig. 11). Genetic silencing and the pharmacologic inhibition of CPT1A potently enhances the ability of DCs to stimulate effector T cell responses. Indeed, genetic silencing of melanoma-expressed Wnts5a significantly promotes T cell tumor infiltration and augments PD-L1 expression in this melanoma model. Consistent with these findings, we further show FAO inhibition to enhance the efficacy of anti-PD-1 therapy while augmenting melanoma antigen-specific T cell responses (Fig. 12).

Conclusions
Our findings implicate the Wnts5a-beta-catenin-PPARg-CPT1A paracrine signaling axis as a driver of DC FAO and functional DC tolerization in the melanoma microenvironment and connect this pathway with the promotion of a “non-inflamed” phenotype in melanoma. This work describes a novel association between DC metabolism and the regulation of IDO enzymatic activity and suggests that this pathway may be a potent pharmacological target for increasing the responsiveness of “non-inflamed” tumors to anti-PD-1 antibody immunotherapy.

Fig. 9 (Abstract O11), Wnts5a Promotes DC FAO in the Melanoma Microenvironment. A. Schematic of tumor-infiltrating DC (TIDC) metabolic analysis. B. Melanoma-derived Wnts5a promotes TIDC OXPHOS. C. Wnts5a promotes DC FAO

Fig. 10 (Abstract O11), Wnts5a-Induced FAO Promotes DC Synthesis of PpIX and Enhances IDO Enzyme Activity. A. Wnts5a stimulates DC PpIX synthesis. B,C. Wnts5a promotes DC IDO activity in a FAO-dependent manner both in vitro and in vivo
Mitochondrial biogenesis is repressed in tumor-infiltrating CD8+ T cells resulting in metabolic insufficiency and T cell dysfunction

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Background
CD8+ tumor-infiltrating T lymphocytes (CD8+ TIL) in the tumor microenvironment (TME) are unable to effectively control their tumor targets due to a variety of immunosuppressive mechanisms, including direct tumor cell-T cell inhibition and soluble immunosuppressive factors. This allows cancer to progress unchecked as T cells are rendered functionally inert. Recently, poor metabolite availability in the TME has been identified as an additional suppressive mechanism exploited by bioenergetically-dysregulated tumors. Because T cell activation also has robust metabolic demands, we hypothesized that CD8+ TIL dysfunction was a result of metabolic insufficiency.

Methods
Metabolic capacity was measured at the single cell level by 2NBDG and MitoTracker FM. Metabolic output was measured by Seahorse extracellular flux analysis. T cell reprogramming was performed by retroviral transduction on OVA-specific transgenic T cells in vitro before adoptive transfer into B16-OVA bearing mice.

Results
We found CD8+ TIL are characterized by dramatic loss of mitochondrial mass in B16, MC38, and LLC implantable mouse tumors. We found increased mitochondrial mass, restored cytotoxic functionality, and dramatically improved tumor regression in mice with reprogrammed CD8+ TIL. To better understand why mitochondrial loss causes T cell dysfunction, we are exploring the importance of biogenesis of mitochondria for T cell functionality, including ATP and nucleotide production, calcium buffering, and ROS production.

Conclusions
Our data support a model in which chronically-activated CD8+ TIL are unable to metabolically support their effector functions. By understanding these metabolic insufficiencies, we can both better understand T cell dysfunction and design metabolic modulation strategies to improve cancer immunotherapy.
Oncolytic Viruses

O14
Phase I/II CANON study: oncolytic immunotherapy for the treatment of non-muscle invasive bladder (NMIBC) cancer using intravesical Coxsackievirus A21
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Background
As a clinical setting in which local live biological therapy is already well established, non-muscle invasive bladder cancer (NMIBC) presents intriguing opportunities for oncolytic virotherapy. Coxsackievirus A21 (CVA21, CAVATAK™) is a novel intercellular adhesion molecule-1 (ICAM-1)-targeted immunotherapeutic virus which exerts potent oncolytic activity against NMIBC cell lines and ex-vivo human bladder tumour. CVA21 in combination with low doses of Mitomycin C enhances CVA21 viral replication and oncolysis by increasing surface expression levels of ICAM-1.

Methods
A two stage Phase I/II study (CANON) was initiated to study the tolerance of escalating intravesicular (IV) doses of CVA21 administered alone or in combination with MitomycinC (10mg) in 16 first-line NMIBC cancer patients prior to TURBT surgery. Cystoscopy photography was performed before and after treatment. Tissues were analysed for CVA21 replication, apoptosis, changes in immune cell infiltrates (multi-spectral imaging) and immune-checkpoint molecules.

Results
IV administration of CAVATAK was well tolerated with no adverse events. Anti-cancer activity including viral induced tumour inflammation was demonstrated by serial cystoscopy including a complete response observed in one of 3 patients in the highest dose monotherapy cohort. Tumour targeting by CVA21 was shown by detection of secondary viral load peaks in the urine and by immunohistochemical analysis of TURBT surgery. Cystoscopy photography was performed before and after treatment. Tissues were analysed for CVA21 replication, apoptosis, changes in immune cell infiltrates (multi-spectral imaging) and immune-checkpoint molecules.

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Methods
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Fig. 15 (Abstract O13). cOTU 1614, which includes members of family Eubacteriaeaceae is associated with decreased relapse after allo-HCT. Cumulative incidence of relapse/POD in the discovery (n = 271) and validation (n = 270) sets stratified by presence or absence of cOTU 1614

Fig. 13 (Abstract O13). Phylogenetic tree of OTUs and clusters of related operational taxonomic units (cOTUs). Each black point is a cOTU. Phylum is color coded along the circumference. Members of the same phyla were largely grouped together, indicating that the tree was broadly concordant with standard taxonomy

Fig. 14 (Abstract O13). Multivariate screening of microbial features for association with relapse. Volcano plot of multivariate p values of cOTUs against the multivariate hazard ratios for relapse/progression of disease in the discovery set. cOTUs are color coded by p value. Multivariate adjustment was performed for Disease Risk Index score, graft source, and conditioning intensity. The most abundant species in each of the labeled cOTUs are 1614: Eubacterium limosum. 2022-3: Streptococcus sinensis. 1638: Eubacterium limosum. 1630-1: Eubacterium limosum. 1790: Parvimonas micra. 0951-3: Leptotrichia hongkongensis 2986: Flavonifractor plautii. 1439: Actinomyces odontolyticus

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Conclusions
The utility of CVA21 as a potent immunotherapeutic agent has been demonstrated by the observed tumour targeting and viral replication. Upregulation of checkpoint molecules following CVA21 exposure may also allow potential sequential combination therapies with checkpoint targeting.

Trial Registration
ClinicalTrials.gov identifier NCT02316171.

Pre-existing immunity to oncolytic virus potentiates its therapeutic efficacy.
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Background
Despite the significant promise of oncolytic viral (OV) therapy in preclinical models, clinical efficacy of systemically-administered viruses has proven to be modest. One major limitation of the systemic OV therapy is neutralization of the virus by pre-existing immunity, or development of neutralizing antibodies shortly after therapy initiation, which limit viral delivery to tumor sites. Recently, we and others have demonstrated that intratumoral therapy with OV can lead to systemic anti-tumor immunity and abscopal effects, and several clinical trials are currently exploring intratumorally administered OVs in patients. The effect of pre-existing anti-viral immunity or the development of new anti-viral immunity on the anti-tumor efficacy, however, is not well defined.

Methods
Using oncolytic Newcastle Disease Virus (NDV) as a model, we explored the effect of pre-existing immunity to the virus on its therapeutic efficacy using syngeneic B16-F10 melanoma and MB49 bladder carcinoma models.

Results
BL6 mice were immunized with NDV and subsequently implanted with B16 or MB49 murine cancer cells. Immunized and naive tumor-bearing mice were treated intratumorally with NDV. As expected, pre-immunized animals demonstrated decreased levels of NDV replication. Surprisingly, pre-existing immunity to the virus did not decrease the antitumor efficacy and led to superior tumor clearance and long-term animal survival. Analysis of tumor-infiltrating lymphocytes from the treated animals demonstrated marked increase in infiltration with CD8+ and CD4 +FOXP3- cells, and significant decrease in CD4+FOXP3+ cells, an effect that was significantly more pronounced in the pre-immunized animals. This was observed in both virus-injected and contralateral flank tumors, in absence of viral spread to distant tumor sites. Concurrent adoptive transfer of luciferase-tagged tumor-specific Trp-1 lymphocytes demonstrated increased intratumoral accumulation of Trp-1 cells in pre-immunized mice. Furthermore, lymphocytes isolated from tumors of NDV-treated pre-immunized mice produced more IFNg than those of NDV-treated naive mice when cultured with tumor cells in vitro, suggestive of antigen spreading. Finally, in an animal model of recurrent cancer after “cure” with NDV, re-treatment with NDV resulted in regression of tumors and long-term animal survival, an effect accompanied by significant increase in tumor-infiltrating immune cells.

Conclusions
Our findings demonstrate that pre-existing immunity to OVs might not deter, and even augment the efficacy of intratumoral OV therapy, which is likely mediated by enhanced tumor-specific immune response. This is a clinically-relevant question, which suggests that prior anti-viral immunity should not be a deterrent to OV therapy with locoregional administration, though it remains to be demonstrated whether such findings would translate to other oncolytic viruses.

Promoting and Measuring Anti-Tumor Immunity

Immunoscore® Colon analytical performance
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Background
The Immunoscore® was validated as a powerful prognostic marker in colon cancer in a study conducted by the Immunoscore® worldwide consortium, led by the Society for Immunotherapy of Cancer (SITC) involving 23 pathology centers from 17 countries, and including more than 3800 patients. HalioDx has developed a standardized version of the test that was used in this study. Here we show the concordance with the research version and present the main analytical performances of the system.

Methods
For each colon tumor block, 2 slides are stained using an automated IHC staining instrument: one with CD3 and one with CD8. Digital images of stained slides are obtained using a whole slide scanner, and analyzed by a software program (Immunoscore® Analyzer, HalioDx). The Immunoscore® Analyzer automatically processes images for tissue detection (core of the tumor, CT and invasive margin, IM). Densities of positive lymphocytes in the CT and IM are reported. For each marker and each zone, densities distributions have been established in the SITC study training set. The Immunoscore® is reported in 5 categories from 0 to 4, or as IS High (IS3 and IS4), Low (IS0 and IS1) & intermediate (IS2). Precision of HalioDx Immunoscore® Colon assay in terms of repeatability and reproducibility was evaluated using commercial FFPE colon cancer blocks, with 152 independent stainings from 4 samples, corresponding to 62 CD3 and CD8 pairs. Intra-block and inter-block variability were assessed from 8 additional blocks. Accuracy based on inter-laboratory concordance was determined using 119 samples. The European Hospital Georges Pompidou (HEGP - center of reference for the SITC study) workflow was used as reference.

Results
The inter-instrument, inter-lot and inter-operator/reader precision in terms of cell density (cells/mm²) CV were below 12%, 22% and 18%, respectively. Only 1 change in Immunoscore® category (out of 62 IS assessments) was observed, from IS1 to IS0. The equivalency between HalioDx and HEGP workflows was assessed in terms of cell densities. Releasing regression slopes were not significantly different from 1 for both CD3 and CD8 antibodies. Pearson correlation coefficients were above 0.89. The concordance table is provided in Fig. 16, corresponding to a weighted Cohen’s kappa coefficient of 0.88.

Conclusions
The Immunoscore® Colon is a robust, easy-to-use and accurate assay. It is the first standardized immune-based assay for the classification of cancer.

References
Mechanisms of chitosan/IL-12 immunotherapy for the treatment of bladder cancer

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Background
Bladder cancer afflicts 430,000 people every year globally and is plagued by recurrence rates as high as 50%. One way to mitigate the risk of recurrence is by engaging adaptive immunity. Our group has been able to direct adaptive immunity via intravesical treatment with CS/IL-12, a coformulation of interleukin(IL)-12 and the biopolymer chitosan. Four twice-weekly administrations of CS/IL-12 routinely eliminate more than 90% of orthotopic bladder tumors in mice while providing systemic protection from recurrence and rechallenge for the duration of the lifespan of treated mice. The purpose of this study is to gain insights into the mechanisms underlying both the initial elimination and later rejection of bladder tumors by exploring the importance of the number of administrations, lymphocyte subtypes, and the immune cell infiltration throughout and following treatment.

Methods
Female C57BL/6J mice were implanted orthotopically with 75,000 MB49 bladder cancer cells. Beginning 7 days after implantation, mice were treated intravesically 2x/week for two weeks with CS/IL-12 (1 μg). The importance of the number of treatments was investigated by monitoring survival while varying the treatment number. The role of lymphocyte subtypes was investigated by monitoring survival after depleting CD4+, CD8+, or NK1.1+ cells prior to and throughout treatment or rechallenge. Cellular responses 24 hours after each treatment were measured in the bladder, bladder draining lymph nodes (BDLNs), and the spleen via flow cytometry.

Results
Varying the number of treatments revealed that a single administration significantly extended survival beyond saline with 4/10, 2/8, 6/9, and 7/8 mice surviving long term after 1, 2, 3, or 4 applications respectively. Depletion studies showed a dependence on CD8+ T cells for tumor elimination (Fig. 17a) and on CD4+ T cells for rejection of subsequent tumor rechallenge (Fig. 17b). Flow cytometry revealed fluctuations in the immune-cell populations over the course of treatment (Fig. 18). The first treatment was characterized by a 54% increase of macrophages in the bladder and a 56% increase in the CD8:Regulatory T cell ratio in the BDLNs. By the third treatment there was an influx of CD4+ and CD8+ T cells in the bladder as well as increased CD8+ T cells in the BDLNs.

Conclusions
Even a single administration of CS/IL-12 eliminates established tumors, though higher rates of survival were possible with 3 or 4 treatments. The initial response is inflammatory and driven by macrophage infiltration and CD8+ T cells while the memory responses is directed by CD4+ T cells.
Survivorship Issues Related to Immunotherapy

O18 Incidence and outcomes of central nervous system metastasis in metastatic melanoma patients treated with anti-PD-1 therapy
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Methods
Under an IRB-approved protocol, outcomes of MM patients treated with anti-PD-1 at The University of Texas MD Anderson Cancer Center from January 2012 to February 2016 were reviewed. The association between development of CNS metastasis and overall survival (OS) was assessed using Cox regression analysis with time to CNS metastasis treated as a time-varying covariate.

Results
We identified 264 MM patients who received anti-PD-1 treatment, including 74 (28%) who had CNS metastasis prior to the first dose of anti-PD-1. With a median follow-up of 10.4 months (range 0-51.6) from the start of this therapy, 37 (19% of patients without prior CNS metastasis) developed CNS metastasis after the initiation of anti-PD-1. Of those, 27 patients were diagnosed with CNS metastasis during anti-PD-1 or within 90 days of treatment discontinuation, and 10 patients were diagnosed with CNS metastases >90 days after last anti-PD-1 dose. The majority of these patients were male (62%), their mean age at new CNS metastasis was 62 years (range 31-86), and most patients had a history of cutaneous primary (59%). Of the 26 patients who were tested for mutations, BRAF was identified in 8 (22%), NRAS in 5 (14%) and KIT in 6 (16%). 86% received at least one CNS directed treatment approach. 62% were treated with stereotactic radiosurgery, 11% received whole-brain radiation and 30% underwent surgery. Median OS from start of anti-PD-1 was 34 months (range 0-51.6 months) for the whole anti-PD-1 treatment cohort. Development of CNS metastasis while on anti-PD-1 therapy was strongly significantly associated with death (HR 3.39, 95% CI 2.06, 5.59, p < .0001).

Conclusions
To our knowledge, this is the first report describing the incidence of CNS metastasis as an initial site of disease progression in MM patients treated with anti-PD-1 and associated with worse OS, despite additional CNS directed therapy.

Tumor Microenvironment

O19 CD8α+ dendritic cells regulate leukemia antigen-specific CD8+ T cell tolerance
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Methods
Following systemic introduction of viable, CellTrace violet-labeled AML cells, leukemia cell-derived fluorescence was observed exclusively within splenic CD8α+ DCs, whereas uptake of proteins from dead AML cells was mediated by CD11b+ macrophages. CD8α+ DCs were also uniquely capable of cross-presenting leukemia antigens to CD8α+ T cells directly ex vivo. Interestingly, antigen
encounter by leukemia-specific CD8+ T cells was severely reduced in Batf3−/− mice, indicating that CD8α+ DCs mediate T cell recognition of leukemia antigens, and that their absence is associated with immunological ignorance of AML antigens. Moreover, leukemia-specific CD8+ T cells in wildtype AML-bearing mice failed to respond following vaccination with the tolerizing antigen, while those in leukemia-bearing Batf3−/− mice expanded vigorously, demonstrating that CD8α+ DCs induce leukemia-specific tolerance in vivo. Activation of CD8α+ DCs with the TRIF-3 agonist, poly(I:C) restored functional anti-leukemia T cell responses and protected mice from disease progression in a Batf3-dependent manner. RNA-seq analysis of "tolerogenic" versus "naive" CD8α+ DCs from leukemia-bearing mice revealed ~200 differentially expressed genes in the former, suggesting that tolerance induction by CD8α+ DCs is an active process.

Conclusions
Our data support a growing body of evidence that has defined a prominent role for Batf3-dependent DCs in regulating anti-cancer immune responses. Batf3-lineage DCs generate functional CD8+ T cell responses against solid tumors, but actively and exclusively induce CD8+ T cell tolerance to systemic leukemia, indicating that the same DC lineage can imprint disparate T cell fates in mice with solid versus hematopoietic malignancies, and suggesting that environmental cues perceived by CD8α+ DCs may dictate their ability to activate or tolerate cancer-specific CD8+ T cells. These results highlight stark differences in the regulation of anti-cancer immunity in hosts with solid versus hematological malignancies.

Adoptive Cellular Therapy

P1
Converting tumor-mediated PD-L1 inhibition into CAR T cell costimulation to potentiate adoptive T cell therapy
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Journal for ImmunoTherapy of Cancer 2016, 4(Suppl 1):P1

Background
To overcome tumor-mediated inhibition of chimeric antigen receptor (CAR) T cells, we herein investigated the impact of tumor PD-L1 upregulation on CAR T cell exhaustion and anti-tumor efficacy, and further developed clinically translatable T cell-extrinsic as well as -intrinsic strategies to overcome PD-L1 inhibition in models of lung cancer (LC) and malignant pleural mesothelioma (MPM).

Methods
Human T cells were transduced with MSLN-specific CAR with CD28 and CD3zeta domains (M28z) were tested in vitro and in clinically-relevant LC and MPM mouse models by bioluminescence imaging (BLI) of tumor burden progression. To counteract PD-1/PD-L1 inhibition in vivo, we evaluated the efficacy of PD-1 blocking antibody or cell-intrinsic genetic-engineering strategies by cotransducing M28z CAR T cells with a PD-1 dominant negative receptor (PD1-DNR) or with PD-1/4-1BB fusion protein.

Results
A single, low-dose of M28z CAR T cells is able to resist the progression of established tumor for 40 days, but mice eventually died with progressing tumor. Tumor harvest analysis demonstrated the PD-1 and PD-L1 upregulation on CAR T cells and tumor cells (Figure Panel A). We then confirmed in vitro that PD-L1 inhibits M28z T cell effector functions (proliferation, cytotoxicity and cytokine secretion). The addition of PD-1 blocking potentiates CAR T cell therapy in vivo but its efficacy requires multiple injections (Panel B). In contrast, a single dose of M28z T cells coexpressing PD-1-DNR restore effector functions, enhance tumor burden control (Panel C) and prolong median survival (56 vs 82 days, p=0.001). Converting PD-L1 inhibition into a positive costimulatory signal by PD-1/4-1BB construct co-transduction into M28z CAR T cells enhanced cytokine secretion and T cell accumulation (Panel D).

Conclusions
Our results demonstrate the therapeutic benefit of providing optimal costimulation and coinhibitory blockade to counteract PD-L1/PD-1 immunosuppression, thus potentiate CAR T cell therapy for lung cancer and mesothelioma.

References
Lack of moesin improves adoptive T cell therapy by potentiating anti-tumor functions

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Background
Moesin is a member of the ezrin-radixin-moesin (ERM) protein family that are crucial for organizing membrane domains [1]. However, the role of ERM proteins in regulating signal transduction activities is still less clear and identifying new target proteins regulated by the ERMs for drug targeting remains an important area within the field due to their increased levels in multiple cancers. Whether ERM proteins play any role during the differentiation of naïve CD4+ T cells to TGF-β-induced Tregs is completely unknown.

Methods
We utilized a combination of knockout (MsnKO) mice, polyribosome profiling, RT-PCR and immunoblotting to demonstrate that a lack of moesin promotes efficient adoptive T cell therapy in mice by controlling translational upregulation of moesin by TGF-β in T cells.

Results
The lack of moesin led to poorer development and function of both peripherally-inducible Tregs and in vitro-induced Treg cells (Fig. 20). We found that the loss of moesin significantly delayed tumor recurrence in a mouse model of melanoma and supported the rapid expansion of adoptively transferred CD8+ T cells against cancer-associated antigens (Fig. 21). Of note, moesin knockout CD4+ T cells exhibited no defects in T cell receptor activation, proliferation or cytokine production, suggesting no alternations in T cell activation in these mice. Instead, our data indicate that moesin interacts with TGF-β receptor II and controls its surface abundance and stability (Fig. 22). Indeed, the lack of moesin significantly impaired optimal TGF-β signaling (Fig. 23) and improved adoptive T cell therapy under cancer setting (Fig. 24).

Conclusions
This finding is important and suggests that modulation of moesin (via inhibitors or agonists), such as developed recently for ezrin [2], could serve as a potential candidates for use in immunotherapy combinations for the treatment of cancer as well as advance our knowledge.

Acknowledgements
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References
Fig. 22 (Abstract P2). a Immunoprecipitation (IP), with anti-TβRII and control immunoglobulin G antibodies of EL4 cell lysates, followed by immunoblot of pulldown and input samples with the indicated antibodies. b Confocal microscopy images of EL4 cells stimulated with TGF-β (5 ng/ml for 1 h) and stained with anti-TβRII and anti-moesin antibodies. c and d HEK293FT cells co-transfected with plasmids encoding wild-type moesin-tagged with CFP at the carboxy terminus (Msn-WT-CFP) and TβRII-tagged with haemagglutinin at the carboxy terminus (TβRII-HA). Immunoprecipitation of solubilised proteins using anti-GFP and anti-HA antibodies and immunoblot of the pull-down samples. Input - whole cell lysate immunoblotting (throughout). e-g Immunoprecipitation and immunoblot (as in c) of HEK293FT cells co-transfected with CFP-tagged wild-type or phosphomimetic moesin mutants and TβRII-HA constructs. Data are representative of at least three (a-c) or four (d-f) independent experiments. h and i Primary CD4+ T cells (h) and B220+ B cells (i) from the spleen of WT and MsnKO mice were treated with cyclohexamide at the indicated times and surface TβRII analyzed by flow cytometry. Data represents the mean ± SD of at least three independent experiments. ***P <0.001 by two-way analysis of variance (ANOVA). j Flow cytometry analysis of primary CD4+ T cells isolated from the spleen of WT and MsnKO mice, and treated with brefeldin A (BFA), 20 μg/ml for up to 5 h and then washed. Cell surface TβRII was left to recover for up to 12 h prior to analysis. Data represents the mean ± SD of at least three independent experiments. ***P <0.001 by two-way analysis of variance (ANOVA).
**P3**

Preclinical evaluation of an optimal-affinity MAGE-A4 T cell receptor for adoptive T cell therapy

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**Background**

Adoptive immunotherapy employing optimal affinity T cell receptor (TCR) engineered T cells is a highly attractive treatment modality for multiple cancer indications. In order to ensure the safety of novel T cell receptor therapies, it is important both that expression of the target antigen is tightly restricted to tumor, and that the TCR does not display off-target activity. Here we describe development of an optimal-affinity MAGE-A4 TCR for adoptive T cell therapy.

**Methods**

Expression profiling of the cancer-germline antigen MAGE-A4 was performed in tumor and normal tissues, determined by analyzing public RNAseq datasets and by in-house qPCR. We then generated an enhanced affinity TCR that recognizes a validated MAGE-A4 HLA-A*02 peptide, selected based on potency and specificity in in vitro testing from panels of engineered TCRs originating from multiple parental TCRs. The selected TCR was subject to full preclinical characterization using Adaptimmune’s extensive preclinical testing process. This process involves potency testing against both tumor cell lines and primary tumor tissue in 2D and 3D, and safety testing consisting of extensive screening of TCR-transduced T cell responses to a wide range of tumor lines, normal human primary cells and induced pluripotent stem cell-derived cells. In addition, the fine specificity of the TCR was characterized to allow the generation of a binding motif and the identification of putative mimotope peptides within the human proteome.

**Results**

The MAGE-A4 antigen was found to be highly over-expressed in several clinically important solid tumor indications, such as lung squamous cell cancer (60%), head and neck cancer (42%), bladder cancer (34%) and esophageal cancer (33%), while expression in non-tumor material was limited to expression in the testes and placenta, both immune-privileged tissues. We generated an enhanced-affinity TCR that demonstrated enhanced potency against MAGE-A4-expressing tumor cell lines and fresh tumor tissue, whilst retaining absence of relevant response against MAGE-A4-negative cells and non-MAGE peptide mimotopes.

**Conclusions**

MAGE-A4 is an attractive target antigen for adoptive T cell therapy using enhanced affinity TCRs. We have generated and characterized an optimal-enhanced-affinity TCR, which shows enhanced potency against MAGE-A4-positive tumor targets whilst maintaining specificity. These data will be used to support an IND for the use of this TCR for investigatory clinical trials.

**P4**

Case report: specific peptide enhanced affinity receptor T cells (SPEAR® T cells) demonstrate long-term persistence and both in vivo and ex vivo tumoricidal activity

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**Background**

SPEAR® T cells reactive against the NY-ESO-1 specific HLA-A02:01 restricted peptide (SLLMWITQC) have demonstrated clinical activity (ORR 50%) in patients (n=12) with advanced synovial sarcoma (SS). The mechanisms underlying tumor relapse in the presence of persisting SPEAR® T cells remain unclear. Here, we report on phenotypic and functional studies on both engineered T cells and tumor biopsies from a patient with a NY-ESO-1 SS treated with NY-ESO-1259 SPEAR® T cells.

**Methods**

Engineered T cell persistence was determined by qPCR for the vector backbone and flow cytometry for HLA-A2:01-SLLMWITQC reactive pentamer+ T cells in post-infusion PBMC samples. Multi-parameter flow cytometric analyses were performed on pre-infusion manufactured product and post-infusion PBMCs to assess memory subsets using CD45RA andCCR7, exhaustion using CD28 and PD-1, and functionality by IFN-γ and Gzmb. Tumor and NY-ESO-1259 T cells from patient PBMCs were isolated at 28 months post-infusion to determine their ex vivo killing capacity against a NY-ESO-1 cell line, A375. Antigen expression and immunomodulatory milieu (e.g. PD-L1) in baseline and post-treatment biopsies were assessed by immunohistochemistry. Serum cytokines were measured by a Luminex based immunoassay. Tumor response was determined by RECIST v1.1.

**Results**

The patient achieved a partial response to NY-ESO-1259 SPEAR® T cells with progression at 9 months post-infusion. Persistence at 28 months with NY-ESO-1259 T cells was observed by qPCR and flow cytometry. Over the course of treatment, the phenotype of the engineered cells changed from a mix of TEMRA (CD45RA+CCR7), TEM (CD45RA+CCR7), and TEMSCM (CD45RA+CCR7) populations at the time of infusion to a predominately TEMSCM (~98.7%) within five months. PBMC derived NY-ESO-1259 SPEAR® T cells 28 months post-infusion exhibited substantial ex vivo killing of NY-ESO-1 A375 cells without additional ex vivo re-stimulation. Pre- and post-infusion biopsies showed NY-ESO-1 expression and exhibited minimal to moderate leukocytic (CD45+) infiltration accompanied by minimal lymphocytic infiltration post-infusion. Of note, PD-L1 expression was exclusive to CD45+ cells.

**Conclusions**

Despite an initial response to NY-ESO-1259 SPEAR® T cells, this patient eventually relapsed despite the persistence of functional SPEAR® T cells and antigen positive tumor. The basis for tumor progression following response remains unclear, but does not appear to result from T cell exhaustion. Other possibilities include loss of antigen expression and/or diminished tumor infiltration, which could result from the large peripheral TEMSCM population, known to traffic to lymphoid tissue rather than tumor.

**Trial Registration**

ClinicalTrials.gov identifier NCT01343043.

**Consent**

Written informed consent was obtained from patient for publication. A copy is available for editor review.

**P5**

Engineering 2nd generation SPEAR® T cells to overcome TGF-β-mediated immunosuppression for adoptive cell therapy

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**Background**

Adoptive cell therapy (ACT) with NY-ESO SPEAR® T cells, is showing promising initial clinical responses in phase II/III trials for both solid and liquid tumors including synovial sarcoma and multiple myeloma. However, the depth and durability of response may be affected by the inhibitory tumor microenvironment. Tumors utilize many different methods to inhibit anti-tumor immunity including secretion of inhibitory cytokines, such as transforming growth factor-β (TGF-β) and...
induction/recruitment of other inhibitory cells including regulatory T cells and myeloid-derived suppressor cells. These inhibitory cells also secrete cytokines such as IL-10 and TGF-β that potentially reduce the efficacy of T cells. TGF-β is expressed at high levels in a range of cancer indications.

Methods

We investigated whether SPEAR® T cells can be engineered to express additional proteins, allowing them to overcome such immune resistance mechanisms, potentially improving clinical responses. TGF-β inhibits T cells by binding to a dimer of TGFβRII, which then recruits a dimer of TGFβRI forming a heterotetrameric complex that activates inhibitory intracellular SMAD signaling pathways. Truncating TGFβRII had a dominant negative effect on TGFβ signaling, resulting in the inhibition of T cells by binding to a dimer of TGFβRII that, although capable of binding TGF-β, is unable to signal. We therefore generated SPEAR® T cells co-expressing enhanced affinity T cell receptors (TCR) that recognize a tumor antigen, and tested their function in vitro.

Results

Firstly we showed that the function of NY-ESO SPEAR® T cells is inhibited with physiologically-relevant concentrations of TGF-β. We further show that dnTGFβRII can be co-expressed with enhanced affinity NY-ESO TCR in SPEAR® T cells. T cells expressing dnTGFβRII had reduced SMAD phosphorylation in response to TGF-β compared with cells expressing TCR alone, indicating that inhibitory signaling in response to TGF-β was reduced. Subsequently we showed that T cells expressing dnTGFβRII were partially or completely resistant to the effects of TGF-β, using assays for T cell proliferation, cytokotoxicity (in 2D and 3D microtissue models) and Th1 cytokine release (IFN-γ and IL-2) in response to antigen positive tumor cells.

Conclusions

Together these data indicate that co-expression of dnTGFβRII may be a viable approach to improve the efficacy of SPEAR® T cells in treating cancer.

P6

Inducible MyD88/CD40 (iMC) costimulation drives ligand-dependent tumor eradication by CD123-specific chimeric antigen receptor T cells

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Background

CD123/IL-3Ra is a promising chimeric antigen receptor (CAR)-T cell target due to its high expression on both acute myeloid leukemia (AML) blasts and leukemic stem cells (AML-LSCs). However, the antigen is also expressed at lower levels on normal stem cell progenitors, presenting a major toxicity concern should CD123-specific CAR-T cells show long-term persistence. Here, we describe a CAR platform, GoCAR-T, that uses a proliferation-deficient, first generation, CD123-specific CAR together with a ligand-dependent costimulatory switch (inducible MyD88/CD40 (iMC)) to provide physician-controlled eradication of CD123+ tumor cells and regulate long-term CAR-T cell engraftment.

Methods

T cells were activated and transduced with a bicistronic retrovirus encoding iMC (MyD88 and CD40 cytoplasmic signaling domains fused with tandem copies of FKBPv36 (binding domain for the dimerizing ligand rimiducid (Rim)) and a first generation CAR targeting CD123 (SGF-iMC-CD123). Ligand dependence for costimulation with iMC was assessed in coculture assays with CD123+ AML cell lines (KG1, THP-1 and MOLM-13) by examination of cytokine production and observation by IncuCyte-based live cell imaging. In vivo efficacy was assessed by i.v. injection of 106 EGFFluc-expressing CD123+ THP-1 tumor cells into immunodeficient NSG mice. After seven days 2.5x10⁶ non-transduced or iMC-CD123,ζ-modified T cells were injected and Rim (1 mg/kg) or vehicle only administered i.p. on days 0 and 15 post-T cell injection. Animals were evaluated for tumor burden using IVIS bioluminescent imaging (BLI) and for T cell persistence by flow cytometry and qPCR at day 30 post-T cell injection.

Results

In coculture assays, both CD123 antigen recognition and Rim-dependent iMC costimulation were required for IL-2 production (285±41 versus 2,541±255 pg/ml for control and 1 nM Rim, respectively), robust CAR-T cell proliferation (87-fold increase with Rim stimulation) and enhanced KG1 cell killing. In NSG mice engrafted with CD123+ THP-1-EGFFluc tumor cells, only animals treated with iMC-CD123,ζ-modified T cells and Rim controlled tumor growth, showing a 2-log reduction in tumor burden with Rim treatment. Two weeks after the second Rim injection, CAR-T cells were infrequent (<1.0%) in the spleen and bone marrow of both CAR groups, suggesting that active costimulation is required for CAR-T persistence.

Conclusions

GoCAR-T, a platform comprising a ligand-dependent activation switch and a proliferation-deficient first generation CAR, efficiently eradicates CD123+ leukemic cells when costimulation is provided by systemic rimiducid administration. Deprivation of iMC costimulation results in reduction of CAR-T levels, providing a user-controlled system for managing persistence and safety of CD123-specific CAR-T cells.

P7

Heterodimeric IL-15 treatment enhances tumor infiltration, persistence and effector functions of adaptively transferred tumor-specific T cells in the absence of lymphodepletion

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Background

Adoptive cell transfer (ACT) is a promising immunotherapeutic approach for cancer. Host lymphodepletion is associated with favorable ACT therapy outcomes, but it may cause detrimental effects in humans. Among the benefits provided by lymphodepletion, ablation of cells forming a cytokine “sink” results in high levels of homeostatic cytokines that support proliferation and survival of the transferred lymphocytes. Interleukin-15 (IL-15) is a lymphocyte growth and activation factor presently in clinical trials for immunotherapy of metastatic cancers. We previously showed that bioactive IL-15 in vivo comprises a stable complex of the IL-15 chain with the IL-15 receptor alpha chain (IL-15Ra), termed heterodimeric IL-15 (hetIL-15). In this study, we tested the hypothesis that hetIL-15 administration enhances ACT in the absence of lymphodepletion.
Methods
We evaluated the effects of the combination regimen ACT +hetIL-15 in the absence of lymphodepletion by transferring melanoma-specific Pmel-1 T cells into B16 melanoma-bearing mice. Tumors were analyzed by both flow cytometry and multi-parameter immunohistochemistry. Tumor-infiltrating transfected Pmel-1 were analyzed for their persistence, proliferation and effector functions.

Results
hetIL-15 treatment delayed tumor growth by promoting infiltration and persistence of both adoptively transferred Pmel-1 cells and endogenous CD8+ T cells into the tumor. In contrast, persistence of Pmel-1 cells was severely reduced following irradiation in comparison to hetIL-15 treatment. Importantly, we found that hetIL-15 led to the preferential enrichment of Pmel-1 cells in B16 tumor sites in an antigen-dependent manner. Upon hetIL-15 administration, tumor-infiltrating Pmel-1 cells showed a “non-exhausted” effector phenotype, characterized by increased IFN-γ secretion, proliferation and cytotoxic potential and low level of PD-1. hetIL-15 treatment also resulted in an improved Pmel-1 to Treg ratio in the tumor.

Conclusions
This study shows that hetIL-15 administration improves the outcome of ACT in immunocompetent hosts and is able to replace the need for lymphodepletion prior ACT for cancer therapy. Applications of heterodimeric IL-15 to ACT will provide new tools and techniques for cancer immunotherapy protocols. Elimination of the need for lymphodepletion will make more patients eligible for cell transfer protocols. In addition, IL-15 could be a general method to place T cells into tumors, increasing the success rate of other immunotherapy interventions.

P8
Withdrawn

P9
Partially differentiated polyfunctional T cells dominate the periphery after tumor-infiltrating lymphocytes therapy for cancer
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Background
Infusion of highly heterogeneous populations of autologous tumor-infiltrating lymphocytes (TILs) can result in tumor regression of exceptional duration. Initial tumor regression has been associated with persistence of tumor-specific TILs one month after infusion, but mechanisms leading to long-lived memory responses are currently unknown. Here, we studied the dynamics of bulk tumor-reactive CD8+ T cell populations in patients with metastatic melanoma following treatment with TILs.

Methods
We analyzed the function and phenotype of tumor-reactive CD8+ T cells contained in serial blood samples of sixteen patients treated with TILs.

Results
Polyfunctional tumor-reactive CD8+ T cells accumulated over time in the peripheral lymphocyte pool. Combinatorial analysis of multiple surface markers (CD57, CD27, CD45RO, PD-1 and LAG-3) showed a unique differentiation pattern of polyfunctional tumor-reactive CD8+ T cells. This subpopulation acquired simultaneously expression of the early differentiation marker CD27, alongside typical features of late effector cells such as loss of CD45RO and up-regulation of PD-1 and CD57. The differentiation and functional status appeared very similar from 1 month to 1 year after infusion. Despite some degree of clonal diversification occurring in vivo within the bulk tumor-reactive CD8+ T cells, further analyses showed that CD8+ T cells specific for defined tumor-antigens had similar differentiation status.

Conclusions
We demonstrated that tumor-reactive CD8+ T cell subsets that persist after TIL therapy are mostly polyfunctional, and display a stable partially differentiated phenotype. These atypical CD27+ incompletely differentiated polyfunctional TILs may have a high capacity for persistence and be crucial in keeping patients tumor-free.

Trial Registration
ClinicalTrials.gov identifier NCT00937625.

P10
Peptide vaccine enhances antitumor effect of adoptive cell transfer using genetically engineered T cells
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Background
Adoptive cell therapy (ACT) has shown promise in tumor eradication in cancer, but current methods require harmful and toxic adjunct procedures. Our laboratory has developed a potent peptide vaccination strategy called TriVax that bypasses the necessity of these adjunct procedures. Previous studies show that retrovirally (RV)-transduced T cells are effective in ACT against various cancers. The present study aimed to determine whether RV-transduced T cells could respond to TriVax specific for melanosomal tumor antigen gp100, and to see if the responses could be enhanced when transduced with a constitutively active form of STAT5 (CA-STAT5), which has been shown to increase survival of CD8 effector/effector memory T cell populations.

Methods
CD8 T cells were purified from B6 mouse spleenocytes and RV-transduced with a gene encoding the clonal T cell receptor (TCRs) for mouse gp100. In some experiments, cells were also co-transduced with a gene encoding for CA-STAT5 (co-expressing Thy1.1). Transduction efficiency was assessed using flow cytometry with gp100 tetramer and Thy1.1 antibody. Functional activity was measured using flow cytometry and EliSpot (IFNγ) assays. These cells were then adoptively transferred (1.0x106 tetramer+ cells) into naïve and tumor-bearing congenic CD45.1 B6 mice, which were then vaccinated with TriVax. Tumor growth was assessed 3 times per week, and immune status was assessed in blood using flow cytometry every 7 days.

Results
TriVax administration selectively expanded the ACT cell population expressing gp100-TCR. Cell numbers in spleen indicate a 14-fold expansion 25 days after vaccination from what was initially transferred. When co-transduced with CA-STAT5, an even higher fold expansion (55-fold) was observed. CA-STAT5+ cells also expanded more robustly than CA-STATs- cells when stimulated with a subsequent vaccine boost, demonstrating a 5000-fold increase in cell numbers with 85% of CD8+ T cells also being positive for gp100 tetramer. ACT of these cell populations into tumor-bearing mice also yielded a dramatic increase in cell numbers, which greatly enhanced the survival of mice in treatment groups.
Conclusions
CD8 T cells RV-transduced to express a gp100 TCR and CA-STATS are capable of expansion in response to TriVax, bypassing the necessity of adjunct procedures. Co-expression of CA-STATS greatly enhances the boost effect of TriVax, leading to a dramatic antitumor effect.

Background
Lion Biotechnologies focuses on the development and commercialization of cancer immunotherapies based on tumor-infiltrating lymphocytes (TIL). Cryopreservation is a beneficial process which allows the final cell product to be shipped in a safe manner with less time constraints [1]. Clinical studies using cryopreserved TIL have not been conducted so far. Freezing and thawing of the cells may cause phenotypic changes such as loss of cell surface receptors [2]. Here, we tested fresh versus frozen/thawed TIL samples and evaluated the expression of phenotypic markers.

Methods
Briefly, PreREP TILs were obtained by culturing melanoma tumor fragments in IL-2 (6000 IU/ml). Rapid expansion protocol (REP) cells were initiated using alogeneic PBMC feeder cells with OKT3 and IL-2 in a Grex-100 flask. When the desired confluency was reached, the cells were cryopreserved in a 5% DMSO solution. We used flow cytometry to phenotype the fresh and thaw/rested TIL at 0h, 24h and 7d following reREP TIL. Flow cytometric analysis was performed using fluorescent antibodies specific for TCRα/b, CD4, CD8, CD27, CD28, CD56, CCR7, CD45RA, CD95, PD-1, and CD25.

Results
No significant differences in CD4, CD8 and TCRα/b expression or memory markers comparing fresh TIL versus thaw/rested TIL at 24h was noted. CD27 expression on TIL was reduced by 50% on thawed TIL, however after a 24h resting period the expression recovered. All other surface antigens that we tested were within 10% difference in their expression levels as compared to baseline.

Conclusions
Cryopreservation did not affect the measured phenotypic characteristics of TIL. We are further investigating the possibility of using cryopreserved TIL in a clinical setting.

References

P11
Stable tumor-infiltrating lymphocytes (TIL) phenotype following cryopreservation
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Comparison of RECIST 1.0 to RECIST 1.1 in the evaluation of adoptive cell transfer
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Background
Adoptive transfer of tumor infiltrating lymphocytes (TIL) can effect long-term durable regression in patients with metastatic melanoma but has not been widely tested in common epithelial cancers. When examining the TIL of successfully treated patients with melanoma, a heterogeneous T cell population can be identified with reactivity against melanoma differentiation antigens, cancer germline antigens, and personalized non-synonymous somatic mutations. Common epithelial cancers, including breast cancer, express far fewer somatic mutations than melanoma, however, in a study of metastatic gastrointestinal cancer, lymphocytes targeting neoantigens were identified in the majority of specimens. This pilot study investigates the ability to grow TIL from breast cancer metastases, to identify personalized non-synonymous somatic mutations and potential neoantigens, and to adoptively transfer TIL into patients with breast cancer.

Methods
Eligible patients were evaluated and treated under IRB-approved protocols for tissue procurement, genomic testing, and adoptive cell transfer. Portions of resected tumors were placed in culture under standard TIL conditions. DNA was extracted from tumor and matched normal peripheral blood samples for whole exome sequencing (WES). Non-synonymous somatic mutations were identified and tested for potential immunogenicity using previously described tandem mini-gene and long (25mer) peptide techniques. Recognition was assessed by IFNγ release on ELISPOT and/or CD137 (4-1BB) upregulation with appropriate controls.

Results
Nine patients underwent surgical resection in this ongoing pilot study, and TIL were successfully grown from the tumors of all patients. All were primarily CD3+ (median 79%) with a small population of natural killer cells. Of the CD3+ cells, 7 of 9 patients had a predominantly CD4+ population (median CD4:CD8 ratio 2.2, range 0.4-5.8). For eight tumors, WES was performed, and non-synonymous somatic mutations were identified as potential neoantigens (median count 96.5, range 71-148). Autologous T cell reactivity has been identified against tumor-specific mutations in 6 of 8 patients.

Conclusions
Tumor-infiltrating lymphocytes derived from metastatic breast cancer can react to tumor-specific non-synonymous somatic mutations in vitro. TIL grown from breast cancers are predominantly CD4+ and can form the basis of an adoptive cell transfer experimental approach to patients with metastatic breast cancer.

Trial Registration
ClinicalTrials.gov identifier NCT01174121.
Methods
Eligible patients were enrolled on an IRB-approved protocol of adoptive cell transfer, randomizing patients to receive one of two lymphodepleting regimens prior to transfer of TIL. This study was reported using RECIST 1.0 criteria, with 24 complete responders and 30 partial responders among 99 treated patients [1]. The official tumor measurements of target lesions and notations of non-target lesions were re-evaluated using RECIST 1.1 criteria, which limits the total number of target lesions, the number of target lesions/organ, and uses short-axis measurements for lymph node disease.

Results
By design, the number of target lesions/patient was decreased, from 4 (range 1-10) to 3 (range 0-5). Thirty-eight lymph nodes did not meet short-diameter criteria for target lesions (10-14mm), and an additional 12 measured <10mm were reclassified as “non-pathologic.” With retrospective application of RECIST 1.1, three patients would not have met eligibility criteria for lack of evaluable disease. In assessing overall response to treatment, 25 patients met CR criteria, with an additional 27 with PR. While there were five patients who achieved CR at an earlier time point, overall time to response was not significantly different (median 16.0 v 19.8 months p=0.19). One patient with lymph node disease did not achieve CR by original criteria, was an early CR in this analysis, but recurred three months later.

Conclusions
Adoption of RECIST 1.1 demonstrated comparable response rates for this trial. A hallmark of our modern studies is the durability of response. The following data will be presented: Highly Sensitive Potency Assay for TILs. A) % cytotoxicity of TIL (from patient M1033T-1) when co-cultured with P815 Clone G6 (with and without anti-CD3 antibody) at different ratios of effector to target cells. B) ELISA data showing amount of IFN-γ released at different ratios of effector to target cells. C) % LAMP1 marker expressed by the M1033T-1 when co-cultured with P815 Clone G6 and anti-CD3 at 1:1 effector to target cells for 4 and 24hr co-culture.

Discussion
Our ‘Bioluminescent Redirected Lysis Assay’ (BRLA) using an engineered P815 cell line can be used as an assay to measure TIL potency. It requires no radionuclides and is more efficient than traditional cytotoxicity assays.
have created a system for sorting spheroids based on size. The spheroids can then be cultured in 2-dimensions for the study of chemotherapy sensitivities, and the efficacy of monocyte and interferon therapies for the treatment of ovarian cancer. Further, we have developed a single-photon confocal microscopy protocol for the multi-parameter imaging of live spheroids and monocytes with and without interferons. To create experimental robustness, we have employed a technique to image multiple conditions (4) over long periods of time (14-16 hours), allowing for the simultaneous imaging of both control and experimental conditions. Post-acquisition analysis of the images can be used to study migration of the individual cells within the spheroids, loss of cell dye viability, and migration of monocytes into the spheroids.

Results
We have found that the size of the spheroid defines, in part its sensitivity to standard chemotherapy agents. Post-acquisition analysis of the images have been used to study migration of the individual cells within the spheroids, loss of cell dye viability, and migration of monocytes into the spheroids. The addition of interferons with or without monocytes significantly reduces the movement of the individual cells within the spheroids. Furthermore, we have found that the addition of interferons slows monocyte migration and initiates monocyte attachment and entry into the spheroids.

Conclusions
The combination of novel cell culturing techniques with modern imaging and post-acquisition data analysis will increase our understanding of ovarian cancer response to both standard chemotherapy and novel cell based therapies.

Acknowledgements
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P16
A novel xenograft model of chimeric antigen receptor-mediated toxicity sheds light on the influence of T cell source on the severity of the toxic sequelae
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Background
Chimeric antigen receptor (CAR)-engineered T lymphocytes are demonstrating striking clinical success, yet these treatments can be accompanied by severe on- and off-tumor toxicities. The availability of murine models to study these toxic phenomenon are currently lacking.

Methods
In our model, human T lymphocytes are engineered with a second-generation CD28-based CAR, targeted with a designed ankyrin repeat protein (DARPin) with picomolar affinity against HER2 (anti-HER2 DARPin CAR-T cells), and adoptively transferred into NRG mice at doses ranging from 6 x 10^5 – 1.2 x 10^6 CAR-T cells/mouse.

Results
Toxicity manifested in a drop in core body temperature and weight and, in some cases, lethality. The onset and severity of the toxicity varied with the source of the T lymphocytes (i.e., donor) used to generate CAR-T cells. We evaluated seven different donors and the toxicities associated with each donor’s cell product was reproducible across multiple experiments and multiple manufacturing runs. Anti-HER2 DARPin CAR-T cells were toxic in tumor-free mice, but toxicities were most severe in the presence of HER2+ tumors demonstrating both on-tumor and off-tumor effects. The CAR-T cells did not respond to murine HER-2 indicating that the toxicity was both off-tumor and off-target. The toxicity was not due to the DARPin itself, as CAR-T cells bearing DARpins with other specificities were not toxic in this model. Further characterization of the model indicated that severity of toxicity was dose-dependent and could be exacerbated by the presence of a HER2+ tumor. Moribund mice were found to have aggregates of T cells in their lungs, liver, and heart and displayed a cytokine storm in the blood. The toxicity was triggered by CD4+ T cells in the anti-HER2 DARPin CAR-T cell product. While anti-HER2 DARPin CAR-T cells generated from donors demonstrating reduced toxicity were able to mediate anti-tumor efficacy in vivo in a xenograft model of ovarian carcinoma at low doses, they exhibited a narrow therapeutic window consistent with data emerging from the clinic where CAR-T therapy is effective.

Conclusions
This model offers a promising avenue to test strategies for the prevention or mitigation of toxicities associated with adoptive CAR-T cell transfer as well as insights into the contribution of T cell source to toxicities. Investigations are ongoing.

Acknowledgements
This work was supported by the Samuel Family Foundation, the Terry Fox Foundation, the Canadian Breast Cancer Foundation, and Triumvira Immunologics.

P17
Ex vivo generation of highly purified and activated natural killer cells from human peripheral blood in accordance with GMP/GCTP for clinical studies
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Background
Natural killer (NK) cells play a crucial role during the innate immune responses, and as such form part of the body of immunological defense against various diseases, including infectious diseases and malignancies. Therefore, adoptive immunotherapy using NK cell is emerging as promising treatments for intractable malignancies; however, there has been still developing because of difficulties in culture, shortage of overall effector numbers, contamination of considerable numbers of T cells, and their limited antitumor potencies. We here established the simple feeder-free method to generate purified (>90%) and highly activated NK cells from human peripheral blood-derived mononuclear cells (PBMCs) in accordance with GMP/GCTP for clinical studies.

Methods
Under approval of the institutional ethical committee, PBMCs were collected from healthy volunteers by using CliniMACS Prodigy® (automati c/closed system). CD3+ cells were depleted by CliniMACS CD3 microbeads, and CD3-depleted PBMCs were cultured at a concentration of 5 x 10^7 cells/ml with high concentration of hIL-2 and 5% human AB serum for 14 days. Fresh medium was added every 4-5 days throughout the culture period. Then, we confirmed the expression of surface markers, CD107a mobilization and cell-mediated cytotoxicity against various tumor cells and normal cells with or without monoclonal antibody drugs in vitro and antitumor effects against K562 in vivo.

Results
Among the several parameters, we found that simply 1) only CD3-depletion, 2) high dose IL-2, and 3) use of specific culture medium were sufficient to obtain the highly purified, expanded (~200-fold) and activated CD3/CD56+ NK cells from PBMCs. Almost all activated NK cells expressed lymphocyte-activated marker CD69, and showed dramatically high expression of NK activation receptors (i.e. NKG2D, Nkp30, Nkp46, etc.), interferon-g, perforin and granzyme B. Importantly, only 2 hours reaction at effector/target ratio=1:1 was sufficient to kill almost all K562 cells, and antitumor activity was also representative on tumor bearing mice in vivo. Cytolysis was specific for various tumor cells, but not for normal cells, irrespective of MHC class I expression.

Conclusions
These findings strongly support that NK cells activated by our method is purified, expanded, and near fully activated. The cells were currently under investigation in clinical trial (phase I/IIa).
T cell antigen couplers (TAC) demonstrate strong efficacy against solid tumors with no measurable toxicities, demonstrating an enhanced therapeutic index

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Background
Engineering T cells with chimeric antigen receptors (CARs) is proving to be an effective method for directing T cells to attack tumors in an MHC-independent manner [1, 2]. Current generation CARs aim to recapitulate T cell signaling by incorporating modular functional components of the TCR and co-stimulatory molecules. We sought to develop an alternate method to re-direct the T cell receptor which exploits the native TCR. To this end, we developed the T cell Antigen Coupler (TAC) technology, a membrane-anchored receptor that redirects the TCR and co-receptor in the presence of tumor antigen.

Methods
The utility of the TAC receptor has been assessed using in vitro and in vivo assays. In vitro assays are based on receptor surface staining, cytokine release and cytotoxicity assays. In vivo studies examined the anti-tumor effect of TAC-engineered T cells against established xenografts.

Results
In vitro testing has demonstrated robust and specific cytokine production and cytotoxicity by TAC-engineered human T cells directed against either HER-2 or CD19. In vivo TAC-engineered T cells revealed strong activity against HER-2 expressing solid xenograft tumor models such as MDA-MB-231 and OVCAR-3. Curiously, the TAC-engineered T cells outperformed a matched CD28-based second generation CAR in these models, demonstrating both increased anti-tumor efficacy and reduced toxicity. Whereas, mice treated with CAR engineered T cells showed serious toxicities that were both donor- and dose-dependent, we did not observe any toxicities arising from the TAC-engineered T cell, even at doses that produced complete tumor regression.

Conclusions
These differences in toxicities and efficacy highlight the biological differences of TAC and CAR receptors and indicated the potential for a superior therapeutic index for TAC engineered T cells. Current TACs in development target lymphatic malignancies and have shown great promise in early in vitro and in vivo assays.

Acknowledgements
Samuel Family Foundation, the Terry Fox Foundation, the Canadian Breast Cancer Foundation and Triumvira Immunologics.

References
P20
haNK, a cytotoxic human high affinity natural killer cell line, exerts enhanced ADCC mediated by avelumab (an anti-PD-L1 antibody) against multiple human tumor cell lines
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Background
Immune checkpoint blocks have been implicated in the down-regulation of antitumor immunity. Anti-PD-1/PD-L1 checkpoint inhibitory monoclonal antibodies (mAbs) can restore immune function in the tumor microenvironment, and have demonstrated clinical benefit in patients with melanoma, Hodgkin’s lymphoma, lung and bladder carcinomas, and other tumor types. In addition to its checkpoint inhibitory function, avelumab, a fully human IgG1 anti-PD-L1 mAb, can mediate antibody-dependent cellular cytotoxicity (ADCC) to lyse human tumor cells in the presence of natural killer (NK) cells [1]. NK cells can be used for cancer therapy. However, obtaining sufficient numbers of functionally active NK cells from patients is technically challenging since only about 10% of the population expresses on NK cells the high-affinity FcR that provides maximum ADCC. One alternative is to use established NK cell lines that have antitumor activity. High affinity NK cells (haNK), provided by NantKwest, derived from the human NK cell line NK-92, are genetically engineered to express high-affinity human CD16 (FcγRIIIA-V158) and transduced with the human IL-2 gene. In addition, haNK cells have little inhibitory killer-cell immunoglobulin-like receptor (KIR) expression, a unique feature that may be a factor in their highly cytotoxic activity against a broad range of malignancies. We report here our investigation of 1) whether haNK cells used in combination with avelumab can lyse human tumor cells via the ADCC mechanism, and 2) the factors that may influence this cytotoxic activity.

Methods
Cell lines used in our experiments included human carcinomas of the head and neck, cervix, bladder, and colon, as well as prostate and pancreatic cancers. haNK cells irradiated with 10 Gy were used as effector cells at various effector-to-target-cell ratios in all experiments. Four- and 18-hour 11In labeled assays were performed to evaluate ADCC activity.

Results
Our results show that 1) haNK cells can lyse a range of human carcinoma cells when avelumab is used to target PD-L1 expression; 2) the addition of anti-CD16 neutralizing antibody significantly inhibits ADCC lysis, implicating CD16 ligation as a major mechanism of action for ADCC lysis mediated by haNK and avelumab; and 3) in combination with avelumab, haNK cells mediate significantly higher levels of ADCC lysis than do NK cells isolated from healthy donor peripheral blood mononuclear cells (PBMC).

Conclusions
These results provide a rationale for using haNK cells in combination with avelumab or other ADCC-mediating cytotoxic mAbs to treat human malignancies.

P21
Adoptive transfer of ex vivo-expanded PD-1+ CD8+ and CD4+ T cells eliminates myeloma in mice
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Background
Adaptive T cell therapy (ACT) has emerged as a potential curative therapy for patients with advanced solid tumors. However, for hematologic cancers, identifying and enriching the cancer antigen-reactive T lymphocytes for ACT remains a challenge. Our lab previously demonstrated that blockade of the PD-1/PD-L1 pathway (in the context of non-myeloablative, lymphodepleting whole body irradiation) was capable of eliminating established myeloma in mice [1, 2]. In the current study, we tested whether PD-1 is a marker for myeloma-reactive T cells.

Methods
C57BL/6 (KalwRij) mice were inoculated with 5x10^6 myeloma cells intravenously, and 28 days later, splenic PD-1+ and PD-1- T cells (CD8+ and CD4+) were isolated by flow cytometric sorting. The purified T cells were expanded in culture for 7 days on plate-bound CD3 and soluble CD28 antibodies plus IL-2, IL-7 and IL-15. Some of the expanded T cells were assayed in vitro for myeloma reactivity. Equal mixtures of expanded CD4+ and CD8+ T cells, or each subset alone, were infused to myeloma-bearing Rag1-deficient recipients, and the mice were followed for myeloma progression.

Results
We found that numbers of cancer antigen-reactive T lymphocytes in myeloma-bearing mice were enriched in PD-1+ CD8+ and CD4+ T cell subsets. PD-1+ T cells could be efficiently expanded ex vivo for adoptive transfer, and the expanded cells maintained their anti-myeloma reactivity. Adoptive transfer of the ex vivo-expanded PD-1+ T cells effectively eliminated established myeloma in Rag1-deficient recipients. In contrast, adoptive transfer of expanded PD-1- T cells failed to demonstrate anti-myeloma efficacy in vivo. Notably, both CD8+ and CD4+ PD-1+ T cell subsets played a role in eradicating myeloma, but combined administration of the ex vivo-expanded subsets was most efficacious.

Conclusions
Our results show that PD-1 is a biomarker for myeloma-specific CD8+ and CD4+ T cells in mice. Furthermore, these PD-1-expressing T cells can be expanded in culture for effective adoptive cell immunotherapy of myeloma-bearing recipients.

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References

P22
Entinostat sensitized osteosarcoma cells for cytotoxic effect of natural killer cells
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Background
The goal of this study is to find an alternate therapy for osteosarcoma (OS) lung metastasis. We previously showed that NK cell therapy significantly decreased, but did not cure, OS lung metastasis in a mouse model. Other studies have shown that histone deacetylase inhibitors (HDACi) sensitize tumor cells to NK cell cytotoxicity, primarily by increasing expression of ligands for NK
cells on tumor cells; thus, to augment NK cell therapy, we combined it with the HDACi, entinostat.

**Methods**

Flow cytometry, western blot analysis, and Q-PCR were used to determine whether entinostat increased expression of NK cell ligands on OS cells. Effects of entinostat on NK cell viability, receptor expression, and cytotoxicity were explored using a viability test, flow cytometry, and calcin release assay, respectively. NK cells were isolated from blood buffy coats and were expanded ex vivo using genetically engineered K562 cells and human IL-2. Q-PCR was used to measure microRNAs expression in OS cells and a CHIP assay was used to determine increased histone acetylation at the MICA/B gene promoters. For the in vivo study, mice were given 10, 5, or 2.5 mg/kg of entinostat orally to determine the subtherapeutic dose of the drug.

**Results**

We demonstrated that 2 μM entinostat for 48 h upregulated expression of NK cell ligands on OS cell lines. Increased expression of ligands on OS cells resulted in increased susceptibility of OS cell lines to NK cell cytotoxicity in vitro. NK cell treatment with up to 2 μM entinostat did not affect NK cell viability or NK cell receptor (NKG2D, Nkp30, Nkp44, Nkp46, and DNAM-1) expression. NK cells pretreated with entinostat for 24 h did not decrease cytotoxicity of NK cells to OS cell lines. We also showed two mechanisms by which entinostat controls MICA/B expression on OS cells: 1) by increasing H4 acetylation at the MICA/B gene promoters and 2) by down-regulating miR-20a, miR-93, and mir-106b expression. We demonstrated that the sub-therapeutic dose of entinostat that significantly increased MICA/B on OS lung metastasis was 5 mg/kg three times a week for 5 weeks. Combining 5 mg/kg entinostat and NK cell therapy is our ongoing in vivo experiment.

**Conclusions**

We demonstrated that entinostat immunosensitized OS cells to NK cell lysis by inducing upregulation of ligands for NK cells on OS cells. Our results suggest that NK cell therapy combined with entinostat provides an innovative approach to enhance the immunotherapeutic effect of NK cells against OS pulmonary metastases.

**P23**

Chimeric antigen receptor macrophages (CARMA) for adoptive cellular immunotherapy of solid tumors

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**Background**

Anti-CD19 chimeric antigen receptor (CAR) redirected T cells have demonstrated profound efficacy in relapsed/refractory B cell malignancies. However, CAR T cells have thus far failed to recapitulate these results in solid tumors. Accumulating evidence suggests that macrophages naturally traffic to and persist in solid tumors. The goal of this study was to evaluate the anti-tumor function of genetically engineered CAR macrophages (CARMA) and assess their potential for translation as a novel immunotherapeutic platform for solid tumors.

**Methods**

To examine the function of CARs in macrophages, first generation CARs with a CD3ζ intracellular domain were introduced into the THP-1 macrophage model. *In vitro* function was assessed via quantitative phagocytosis and luciferase-based specific killing assays. To assess the function of the CD3ζ-domain, CD3ζ-null CAR constructs were compared. Ad5F35 was used to transduce primary human monocyte derived macrophages with an anti-HER2 CAR construct, and anti-tumor function was tested in vitro and *in vivo*. The impact of inhibiting the CD47/SIRPα “do-not-eat-me” signal was tested with blocking antibodies and CRISPR-Cas9 mediated SIRPa knockout. Macrophage M1/M2 phenotype was determined by flow cytometry. Immunodeficient mouse xenograft models of a human HER2(+) ovarian cancer cell-line (SKOV3) were used for *in vivo* efficacy studies.

**Results**

CAR19, but not untransduced macrophages, phagocytosed CD19+ (but not CD19-) K562 cells. Deletion of CD3ζ in CAR19 macrophages abrogated phagocytosis and significantly reduced specific killing. Phagocytosis was inhibited by pharmacologic blockade of phagocytic signaling - suggesting that CAR activation drives productive cell-signaling in macrophages. Phagocytosis was also demonstrated in HER2 and mesothelin CARMA models. Blockade of the inhibitory CD47/SIRPa “do-not-eat-me” signal enhanced CARMA phagocytosis of antigen-bearing target cells in a dose-dependent manner (Fig. 27). We identified Ad5F35 as an efficient transduction approach for engineering primary human macrophages, resulting in >70% CAR transduction efficiency. Primary human anti-HER2 CARMA demonstrated targeted phagocytosis and specific killing. Ad5F35 transduction polarized human macrophages to the pro-inflammatory M1 phenotype, and rendered them resistant to downstream M2 subversion by immunosuppressive cytokines and cell lines. Anti-HER2 CARMA was evaluated *in vivo* in an ovarian cancer xenograft model. Mice that received CARMA had a decrease in tumor burden of approximately two orders of magnitude and had a 30-day survival benefit relative to untreated or control macrophage treated mice (p=0.018, Fig. 28).

**Conclusions**

Here, we introduce for the first time that human macrophages engineered with a CAR exert antigen-specific tumor phagocytosis and killing, and propose a novel immunotherapeutic platform for the treatment of diverse solid tumors.

[Fig. 27 (Abstract P23). Timelapse of a CAR19 macrophage (mRFP+) phagocytosing a CD19+ K562 cells (GFP+) cell (A). CARMA but not Wt macrophages phagocytosed CD19+ but not CD19- tumor cells (B). Phagocytosis was confirmed by Imagestream cytometry, gating on mRFP+GFP+ events (C). Blockade of CD47 and SIRPα led to a dose dependent increase in CARMA phagocytosis. Non-blocking anti-CD47 clone 2D3 (opsonization control) did not enhance phagocytosis (D).]

[Fig. 28 (Abstract P23). Anti-HER2 primary human CARMA were tested in immunodeficient mouse models of human HER2+ ovarian cancer (schema, a). CARMA but not control untransduced macrophages reduced tumor burden (b,c) and prolonged survival by 30 days (p=0.018, d).]
P24
Regulation of T cell sensitivity by TCR-proximal signaling components during anti-melanoma responses
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Background
Immunotherapies for cancers have made great strides in recent years, yet new and improved approaches are required to achieve more durable responses in a greater number of patients. The in vitro expansion phase of adoptive T cell therapy prior to reinfusion into the patient present opportunities to genetically enhance T cell subsets to improve in vivo performance. While the most common genetic modification is the incorporation of engineered antigen-specific TCRs or chimeric antigen receptors, modification to signaling pathways in T cell memory subsets in order to enhance T cell sensitivity is an underexplored strategy. This is mainly because contributions of TCR signaling components that confer differences in activation sensitivity and functional outcomes between CD8+ Tcm and TEM are unclear.

Methods
To understand how TCR-proximal signaling differs significantly between T cell memory subsets, we derived Tcm and TEM from the humanized TCR-transgenic melanoma mouse model (JR209). We quantified differences in TCR activation and feedback regulation by novel live-cell imaging technologies, phospho-specific protein assays and used modeling of early TCR signaling to reveal the physiological significance of these differences.

Results
One of the critical steps of T cell triggering is the coordinated phosphorylation and binding of CD3 and Zap-70 by Lck following TCR ligation by pMHC. Here, we show that Tcm and TEM possess differential constitutive Lck activities. Immediately proximal to Lck signaling, we observed enhanced Zap-70 phosphorylation in TEM following TCR ligation compared with TCM. Further, we observed increased intracellular calcium influx and cytotoxic effector function in TEM compared with TCM, and provide evidence that this results from a lower probability of TCM reaching threshold activation signaling due to the decreased magnitude of TCR-proximal signaling. We show that the differences in Lck constitutive activity between CD8+ Tcm and TEM are driven in part by differential regulation by SH2 domain-containing phosphatase-1 (Shp-1) and C-terminal Src kinase (Csk). We demonstrate that inhibition of Shp-1 results in increased constitutive Lck and cytotoxic activity in TCM to levels similar to that of TEM.

Conclusions
Together, this work demonstrates that differential activities of TCR-proximal signaling components may contribute to establishing the divergent effector properties of TCM and TEM. Inhibition of negative regulatory molecules, for example Shp-1 or Csk, or generalized augmentation of T cell sensitivity with miRNA offer potential therapeutic approaches in T cell immunotherapy but must be considered in the context of specificity and optimal targeting.

P25
Co-expression of synthetic PD-1 fusion proteins augments HER2 CAR T cell functionality against glioblastoma
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Background
HER2-CAR T cells home to the central nervous system (CNS) and induce tumor regression in patients with glioblastoma (GBM) [1]. However, most responses are transient, as CAR T cells fail to expand and have limited persistence. Ex vivo analyses of tumor infiltrating lymphocytes demonstrate high levels of inhibitory receptors, including PD-1. Monoclonal antibodies blocking PD-1/ PD-L1 induce responses in some patients with solid tumors and potentiate anti-tumor T cell activity. However, because antibodies exhibit erratic CNS pharmacokinetics, combining this approach with CAR T cells is not optimal for CNS tumors. We hypothesize that co-expression of a synthetic PD-1 fusion protein will convert PD-L1 into a costimulatory T cell signal, improving expansion, persistence, and anti-tumor activity of adoptively transferred HER2-CAR T cells.

Methods
We generated an array of bicistronic vectors encoding our clinically utilized 2nd generation HER2-CAR (CD28-endodomain) and a PD-1 fusion protein. All PD-1 fusion proteins contained the native PD-1 ectodomain fused to either the CD28 transmembrane and endodomain (PD-1/CD28) or CD8α-transmembrane and 4-1BB-endodomain (PD-1/4-1BB). T cell expansion, persistence, and exhaustion (LAG3, TIM3, PD-1) were measured using flow cytometry following coculture with autologous HER2+/PD-L1+ GBM cells for 2-4 weeks. Cytokine release at 24 hours was measured using standard ELISA, and the xCELLigence impedance-based system was used to evaluate cytolytic activity. Using high-resolution immunofluorescence imaging, we interrogated differences at the CAR T cell/GBM contact point, also referred to as the immunologic synapse (IS).

Results
Compared to conventional HER2-CAR T cells, PD-1/CD28 T cells expanded more quickly with significantly higher IL2/IFNγ-release at 24 hours (Fig. 29), whereas PD-1/4-1BB T cells demonstrated enhanced cytolytic ability against autologous GBM cells in prolonged killing assays (Fig. 30) and better long-term persistence. Inhibitory receptor expression following coculture was comparable among T cell products, but PD-1/4-1BB T cells maintained a more durable killing profile (Fig. 29).

Conclusions
Co-expression of synthetic PD-1 fusion proteins augments HER2 CAR T cell functionality against glioblastoma.
confocal images of the CAR T cell/tumor interface revealed increased stability of the IS between the HER2-CAR and the HER2 molecule on GBM.

**Conclusions**

We conclude that PD-L1 can be converted into a costimulatory signal using synthetic PD-1 fusion molecules to drive key T cell activation pathways and enhance stability of the CAR-target antigen interface, leading to improved HER2-CAR T cell functionality against GBM.

**References**


**Fig. 29 (Abstract P25).** 2nd generation HER2 CAR T cells with and without PD-1/CD28 fusion proteins were incubated with autologous GBM cells at a 1:1 ratio. Cytokine concentrations from culture supernatant collected at 24 hours were measured using standard ELISA.

**Fig. 30 (Abstract P25).** The xCELLigence platform uses impedance across resistor-coated plates to measure adherent tumor cell viability. Once 10,000 tumor cells became adherant and confluent, 1,000 T cells were added that expressed either a HER2 CAR only (H2) or a HER2 CAR with a truncated PD-1 protein (X), PD-1/CD28 protein (28), or PD-1/4-1BB protein (88). Non-transduced T cells and tumor only wells served as controls.

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**P26**

Adoptive therapy with tumor-infiltrating lymphocytes for melanoma: interim results of a phase II single-institution study

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**Background**

Adoptive cell therapy using tumor-infiltrating lymphocytes (TIL) has been established as an effective treatment option for metastatic melanoma but is available at only a small number of institutions. We have developed a TIL program at the Fred Hutchinson Cancer Research Center and present our initial results in melanoma patients.

**Methods**

Patients with metastatic melanoma were enrolled in a two-step fashion to a nonrandomized, phase II TIL trial: Step 1 for TIL generation and Step 2 for TIL treatment. TIL are cultured from tumor fragments, using standard methodologies developed at the NCI. At a time of future disease progression, patients are given cyclophosphamide 60mg/kg/day x 2 days, fluorouracil 25mg/m2/day x 5 days, then TIL up to 1.5 x 10^11, followed by high-dose IL-2, 600,000 units/kg every 8 hours, up to 14 doses as tolerated. TIL populations are selected for infusion based on growth, phenotype, and MHC-class-I-dependent autologous tumor recognition, as assessed by interferon-gamma release.

**Results**

Thirty-two patients have been enrolled on Step 1 for TIL generation. Of these, 23(72%) achieved adequate TIL number of >40 x10^6 by 5 weeks of culture. TIL in 17(53%) patients also demonstrated positive autologous tumor reactivity. To date, 7 patients have received TIL therapy on Step 2; all had progressed on prior immunotherapy (Table 3). In these 7 patients, the RECIST 1.1 responses are: 2 CR, 1 PR, 3 SD, 1PD. All patients experienced tumor regression of some or all baseline target lesions; the patient with PD progressed in the brain, but experienced a partial response of extracranial sites. Two patients were on concurrent BRAF therapy and had reached a plateau in their response to BRAF inhibitors prior to start of TIL treatment, and both experienced further tumor reduction after TIL. Responses were associated with a higher percent CD8+TIL, a lower percent Treg(CD4+CD25+CD127-), and TCR oligoclonality, while functional markers PD-1 and TIM3 did not associate with response.

**Conclusions**

Our single-institution study validates the utility of TIL therapy in patients with advanced melanoma, refractory to other immunotherapy. TIL can induce durable CRs and can also mediate additional tumor regression in patients on active BRAF inhibition. The replication of TIL methodology across different institutions, with reproducible clinical efficacy, supports the feasibility of its widespread application, as well as further investigation into optimizing elements of this treatment modality.

**Acknowledgements**

We wish to thank the NCI Surgery Branch and MD Anderson for their generous guidance with the development of our TIL therapy program, and Prometheus for their generous IL-2 support.
Table 3 (Abstract P26). Characteristics and responses of treated patients

<table>
<thead>
<tr>
<th>Age/Gender</th>
<th>Prior Treatments</th>
<th>M Classification and Metastatic Sites</th>
<th>Response</th>
<th>Response Duration (months)</th>
</tr>
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<tbody>
<tr>
<td>1 54 F</td>
<td>Anti-CLL-4, anti- interferon (FN)</td>
<td>M1c: Mesentery, small bowel, lymph nodes (LN)</td>
<td>PR</td>
<td>8</td>
</tr>
<tr>
<td>2 34 F</td>
<td>Anti-PDL1, anti- CLA-4, IFN, IL-2 + radiation (XRT), temozolomide</td>
<td>M1c: Brain, kidneys, adrenals, bone, liver, LN, subcutaneous (SC)</td>
<td>SD</td>
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</tr>
<tr>
<td>3 61 M</td>
<td>IL-2, BRAF inhibitor</td>
<td>M1c: Brain, liver, lungs, SC, mesentery, chest wall</td>
<td>PD (Progression in brain, but PR in extracranial sites)</td>
<td>B, for extracranial response</td>
</tr>
<tr>
<td>4 27 F</td>
<td>IL-2, anti-CTLA-4 +IL-21, anti-CTLA-4 + XRT, recombinant IL-15</td>
<td>M1a: Brain, SC, LN, pleura, kidneys, peritoneum</td>
<td>SD</td>
<td>3</td>
</tr>
<tr>
<td>5 63 M</td>
<td>Anti-CTLA-4</td>
<td>M1a: Intramuscular, LN</td>
<td>CR</td>
<td>17, ongoing</td>
</tr>
<tr>
<td>6 52 M</td>
<td>Anti-CTLA-4</td>
<td>M1a: SC, LN</td>
<td>CR</td>
<td>20, ongoing</td>
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<tr>
<td>7 31 F</td>
<td>Anti-PDL1, anti- CTLA-4, decarbazine, BRAF/MEK inhibitor</td>
<td>M1c: Brain, lungs, liver, SC</td>
<td>SD</td>
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</table>

Stimulation with other CD19+ cell lines induced a different cytokine production profile of CD19 CAR T cells.

Conclusions
CD4+ and CD8+ CD19 CAR T cells display comparable differentiation phenotype, cytokine production and cytotoxic capacity. The presence of a high frequency of CD19+ B cells during the generation of CAR T cells did not have an impact on CAR T cell phenotype. However CAR T cells from patients with CLL produced more cytokines when stimulated with CD19+ target cells suggesting that activation of CD19 CAR T cells by B cells during cell expansion impacts the cytokine profile. Furthermore, our data show that the level of CD19 cell surface expression modulates CAR T cells cytokine production.

P28
Directing traffic: fostering chemokine receptor expression on tumor-infiltrating lymphocytes improves re-trafficking in vivo
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Background
Previously, a critical role for chemokines was found in a unique immune-related gene expression signature (GES) for immune cell recruitment and tertiary lymphoid structure formation in metastatic melanoma [1]. Regarding adoptive cell therapy (ACT) of tumor-infiltrating lymphocytes (TIL), we hypothesized that expression of chemokine receptors (CCR2, CCR5, and CCR7, which bind multiple GES chemokines, favor TIL re-trafficking to the tumor, thereby bolstering ACT efficacy.

Methods
We utilized a preclinical ACT model in which TIL from syngeneic MC-38 colon carcinomas grown in wild-type (WT) C57BL/6 mice, or gene knock-out (KO): C57BL/6 mice lacking CCR2, CCR5, or CCR7, were expanded ex vivo, and adoptively transferred into MC-38-bearing C57BL/6 recipient mice bearing congeneric CD45.1. After seven days, tumor burden was assessed, and Spanning-tree Progression Analysis of Density-normalized Events (SPADE) of 18-parameter cytometry data was used to identify and quantify TIL subsets, and 51Cr-release assays quantified cytotoxicity among sorted TIL populations.

Results
ACT of WT TIL reduced tumor burden by 50% compared to untreated mice (p<0.0098). This benefit was lost when transferring CCR5KO or CCR7KO TIL, and reduced when transferring CCR2KO TIL (all p < 0.05). MC-38 tumors contained 90%, 85%, and 70% less transferred TIL with CCR7KO TIL, and reduced when transferring CCR2KO TIL (all p < 0.05). During TIL expansion, IL-2 up-regulated CCR2, CCR5, and CCR7 in a cell density-dependent fashion (all p < 0.007). ACT of TIL with up-regulated CCRs increased tumor re-trafficking three-fold (p < 0.05), and decreased tumor burden by an additional 25% (p < 0.05) versus WT TIL without up-regulated CCRs.

Conclusions
CCR2, CCR5, and CCR7 are vital for TIL re-trafficking to MC-38 tumors following ACT, including cytotoxic subsets among eight novel TIL phenotypes. Fostering CCR expression during TIL expansion augments ACT efficacy in murine MC-38 colon carcinoma. Translational studies to human TIL ACT are currently underway.

Acknowledgements
Supported by NCI-NIH (CA148895-01); P30CA07639; P50CA168536); V Foundation, Dr. Miriam and Sheldon G. Adelson Medical Research Foundation, and the Chris Sullivan Foundation.
References

Table 1: Marker Expression on Novel TIL Subsets

<table>
<thead>
<tr>
<th>Subset</th>
<th>Marker</th>
<th>Expression</th>
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<tr>
<td>CD8+</td>
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<td>++</td>
</tr>
<tr>
<td>CD4+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD16+</td>
<td>+</td>
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Fig. 31 (Abstract P28).

P29
Provision of inducible MyD88 and CD40 co-stimulation in CAR T cells results in potent antitumor activity in preclinical solid tumor models
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Background
Although adoptive immunotherapy using T cells expressing chimeric antigen receptors (CAR) is successful in refractory hematological malignancies, limited clinical responses have been observed in solid tumors. We reasoned that introducing an inducible co-stimulation (iCO-STIM) gene into T cells would allow for improved activation of CAR T cells, resulting in enhanced antitumor activity. Due to the co-stimulatory properties of MyD88 and CD40 in T cells, we explored whether CAR T cells expressing an iCO-STIM molecule consisting of a myristoylation-targeting sequence, two FKBPs dimerizing domains, MyD88ATIR, and the intracellular domain of CD40, have superior effector function relative to standard CAR T cells in vitro and in vivo.

Methods
T cells expressing a HER2-CAR and iCO-STIM (HER2-CAR/iCO-STIM T cells) were produced as described by transduction with a retroviral vector encoding FRP5Δ (HER2-CAR), a 2A peptide, and iCO-STIM. HER2-CAR/iCO-STIM T cell effector function was then evaluated in vitro and in murine xenograft models.

Results
In the presence of Cid (AP20187), HER2-CAR/iCO-STIM T cells produced significantly higher levels of IL2 (p < 0.05) compared to HER2-CAR/iCO-STIM T cells in the absence of Cid or HER2-CAR/CD28Δ, T cells in co-culture assays with HER2+ tumor cells (MDA-HER2, LM7, A549). In contrast, HER2-negative tumor cells (MDA) did not induce IL2 production by HER2-CAR/iCO-STIM T cells +/- Cid or HER2-CAR/CD28Δ, T cells. In repeat stimulation assays, HER2-CAR/iCO-STIM T cells showed robust antigen-dependent expansion in the presence of Cid and were able to lyse HER2-positive LM7 cells after 4 restimulations compared to HER2-CAR/CD28Δ, T cells (p < 0.0001). In vivo, a low dose of HER2-CAR/iCO-STIM T cells (3x10^4) + Cid had superior antitumor activity in the metastatic LM7 osteosarcoma murine xenograft model compared to HER2-CAR/iCO-STIM T cells without Cid or HER2-CAR/CD28Δ, T cells, resulting in a significant survival advantage (p < 0.0001). In 3 mice that developed late recurrences after HER2-CAR/iCO-STIM T cell + Cid therapy, a second dose of Cid, given 100 days post T cell injection, eliminated 2/3 tumors. Superior antitumor activity of HER2-CAR/iCO-STIM T cell + CID therapy was confirmed in the HER2+ A549 lung cancer murine xenograft model.

Conclusions
CID-mediated activation of MyD88 and CD40 co-stimulatory signals in HER2-CAR T cells results in superior effector function compared to standard HER2-CAR T cells. Thus, expressing iCO-STIM molecules in CAR T cells has the potential to improve the efficacy of CAR T cell therapy approaches for solid tumors. In addition, our results indicate that the CID/iCO-STIM system will enable the ‘remote control’ of infused T cells.

P30
In vivo administration of immune checkpoint inhibitors prior to tumor harvest enhances the function of tumor-infiltrating T lymphocytes expanded for adoptive T cell transfer
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Background
T cell reactivity against mutated antigens, derived from cancer genomic alterations, is a key mediator of immunotherapy efficacy. Adoptive cell transfer (ACT) of mutation-reactive tumor-infiltrating T lymphocytes (TILs) however has been only effective in a minority of patients with metastatic gastrointestinal cancers. We hypothesize that the low frequency of mutation-reactive TILs and their exhaustion features may contribute to the lack of efficacy of TIL ACT, and that these factors can be overcome by in vivo administration of blocking antibodies against CTLA-4 or PD-1 prior to tumor harvest and TIL expansion.

Methods
We selected the mouse MC-38 colorectal cancer model to study CD8+ T IL reactive to 2 mutated, 2 self, and 1 retroviral antigens using cocultures with peptide-pulsed splenocytes followed by IFN-γ intracellular staining. MC-38 cancer cells were inoculated subcutaneously and allowed to grow until 20mm^2. Then anti-CTLA-4 (9H10) and/or anti-PD-1 (RPM1-14) antibodies were administered twice (4 days interval). Three days following the last antibody injection, tumors were harvested and analyzed for immune cell infiltration, phenotype and functionality. TILs were also expanded in vitro and characterized regarding the specificity and the functionality against the known antigens.

Results
The administration of anti-CTLA-4 prior to tumor harvest increased immune cell infiltration, decreased the proportion of myeloid derived suppressor cells and regulatory T cells in the tumors and enhanced CD8+ T cell IFN-γ and TNF-α production. Administration of both anti-CTLA-4 and anti-PD-1 was more effective to eliminate the tumor burden and recapitulated the effects observed following anti-CTLA-4 injection alone. Anti-CTLA-4 and/or anti-PD-1 alone did not appear to change the relative frequency of TIL reactive to mutated, self, or viral antigens but increased their polyfunctionality.

Conclusions
In vivo pre-conditioning of MC-38-bearing mice with immune checkpoint blocking antibodies may generate TILs more fit for ACT immunotherapy. Experiments are underway to compare the efficacy of this approach to conventional TIL ACT in this mouse model.

P31
Redirecting gene-engineered T cells through covalent attachment of targeting ligands to a universal immune receptor
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Background
Infusion of T cells genetically engineered to express a chimeric antigen receptor (CAR) is a promising approach for the treatment of certain cancers. Though CAR T cells are highly efficacious against CD19+ hematological malignancies, limitations exist in broadening their use. Conventional CAR T cells target a single tumor associated antigen (TAA), limiting their effectiveness against tumors with heterogeneous TAA expression as well as emerging antigen loss variants, as observed in CD19 CAR trials. Additionally, stably engineered CAR T cells may continually proliferate and activate in the presence of antigen, potentially causing fatal toxicity, without a method of elimination. To overcome these issues, we and others have developed a variety of universal immune receptors (UIRs) that allow for targeting of multiple TAAs with a single receptor-expressing T cell. Although these UIRs are a promising new technology, their reliance on noncovalent interactions between receptor and targeting ligand can lead to potential issues of affinity, specificity and activity.

Methods
Our UIR platform employs the use of the SpyCatcher and SpyTag proteins that, when combined, can form a covalent bond with high efficiency both in vitro and in vivo. Our SpyCatcher immune receptor is composed of an extracellular SpyCatcher domain attached to intracellular T cell signaling motifs in a lentiviral expression vector. Anti-TAA antibodies conjugated to SpyTag are used to confer redirected specificity to SpyCatcher expressing T cells. Measurements of T cell effector function include T cell cytokine secretion, activation, and targeted tumor cell lysis in vitro.

Results
In this study, we demonstrate the first universal immune receptor platform that allows for the endowment of function through post-translational covalent attachment of targeting ligands, securing their loading on the T cell surface while expanding recognition specificity. We demonstrate that the SpyCatcher immune receptor is expressed in primary human T cells and allows for specific T cell activation and cytokine secretion against plate bound SpyTag. Notably, in the presence of SpyTag-labeled targeting antibody, SpyCatcher T cells recognize and lyse antigen-expressing human tumor cells in a target-specific and dose-dependent fashion.

Conclusions
The SpyCatcher immune receptor is the first universal immune receptor designed for its capacity to covalently bind targeting ligands and redirect T cells against a diverse array of antigens, addressing current limitations of conventional CAR T cell therapy.

P32
Simple automated manufacturing of gene engineered T cells under serum free conditions for adoptive T cell therapy
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Correspondence: Nadine Mockel-Tenbrinck (nadinet@miltenyibiotec.de) Journal for ImmunoTherapy of Cancer 2016, 4(Suppl 1):P32

Background
Adoptive immunotherapy using gene-modified T cells redirected against cancer has proven clinical efficacy and tremendous potential in several medical fields. However, such personalized medicine faces several challenges in the complexity associated with the current clinical manufacturing methods. The processes are mostly suboptimal requiring cell manufacturers to deal with open steps, liquid handlings between devices used, manual interventions, removal of activation beads and often the use of reagents for which commercial availability is not in line with the high and increasing demand. GMP compliant human AB serum is one of such reagents. Therefore, developing improved methods to generate gene-engineered T cells suitable for clinical use that do not require serum are essential for the commercial scalability of such therapies.

Methods
We have developed a robust and reproducible automated manufacturing process for the lentiviral gene-modification and expansion of selected T cells. The CliniMACS Prodigy TCT (for T Cell Transduction) process software allows automated purification and polyclonal T cell stimulation followed by gene-modification and expansion of T cells in a single-use closed tubing set.

Results
Here we show that the TCT process enables the manufacturing of gene-modified T cells without the need for serum supplementation (human AB serum) when using TexMACS GMP Medium. Furthermore, implementation of a humanized recombinant activation reagent TransAct allows for a simplification of the process whereby the “bead removal” step is unnecessary. Comparable results from healthy donor or patients starting cells are demonstrated.

Conclusions
Taken together the TCT process in combination with the CliniMACS Prodigy and minimal user interactions enables the preparation of gene-modified T cells in serum free conditions. Process risks, due to use of different devices, unnecessary manipulations, or potential shortage of human AB serum availability can be minimized by automation of the entire process as developed and shown here. Overall, these improvements are meant to fully support commercial treatment of a large number of patients.

P33
Tumor Infiltrating lymphocytes from soft tissue sarcoma have tumor-specific function
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Correspondence: John Mullinx (john.mullinx@moffitt.org) Journal for ImmunoTherapy of Cancer 2016, 4(Suppl 1):P33

Background
Adoptive Cell Transfer (ACT) using Tumor Infiltrating Lymphocytes (TIL) for unresectable metastatic melanoma results in a median overall survival of 52 months at our institution. This stands in contrast to the median overall survival for metastatic soft tissue sarcoma (mSTS) which is 12 months. The purpose of this report is to describe the phenotype and function of TIL from fresh surgical sarcoma specimens as a rationale for applying ACT to mSTS.

Methods
Fresh surgical sarcoma specimens were acquired under an IRB-approved protocol. Half of the specimen was digested and phenotyped by flow cytometry. The remaining half was plated as fragments for the isolation of TIL, which were expanded in vitro with conditions validated for melanoma-derived TIL. Cultured TIL were phenotyped by flow cytometry and propagated further with a rapid expansion protocol (REP). Tumor-specific reactivity was assessed by co-culture of TIL with autologous tumor or HLA-matched cell lines with measurement of IFN-gamma using ELISA.

Results
Sixteen patient-derived sarcoma specimens were collected. Histology of primary tumor included dedifferentiated liposarcoma (9), well-differentiated liposarcoma (2), undifferentiated pleomorphic sarcoma (2), and one each of gastrointestinal stromal tumor, myxofibrosarcoma and synovial sarcoma. Analysis of tumor digests indicated an average of 48% of cells from the lymphocyte gate were CD3+ (range 36%-76%), TIL were grown from all specimens, with TIL observed in 152 out of 192 (79%) fragments. The phenotype of the CD3+ subpopulations from TIL cultures included a median of 90.8% (range 2-99.9%) CD8+ and 2.3% (range 0-96.4%) CD4+ T cells. TIL were expanded in a REP to clinically meaningful numbers (mean 1385-fold) with no change in the CD8+ T cell proportion.
Tumor-specific function of TIL generated from fragments was measured in two patients. There was tumor-specific IFN-gamma release (mean 148.8±13.5 pg/ml) in TIL co-cultured with HLA-matched cell lines and also in TIL co-cultured with autologous tumor digest (mean 259.9±14.7 pg/ml). The degree of IFN-gamma release was significantly greater when TIL were co-cultured with autologous digest compared to an HLA-mismatched cell line (p=0.0295).

Conclusions
CD3+CD8+ TIL can be isolated from human sarcoma tumors in vitro, expanded to meaningful numbers for therapeutic use, and demonstrate reactivity to the tumor from which they were cultured. These data form the basis for efforts to develop a clinical trial using ACT for patients with advanced sarcoma.

Acknowledgements
Work funded by a grant from the Chotiner Family Foundation.

References

P35
Human natural killer cells engineered to express a chimeric NK activating receptor have activity against highly suppressive cells of the solid tumor microenvironment
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Background
The suppressive microenvironment of solid tumors inhibits the anti-tumor activity of endogenous and chimeric antigen receptor (CAR)-bearing T cells, thereby limiting the efficacy of adoptive T cell therapies. Myeloid-derived suppressor cells (MDSCs; CD33+ CD11b+ HLA-DRlow) and regulatory T cells (Tregs; CD4+ CD25high FoxP3+ Helios+) contribute to the inhibitory tumor microenvironment (TME) through secretion of suppressive cytokines, expression of inhibitory ligands, and by promoting tumor neo-vascularization. NKG2D is an activating surface receptor expressed on natural killer (NK) cells, whose ligands are highly expressed by human Tregs and MDSCs. We genetically modified primary human NK cells to express a chimeric NKG2D molecule (ectodomain of endogenous NKG2D fused to the cytotoxic CD3-zeta chain; called NKG2D.z) in order to promote NK cell activation against Tregs and MDSCs. We hypothesized that NKG2D.z NK cells would exhibit enhanced cytolytic activity against suppressive autologous Tregs and MDSCs via the chimeric NKG2D, as well as secrete chemokines and cytokines that recruit and activate tumor-specific T cells. The objective of the current study was to compare the activity of NKG2D.z NK cells vs. non-transduced (NT)-NK cells on Tregs, MDSCs, or the combination.

Methods
We isolated MDSCs and Tregs from normal donors and patients with solid tumors, confirmed their phenotype by flow cytometry and their suppressive activity in T and NK cell proliferation assays, and used them as targets in NK cell cytotoxicity and co-culture assays.

Results
NT (endogenous) and mock-engineered (empty vector control) NK cells were unable to mediate cytotoxicity or release pro-inflammatory cytokines in response to autologous MDSCs or Tregs, either alone or in combination. In contrast, NKG2D.z NK cells exhibited enhanced cytolytic and secreted T cell-recruiting and -activating cytokines in response to both suppressive cell types. Further, when NKG2D.z NK cells were co-cultured in a highly suppressive environment containing both MDSCs and Tregs, their cytolytic and cytokine-secreting activities against either cell type were unimpaired. We have established an in vivo TME model comprising tumor cells with MDSCs and Tregs, and experiments testing the ability of NKG2D.z NK cells to eliminate suppressive cells and recruit endogenous or CAR-T cells in vivo are underway.

Conclusions
Our results suggest that our modified NK cells may reverse immune suppression by the TME and improve T cell-based immune therapies for solid tumors.

Acknowledgements
Authors thank Charles L. Sentman, PhD for initial use of the NKG2D.zeta sequence.
Cytokines induced by pre-B leukemia progression mediates irreversible T cell dysfunction

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Background
Acute lymphoblastic leukemia (ALL) is the most common childhood malignancy. The cure rate has reached up to 90% with modern therapy, outcomes are still very poor for patients who relapse. Immunotherapy with donor lymphocyte infusions for ALL has not demonstrated the success seen in other hematologic malignancies, suggesting there are characteristics inherent to ALL that make it less amenable to detection and elimination by the immune system. T cell dysfunction in the setting of leukemia has been well described, but the mechanisms have not been fully elucidated. It is also unclear if T cell dysfunction underlies some of the treatment failures seen in patients receiving adoptive therapy with chimeric antigen receptor (CAR) expressing T cells. Immune checkpoint blockade has led to advances in the treatment of many solid tumors, but it has yet to demonstrate similar success in ALL.

Methods
Using two preclinical models of pre-B cell ALL, we studied the negative impact of ALL progression on T cell function and the efficacy of CAR T cell therapy and immune checkpoint blockade. We also dissected the mechanism underlying the observed T cell dysfunction.

Results
Prophylactic vaccination protects mice against the murine pre-B ALL in a T cell-dependent manner. However, therapeutic vaccination is ineffective, even in the setting of low disease burden. Adoptive transfer of primed T cells from immunized donors can completely eradicate established leukemia; however, this efficacy is not seen with the adoptive transfer of T cells from leukemia-bearing mice. Expression of a CD19 CAR in T cells from leukemic mice fails to eradicate ALL, while the CAR T cells derived from naive mice can. T cells from leukemic mice express markers of T cell dysfunction. The expression of these molecules was associated with elevated levels of IL6, TNFa and IL10 in the serum of leukemia-bearing mice. Incubation of naive T cells in these cytokines recapitulated the upregulation of exhaustion markers seen in vivo, suggesting these cytokines play a role in the observed T cell dysfunction. Blockade of PD-1, its ligand, PD-L1, or Tim3 were ineffective at reversing T cell dysfunction and preventing leukemia progressions in vivo, suggesting other mechanisms must be targeted to restore immune function in leukemia bearing hosts.

Conclusions
Cytokines induced by pre-B Leukemia progression mediates irreversible T cell dysfunction. These findings underscore the need to elucidate the mechanisms of leukemia-induced immune suppression to fully optimize the use of CAR-expressing T cells in the treatment of ALL.

Identification of a recurrent high-affinity MHC class I restricted neoepitope in neuroblastoma using ProTECT

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Background
T cells are trained to differentiate between cell-surface MHC-displayed peptide sequences from self- and non-self proteins and act on the latter. The numerous mutations often associated with cancers can occur in coding regions of the genome and modify the sequence of wild-type proteins, potentially creating targets for immunotherapies. We have developed an analysis pipeline ProTECT (Prediction of T cell Epitopes for Cancer Therapy) to identify and rank neo-epitopes in terms of immunogenicity. Running ProTECT on a set of recurrent neuroblastomas showed that recurrent tumors share neo-epitopes with their corresponding primary tumors suggesting that immunotherapies could provide long-term protection.

Methods
ProTECT accepts purged tumor and normal DNA sequencing fastq files, and tumor RNA sequencing fastq. Mutations are called using a panel of callers, and are annotated to identify coding mutations. Prediction of self-MHC:mutated-peptides is carried out and the final binding predictions are ranked using an in-house algorithm. We support both MHC-I and MHC-II predictions.

Results
Running ProTECT on 6 recurrent neuroblastoma samples (NBL) from the TARGET (Therapeutically Applicable Research To Generate Effective Treatments) project revealed that the relapsed tumor inherits neo-epitopes predicted in the primary tumor. We also predicted neo-epitopes from 2 well-known hotspot mutations in NBL (NRAS Q61K and ALK R1275Q) that bind to common MHC alleles (HLA-A*01:01, HLA-A*03:01 and HLA-B*15:01). We carried out in vitro refolding and crystallization assays [1] for the five highest-ranking mutant NRAS and ALK predictions. Properly conformed MHC trimers were verified by a single, monodisperse peak after anion exchange chromatography and validated by SDS gel electrophoresis. Mass spec confirmed bound peptide for 4/5 tested predictions and 3 of these were used to set up hanging-drop crystallization trials in various conditions. Positive hits were obtained for one (MAQDIYRASY::HLA-B*15:01). ProTECT has also been run on a large subset of The Cancer Genome Atlas (TCGA). We aim to reveal clinically relevant hotspot-mutation/MHC pairs.

Conclusions
We have described a pipeline for identification and ranking of therapeutically relevant neo-epitopes. We have predicted potentially therapeutic targets for NBL that have been validated in vivo.

References

A higher-affinity variant of a GD2-specific CAR significantly enhances potency in vivo and allows for a novel model of on-target off-tumor toxicity

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Background
As many potential targets of chimeric antigen receptor (CAR) T cell immunotherapy are self-antigens that are over-expressed in tumors but also present at lower levels on some normal tissue, understanding the nature of on-target off-tumor toxicity and how to overcome it is important in the development of new CAR T cell therapies. Preclinical modeling of such toxicity is complicated by the fact that most antigens are not shared between humans and mice, and strategies have largely relied on co-injection of antigen-low tumors or
Introduction of human antigen into mouse tissue by viral gene delivery. The GD2 tumor antigen would provide an excellent model in which to study on-target off-tumor toxicity as the exact glycolipid antigen is naturally shared between mice and humans. However, as of yet, GD2-specific CAR T cells have yielded modest efficacy and little toxicity in preclinical studies. Here we have engineered a higher potency GD2 CAR by introducing an affinity-enhancing mutation (E101HK), previously described by Horwacki et al. et al. We show significantly enhances CAR T cell activity and provides a model for toxicity.

Methods
Primary human T lymphocytes were transduced with lentivirus encoding either wild-type 14G2a-based anti-GD2 CAR, E101K mutant GD2 CAR, or a negative control CAR. After standard stimulation and expansion, T cells were analyzed for CAR surface expression by flow cytometry and for in vitro effector function by chromium release and IFN gamma ELISA. To evaluate in vivo, NOD-SCID-IL2Rγc-/-(NSG) mice were injected with the luciferase-expressing GD2-high human neuroblastoma cell line SYSY, and four days later, 3,000,000 CAR+ T cells were injected via tail vein. Tumor burden was measured using in vivo bioluminescence, and tumor and normal tissues were evaluated histologically by H&E staining and immunohistochemistry.

Results
The higher affinity mutant displayed comparable surface expression and T cell expansion but significantly enhanced GD2-specific cytokotoxicity and cytokine release in vitro and tumor control in vivo. However, this enhanced efficacy was associated with severe CNS toxicity causing neurologic symptoms and death, and post-mortem evaluation of tissues revealed extensive CAR T cell infiltration into certain brain structures, particularly cerebellum, known to contain GD2.

Conclusions
The introduction of an affinity-enhancing mutation into the GD2-specific CAR dramatically increases CAR T cell potency and permits off-tumor CAR T cell activity in areas of the brain containing GD2. This scenario provides a new opportunity to investigate the mechanism of this toxicity and test strategies to achieve a therapeutic window.

P39
Evaluating the potential of Müllerian inhibiting substance type II receptor (MISIIR) as a target for CAR T cell therapy against ovarian cancer
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Background
Ovarian cancer is responsible for 5% of cancer-related deaths among women, and the majority of the cases are diagnosed at a late stage, accounting for a 5-year survival rate of 27%. Therefore, there is a dire need for effective therapies. The recent success of adoptive cell therapy using T cells engineered to express anti-CD19 chimeric antigen receptors (CARs) for the treatment of hematologic malignancies, rationalizes the development of similar strategies for solid tumors such as ovarian cancer. The achievement of safe, effective therapy requires the selection of a target antigen that is overexpressed in malignant cells but present in few to no normal cells. The Müllerian inhibiting substance type 2 receptor (MISIIR) is a member of the TGF-β receptors family involved in the regression of the primordial female reproductive tract in male embryos. This action is exerted through its interaction with soluble Müllerian inhibiting substance (MIS), triggering a downstream signaling cascade that induces apoptosis. MIS signaling through MISIIR has been shown to cause growth inhibition in ovarian, breast, prostate and endometrial cancer cell lines in vitro. In humans, MISIIR is expressed at very low levels in a restricted set of healthy tissues but is overexpressed in gynecologic cancers, including 69% of epithelial ovarian cancers, making it a candidate target antigen.

Methods
Here, we evaluate for the first time the potential of MISIIR as a target for CAR T cell therapy. In this work, we generated and functionally tested 5 distinct CARs comprised of different human MISIIR-specific single-chain antibody variable fragments (scFv) isolated from a phage display library coupled to the T cell signaling domains from CD27 and CD3Z.

Results
All the CARs were efficiently expressed primary human T cells transduced using recombinant lentivirus technology and showed specific binding and reactivity against recombinant MISIIR protein. Interestingly, when co-cultured with target cells engineered to overexpress MISIIR, just one of the CARs, GM7-27Z, showed specific reactivity in terms of cytolytic function and proinflammatory cytokines secretion. The activity of this CAR was further evaluated in vitro and in vivo in a panel of tumor cells lines expressing different levels of the target antigen.

Conclusions
Although the assessment of CAR-mediated antitumor activity and on-target off-tumor toxicity potential in vivo is required, the results obtained do far support the further exploration of an anti-MISIIR CAR-based therapy for the effective treatment of ovarian cancer as well as other gynecologic malignancies.

P40
IL-2 in adoptive cell therapy-local production from an adenovirus vector instead of systemic administration results in safety and efficacy gains
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Background
The use of interleukin-2 (IL-2) has been a major asset to boost the therapeutic anti-tumor efficacy of adoptive cell therapy, including tumor infiltrating lymphocyte (TIL) therapy in the context of solid tumors. However, clinical assessments have revealed that its systemic administration results in poor accumulation at solid tumor sites. Additionally, the half-life of this recombinant molecule is short. High dose administration has therefore been used but this results in severe adverse events, including mortality. Hence, local production at the tumor is an attractive concept which might retain or even increase the useful aspects of IL-2, while reducing systemic side effects.

Methods
We aimed to evaluate the efficacy and safety of, intratumoral delivered IL-2 armed adenoviruses combined with T cell transfer in rodents. Experiments were set up using the syngeneic CB57BL/6 mouse B16.OVA melanoma tumor model infused with OVA-specific T cells, and the syngeneic Syrian hamsters Hap1 pancreatic tumor model infused with TILs. Both therapeutic schedules involved once-a-week intratumoral administration of replication deficient serotype 5 (mice) or oncolytic serotype S/3 chimeric (hamsters) IL-2 armed adenoviruses comparing with weekly-continuous systemic administration of recombinant IL-2.

Results
In both models, local production of IL-2 successfully replaced that need for systemic IL-2. In fact, efficacy was even higher than with systemic IL-2. Furthermore, the vectored delivery of IL-2 significantly potentiated the infiltration of CD8+ T cells and, significantly, decreased the percentage of regulatory T cells. In animals that received systemic recombinant IL-2 therapy, significant histological changes...
were observed in the lungs, liver, heart, spleen and kidneys that should be considered as side-effects of the treatment.

Conclusions
In summary, local production of IL-2 seems appealing from the point of view of efficacy and safety in the context of adoptive cell therapy. This preclinical assessment provides the rationale for clinical translation, which is ongoing by TILT Biotherapeutics Ltd.

Table 4 (Abstract P41).

<table>
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<th>Tumor</th>
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<tr>
<td>Bladder</td>
<td>2</td>
<td>290 (97-600)</td>
</tr>
<tr>
<td>Cervical</td>
<td>4</td>
<td>360 (147-800)</td>
</tr>
<tr>
<td>H&amp;N</td>
<td>7</td>
<td>539 (132-738)</td>
</tr>
<tr>
<td>Lung</td>
<td>8</td>
<td>688 (50-915)</td>
</tr>
<tr>
<td>TNBC</td>
<td>13</td>
<td>429 (81-665)</td>
</tr>
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</table>

were successful in culturing and expanding TILs from various non-melanoma solid tumors. We will initiate REP from pre-REP TILs from non-melanoma tumors to enable product development for subsequent possible clinical trials. Efforts are currently focused on culturing TILs from smaller tumor specimens/biopsies to assess utility in promoting expansion of TILs with central and effector memory phenotypes and selecting for mutanome reactive TILs.

References

P42
A tumor-penetrating recombinant protein anti-EGFR-iRGD enhances efficacy of antigen-specific CTL in gastric cancer in vivo
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Background
Strategies that enhance the function of T cells are critical for immunotherapy. Targeted delivery of T cells through BiTE (bispecific T cell engager) platform to cancerous tissues shows potential in sparing unaffected tissues. However, it has been a major challenge for cells penetration in solid tumor tissues due to the complicated tumor microenvironment. Activated T cells expression integrin, which is the target of peptide RGD. Peptide iRGD (CRGDK/RGPD/EC) increased vascular and tissue permeability in a tumor-specific and neuropilin-1-dependent manner, allowing co-administered drugs to penetrate into extravascular tumor tissue. Recombinant protein anti-EGFR-iRGD was purified and examined.

Methods
Recombinant protein anti-EGFR-iRGD consisting of an anti-EGFR VH1 (the variable domain from the heavy chain of the antibody) fused to iRGD, a tumor-specific binding peptide with high permeability were expressed in E. coli BL21 (DE3) and purified by nickel-nitrilotriacetic acid affinity chromatography. We use tumor cell lines and mice to analyze the targeting, penetrating and antitumor activity of antigen-specific T cells together with recombinant protein.

Results
We have successfully constructed a recombinant protein named anti-EGFR-iRGD, a dual targets of EGFR and integrin and high permeable protein. It could spread extensively throughout both the multicellular spheroids and the tumor mass. The recombinant protein anti-EGFR-iRGD could help more T cells infiltrating into tumor mass and also exhibited antitumor activity in tumor cell lines and mice.

Conclusions
Our results provide impetus for further studies for potentially using iRGD based fusion protein anti-EGFR-iRGD with immune therapy regimens for enhancing therapy of gastric cancer patients.

Fig. 32 (Abstract P42). Purification and verification of recombinant protein
P43

Immunodominance of cancer neoantigen and cancer-germline antigen T cell reactivities in successful immunotherapy of virally-induced epithelial cancer
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Background
Immunotherapy has clinical activity in human papillomavirus (HPV)-induced epithelial cancers, but the tumor antigens targeted by T cells resulting in cancer regression are poorly defined. The viral proteins expressed by these malignancies are generally considered the primary targets of T cell based immune attack. However, HPV+ cancers also harbor somatic mutations and express cancer-germline antigens that may be targets of tumor-specific T cells. Here, we aimed to elucidate the landscape of tumor antigens targeted by T cells in two patients with metastatic HPV+ cervical cancer who experienced durable complete tumor regression after adoptive transfer of tumor-infiltrating lymphocytes (TIL).

Methods
To this end, reactivity of therapeutic TIL was assessed in immunological assays against HPV-derived antigens (L1, L2, E1, E2, E4, E5, E6 and E7), mutated neoantigens and cancer-germline antigens identified by whole-exome and/or RNA sequencing of patient's tumors. T cell receptor (TCR) clonotypes conferring specificity to tumor antigens were elucidated and quantified, and their in vivo persistence was profiled by TCR deep sequencing.

Results
T cell reactivity against the HPV-E6 and/or -E7 antigens was detected in both patient's infused TIL, consistent with previously reported results. No T cell reactivity was detected against other six HPV-derived antigens. However, our data indicated that these patient's infused TIL distinctly recognized mutated neoantigens or a cancer-germline antigen. Detailed TCR clonotype analysis showed that in one patient multiple CD8+ clonotypes (35% of infused TIL) recognized somatically mutated gene products (n=3) unique to patient's tumor in addition to several CD8+ and/or CD4+ clonotypes (14% of TIL) targeting HPV-E6 and/or -E7. In the other patient, one CD8+ clonotype (67% of TIL) recognized the cancer-germline antigen Kita-kyushu lung cancer antigen 1 in addition to one CD4+ clonotype (14% of TIL) that targeted HPV-E7. Administered viral and non-viral tumor antigen-specific T cells in both patients remained functional and persisted at elevated levels in the circulation for months during ongoing remission.

Conclusions
Our data show that both patients who experienced complete tumor regression received TIL that contained low frequency of HPV-targeted T cells but a high frequency of mutated neoantigen- or cancer-germline antigen-targeted T cells. These results reveal a previously unappreciated role for T cells targeting non-viral antigens in HPV+ cervical cancer. By expanding the categories of potential tumor regression antigens for cervical cancer and possibly other HPV-induced malignancies, our findings provide new targets for personalization of cancer vaccines and/or adoptive T cell therapies as well as for immune-monitoring of various cancer immunotherapies.

Consent
Written informed consent was obtained from patients.

P44

Adaptive cellular therapy (ACT) with allogeneic activated natural killer (aNK) cells in patients with advanced Merkel cell carcinoma (MCC): preliminary results of a phase II trial
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Background
MCC is an aggressive skin cancer associated with Merkel cell polyomavirus (MCPyV). Downregulation of class I major histocompatibility complex (MHC) expression in >80% of MCC tumors suggests potential susceptibility to adoptively transferred NK cells. aNK cells are derived from a NK cell line that is highly cytotoxic to a broad range of tumor cells. Phase I studies suggest that aNK cell therapy is well tolerated and has antitumor activity in patients with advanced hematologic or solid cancers. This study seeks to determine the efficacy of aNK cell therapy in patients with advanced MCC.

Methods
In this open-label phase II study, advanced MCC patients (N=24) receive aNK cells (2 × 10^9 cells/m²) intravenously on two consecutive days (1 cycle) every 2 weeks. Key eligibility criteria include age ≥18 years, unresectable stage III or IV MCC, and ECOC performance status ≤2. Up to two prior systemic chemotherapies and/or immunotherapies are allowed. The study uses a Simon optimal two-stage design. The primary efficacy endpoint is 4-month progression-free survival (PFS) rate. Secondary endpoints include objective response rate by RECIST 1.1, time to progression, overall survival, safety, and quality of life assessment (FACT-G). Planned correlative studies include genomic and proteomic tumor profiles, MCPyV status, and immunohistochemical assessment of MHC-1, correlated to treatment outcome.

Results
As of August 2016, 3 patients have been enrolled. Treatment-related adverse events have been grade 2 or milder with no serious adverse events. The efficacy criteria for the first stage of the study has been met, with a patient with advanced MCC refractory to multiple prior therapies including PD-1 blockade demonstrating an impressive partial response (PR) with >70% regression, ongoing at 20 weeks. Intriguing changes were observed clinically in another patient’s superfi-
Background

Anti-CD19 CAR T cell therapy has shown promising clinical efficacy. Recent evidence points to a critical role for non-myeloablative conditioning chemotherapy, influencing the expansion, persistence and activity of CAR T cells. To diminish toxicities, Kochenderfer, et al. pioneered a conditioning chemotherapy regimen with low dose cyclophosphamide (300-500mg/m^2) and fludarabine (30mg/m^2) administered daily for 3 days [1]. This resulted in a response rate of 73% in patients with advanced Non-Hodgkin lymphoma, with lower rate of hematologic toxicities. Post CAR T cell treatment peak levels of cytokines in blood were associated with T cell expansion, clinical efficacy or neurotoxicity [1].

Methods

We analyzed 41 blood biomarkers in 22 patients treated with anti-CD19 CAR T cells, preceded by low dose conditioning chemotherapy. We also measured cytokine levels produced by CAR T cells ex vivo, upon culture with CD19-expressing target cells. The expansion of CAR T cells in blood was measured by quantitative PCR.

Results

Conditioning chemotherapy enhanced IL-15 and decreased lymphocytes and perforin blood levels. Several cytokines peaked sequentially in blood post CAR T cell infusion: among those, IL-15 and GM-CSF at days 2-3, followed by IL-10 and Granzyme B, at day 6. CAR T cell expansion in blood occurred within 7-14 days. IL-15 blood levels associated with T cell expansion, clinical response and toxicities. In addition, early post-CAR T cell treatment levels GM-CSF and peak blood levels of IL-10 and Granzyme B, were associated with clinical efficacy or neurotoxicity. When stimulated with CD19-expressing cells, CAR T cells produced a broad range of cytokines and perforin blood levels. Several cytokines peaked sequentially in blood post CAR T cell infusion: among those, IL-15 and GM-CSF, followed by IL-10 and Granzyme B, at day 6. CAR T cell expansion in blood occurred within 7-14 days. IL-15 blood levels associated with T cell expansion, clinical response and toxicities. In addition, early post-CAR T cell treatment levels GM-CSF and peak blood levels of IL-10 and Granzyme B, were associated with clinical efficacy or neurotoxicity. When stimulated ex vivo with CD19-expressing cells, CAR T cells produced a broad range of cytokines including GM-CSF, IL-10 and Granzyme B, but not IL-15.

Conclusions

In conclusion, these preliminary findings suggest that three immune programs impact clinical outcome of CAR T cell treatment: a T cell proliferative program initiated by conditioning chemotherapy, together with an inflammatory and a cytotoxic program deployed by CAR T cells. In addition, this analysis highlights the need to carefully optimize the conditioning chemotherapy regimen.

Acknowledgements

This study was conducted under a CRADA between the NCI and Kite Pharma.

Trial Registration

ClinicalTrial.gov identifier NCT00924326.

References


Fig. 34 (Abstract P46). Rapid expansion of TILs using irradiated engineered MOLM14 or PBMC feeders. TIL were co-cultured with PBMC Feeders or MOLM14 (CD86/41BBL) at 1:100 ratios plus OKT3 (30ng/ml) and IL-2 (3000 IU/ml). Cells were counted and split on Day 6 and 11. Each dot represents cell numbers determined on Day 14.
P47
Blocking vasoactive intestinal peptide signaling modulates immune checkpoints and graft-versus-leukemia in allogeneic transplantation in mice
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Background
The goal of allogeneic bone marrow transplantation (allo-BMT) is the elimination of leukemia cells through the graft-versus-leukemia (GvL) activity of donor cells, while limiting graft-versus-host disease (GvHD). Immune checkpoint pathways regulate GvL and GvHD activities, but blocking these pathways can cause lethal GvHD. Vasoactive intestinal peptide (VIP) is an immunosuppressive neuropeptide that regulates co-inhibitory pathways.

Methods
Murine models of MHC-mismatched allogeneic bone marrow transplantation were used to evaluate the effect of blocking VIP-signaling on the graft-versus-leukemia (GvL) and graft-versus-host disease (GvHD) effect of donor T cells. Mice were transplanted with donor grafts from VIP-KO mice or recipients of wild-type grafts were treated with seven daily injections of a peptide antagonist to VIP (VIPhyb). Survival, GvHD and the growth of luciferase+ LBRM, a T cell lymphoblastic leukemia cell line, or C1498, a myeloid leukemia cell line, were monitored by bio-luminescent imaging. Expression of chemokine receptors, cytokines and checkpoint molecules were measured by flow cytometry. VIP expression on donor and host cells was visualized using a transgenic mouse in which GFP expression is driven by the VIP promoter. T cell repertoire from T cells in mice with GvHD or GvL was analyzed by deep sequencing.

Results
VIP is expressed transiently in donor NK, NKT, dendritic cells, and T cells after allo-transplant, as well as in host leukocytes. A peptide antagonist of VIP signaling (VIPhyb) increased T cell proliferation in vitro and reduced IL-10 expression in donor T cells. Treatment of allo-BMT recipients with VIPhyb, or transplanting donor grafts lacking VIP (VIP-KO), activated donor T cells in lymphoid organs, reduced T cell homing to GvHD target organs, and enhanced GvL without increasing GvHD in multiple allo-BMT models. Genetic or ex vivo deletion of donor NK cells or CD8+ T cells from allografts abrogated the VIPhyb-enhanced GvL activity. VIPhyb treatment led to downregulation of PD-1 and PD-L1 expression on donor immune cells, increased effector molecule expression, and expanded oligoclonal CD8+ T cells that protected secondary allo-transplant recipients from leukemia.

Conclusions
VIP production by donor immune cells is dynamically regulated after allo-BMT, and transplanting VIP-KO cells, or daily treatment with VIPhyb, significantly enhanced survival of leukemia-bearing transplant recipients via a CD8+ T cell dependent GvL effect without increased GvHD in murine models of MHC mis-matched allo-BMT. Blocking VIP-signaling thus represents a novel pharmacological approach to separate GvL from GvHD and enhance adaptive T cell responses to leukemia-associated antigens in allo-BMT.

Fig. 35 (Abstract P47). Treatment of allo-BMT recipients with a VIP antagonist induces a CD8+ donor T cell-dependent GvL response associated with down-regulation of PD-L1 on donor pDC and expansion of oligoclonal donor CD8+ T cells. A: Survival curves and GVHD clinical scores for B10.BR→B6 allo-BMT harboring C1498 leukemia cells. Donor T cell subsets were depleted of specific populations by MACS prior to transplantation as shown. B: Expression of PD-L1 on donor pDC in B6→B10.BR transplants. C: TCR Vbeta and J gene segments present in donorCD8+ T cells (left), CD8+ T cells from mice with GvHD (middle) and CD8+ T cells from mice with GvL (right). D: Lack of correspondence between TCR Vbeta and J genes sequenced in mice with GvHD and mice with GvL.

P48
Bortezomib sensitizes cancer stem cells from solid human tumors to natural killer cell-mediated killing
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Background
Cancer stem cells (CSCs) from solid and hematopoietic tumors resist conventional cytotoxic therapies that target rapidly proliferating cells. Thus, residual CSCs can hide within the tumor niche and seed relapse and metastasis. Due to their relapse potential there is an urgent need to identify ways to therapeutically target CSCs. We previously found that cells expressing high amounts of the stem cell associated protein aldehyde dehydrogenase (ALDH) are effectively killed by activated natural killer (NK) lymphocytes. NK cells are known to kill malignant cells though apoptotic processes inherent to the target cell, such as TRAIL-DR5 or Fas-FasL binding, without prior immunization. We and others have also found that the FDA
approved proteasome inhibitor, bortezomib, sensitizes tumor cells to NK cell killing by upregulating DRs and intracellular machinery associated with apoptosis. Based on this previous work, we investigated the effects of bortezomib to promote NK cell killing of ALDH<sup>bright</sup> CSCs. We evaluated CSCs derived from solid tumors, in vitro and in vivo, for the induction of receptors associated with NK cell mediated killing, and for their susceptibility to NK killing after treatment.

**Methods**

In vitro sensitization and cytotoxicity assays were performed using cultured NK cells isolated from human peripheral blood. The glioblastoma and sarcoma cell lines, U87 and A673, respectively, were first treated with bortezomib, then co-cultured with activated NK cells at serial effector:target ratios. Target tumor cells were analyzed using flow cytometry for cell survival, and expression of Fas, DR4, DR5, and MICA/B on both ALDH<sup>bright</sup> and ALDH<sup>dim</sup> cells.

**Results**

Bortezomib led to a 3-fold increase in the percentage of viable ALDH<sup>bright</sup> glioblastoma and sarcoma cells, in vitro, compared to untreated controls. Bortezomib enhanced the expression of Fas and DRs by 10% and 40%, respectively, in ALDH<sup>U87</sup> U87 cells. It increased the expression of DR4 by 20% in ALDH<sup>A673</sup> A673 cells. However, bortezomib had little effect on ALDH<sup>dim</sup> cells. Bortezomib pretreatment led to a 98% decrease in viable ALDH<sup>bright</sup> cells following NK cytotoxicity assays in vitro. In vivo, bortezomib improved the efficacy of adoptive NK cell therapy in multiple mouse xenograft models.

**Conclusions**

ALDH<sup>bright</sup> CSCs are resistant to the cytotoxic effects of bortezomib. Bortezomib resistance is marked by increases in the expression of Fas, DR4, and DR5 and leads to increased susceptibility to lysis by activated NK cells. The combined use of bortezomib with activated natural killer cells could act as a potential anti-CSC strategy to improve outcomes for patients with solid tumors.

**P49**

**Targeted NK cells display potent activity against glioblastoma and induce protective antitumor immunity**

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**Background**

Significant progress has been made over the last decade towards realizing the potential of natural killer (NK) cells for cancer immunotherapy. In addition to donor-derived primary NK cells, also continuously expanding cytotoxic cell lines such as NK-92 are being considered for adoptive cancer immunotherapy. High cytotoxicity of NK-92 has previously been shown against malignant cells of hematologic origin in preclinical studies, and general safety of infusion of NK-92 cells has been established in phase I clinical trials. To enhance their therapeutic utility, here we genetically modified NK-92 cells to express a chimeric antigen receptor (CAR), consisting of an ErbB2 (HER2)-specific scFv antibody fragment fused via a linker to a composite CD28-CD3ζ signaling domain. GMP-compliant protocols for vector production, lentiviral transduction and expansion of a genetically modified NK-92 single cell clone (NK-92/S.28.2ζ) were established.

**Methods**

Functional analysis of NK-92/S.28.2ζ cells revealed high and stable CAR expression, and selective cytotoxicity against ErbB2-expressing but otherwise NK-resistant tumor cells of different origins in vitro. Ongoing work focuses on the development of these cells for adoptive immunotherapy of ErbB2-positive glioblastoma (GBM). ErbB2 expression in primary tumors and cell cultures was assessed by immunohistochemistry and flow cytometry. Cell killing activity of NK-92/S.28.2ζ cells was analyzed in *in vitro* cytotoxicity assays. *In vivo* antitumor activity was evaluated in NOD-SCID IL2Rnull (NSG) mice carrying orthotopic human GBM xenografts and C57BL/6 mice carrying orthotopic ErbB2-expressing murine GBM tumors.

**Results**

We found elevated ErbB2 protein expression in >40% of primary GBM samples and in the majority of GBM cell lines investigated. In *in vitro* assays, NK-92/S.28.2ζ in contrast to untargeted NK-92 cells lysed all ErbB2-positive established and primary GBM cells analyzed. Potent *in vivo* antitumor activity of NK-92/S.28.2ζ was observed in orthotopic GBM xenograft models in NSG mice, leading to a marked extension of symptom-free survival upon repeated stereotactic injection of CAR NK cells into the tumor area. In immunocompetent mice, local therapy with NK-92/S.28.2ζ cells resulted in cures of transplanted syngeneic GBM in the majority of animals, induction of endogenous antitumor immunity and long-term protection against tumor rechallenge at distant sites.

**Conclusions**

Our data demonstrate the potential of ErbB2-specific NK-92/S.28.2ζ cells for adoptive immunotherapy of glioblastoma, justifying evaluation of this approach for the treatment of ErbB2-positive GBM in clinical studies.

**P50**

Shared T cell receptor sequences between HLA-A2+ patients vaccinated against a Melan-A epitope correlate with clinical benefit

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**Background**

Adaptive T Cell Therapy (ACT) of *in vitro* expanded T cell clones or transduced T cells redirected against defined tumor antigens has shown therapeutic efficacy in some patients. Ideas to improve upon this therapy are multifaceted, including combining ACT with checkpoint blockade, increasing the number of defined T cell receptors (TCR) against defined antigens, identifying new tumor-specific somatic mutations to target, and engineering TCGRs to have increased avidity. However, even for well characterized antigens such as Melan-A, the optimal TCR is not known. Some engineered TCGRs have shown off-tumor toxicity, and so selecting TCGRs with maximal therapeutic efficacy but at the same time giving minimal side effects remains an important goal.

**Methods**

We reasoned that one strategy for selecting optimal TCGRs might be to identify T cells expanded after active immunization against defined epitopes in patients who experienced clinical benefit but no apparent side effects. To this end, we performed deep TCR sequencing of HLA-2/Melan-A<sup>CD8</sup> T cells from 16 metastatic melanoma patients vaccinated against a Melan-A epitope.

**Results**

While changes in overall TCR clonality measured before and after vaccination did not correlate with clinical benefit, many TCGRs showed a significant increase in representation of the total TCR repertoire after vaccination. Of the 6 patients that received a clinical benefit we found 122 public TCGRs and 124 public TCR<sub>αβ</sub> sequences. 105 of these sequences showed expansion after vaccination in 2 or more patients. Surprisingly, we did not observe the defined Melan-A-specific TCGRs used previously in redirected ACT clinical trials, designated DMF4 and DMF5. Mapping of public sequences by frequency per patient and aligning TCR<sub>αβ</sub> sequence clusters highlighted several potential TCR<sub>αβ</sub>/TCGR<sub>αβ</sub> pairings. One patient was of particular interest as he had participated in two vaccine trials, with a 32-month interim between trials and clinical benefit each time. By the end of the second treatment period, the patient’s TCR repertoire contained 55 public
sequences. Interestingly, 7 of these sequences showed an initial contraction at the end of the first trial followed by a significant expansion by the end of the second trial, suggesting a strong clonotypic response to Melan-A.

Conclusions

Together, these data highlight multiple TCRα and TCRβ sequences correlating with clinical benefit in the setting of no treatment-related toxicities. Similar results have been observed in other trials utilizing CEA peptide or WT1 peptide immunization. These sequences should enable full-length cloning of TCRs to be used in redirected adoptive cell therapy.

Trial Registration

ClinicalTrials.gov identifier NCT00515528.

P51

T cells redirected to TEM8 have antitumor activity but induce 'on target/off cancer toxicity' in preclinical models

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Background

Targeting the tumor vasculature holds promise to improve the outcome for patients with refractory solid tumors. Tumor endothelial marker (TEM) 8 is an attractive target for T cell therapies since it is expressed at higher levels in malignant cells and the tumor endothelium as judged by studies using monoclonal antibodies (mAbs). However, T cells do not require high expression of the targeted antigen for activation, because of the higher overall avidity of a multivalent T cell compared to bivalent mAbs. Thus, the aim of this project was to determine the safety and antitumor activity of T cells expressing TEM8/CD3-specific T cell engagers (TEM8-ENG).

Methods

qPCR and FACS analysis was used to determine the expression of TEM8 in solid tumor and endothelial cells. TEM8-ENG T cells were generated by transducing T cells with a retroviral vector encoding a TEM8-ENG consisting of the TEM8-specific scFv L2 linked to a scFv recognizing CD3. TEM8-ENG T cell effector function was evaluated in vitro and in vivo. Appropriate controls were used including ENG T cells specific for an irrelevant antigen (CD19).

Results

To confirm the specificity of TEM8-ENG T cells we used targets that did not express TEM8 (BV173) or BV173 cells that were genetically modified to express human TEM8, murine TEM8, or murine TEM1. TEM8-ENG T cells recognized TEM8+ cells (BV173) as well as TEM8+ (BV1.73, TEM8) cells as judged by their ability to secrete IFNγ in coculture assays and kill both targets in cytotoxicity assays; in contrast, TEM8-specific cells (BV173, BV173, TEM8) were not recognized. Specificity of TEM8-ENG T cells was further confirmed with TEM8+ solid tumor cells (A431, A549, LM7, LAN1, U87) and primary endothelial cells (HHSEC, HPAEC) in contrast to TEM8neg tumor cells (KG1a, Daudi). In vivo, intratumoral administration of TEM8-ENG T cells induced regression of U373 gliomas in an orthotopic xenograft model. Intravenous administration of 1x10⁷ TEM8-ENG T cells resulted in antigen-dependent expansion and death of 7/10 mice; no toxicity was observed after the injection of 1x10⁸ TEM8-ENG T cells.

Conclusions

TEM8 is expressed in tumor endothelium, normal endothelial cells and solid tumor cells as judged by qPCR, FACS, and functional assays. TEM8-ENG T cells had antitumor activity in vivo, but displayed dose-dependent toxicity. Our studies highlight that mAbs and T cells may have different toxicity profiles, most likely due to differences in their avidity for the targeted antigen. TEM8-ENG T cell xenograft models represent an ideal model to study genetic approaches to prevent 'on target/off cancer toxicities' of cell therapies.

P52

A pathogen boosted adoptive cell transfer immunotherapy to treat solid tumors

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Background

Despite the remarkable success in treating hematological malignancies, adoptive cell transfer (ACT) still faces several challenges in treating solid tumors. The main stumbling blocks include insufficient quantity of tumor-specific T cells for transfer, impaired migration of transferred T cells into the tumor and the immunosuppressive microenvironment within the tumor.

Methods

To overcome these problems, we designed an innovative approach that not only overcomes immunosuppression, but also induces robust anti-tumor T cell responses in the tumor. We first genetically engineered dual-specific CD8 T cells that can recognize both a tumor associated antigen and a bacterial antigen in vitro. Then, we treated tumor-bearing mice with ACT using a small number of the dual-specific CD8 T cells. This was accompanied by intratumoral injection of a low dose of the bacteria, which was sufficient to break local immunosuppression.

Results

The dual-specific CD8 T cells expanded robustly and migrated to the tumor bed in response to the infection. At the same time, the second TCR of these效应or CD8 T cells recognized tumor antigen and executed effector function, causing tumor regression. As a result of this enhanced anti-tumor effect, 60% of the treated mice successfully eradicated their solid tumor at the primary site.

Conclusions

Our approach not only overcomes immunosuppression, but also recruits robust anti-tumor T cell responses to the tumor. Overall, our study harnesses the power of multiple arms of the immune system with promising translational value, which can be used to target many types of solid tumors.

P53

Pharmacologic rejuvenation of exhausted T cells to improve adoptive TIL therapy

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Background

Immunotherapy has emerged as a strategy for the treatment of cancer. One of these immunotherapies is adoptive tumor-infiltrating lymphocyte (TIL) therapy, in which T cells from resected tumors are expanded in vitro and then given to patients. However adoptive TIL therapy has little efficacy for many patients, because the tumor microenvironment creates an extreme environment for T cells. Our lab has revealed that T cells display metabolic defects, especially a loss of mitochondria, when they infiltrate the tumor microenvironment. This loss is related to T cell exhaustion. We hypothesize that these exhausted T cells were the most functional cells as they responded to tumor earliest and strongest. However their
loss of mitochondria prevents them from further expansion when removed and cultured in vitro. Thus, we are utilizing what we have identified about their metabolic dysfunction to rejuvenate those T cells during ex vivo expansion. Our goal is to make exhausted T cells more metabolically active and provide a potent method for TIL therapy.

Methods

Tumor injection: mice were given either 250,000 B16 or MC38 tumor cells injected intradermally in the center of the back. T cell activation: TILs are activated with 3 ug/ml anti-CD3 (plate bound), 2 ug/ml anti-CD28, 50 units/ml IL-2. Adaptive TIL transfer: treated and non-treated TIL are given to the mice that bear tumors by intravascular injection.

Results

PD-1hi cells remain mitochondrial deficient and fail to proliferate ex vivo. Rosiglitazone can rescue mitochondrial mass and proliferation. PD-1 Tim-3hi cells are over proliferated by PD-1 Tim-3lo cells. Preliminary data has shown that glitazone compounds to long-term expansion protocols prevents loss of the previously-exhausted T cells during expansion.

Conclusions

Cells expressing high levels of PD-1 and Tim-3 have low mitochondrial mass and fail to proliferate effectively in vitro. Mixing congenically marked cells from the non-exhausted or exhausted compartment shows exhausted cells are quickly overtaken by the non-exhausted (less than 1 week). Adding glitazone compounds to stimulate mitochondrial biogenesis results in short-term improvement of T cell proliferation in vitro. Preliminary data has shown that glitazone compounds to long-term expansion protocols prevents loss of the previously-exhausted T cells during expansion.

Acknowledgements

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References

Biomarkers and Immune Monitoring

P55

Map of targets on dendritic cells (DC) in human tonsils and lymph nodes potentially facilitating antigen cross-presentation

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Background

Dendritic cells (DCs) orchestrate adaptive and innate immune responses and are therefore key targets for immunotherapy (IT). Of special interest for IT directed against cancer are DCs with a high capacity of antigen cross-presentation, potentially resulting in cell-mediated tumor antigen-specific effects. This study reports an update on the map of DC subtypes and phenotypical aspects that may be reached by adjuvant measures in human tonsils and lymph nodes, both potential sites for “vaccine” deposition.

Methods

From biopsies of tonsils (n=23) and neck lymph nodes (n=16), single cell suspensions were prepared by enzymatic digestion and DCs were identified by an 8-color flow cytometry Ab panel. DC subsets frequencies (CD1c+, CD123+, CD141+, CD1c- CD141-), maturity status, and PRR expression. However, a higher frequency of CD141+ cells in tonsils compared to lymph nodes. No maturity differences were found among the DC subsets in tonsils and lymph nodes based on expression of CD80 and CD86. DC subsets expressing XCR1, TLR2, CLECSP14, CD206, DEC205, and Dectin-1 were observed with similar frequencies in tonsils and lymph nodes. However, among the DC subsets studied, CD141+ DCs showed a higher frequency in tonsils compared to lymph nodes. No maturity differences were found among the DC subsets in tonsils and lymph nodes based on expression of CD80 and CD86. DC subsets expressing XCR1, TLR2, CLECSP14, CD206, DEC205, and Dectin-1 were observed with similar frequencies in tonsils and lymph nodes.

Conclusions

DCs in tonsils and lymph nodes largely share similar features in terms of frequency, maturation, and PRR expression. However, a higher frequency of CD141+ cells in tonsils may be of interest considering this subset’s capability in antigen cross-presentation. Our work suggests tonsils as well as lymph nodes as vaccine deposition sites in DC-mediated IT. Furthermore, specific adjuvant measures directed at C-type lectin receptors, TLR2, and

P54

A turbocharged chimeric antigen receptor against prostate cancer

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Background

Both CD28- and 4-1BB-based second-generation CAR T cells elicit dramatic clinical responses in patients with refractory/relapsed CD19 positive malignancies, especially patients with acute lymphoblastic leukemia. We recently demonstrated that co-expressing the second-generation 19-28Z CAR with 4-1BBL yields balanced tumoricidal function and T cell persistence, resulting in the greater therapeutic efficacy (Turbocharged CAR). However, due in part to their tumor microenvironment, solid tumors often resist CAR T cell therapy. We hypothesized that CD28-based second-generation CAR T cells coexpressing 4-1BBL would have better therapeutic efficacy against solid tumors than current second-generation CARs, owning to their unique intrinsic and immunomodulatory qualities.

Methods

Prostate-specific membrane antigen (PSMA) is a dimeric type II integral membrane glycoprotein, which is overexpressed in castrate-resistant, metastatic prostate cancer. We constructed a tricistronic PSMA-targeted CAR vector encoding the Pd28z CAR, 4-1BBL and a truncated, nonfunctional EGFR as a safety control (Pd28z-4-1BBL-EGFRT). Two second-generation CARs (Pd28z and PdB8z) served as controls.

Results

In a high tumor burdened model of disseminated prostate cancer, we used the in vivo “stress test” in which the T cell dose is gradually lowered to levels where CAR therapy begins to fail, in order to compare the relative functionality and persistence of these CAR T cells. CAR T cells coexpressing Pd28z with 4-1BBL exhibited higher tumor eradication and T cell persistence in NSG mice bearing diffuse metastatic prostate cancer, compared to both second-generation CARs Pd28z and PdB8z.

Conclusions

4-1BBL Turbocharged CAR T cells thus seem to possess striking therapeutic potential against solid tumors.
XCR1 may be employed to achieve cross-presentation of antigen and cell-mediated tumor antigen-specific effects.

| P56 | PD-L1 and immune infiltrates are prognostic and differentially expressed in distinct subtypes of gastric cancer
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**Background**
Gastric cancer (GC) is often diagnosed at an advanced stage, for which therapeutic options are largely limited to cytotoxic chemotherapy and five-year survival is less than 20%. Immune checkpoint blockade with anti-programmed cell death-1 (PD-1) or anti-programmed cell death ligand-1 (PD-L1) antibodies is emerging as a promising therapeutic approach for several cancer types. An important question is whether the clinical efficacy of PD-1/PD-L1 checkpoint blockade can be improved through combination with targeted agents, such as trastuzumab, for use in human epidermal growth factor receptor 2 (HER2)-positive disease and olaparib, a poly(ADP-ribose) polymerase (PARP) inhibitor. This study determines the association of PD-L1 expression and immune cell infiltrates with clinical outcome and investigates the overlap of these with microsatellite instability (MSI)-high, ATM low and HER2 high segments.

**Methods**
PD-L1 membrane expression on tumour cells (TC) and infiltrating immune cells (IC), CD3+ T lymphocytes, CD8+ cytotoxic T cells, ATM and HER2 were assessed by immunohistochemistry (IHC) in a cohort of 380 Korean gastric cancer patients. PD-L1 positivity was assessed by a pathologist (positive <0%). CD3 and CD8 were quantified by HALO® image analysis (cells/mm²). EBV status was determined using in situ hybridization and MSI status was performed using PCR and MLH1 IHC.

**Results**
The ATM-low and HER2-high segments are mutually exclusive and differ markedly in their immune profile; the ATM-low segment being enriched for MSI (p < 0.01), PD-L1 TC positivity (p < 0.01) and CD8+ cytotoxic immune infiltrates (p=0.033), while the HER2 segment is enriched for MSS, with no enrichment for immune markers. The PD-L1 segment is associated with increased T cell infiltrates: CD3 (p < 0.01) and CD8 (p < 0.01), while the MSI-high segment is enriched for PD-L1 TC (p < 0.01), PD-L1 IC (p < 0.001), CD3 (p < 0.05) and CD8 (p < 0.01), and has significant overlap with the ATM-low but not HER2 segments. Multivariate analysis confirmed PD-L1 TC positivity (p < 0.01), high CD3 (overall survival OS P < 0.01; disease-free survival [DFS] p=0.021) and high CD8 (OS p < 0.01; DFS p=0.027) as independent prognostic factors for both DFS and OS. Patients with MSI-high tumours had better overall survival by both univariate (p < 0.01) and multivariate (p < 0.05) analysis.

**Conclusions**
Here we present evidence for segmentation of gastric cancers into four distinct molecular segments, namely ATM-low, HER2-high, PD-L1 positive and MSI-high. This illustrates the potential for subsets of GC patients to respond differently to immune therapy and the opportunity to employ different strategies for maximizing the benefit from immune therapies in these segments.

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| P57 | Four color T and B cell ELISPOT assays for simultaneous detection of analytes
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**Background**
ELISPOT assays are a key research tool for enumerating antigen-specific T and B cells in PBMC. As both T and B cells occur in major classes, immune monitoring has to be concerned with identifying these as well. So far, ELISPOT assays have been primarily done single or double color. In this report, we demonstrate the development of four color T and B cell ELISPOT assays.

**Methods**
PBMC were cultured for 4 days with a peptide pool of CMV-, EBV- and Flu- viruses for T cell assays or polyclonal B cell activators for B cell assays. On day 4, cells were washed, counted and plated in a low autofluorescence PVDF plate. Plates were precoated with capture antibodies for detection of IFN-γ, IL-2, G2b, or TNF-a (T cell assays) or Ig secretion (B cell assays). During a 4h culture, the cytokine or antibodies secreted by the individual T or B cells respectively was captured on the membrane. The plate-bound “spots” were visualized using cytokine-specific or IgG subclass- or Ig class-specific detection reagents, whereby each detection reagent is distinguished from the other 3 reagents through its unique fluorescence. The four-color assays were analyzed using an ImmunoSpot® S6 Analyzer.

**Results**
We show that the four color assay has the same sensitivity for detecting individual cells secreting analytes as the respective single color assays, and that the four fluorescent colors can be discerned unambiguously, without overlap. Cells secreting any of the four analytes can therefore be identified unambiguously in an automated fashion, without the need for compensation. Cells co-expressing analytes can be identified by superimposing the individual colors. Studying B cells and T cells experimentally has permitted us to verify the accuracy of co-expression measurements. Each B cell secretes only one type of Ig class/subclass. T cells, in contrast, frequently coexpress cytokines. Serial dilution experiments showed that for T cells the numbers of co-expressors linearly decreased with the numbers of cells plated. For B cells, no coexpressers were found.

**Conclusions**
The feasibility of four color T and B cell assays have been shown here. This is particularly important when conserving cell material thereby allowing researchers the opportunity for comprehensive immune monitoring spanning multiple cytokines.

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| P58 | A positive control for the detection of functional CD4 T cells in human PBMC – CPI protein pool
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**Background**
Testing of PBMC for immune monitoring purposes requires verification of their functionality. This is of particular concern when testing cryopreserved PBMC or cells that have been shipped, stored for prolonged periods of time. The CEF peptide pool has been developed as a positive control for CD8 cell functionality. A positive control for detecting CD4 memory cell functionality so far is lacking.
Methods
Protein antigens from infectious/environmental organisms have been selected that are ubiquitous. T cell reactivity to these antigens has been tested in an IFN-g Immunospot® assay from CTL. Cryopreserved PBMC from 100 Caucasian donors were selected from CTL’s ePBMC database for testing. Magnetic bead depletion experiments were performed to verify CD4 or CD8 response.

Results
Of an initial selection of 12 antigenic systems, (Varicella, Influenza, Parainfluenza, Mumps, Cytomegalovirus, Streptococcus, Mycoplasma, Lactobacillus, Neisseria, Candida, Rubella, and Measles) 3 were selected as a) eliciting CD4 cells exclusively and b) eliciting recall responses in the majority of donors. While individually none of the antigens triggered recall responses in all of the donors, the pool of these three antigens did. Only 2 of 100 donors did not respond to the C (Cytomegalo-, Epstein Barr-, and Influenza- virus) protein pool. These two however were impaired functionally non-viable cells with increased numbers of dead and apoptotic cells and showed increased apoptotic progression. Comparisons with CEF peptide pool, CEF-7 peptide, pp65 (495-503) at 100,000 cells per well in IFH- ELISPOT assays. However, we selected donors whose PBMC, (CEF-7 peptide, pp65 (495-503)) at 100,000 cells per well and challenged with the CEF-A2-0201-restricted HCMV peptide, pp65(495-503) did not display spot counts over medium background. We tested the PBMC for pp65 reactivity in 96 replicate wells to establish the HLA-A2-0201-restricted HCMV peptide, pp65 expression also correlated with mutational load. Inferred MSI-H status was identified across multiple tumor types (aggregate prevalence 3.2%) in the Moffitt database (Fig. 36). In KEYNOTE-012 (n=96) and KEYNOTE-028 (n=265), MSI-H status was identified in 6% and 2% of patients, respectively.

Conclusions
The Normal distribution of ELISPOT counts permits us to make precise predictions regarding the numbers of replicate wells needed, and cut off values, especially when responses from donors are low.
Whole-blood RNA transcript-based signatures predict pre- and post-treatment response in two large independent clinical studies of patients with advanced melanoma treated with tremelimumab

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Background

Tremelimumab is a cytotoxic T lymphocyte-associated antigen-4 blocking monoclonal antibody. An unmet clinical need exists for blood-based response-predictive gene signatures to facilitate use of cancer immunotherapy in the most clinically effective and cost-efficient manner.

Methods

Pre- and post-treatment (30 days) peripheral blood samples were taken from 210 treatment-naive melanoma patients receiving tremelimumab in a worldwide, multicenter phase III study. Objective response was determined by an expert panel of radiologists using RECIST criteria. 169 mRNA transcripts were measured for the n=210 patients using reverse transcription polymerase chain reaction (RT-PCR) [1]. Pre- and post-treatment response-predictive signatures were identified in the n=210 training dataset. An independent population of n=150 refractory/relapsed melanoma patients receiving tremelimumab after chemotherapy enrolled in a worldwide, multicenter phase II study [2] was the test dataset.

Results

A 16-gene pre-treatment and 8-gene post-treatment mRNA signatures were identified in the n=210 training dataset. These pre- and post-treatment signatures were tested in the n=150 test dataset first, for objective response as determined by RECIST criteria, and second for one-year survival after treatment. The same genes, coefficients and constant from the training dataset were used in the test cases for one-year survival criteria also validated with even higher AUC when compared to objective response in the test dataset. The one-year survival training gene signatures validated in the n=150 test dataset for one-year survival after treatment. The same genes, coefficients and constant from the training dataset were used in the test cases for one-year survival criteria also validated with even higher AUC when compared to objective response in the test dataset.

Conclusions

This is the first large clinical study of tremelimumab, independently validated in a second large clinical study, to show both pre- and post-treatment response-predictive mRNA signatures in blood. The pre-treatment biological signature may represent expression levels of particular immune system genes that are needed for a robust immune response against cancer. They may identify patients whose immune systems are already primed to fight the cancer and are particularly amenable to a boost in effectiveness provided by immunotherapy. The 30-day post-treatment biological signature represents a timely way to determine whether the patient is responding positively to the immunotherapy.

Trial Registration

ClinicalTrials.gov identifier NCT00257205.

References


Table 5 (Abstract P61).

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Extensive analysis of PD-1 and CTLA-4 in HVs and GBM patients: implications for monitoring patients on checkpoint inhibitors

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Background

Checkpoint inhibitors are becoming widely used for immunotherapy but methods to monitor dosing and duration for each individual patient need to be more fully understood. Immune monitoring by flow cytometry is a tool that can be utilized for measuring responses to immunotherapy in patients. In this study we assessed the expression of PD-1 and CTLA-4 on numerous cell types in healthy volunteers (HVs) and glioblastoma (GBM) patients enrolled in a dendritic cell clinical trial.

Methods

Peripheral blood was collected from 20 HV and 20 GBM patients receiving a DC vaccine in a clinical trial. Whole blood was stained using a previously established method for the identification of multiple cell populations by flow cytometry and novel analysis that captures data on over 120 phenotypes [1]. An extended analysis focused on T cell phenotypes was performed using markers for CD154, CD45RO, CD56, CD3, CD8, CD28, CD4, and CD45. T cell parent populations were characterized by SS, FS, CD8, CD45RO, CD3, CD4+, CD8+, CD4+/CD8+ sub populations. Non-T cell populations were assessed by various gating strategies. These populations were measured for PD-1, CTLA4, DP, and DN populations.

Results

We identified 15 parent populations, of which 11 expressed PD-1 and 9 expressed CTLA-4. Within subsets of the parent populations we found statistically significant differences (p <0.001) in PD-1 between CD8+ memory and CD8+ naive cells, CD4+ memory and CD4+ naive cells, CD8+ NKT and CD8+CD3+ cells, as well as NKT and NK cells. These statistical differences hold true for both HV and GBM patients. We also found HVs to have higher levels of CTLA-4 on CD4+CD8+ cells and B cells compared to GBM patients, and lower levels of PD-1 on CD8+ and naive CD8+ cells.
Conclusions
This panel allows us to measure approximately 60 phenotypes related to checkpoint proteins. The data presented here identify PD-1 and CTLA-4 phenotypic differences within parent populations, within subsets of parent populations, and differences in healthy volunteers compared to GBM patients. These results may help optimize the targeting of checkpoint proteins as well as other immunotherapeutic approaches in clinical trials.

Acknowledgements
This study is funded in part by the Ivy Foundation.

Trial Registration
ClinicalTrials.gov identifier NCT01957956.

References

P64
CD8+ T cell subsets may be associated with response to anti-CD137 agonist antibody treatment
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Background
Cancer immunotherapies historically, have low success rates. One way to increase these success rates involves investigating baseline or early response biomarkers that predict success. Selecting patients with early biomarkers for success and competent immune status are essential when choosing the best therapeutic strategy. CD137 agonists, activators of T and NK cells and a promising newer immunotherapy, have dramatically reduced tumor size and disease in murine tumor models [1] and are currently being examined in clinical trials [2].

Methods
This study investigated the immune response and potential early immune biomarkers in both solid tumor and hematologic cancer patients participating in the phase I BMS-663513 NCT01471210 clinical trial of urelumab monotherapy. We used peripheral blood mononuclear cells to determine the circulating immune competence of patients (n=8) before treatment (baseline/C1D1), 24 hours after the first treatment (C1D2), before the second treatment (C2D1), and up to 30 days after the third treatment (C3R). Analysis was performed using mass cytometry, surveying 38 different immune proteins simultaneously.

Results
At all time points, we observed a trend toward higher central memory and naive CD8+ T cells in patients with stable disease (n=3) or partial response (n=1) vs. progressors (n=4), while the opposite was true in effector and effector memory RA cells. The most striking difference was seen when considering all CD8+CD27+ T cells, which were higher in those with stable disease or partial response, at all time points. CD8+FoxP3+CD28- T cells showed a similar trend, albeit to a lesser extent, while CD57+CD8+ T cells showed the opposite trend. CD8+ T cells in both groups were comparably responsive to PMA/ionomycin stimulation, producing multiple cytokines. These trends were not seen in CD4+ T cells or with head and neck solid tumor patients treated with cetuximab.

Conclusions
The aforementioned trends suggest that CD27+CD8+ T cells, and possibly other subsets of CD8+ T cells, should be further explored to determine whether they predict response to anti-CD137 agonist therapy. They also suggest that potential predictive measures of immune status prior to immunotherapy are detectable in peripheral blood.

References

P65
Regulation of PD-L1 expression in melanoma and immune cells
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Background
The therapeutic effects of PD-1/PD-L1 inhibition in multiple cancers indicates a critical role for this pathway in immunosuppression in the tumor microenvironment (TME), but factors regulating PD-L1 expression on tumor and immune cells are poorly understood. The dichotomous transcription factors STAT1 and STAT3 have both been reported to bind the PD-L1 promoter. The current study investigates the role of STAT1/3 and other signaling in cytokine-induced PD-L1 expression on human melanoma (MEL) cells and monocytes.

Methods
Seventeen cultured MELs or short-term monocyte cultures were treated with recombinant cytokines including IFN-g, IL-6, IL-10, IL-32g, or TNF-a. PD-L1 cell surface protein expression was detected by flow cytometry, and mRNA by quantitative real-time RT-PCR (qRT-PCR). STAT1 and STAT3 were knocked down by small interfering RNAs (siRNAs). Total and phosphorylated STAT1/3 proteins were quantified by Western blotting.

Results
While PD-L1 is expressed on 35-40% of MELs in situ, it was not expressed on 17 cultured MELs. IFN-g significantly enhanced PD-L1 protein expression on MELs (p=0.0003), increasing PD-L1 mRNA expression (qRT-PCR) in association with PD-L1 cell surface protein expression (FACS) in all MELs tested (p=0.0004). This suggests that IFN-g regulates PD-L1 expression primarily at the transcriptional level and not via translocation of intracellular protein stores. Enhanced PD-L1 expression in IFN-g-treated MELs correlated with increased STAT1 phosphorylation (p=0.05). Consistent with this, siRNA knockdown of STAT1 reduced PD-L1 expression by 40-70% in 2 MELs after 24-48hr IFN-g exposure. In contrast, STAT3 knockdown reduced IFN-g-induced PD-L1 expression by only 12-15%, on one of two MEL lines. In cultured monocytes from two donors, PD-L1 mRNA expression was induced by IFN-g, IL-10, IL-32-g and TNF-a, and significantly correlated with PD-L1 cell surface protein expression, suggesting that these cytokines acted at the transcriptional level. In monocytes, IFN-g was associated with markedly increased pSTAT1, and IL-10 with increased pSTAT3. These cytokines were not associated with increased pp65 or focal adhesion kinase (FAK) phosphorylation in monocytes.

Conclusions
In addition to IFN-g, other cytokines in the TME may play important, coordinate and selective roles in promoting PD-L1 expression on different cell types including tumor and stromal cells. pSTAT1 and pSTAT3 are associated with PD-L1 protein expression in response to different cytokine stimuli. Future studies will further characterize cytokine-triggered transcription factors and signaling pathways responsible for PD-L1 expression on tumor cells and immune cells. Understanding mechanisms regulating PD-L1 expression will help guide the development of more optimal predictive biomarkers and combinatorial therapies based on anti-PD-1.

P66
Gene expression markers of tumor infiltrating leukocytes
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Background
Assays of the abundance of immune cell populations in the tumor microenvironment promise to inform immune oncology research and the choice of immunotherapy for individual patients. We propose to measure the intratumoral abundance of various immune cells populations with gene expression. In contrast to IHC and flow cytometry, gene expression assays yield high information content from a clinically practical workflow. Previous studies of gene expression in purified immune cells have reported hundreds of genes showing enrichment in a single cell type, but the utility of these genes in tumor samples is unknown. We describe a novel statistical method for using co-expression patterns in large tumor gene expression datasets to validate previously reported candidate cell type marker genes.

Methods
We used co-expression patterns in 9986 samples from The Cancer Genome Atlas (TCGA) to validate previously reported cell type marker genes. We compared immune cell scores derived from these genes to measurements from flow cytometry and immunohistochemistry. We characterized the reproducibility of our cell scores in replicate runs of RNA extracted from FFPE tumor tissue.

Results
We identified a list of 60 marker genes whose expression levels quantify 14 immune cell populations. Cell type scores calculated from these genes are concordant with flow cytometry and IHC readings, show high reproducibility in replicate RNA samples from FFPE tissue and reveal an intricate picture of the immune infiltrate in TCGA. Most genes previously reported to be enriched in a single cell type have co-expression patterns inconsistent with cell type specificity.

Conclusions
Due to their concise gene set, computational simplicity and utility in tumor samples, these cell type gene signatures may be useful in future discovery research and clinical trials to understand how tumors and therapeutic intervention shape the immune response.

Fig. 37 (Abstract P66). Comparison of gene expression and flow cytometry cell type measurements in PBMCs
Quantitative real-time PCR based diagnostic to assess NKT cell function in breast cancer patients
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Background
Breast cancer is a leading cause of cancer-related death among women worldwide. Although, surgery, radiotherapy, and chemotherapy have improved the 5-year survival rate, new treatment methods are needed to combat this disease. To date, significant efforts have been invested into harnessing the therapeutic potential of the immune system for the treatment of cancer. However, tumor tolerance and immune suppression can severely limit its therapeutic efficacy. In fact, natural killer T (NKT) cells play an important role in cancer immune surveillance, but are reduced in cancer patients. In order to assess which patients will likely benefit from immune cell-based therapies, we have developed a quantitative method to rapidly assess the baseline function of NKT cells using stimulation with artificial antigen presenting cells followed by a quantitative real-time PCR (aAPC-qPCR).

Methods
In this study, we assessed NKT cell number and function in healthy donors and breast cancer patients by flow cytometry, ELISA, and qPCR. In addition, we assessed the percentage of tumor-infiltrating lymphocytes and PD-L1 expression within the tumor microenvironment by immunohistochemistry.

Results
Although % circulating NKT cell were significantly reduced in breast cancer patients (BCP), compared to healthy donors (HD), we detected NKT cell function in 82% HD (n=22) and 70% BCP (n=30). We compared high responders (high IFN-γ induction) to low responders and found that there was no significant difference in NKT cell number between these two groups. Following further characterization of these groups, it was found that low responders had a significant reduction in the induction of TNFα, LAG3, and LIGHT.

Conclusions
In summary, this data we have developed a novel diagnostic platform using aAPC-qPCR to determine NKT cell function in patients. This technology is important because NKT cell number did not correlate with function in our breast cancer patient cohort. Thus, our studies demonstrate that there is a critical need to assess baseline immune function prior to the initiation of immunotherapy. Future studies can focus identifying new breast cancer classifications according to immune gene expression patterns, and these tumor subtypes may provide a basis for new therapeutic opportunities.

Acknowledgements
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Arming of CD56dim and CD56bright NK cells in IL-15-infused cancer patients
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Background
Survival and expansion of NK cells depends on interleukin (IL)-15. It has recently been shown that IL-15 infusions caused NK cell expansions in cancer patients. The objectives of our study were to assess the effects of IL-15 on functions of CD56dim and CD56bright subpopulations of NK cells in IL-15-treated cancer patients.
CD56dim NK cells, and IL-15 infusions increased the percentage of CD94 and the IL-18 receptor, while CD56 bright NK cells remained essentially unchanged. CD56bright NK cells retained their superior ability to produce IFNγ in responses to IL-12/IL-18 when compared with CD56dim NK cells, and IL-15 infusions increased the percentage of cytokine-producing CD56bright NK cells. The cytotoxic capacity of CD56dim NK cells remained superior to CD56bright NK cells even after IL-15 infusions. However, cytotoxic competencies were increased for both subpopulations after IL-15 infusions that resulted in substantial lytic activities via natural cytotoxicity receptors, stress receptors, and antibody-dependent cytotoxicity even among CD56bright NK cells.

Conclusions
These data show that IL-15 infusions increase the functional abilities of both types of NK cells in cancer patients.

P69
Identification of a novel subset of tumor-resident human CD8+ T cells, marked by dual expression of CD103 and CD39
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Background
Homing and retention of T cells in tissues is mediated by the interaction of adhesion molecules with their respective ligands. Among these, the integrin CD103 interacts with its ligand E-cadherin and allows T cells to interact with epithelial tissues. T cells expressing high levels of CD103 have been associated with tissue residence and tumor infiltration. Recent studies have identified a subset of CD103+ CD8+ T cells that preferentially home to epithelial tissues. These cells express high levels of CD103 and are thought to play a crucial role in protecting epithelial tissues against viral infections. Previous reports have shown that CD103+ CD8+ T cells are present in some but not all human solid malignancies.

Methods
Cell sorting, gene expression analysis, and TCR sequencing.

Results
Here, while confirming these data, we identify a subset of CD103+ CD8+ T cells that co-express the ectonucleotidase CD39. This subset is enriched in primary tumors and metastatic lymph nodes but absent in the blood and normal lymph nodes of cancer patients. We compared several tumor histologies and found highest frequencies in head and neck squamous cell carcinomas (HNSCC), ovarian, lung and rectal cancers (ranging from 20-80% of tumor-infiltrating CD8+ T cells), whereas those cells were absent or low in primary colon cancer and colorectal liver metastasis. Gene expression analysis of CD103/39 double positive CD8+ T cells revealed a gene signature reminiscent of Tumors (Tumors) cells (CCR7+L-selectin+L1P1+CD69+), and their activated phenotype (HLA-DR+, KIR+) Granzyme B+ implies strong tumor reactivity. Furthermore, TCR repertoire analysis shows high clonality and distinct CDR3 sequences in this subset compared to other CD8+ T cells present in the tumor. Based on this phenotype, gene signature, circulation pattern and clonality, we believe that CD103/39 double positive CD8+ T cells are being chronically stimulated within the tumor microenvironment, and may recognize neoantigens. In support of this finding, our in vitro data suggests that expression of CD39 is upregulated as a result of strong, sustained TCR stimulation in naive CD8+ T cells. Finally, to better address the role of those cells in vivo we examined 9 different solid murine tumor models. Unexpectedly, CD103/39 double positive CD8+ T cells were only found in the transgenic MMTV-PyMT breast cancer model. Utilizing this model, we plan to study their differentiation and function in vivo and address their antigenic specificity and role in tumor development.

Conclusions
Taken together our findings suggest that targeting tumor-resident CD103/39 CD8+ T cells may be a promising approach to enhance immune-mediated tumor regression.

P70
Characterization of tumor infiltrating T cell receptor (TCR) repertoire in non-muscle invasive bladder cancer (NMIBC) patients treated with Bacillus Calmette-Guérin (BCG)
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Background
Bladder cancer is the 5th most common malignancy in the US, and the majority of bladder cancer is diagnosed while confined to the most superficial layer (non-muscle invasive bladder cancer, NMIBC) [1]. Treatment goals in this minimal residual disease setting are ideal for immunotherapy: reduce local disease recurrence following surgical resection and prevent progression to muscle invasive bladder cancer (MIBC). Bacillus Calmette-Guérin (BCG), an attenuated form of Mycobacterium bovis, is an intravesical immunotherapy which remains the mainstay of NMIBC treatment since 1976. Despite the tenure in bladder cancer treatment, full characterization of the immunologic mechanism of action of BCG is still lacking [2]. In a pilot study, we sought to investigate the diversity of T cells infiltrating bladder tumors and compare the changes in T cell diversity among patients who were responders and unresponsive to BCG.

Methods
Six patients were selected from the IRB-approved Johns Hopkins bladder cancer tumor repository. All patients had T1 disease without concurrent carcinoma in situ (CIS) and received standard of care BCG induction and maintenance. Three patients were classic “responders” to BCG, with disease-free intervals of 8, 28, and 34 months. Three patients were unresponsive to BCG: two patients progressed to MIBC and ultimately died of metastatic bladder cancer and one had recurrent disease requiring cystectomy. Pre-treatment and post-treatment tumor tissue samples were compiled; genomic DNA was isolated, amplified, and sequenced using Adaptive Biotechnologies’ ImmunoSeq assay.

Results
ImmunoSeq technology surveyed the T cells in all 12 samples. Median fraction of T cells in the samples pre-treatment was 0.16 (95% CI, 0.1419 to 0.731) while post-treatment median was 0.36 (95% CI, 0.184 to 0.532), indicating a trend towards a higher T cell fraction after treatment. Clonality, an objective measure of T cell diversity, was low in most patients and unchanged post-treatment: some T cell clones in the tumor samples expanded or contracted, though most clones were stable post-treatment, and no clones expanded to be dominant. Clonality likewise did not appear to correlate with patient response to BCG.

Conclusions
Tumor infiltrating T cell kinetics do not appear to correlate with response to BCG in this pilot sample of patients. These data suggest that an alternative mechanism involving the innate immune cell population may be the primary driver of BCG response.

References
An immune-related gene expression profile delineates features of the tumor microenvironment required for clinical response to PD-1 blockade

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Background
PD-L1 expression, which predicts response to PD-1-directed therapies such as pembrolizumab in many cancer types, is often associated with an active T cell infiltrate in the tumor microenvironment, suggesting that the presence of activated intratumoral T cells may be a determinant of response to PD-1 checkpoint blockade. This study describes the stepwise derivation of an immune-related gene expression profile (GEP) that identifies key biological features of a T cell inflamed tumor microenvironment and predicts clinical response to pembrolizumab across a wide variety of solid tumors.

Methods
Associations between clinical response to pembrolizumab and gene expression signatures of IFN-γ signaling and activated T cell biology were evaluated using RNA isolated from formalin-fixed paraffin-embedded baseline samples of patients with multiple tumor histologies. Gene expression was analyzed on the NanoString nCounter system.

Results
Preliminary signatures, comprised of genes associated with IFN-γ and activated T cell biology, were initially evaluated in a discovery set of 19 patients with melanoma, and subsequently validated in an independent cohort of 62 melanoma patients [1, 2]. Reﬁned versions of these signatures were independently tested and shown to predict objective response and progression free survival (PFS) in 40 patients with head and neck squamous cell carcinoma (HNSCC) and 33 patients with gastrointestinal cancer [3, 4]. Using data combined from 220 pembrolizumab-treated patients across 9 cancer types, a ﬁnal 18-gene GEP was derived that included immune-related genes related to antigen presentation, chemokine expression, cytotoxic activity, and adaptive immune resistance. The predictive value of the GEP compared favorably with that of PD-L1 immunochemistry when evaluated in an additional independent cohort of PD-L1-unselected HNSCC patients (n=96).

Conclusions
The pan-tumor GEP described in this study, typiﬁed by indicators of a T cell inflamed microenvironment, captures hallmark characteristics of tumors that are responsive to anti-PD-1 therapy. Our data suggest that these immune-related components are generally necessary, but not always sufﬁcient, for clinical response to pembrolizumab. The GEP represents a potential tumor type-agnostic determinant of response to PD-1 checkpoint blockade, and has undergone analytical validation as a potential diagnostic assay with a clinical utility proﬁle that suggests good performance for maintaining high negative-predictive value and sensitivity [5].

Acknowledgements
Joanne Tomassini, Merck & Co., Inc., writing support.

Trial Registration
ClinicalTrials.gov identiﬁer NCT01295827, NCT01848834, and NCT02054806.

References
P73
Next generation techniques for biomarker development, validation and implementation reveal the importance of non-coding RNAs in predicting response to immunotherapy
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Correspondence: Alex Forrest-Hay (alex_forrest-hay@sffymetrix.com) Journal for ImmunoTherapy of Cancer 2016, 4(Suppl 1):P73

Background
Cancer immunotherapies are changing treatment paradigms and expanding the therapeutic landscape for cancer patients. The current success of these therapies is well documented, however not all patients respond to immunotherapy and those that do can experience toxicities. There is a growing need to identify predictive and prognostic biomarkers to enhance understanding of the mechanisms underlying the interactions between the immune system and cancer. This presentation will discuss emerging biomarker techniques.

Methods
Several new technologies will be showcased in this presentation: (1) The ability to obtain deep transcriptomic data including both coding and non-coding transcripts from a single FFPE section - less than 1ng of RNA and from 50pg of RAN from exosomes; (2) RNA in situ methodologies to explore ncRNAs and secreted cytokines/chemokines and quantify their expression levels in the tumor micro-environment; and (3) immuno-assays for assessing soluble checkpoint markers from 25ul of serum/plasma.

Results
Data will be showcased that demonstrates the importance of exploring different isoforms of genes and non-coding RNA when searching for biomarkers. A new ncRNA discovered through single cell sequencing and microarray work is a surrogate marker for CD8+ T cell infiltration in a melanoma cohort and correlates with response to nivolumab in this initial small dataset (n=13). Additional data will be available by November. Finally, data will be presented that demonstrates that it is possible to detect “cleaved” or soluble PD-L2 upregulation in the serum of a glioblastoma patient who has undergone immunotherapeutic vaccine therapy via a novel multiplexed Luminex assay.

Conclusions
Biomarkers are critical as we move further into the age of combination immunotherapies. It is important to cast the net as broadly as possible and not restrict studies to small subsets of known genes as the likely next generation of clinically useful biomarkers will be non-coding elements or endogenous retroviruses. It is also critical to understand which spliced variant of any given transcript is differentially expressed to fully understand the mechanism that drives any given cancer or response to treatment. New technologies are now available to do this cost effectively from clinical samples such as fine needle aspirates, FFPE tissue sections and blood. This presentation demonstrates the importance of using the latest molecular tools to advance the field of immunotherapy to facilitate the next wave of therapeutic advances.

References

P74
Rapid generation of new specificity MHC tetramers for the detection of antigen-specific T cells using a novel peptide exchange tetramer kit that allows for quantification of peptide exchange
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Background
Major histocompatibility complex (MHC)-encoded glycoproteins bind peptide antigens through non-covalent interactions to generate complexes that are displayed on the surface of antigen-presenting cells for recognition by T cells. Peptide-binding site occupancy is necessary for stable assembly of newly synthesized MHC proteins and export from the endoplasmic reticulum. During this stage peptides produced in the cytosol compete for binding to MHC molecules, resulting in extensive peptide exchanges.

Methods
We have developed a kit based on the principle of peptide exchange to generate novel specificity MHC tetramer reagents in a four-hour assay. While alternate methodologies rely on UV cleavage of exiting peptide on monomeric MHC complexes and a subsequent lengthy tetramerization procedure, we have developed a fully formed fluorescently labeled MHC tetramer that requires only the addition of the peptide of interest and a proprietary peptide exchange factor to complete the reaction.

Results
A human HLA-A*02:01 tetramer made from monomer units folded with an irrelevant exchangeable peptide, along with peptide exchange factor, was used with exogenous peptides to generate new specificity tetramers. The efficiency of peptide exchange was quantified using a novel flow cytometry-based sandwich immunoassay using magnetic beads conjugated with an anti-HLA-A,B,C capture antibody and a FITC conjugated antibody reacting against the exiting peptide. Exogenous peptide exchange rates correlated with their theoretical binding affinity to HLA-A*02:01. Tetramers generated using peptides specific for CMV and Influenza demonstrated efficient exchange and detected similar percentages of antigen-specific CD8+ T cells as classically folded MHC tetramers in flow cytometry staining assays.

Conclusions
The application of this technology to screening and neoantigen cytotoxic T lymphocyte (CTL) epitope discovery will be discussed.

References

P75
Selection of indications for JTX-2011 ICONIC clinical trial
Heather A Hirsch, Amit Deshpande, Jason Reeves, Jenny Shu, Tong Zi, Jennifer Michaelson, Debbie Law, Elizabeth Trehu, Sriram Sathyarayanan
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Selection of indications for JTX-2011 ICONIC clinical trial
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Correspondence: Heather A Hirsch (hhirsch@jouncetx.com) Journal for ImmunoTherapy of Cancer 2016, 4(Suppl 1):P75

Background
Cancer immunotherapies are changing treatment paradigms and expanding the therapeutic landscape for cancer patients. The current success of these therapies is well documented, however not all patients respond to immunotherapy and those that do can experience toxicities. There is a growing need to identify predictive and prognostic biomarkers to enhance understanding of the mechanisms underlying the interactions between the immune system and cancer. This presentation will discuss emerging biomarker techniques.

Methods
Several new technologies will be showcased in this presentation: (1) The ability to obtain deep transcriptomic data including both coding and non-coding transcripts from a single FFPE section - less than 1ng of RNA and from 50pg of RAN from exosomes; (2) RNA in situ methodologies to explore ncRNAs and secreted cytokines/chemokines and quantify their expression levels in the tumor micro-environment; and (3) immuno-assays for assessing soluble checkpoint markers from 25ul of serum/plasma.

Results
Data will be showcased that demonstrates the importance of exploring different isoforms of genes and non-coding RNA when searching for biomarkers. A new ncRNA discovered through single cell sequencing and microarray work is a surrogate marker for CD8+ T cell infiltration in a melanoma cohort and correlates with response to nivolumab in this initial small dataset (n=13). Additional data will be available by November. Finally, data will be presented that demonstrates that it is possible to detect “cleaved” or soluble PD-L2 upregulation in the serum of a glioblastoma patient who has undergone immunotherapeutic vaccine therapy via a novel multiplexed Luminex assay.

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Biomarkers are critical as we move further into the age of combination immunotherapies. It is important to cast the net as broadly as possible and not restrict studies to small subsets of known genes as the likely next generation of clinically useful biomarkers will be non-coding elements or endogenous retroviruses. It is also critical to understand which spliced variant of any given transcript is differentially expressed to fully understand the mechanism that drives any given cancer or response to treatment. New technologies are now available to do this cost effectively from clinical samples such as fine needle aspirates, FFPE tissue sections and blood. This presentation demonstrates the importance of using the latest molecular tools to advance the field of immunotherapy to facilitate the next wave of therapeutic advances.

References
Background
ICOS (inducible co-stimulator of T cells) is a co-stimulatory molecule expressed primarily on T lymphocytes. Clinical data identified ICOS as a potentially key molecule in providing optimal anti-tumor benefit following anti-CTLA-4 therapy, with preclinical data confirming that engagement of the ICOS pathway plays a significant role in mediating anti-CTLA-4 driven anti-tumor responses. JTX-2011 is an ICOS agonist antibody that will be entering early phase clinical development for the treatment of advanced solid tumors in the ICONIC trial. JTX-2011 is designed to generate an anti-tumor immune response through stimulation of T effector cells and preferential reduction of intratumoral T regulatory (Treg) cells. Efficacy of an ICOS agonist in mouse syngeneic tumor models is greatest in tumors with the highest levels of intra-tumoral ICOS, suggesting a potential predictive biomarker approach for clinical development. In this study we report assessment of indications for enrollment in the clinic trial using in silico and IHC analyses across multiple tumor types.

Methods
Integrated analyses of RNA, DNA, and clinical data from the Cancer Genome Atlas (TCGA) were performed within multiple indications to understand the context in which ICOS is expressed. Using a proprietary ICOS IHC assay, ICOS expression analysis was performed on a subset of indications based on ranking from in silico analysis. ICOS expression was also compared to histology and molecularly defined indication subtypes as well as signatures of immune cell infiltrates to understand context of ICOS positivity.

Results
We analyzed ICOS mRNA expression in ~10,000 solid tumors samples across ~30 indications. These data were used to rank indications, and ICOS expression in key indications was orthogonally confirmed using IHC. Consistent with previous data showing ICOS protein expression on Treg cells, ICOS RNA expression significantly correlated with Treg marker expression across multiple indications in TCGA tumor samples. Based on frequency of high ICOS expression, we determined non-small cell lung cancer, head and neck squamous cell carcinoma, triple negative breast carcinoma, gastric adenocarcinoma, and melanoma to be potential indications for JTX-2011 therapy. We further compared ICOS expression to PD-L1 expression to understand if there is a distinct population within an indication that is not a candidate for PD-1/PD-L1 therapy but could benefit from JTX-2011 therapy. A subset of patients in multiple indications exhibit high ICOS levels but low PD-L1 expression.

Conclusions
In conclusion, these data support the prioritization of specific tumor types for treatment with JTX-2011 in the ICONIC trial.

References
antibody responses to both the mutant and wild-type variant site peptides in combination vaccinated animals versus adjuvant alone (p=0.0002). Surprisingly, we found that these antibody response increases strongly correlated with a higher maximum predicted MHCI binding score from NetMHCpan. To investigate T cell responses, we performed an ex vivo screen with a selection of predicted MHCI binding minimal peptides. IL2 expanded splenocytes from vaccinated animals were more likely than those from naïve animals to make an interferon gamma response to the SNP peptides whose parent proteins we had previously identified in the vaccine by mass spectrometry (p=0.01). A similar trend was seen for mass spectrometry identified wild-type variant site peptides; these results are from a pool of three independent biological experiments.

Conclusions
This study identifies a previously unknown link between predicted MHCI binding affinity and the anti-tumor antibody response, a link which correlates with pooled T cell response data found in screening assays. We are confirming these data with larger scale T cell studies of screening assay hits ex vivo.

Acknowledgements
Chiles Foundation, Robert W. and Elise Franz, Lynn and Jack Loacker, Wes and Nancy Lematta, M.J. Murdock Charitable Trust, Harder Family, OCTRI-OSLER TL1, the Providence Medical Foundation, and the ARCS Foundation – Portland.

P78
miRNA a real kid for early recognition for PANcreatic adenocarcinoma (MARKER PAN study)
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Background
Pancreatic ductal adenocarcinoma constitutes the majority of malignant pancreatic tumors. Pancreatic adenocarcinoma is one of the deadliest cancers for humankind to tackle. More so over, its lethality has increased in the presence of late presentation. The median survival in advanced stage is 5-6 months. The present state CA 19.9 is the most commonly used marker though its sensitivity is questionable regarding terms of any help in early diagnosis. One of the promising evolving entities is miRNA, which has recently come to light, as a possible biomarker and cellular target for pancreatic cancer. The main aim of this study is to create a potential pool of circulatory miRNA panel for diagnosis of pancreatic cancer.

Methods
We have evaluated the current literature for various reported miRNA of diagnostic value in the serum by using MeSH terms: pancreatic adenocarcinoma, serum miRNA, and urinary miRNA. Six studies related to miRNA were evaluated in detail and discussed here.

Results
Details analysis of various studies have outlined many promising miRNA as potential candidates for the biomarker pool. The most notable ones are miRNA 143, miRNA 223, miRNA 30e, miRNA 204, miRNA 486, miRNA 145, miRNA 150, miRNA 223, miRNA 200, miRNA 21, miRNA 155 are few notable ones. Details of all potential miRNA are outlined in the Table 6.

Conclusions
miRNA will be a great potential tool to help in the disease diagnosis in the very early stage of a disease. Creating the database of these circulatory miRNAs will be of great potential in creating a panel that will aid in the development of a screening tool.

References

Table 6 (Abstract P78). Table showing pool of potential miRNA with diagnostic values

<table>
<thead>
<tr>
<th>Study</th>
<th>Body Fluid Examined</th>
<th>Sample Size of pancreatic cancer</th>
<th>miRNA</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Debernardi et al. 2016</td>
<td>Urine</td>
<td>59</td>
<td>miRNA 143, miRNA 223, miRNA 30e, 204</td>
<td>Sensitivity-83.3%, Specificity-96.2%</td>
</tr>
<tr>
<td>Xu et al. 2016</td>
<td>Plasma</td>
<td>192</td>
<td>miRNA 486</td>
<td>AUC-0.865, Sensitivity-75%, Specificity-87%</td>
</tr>
<tr>
<td>Schultz et al. 2014</td>
<td>Blood</td>
<td>409</td>
<td>miRNA 145, miRNA 150, miRNA 223, miRNA 636</td>
<td>AUC-0.86, Sensitivity-85%, Specificity-64%</td>
</tr>
<tr>
<td>Liu et al. 2012</td>
<td>Serum</td>
<td>197</td>
<td>miRNA 20a, miRNA 21, miRNA 24, 25, miRNA 99a, miRNA 99b, miRNA 185, miRNA 191</td>
<td>AUC-0.992, Sensitivity-89%, Specificity-100%</td>
</tr>
<tr>
<td>Bauer et al. 2012</td>
<td>Blood</td>
<td>345</td>
<td>miRNA 320, miRNA 159, miRNA 225</td>
<td>AUC-0.973</td>
</tr>
<tr>
<td>Liu et al. 2012</td>
<td>Plasma</td>
<td>140</td>
<td>miRNA 16, miRNA 21, miRNA 155, miRNA 181a, miRNA 181b, miRNA 19b, miRNA 210, miRNA 199</td>
<td>AUC-95.6</td>
</tr>
</tbody>
</table>

P79
Neutrophil lymphocyte ratio as a biomarker predictive of clinical outcome with nivolumab therapy in RCC
Ghayathri Jeyakumar1, Seongho Kim2, Heejin Kim3, Cynthia Silski1, Stacey Suisham1, Elisabeth Heath1, Ulka Vaishampayan1
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Background
With numerous therapies available for treatment of RCC, there is a need to evaluate factors predictive of response to enable patient selection. We are reporting a preliminary analysis evaluating the neutrophil lymphocyte ratio (NLR) and response to prior vascular endothelial growth factor (VEGF) inhibitors as factors predictive of response and clinical outcome in RCC patients treated with nivolumab. We also evaluated factors such as race, smoking status and prognostic scoring by MSKCC (Memorial Sloan Kettering Cancer Center) and Heng criteria.

Methods
Regulatory approval was obtained. A retrospective chart review of RCC patients at Karmanos Cancer Institute treated with PD-1/PD-L1 inhibitors was conducted. Data was collected on demographics, smoking status, prognostic scoring (MSKCC and Heng), NLR pre and post 4 doses of nivolumab, response to prior therapies and correlated with clinical
outcomes on immunotherapy therapies. Univariable and multivariable analyses were performed to evaluate any association with response rate (RR), progression free survival (PFS) and overall survival (OS).

Results
Twenty-six patients were evaluated; 25 received nivolubam and 1 received nivolubam and ipilimumab. The median age was 61 years (39-82). 7 patients (27%) were African American (AA) and 12 patients (46%) were smokers. Pretherapy NLR > 6 months and 2 prior therapies. On univariable Cox analysis, pretherapy NLR > 4 was not a significant predictor of response, but approached borderline statistical significance in the prediction of shorter PFS and OS (PFS: p=0.06, HR 2.532; OS: p=0.058, HR 4.926). Median OS was 2.79 months in the group with NLR > 4 and 18.39 months in the group with NLR > 6 months had a negative impact on PFS and OS (PFS: p=0.067, HR= 2.98; OS: p=0.028, HR=10.834). In univariable logistic analysis, AA patients had higher risk of non-response (OR 10.5, p=.018), and demonstrated shorter OS (HR15.81, p=0.01) with nivolubam therapy in RCC.

Conclusions
NLR is worthy of future investigation as a predictor of clinical outcomes with immune checkpoint inhibition therapy in RCC. Prior therapy that was > 6 months had a negative effect on the PFS and OS with immunotherapy. Validation of this preliminary observation is required in a larger sample size.

P80
CD47 is overexpressed on Merkel cell carcinoma and a target for SIRPα antiphagy therapy
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Background
Merkel cell carcinoma (MCC) is an aggressive, neuroendocrine skin cancer with currently no effective, durable treatments for advanced disease. CD47 delivers an anti-phagocytic (“do not eat”) signal by binding SIRPs on the surface of macrophages. While CD47 has recently emerged as a promising drug target in oncology, its role in MCC is unknown. In this study, we evaluated CD47 expression in MCC tumor samples in conjunction with markers of immune infiltrate, Merkel cell polyomaviral (MCPyV) status, and survival. In addition, we assessed the ability of TTI-621 (SIRPαFc), a CD47-blocking decoy receptor, to enhance macrophage-mediated phagocytosis of MCC tumor lines.

Methods
A formalin-fixed paraffin-embedded (FFPE) tissue microarray (TMA) from 23 MCC patients (Fig. 41) was simultaneously stained for DAPI, CD3, CK20, CD47, CD64, CD68, and CD163 and analyzed using multiplex imaging and inForm software (Perkin Elmer). An in vitro assay was used to determine the effect of TTI-621 on the phagocytosis of the MCC tumor cell line MCC26. Monocyte-derived macrophages were primed with IFN-γ and co-cultured with violet proliferation dye (VPD) labeled MCC26 cells +/− TTI-621 or control Fc for two hours. Phagocytosis was determined by flow cytometry as the percentage of VPD+CD14+ macrophages.

Results
Mean membranous CD47 levels on CK20+ tumor cells ranged from 0.55-17.63 (median 4.5) compared to 0.55-9.55 (median 3.33) on CD3+ cells. Using median CD47 expression on MCC cells, Kaplan-Meier analysis suggested that patients with lower CD47 expression have improved survival rates however, this did not reach statistical significance (p=0.121) in this small number of patients. There were few intratumoral T cells (146 ± 434.9 CD3+imm−/mm3) compared to infiltrating macrophages (5191±1781 CD68+imm−/mm3). Spearman correlation analysis indicated a weak correlation between higher expression of tumor CD47 and decreased numbers of infiltrating T cells (r=0.352, p=0.012). Additionally, MCPyV+ patients (17/23) had slightly higher mean expression levels of CD47 (6.08), and it was not significantly higher than the small number of MCPyV negative (6/23) patients represented on the TMA (mean = 4.17; p = 0.223). Phagocytosis of MCC26 was increased in the presence of TTI-621, in a dose-dependent manner.

Conclusions
CD47 is over-expressed in MCC, regardless of MCPyV status, suggesting that blockade of CD47 by TTI-621 may be particularly beneficial to patients with sparse T cell infiltrate. A phase I dose escalation clinical trial of TTI-621 in patients with solid tumors, including MCC, will initiate enrollment this year. This study will examine changes in the tumor microenvironment in response to TTI-621.
fixed, paraffin-embedded non–small cell lung cancer tissues. Similar to analytical validation studies for monoplex IHC assays, this study utilized an appropriate reference method and multiple days of staining. Distinct to this assay validation study, however, was the inclusion of an additional step in the reference standard comparison due to the potential for false-positive and false-negative results linked to the dual-staining methodology. Specifically, a reference method was used to qualify Ki67 and CD8 monoplex IHC assays that were then used as the reference standard to assess duplex assay performance. Five performance criteria were evaluated: reportable range, analytical sensitivity, analytical specificity, accuracy, and precision. These performance criteria were selected based on Clinical Laboratory Standards Institute guidelines.

**Results**

To ensure accurate and consistent measurement of chromogenic staining in the duplex setting, we quantified the percentage of cells positive for Ki67 nuclear staining and/or CD8 membrane staining, using our Computational Tissue Analysis (cTA) technology. Performance of the Ki67/CD8 chromogenic duplex IHC-cTA assay was considered acceptable for the performance criteria evaluated.

**Conclusions**

The multiplex setting requires additional assay performance assessments due to the complexity of the staining interpretation when multiple IHC stains are present on one tissue section. Flagship’s cTA technology allows for more consistent quantification of individual analytes on dual-stained tissue sections.

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**P83**

Combining the best of both worlds: immune profiling the tumor microenvironment with RNA and protein biomarkers by fluorescence multiplex RNA in situ hybridization and immunohistochemistry

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**Background**

Recent triumphs in cancer immunotherapy have benefited many cancer patients across multiple malignancies, generating much interest across all sides. At the same time, there is an urgent need to develop predictive biomarkers to identify patients who are most likely to benefit from all sides. At the same time, there is an urgent need to develop predictive biomarkers to identify patients who are most likely to benefit from various immunotherapeutic strategies. While many biomarker analysis technologies are available, most do not provide spatial and cell type-specific information critical for assessing the specific immune cell types with lineage and functional information in the evolving microenvironment of each tumor. Furthermore, multiplexing capabilities are highly desirable in order to obtain comprehensive single cell-level co-expression information and to maximize the use of limited biopsied sample material.

**Methods**

In this study, we demonstrate the development of an improved fluorescence multiplex in situ hybridization (ISH) method to detect three RNA biomarkers simultaneously in FFPE and fresh frozen tissues on the Leica BOND RX automated slide staining system. The presented method detects RNA biomarkers in a highly specific and sensitive manner, overcoming the inherent challenge of auto-fluorescence in FFPE tissues. Individual RNA molecules are visualized as distinct bright dots using any fluorescence microscopy or multi-spectral fluorescence imaging system.

**Results**

We applied this technique to archived non-small cell lung cancer FFPE tissues to detect (1) the co-expression of various immune checkpoint markers (such as PD-1, LAG-3, TIM-3) in PD-L1 positive and negative tumor environments and (2) immune functional markers such as cytokines and chemokines in combination with cell lineage markers. We further demonstrate the flexibility of this technique to allow for detection of both RNA and protein biomarkers, where immune checkpoint and functional markers are detected by ISH and cell lineage markers (such as CD3, CD8) by IHC. The data on the co-expression and localization of multiple combinations of markers derived from serial sections of FFPE tissues provide comprehensive information regarding the immune network in each tumor microenvironment.

**Conclusions**

The newly developed multiplex fluorescence RNAscope assay and its combination with IHC presents a powerful tool to interrogate the various cell types and spatial heterogeneity within tumor tissues. Information revealed through simultaneous detection of multiple RNA and/or protein markers may provide new insights to maximize the benefits of current therapeutic approaches. This fully automated assay platform is well suited for developing and validating clinically relevant biomarkers in FFPE tissue.
**Background**

The promising advances of immunotherapy in cancer patient suggest a need of parallel innovative solution to facilitate study and manipulation of immune cells. The design of cell based assays for studying patient T or B cells in conventional microtiter plate suffers from key limitations. The large microwell volume of classical microtiter plate is poorly suited to the limited amount of primary immune cells available and especially when a rare subpopulation study is needed. Furthermore, a cell based assay with multi-step staining procedures and immune cells often leads to significant cell loss and require a complex washing process optimization. Here, we present the use of DropArray wall-less microtiter plate for cell based assays with immune cells.

**Methods**

DropArray 384 well plates are designed with hydrophobic/hydrophilic patterning and hold an array of 2 μl drops in which cell based assays can be conducted conveniently and where cells and reagent consumption can be minimized by up to 90%. DropArray wall-less plates employ surface tension to retain suspension cells efficiently on the plate surface during a wash process with a convenient automatic washing station with no optimization required. DropArray plates are used in conjunction with common high content imaging platforms for analysis.

**Results**

DropArray plates display efficient retention of suspension cells such as PBMC, plasmacytoid dendritic cells, and B or T cells in a range of 70 to 90% after multiple wash steps. Among few cell based assays, we highlight how the DropArray plate is used to visualize/quantify events of cytotoxic T cells mediating tumor cell killing and where bi-specific antibody efficacy is evaluated with high content imaging platforms in a real time assay.

**Conclusions**

DropArray wall-less plates constitute the next generation microtiter plate for running immune cell based assay with suspension cells.

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**P86**

**Miniaturization of Luminex based multiplex cytokine assay with 96 and 384 DropArray plates**

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**Background**

Luminex®, magnetic bead-based assays have contributed to the expansion of biomarker discovery by providing tools for simultaneous measurement of multiple analytes. However, high cost and sample volume requirements limit the use of this technology in drug discovery and research, especially when analyzing cerebrospinal fluid, tears and limited organic and body fluids. In this poster, we present the use of DropArray technology to reduce Luminex reagents and sample use by 80% while generating similar or enhanced data as obtained by traditional Luminex® methods.

**Methods**

DropArray 96 (DA-96) plate is a wall-less plate that can accommodate 5 to 20 μl drops in each of its 96 circular imprints. DA-96 plate follows standard SLAS/ANSI format, compatible with standard microtiter instruments and follows the same Luminex workflow for magnetic bead-based assays. Each circular hydrophilic area of the DA-96 plate can hold 5μl of each Luminex reagent and samples thus effectively miniaturizing the Luminex assay by at least 80%. DA-96 plate is used sequentially on vortex/shakers, eluted out in a 384 well plate in a highly convenient manner which avoids centrifugation of beads and laborious plate transfer before acquisition into Singulex reader.

**Results**

Using Milliplex®, Procartaplex™ or Bio-Plex® based kits coupled with DA-96 plates for cytokine analysis.

**Conclusions**

With similar performance as conventional plates, faster and streamlined workflow, DA-96 plate is the next generation plate for Singulex assay providing similar or improved sensitivity and a smaller sample volume requirement.

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**P87**

**Miniaturization of Singulex/Erenna based multiplex cytokine assay with wall-less DropArray 96 plates**

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**Background**

Recent expansion in biomarker discovery has been possible by the use of bead-based assays and single molecule detection capabilities such as the Singulex assay. Minimal volume requirement with the Singulex methodology requires 50-100μl per well and can be a major bottleneck with limited clinical samples. Furthermore, requirement of multiple plate transfers with the Singulex workflow present a significant delay and obstacle during the workflow when multiple plates are processed simultaneously. Here, we combine DropArray 96 plates (DA-96) and the Singulex assay to achieve similar performance with 80% less volume than the current method.

**Methods**

DA-96 plate is a wall-less plate with 96 circular hydrophilic imprints on a hydrophobic/plastic surface displayed in SLAS/ANSI format, compatible with standard microtiter instruments. Each circular hydrophilic area can hold conventional Singulex reagents such as magnetic beads/antibody/sample/standards up to a total of 20μl volume. Following a highly similar Singulex workflow and using conventional multichannel pipettes, DA-96 plates are used sequentially on vortex shaking and washed in a fully automated wash station. DA-96 to DA-96 plate bead transfer is performed conveniently with a Magnet Transfer Jig™ tool to bypass the need for multiple pipetting steps required in the conventional method. Finally, Beads on DA-96 plate are eluted out in a 384 well plate in a highly convenient manner which avoids centrifugation of beads and laborious plate transfer before acquisition into Singulex reader.

**Results**

Using Singulex Erenna based kits for cardiac Troponin-I, DA-96 plate miniaturized workflow displayed precision and accuracy analysis in line with conventional methods with intra assay CV% below 20% and recovery within acceptable range of 80-120%. Reliable sensitivity LLOQ reached 0.35pg/ml. DA-96 plate and washer offer excellent reproducibility well to well and plate to plate with 20μl of sample volume per well.

**Conclusions**

With similar performance as conventional plates, faster and streamlined workflow, DA-96 plate is the next generation plate for Singulex assay providing similar or improved sensitivity and a smaller sample volume requirement.
To study transcriptional signatures, FACS-purified peripheral blood T cells of AML-patients before and after induction-chemotherapy. The aim was to genotypically, phenotypically and functionally characterize T cell dysfunction in AML, which remains poorly understood. Therefore, we aimed to study the effects of AML blast/T cell interactions on T cell function and gene expression.

Methods
To study transcriptional signatures, FACS-purified peripheral blood T cells from 69 AML-patients before and after induction-chemotherapy, and from 55 HCs, were analyzed by flow cytometry (FLC). Cultured T cells and primary AML-blasts from newly diagnosed patients for 3 days. T cells were cultured alone or in co-culture with blasts (1:10) and analyzed by FLC.

Results
The transcriptional profile of CD8+ T cells at AML diagnosis included significant upregulation of the immune inhibitory receptors genes B2G1L, KLRG1, CD160 and TIGIT compared to HCs. In contrast, co-stimulatory receptor genes were downregulated, including CD28LG, CD28 and CD30LG. Ingenuity pathway analysis (IPA) revealed that the co-stimulatory CD28, ICOS and OX40 signaling pathways were downregulated. We performed confirmatory T cell phenotype characterization by FLC in a larger patient cohort (n=69). CD8+ T cells were phenotypically senescent (CD27 CD28 CD57+) and %T cells co-expressing 2-4 co-inhibitory receptors (2B4/KLRG1/CD160/CD57) was significantly higher in AML patients compared to HCs. Next, we compared R to NR after induction chemotherapy. R-patients upregulated immune-stimulatory receptor genes like ICOS, whereas NR-patients upregulated immune-inhibitory receptor TIM3; LST1 (inhibits lymphocyte proliferation); TWEAK-APRIL (T cell apoptosis); and CD39 (terminally exhausted T cells). In accordance with these findings, IPA showed enrichment of the co-stimulatory ICOS and OX40 signaling pathways in R-patients. In the confirmatory patient cohort, %senescent T cells and T cells co-expressing 2-4 co-inhibitory receptors was significantly decreased in R-patients (n=52), but unchanged in NR-patients (n=17) compared to pretreatment levels. The co-culture assay showed that the presence of AML blasts also significantly decreased the %primary AML T cells expressing co-stimulatory receptors 41BB, ICOS and OX40, while it increased the frequency of HC T cells expressing 2B4 and CD57.

Conclusions
Our study provides insight into AML-associated phenotypical and transcriptional changes in T cells. Our data suggest that the AML-blasts influence the T cell phenotype and genotype. Response is associated with reversion to HC pattern, whereas NR patient remain in an exhausted/senescent state. Identification of their immune signature will hopefully help to rationally designing future clinical trials of immune-modulating strategies in AML.
P90
Persistance and turnover of therapy-induced peripheral CD4+ T cell clones in patients with metastatic melanoma upon ipilimumab therapy
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Background
Ipilimumab is a human monoclonal antibody targeting CTLA-4, which is expressed in activated T cells and involved in negative regulation. 22% of patients with metastatic melanoma who received ipilimumab monotherapy are alive 3 years after treatment and the majority of these patients have had a durable response lasting ten years or more [1]. It is important to uncover underlying resistance mechanisms as most patients still fail to benefit from ipilimumab or other immunotherapies.

Methods
We assessed whether activation and/or proliferation of peripheral T cells correlated with clinical outcome of patients with metastatic melanoma treated with ipilimumab. PBMCs from patients at baseline and 12 weeks following initiation of treatment participated in a compassionate use trial of ipilimumab [2] were evaluated for ICOS+ or Ki-67+ frequencies on CD4+ and CD8+ T cells, as well as regulatory T cells, using flow cytometry. We conducted T cell receptor (TCR) CDR3 sequencing for sorted bulk CD4+ T cells from baseline, weeks 7 and 12 of ipilimumab treatment to understand their clonal behaviors in patients with metastatic melanoma upon treatment.

Results
Ipilimumab treatment significantly increased the frequency of ICOS+ T cells regardless of clinical outcome as previously reported by others [3]. Increased Ki-67+ CD4+ T cells upon ipilimumab treatment (>1.7 fold) was associated with prolonged overall survival. Our results suggest that ipilimumab’s anti-tumor effects may be mediated in part by proliferation of CD4+ T cells. However, some patients with melanoma progresed showed increased Ki-67 expression in CD4+ T cells after ipilimumab, implying that CD4+ T cell proliferation alone is not a predictive biomarker. When we tracked destiny of newly emerging CD4+ T cell clones upon treatment (present at weeks 7 but not present at baseline), we found prominent turnover at weeks 12 in patients who progressed upon treatment. In contrast, a patient who showed complete response and later survived longer than 3 years showed persistence of new CD4+ T cell clones upon treatment.

Conclusions
Our results suggest that successful treatment with ipilimumab may be, in part, explained by appearance and persistence of potentially tumor-specific CD4+ T cell clones. We will be conducting TCR CDR3 sequencing for DNA extracted from tumor FFPE blocks to see whether clones of interests can be detected in tumor.

Trial Registration
ClinicalTrials.gov identifier NCT00495066.

References
Antigen recognition avidity dependent miR-155 upregulation in melanoma tumors correlates with increased CD8+ T cell infiltrates

Amaia Martinez-Usatorre1, Camilla Jandus1, Alena Donda1, Laura Carretero-Iglesia2, Daniel E. Speiser2, Dietmar Zehn3, Nathalie Rufer4, 1Department of Fundamental Oncology, Ludwig Cancer Research Project seeks to facilitate harmonization of PD-L1 tests in order to enable their practical use, with the goal of building a method for objective, consistent, proper test interpretation. In response, we have developed Computational Tissue Analysis (cTA™) approaches that enable objective, consistent assessments of staining. In addition to evaluating staining concurrently in multiple cell types (e.g., tumor epithelium, immune infiltrate) and ensuring reproducible scoring, these approaches may help clarify the differences between various PD-L1 assays. The present study evaluated Flagship’s cTA™ approaches to scoring samples stained with the Dako PD-L1 pharmDx (28-8) immunohistochemistry complementary diagnostic assay. The current manual scoring method considers any patient with at least 1% PD-L1-positive tumor cells a candidate for Opdivo treatment. However, a trend toward increased overall survival was observed in patient groups with greater PD-L1 positivity (5% and 10%), suggesting the importance of reliably distinguishing between positivity thresholds. We developed a cTA™ approach to test the hypothesis that it could provide more consistent scoring around these low thresholds, which are challenging for pathologists. We stained 40 formalin-fixed, paraffin-embedded nonsquamous non-small cell lung cancer samples with the aforementioned Dako assay; a subset of these we strained on consecutive days to evaluate assay and scoring precision. The cTA strategy digitally identified tumor cells and quantified membrane staining intensity consistent with the manufacturer’s scoring guidelines. We also evaluated scoring of PD-L1 expression in immune infiltrate in the same analysis. This study demonstrated the utility of cTA™ approaches for consistently and objectively scoring PD-L1 expression in tumor cells within the CLIA environment. It also highlighted the challenge pathologists face in differentiating between 1%, 5%, and 10% positivity. cTA™ approaches could be used for consistent scoring of a single assay or to understand differences between assays in performance or relationship to clinical response.

Background

The PD-1/PD-L1 pathway mediates immunosuppression in the tumor microenvironment. Therapies targeting this pathway have been approved along with corresponding diagnostic assays for selected indications. These assays predict patient responses to therapy by measuring the percentage of tumor cells (Keytruda, Opdivo) or immune cells (durvalumab) that stain positive for PD-L1. The AACR-ASCO-FDA Blueprint Project seeks to facilitate harmonization of PD-L1 tests in order to enable their practical use, with the goal of building a method for objective, consistent, proper test interpretation. In response, we have developed Computational Tissue Analysis (cTA™) approaches that enable objective, consistent assessments of staining. In addition to evaluating staining concurrently in multiple cell types (e.g., tumor epithelium, immune infiltrate) and ensuring reproducible scoring, these approaches may help clarify the differences between various PD-L1 assays. The current manual scoring method considers any patient with at least 1% PD-L1-positive tumor cells a candidate for Opdivo treatment. However, a trend toward increased overall survival was observed in patient groups with greater PD-L1 positivity (5% and 10%), suggesting the importance of reliably distinguishing between positivity thresholds.

Methods

We developed a cTA™ approach to test the hypothesis that it could provide more consistent scoring around these low thresholds, which are challenging for pathologists. We stained 40 formalin-fixed, paraffin-embedded nonsquamous non-small cell lung cancer samples with the aforementioned Dako assay; a subset of these we stained on consecutive days to evaluate assay and scoring precision. The cTA strategy digitally identified tumor cells and quantified membrane staining intensity consistent with the manufacturer’s scoring guidelines. We also evaluated scoring of PD-L1 expression in immune infiltrate in the same analysis.

Results

The cTA™ membrane scoring approach for tumor cells met Flagship’s Clinical Laboratory Improvement Amendments (CLIA) validation criteria, with the manually scored assay as the reference standard. This approach was accurate and provided greater reproducibility of scoring over the dynamic range of PD-L1-positive cell frequencies.

Conclusions

This study demonstrated the utility of cTA™ approaches for consistently and objectively scoring PD-L1 expression in tumor cells within the CLIA environment. It also highlighted the challenge pathologists face in differentiating between 1%, 5%, and 10% positivity. cTA™ approaches could be used for consistent scoring of a single assay or to understand differences between assays in performance or relationship to clinical response.

References


P94 Immune activation and prolonged benefit to avelumab (anti-PD-L1) therapy in a patient with metastatic EBV+ gastric cancer

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Background

The molecular basis for response to immune checkpoint therapy in different cancers is not well understood. In some cases response correlates with high tumor mutation burden, due to environmental exposure to carcinogens (UV exposure/tobacco), or intrinsic DNA repair defects (mismatch repair defects/POLE mutations) leading to generation of immunogenic neoantigens. However, some tumors with low mutation burdens are sensitive to immune checkpoint therapy, suggesting other mechanisms of immune activation.

Methods

Exceptional clinical benefit was observed in a patient with advanced gastric cancer treated with the PD-L1 inhibitor avelumab. Informed consent was obtained and she was enrolled to the Rutgers Cancer Institute of New Jersey genomic tumor-profiling protocol. Comprehensive genomic profiling was performed using the FoundationOne platform. In situ hybridization was performed to evaluate expression of Epstein Barr Virus (EBV)-encoded RNA. Data from The Cancer Genome Atlas (TCGA) dataset of gastric cancer was analyzed to review associations between EBV status, mutation burden, gene expression based immune signatures and histologic lymphocytic infiltration.

References

Results
This 57-year-old female presented with chemotherapy refractory metastatic gastric cancer causing complete esophageal obstruction, cervical and thoracic lymphadenopathy, and chronic anemia from tumor blood loss. Avelumab 10 mg/kg every 2 weeks was administered in a clinical trial. She experienced tumor reduction after 4 cycles. After 10 cycles, further tumor regression, improved anemia, and improved ability to ingest solid foods with >15 lb weight gain was observed and continues at 20+ cycles. Genomic analysis of a pre-treatment tumor specimen did not show a high mutation burden or evidence of mismatch repair defects, but was strongly positive for EBV-encoded RNA. Approximately 9% of gastric cancers in the TCGA dataset have evidence of EBV RNA expression. These EBV+ cancers were micro-satellite stable with relatively low mutation burden, but high expression of immune checkpoint genes including PD-1 and PD-L1, and gene expression evidence of immune infiltration.

Conclusions
These data suggest that EBV+ gastric cancers are a subset of micro-satellite stable gastric cancers with low mutation burden that respond to immune checkpoint therapy. Tumor EBV expression may be a marker of sensitivity to immune checkpoint therapy that is independent of total mutation burden and microsatellite instability. A phase III trial of avelumab in patients with recurrent gastric cancer is ongoing.

Acknowledgements
We acknowledge our patient’s participation.

Trial Registration
ClinicalTrials.gov identifier NCT01772004.

Consent
Written informed consent was obtained from the patient for publication of protocol data in this abstract and accompanying images. A copy of the consent is available for review.

P95
Immune-enriched NSCLC biopsy tissue microarrays demonstrate that proliferating and checkpoint expression of TIL correlate with positive outcome
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Background
Immune checkpoint (ICP) blockade therapies using anti-PD-1 have elicited positive responses in non-small cell lung cancer (NSCLC) patients, where non-responders are suspected to arise from the downstream expression of additional ICP on effector TIL. In this age of companion diagnostics, during the expanding list of predictive biomarkers like PD-L1, it will become invaluable to: 1) define which ICP impact prognosis; 2) determine whether ICP expression is co-dependent, ordered, and can be used to stratify patients; and 3) develop standardized methods for their use in scoring patients ahead of ICP blockade therapies.

Methods
An immune-cell-specific biopsy-based tissue microarray (TMA) from untreated NSCLC patients (n=81) was fabricated to profile TIL-ICP using multiplex immunofluorescence (MP-IF). ICP distributions and coexpression were correlated with clinical data, and results were compared to The Cancer Genome Atlas (TCGA) RNA-Seq lung adenocarcinoma (LUAD; n=520) and lung squamous cell carcinoma (LUSC; n=504) datasets. Using microfluidic qRT-PCR, coexpression of ICP with numerous effector TIL genes was assessed in CD8+ and CD4+ TIL isolated from freshly resected untreated tumors, normal tissues, and peripheral blood mononuclear cells (PBMC).

Results
TIL that are proliferating and expressing certain ICP positively correlate with the overall survival (OS) of NSCLC patients. From TMA staining data, ICP expression is increased (CTLA4) or decreased (PD-1, CD26, CD57, CD244) relative to patient mortality, and ICP coexpression (CD57-CD39, CD26-CD39) or TIL-ICP expression (CD3-CTLA4, CD3-TIM-3, increased; CD3-CD26, CD3-CD73, decreased) enhance statistical significance. Increased expression of ICP (TIM-3, CD26, LAG-3) or TIL-ICP (CD3-TIM-3, CD3-BTLA, CD3-LAG-3, CD3-CD26, CD3-CD39) correlates with improved OS. From both TMA results and TCGA datasets, ICP subset expression is observed to have heightened importance relative to OS at earlier time points, suggesting an ordered accruing of ICP by TIL. Hierarchical cluster analysis reveals ICP groupings (CD26, CD39, TIM-3; TIGIT, CTLA4, PDCD1; CD244, CD57) reflected by qRT-PCR results also revealing ICP distributions on CD8+ and CD4+ TIL and PBMC. TCGA datasets also provide evidence that overall ICP expression positively correlates with OS, and that advanced cancer stages have lowered ICP expression. Finally, ICP expression analysis defines an ordered build of ICP on NSCLC TIL.

Conclusions
This multi-cohort analysis performed using an array of techniques has resulted in the discovery of TIL ICP expression having the greatest benefit for different NSCLC subtypes. ICPs are coexpressed, and their early, stepwise acquisition may be an important determinant of OS. In this era of personalized medicine, use of ICP MP-IF panels may better stratify patients for ICP blockade therapies.

P96
High sensitivity detection of low expressing interleukins and interferons for biomarker research analysis from FFPE samples using multiplexed NGS
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Background
There is growing evidence supporting the association of tumor infiltrating lymphocytes, inflammatory signaling molecules, and drug sensitivity to cancer checkpoint blockade therapy. At the same time, the exact markers that are predictive of response for each therapeutic agent are still the subject of active investigations. To address this need for better understanding of the effect of different T cell subsets, antigen presentation, and tumor killing, gene expression profiling presents an attractive means to simultaneously evaluate the tumor microenvironment and cancer cells. Furthermore, as most of the samples available for drug sensitivity research studies are derived from formalin-fixed paraffin-embedded (FFPE) slides with typically low RNA quality, target sequencing offers a cost-effective solution that provides significantly higher sensitivity and specificity over whole transcriptome sequencing or other gene expression profiling methods.

Methods
Here we report the results of a 395-gene expression panel profiling FFPE non-small cell lung cancer (NSCLC) and melanoma tumor research samples. The panel uses Ion AmpliSeq™ technology to measure the expression of genes involved in T cell activation, markers of different leukocyte subsets, antigen presentation, and tumor characteristics (proliferation, adhesion/migration, epithelial-to-mesenchymal transition, etc.).

Results
Gene expression measured by the panel stratified NSCLC samples into groups that are consistent with histopathology classification and tumor infiltrating levels provided by pathologists. The panel offers reproductibility between replicates and high sensitivity of detection for low expressing genes such as IL-2, IL-10, IL-21, and IFNG, among others. We further demonstrated excellent concordance between
fresh frozen and FFPE samples (correlation >0.95) as well as concordance with TaqMan® qPCR. With a series of limiting dilution experiments between two cell lines, the assay showed broad dynamic range and linearity up to 200 fold. This Oncomine™ Immune Response Research Assay® is accompanied by a Torrent Suite™ software package that provides run quality metrics, normalized gene expression, and hierarchical clustering among multiplexed samples.

Conclusions

In summary, the current gene panel offered an accurate and accessible tool for evaluating biomarkers that may be relevant to cancer immunotherapy.* For Research Use Only. Not for use in diagnostic procedures.

P97

Validation of a custom RNA-Seq approach to cancer immune profiling

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Background

Immune checkpoint blockade with monoclonal antibodies directed at the inhibitory immune receptors has emerged as a successful treatment for cancer patients. Evaluation of tumor checkpoint blockade by IHC is subjective, not reproducible, and importantly not scalable. We have designed a custom 362 gene RNA-Seq immune profiling panel that uses a multi-analyte algorithmic analysis (MAAA) to evaluate checkpoint blockade, tumor infiltrating lymphocytes (TILs), and cytokine/chemokine interactions. Additionally, we have previously profiled several hundred samples that are used for ranking of expression of each gene.

Methods

269 FFPE cancer samples of diverse histologies were evaluated by RNA-Seq with a custom 362-gene Ion AmpliSeq Immune Response Profiling Assay using the Ion Chef™ and Proton. RNA-Seq analysis was performed with the Torrent Suite™ followed by normalization. Results for each sample were then evaluated with a MAAA to provide a prediction score for 61 known immunotherapy drugs. Predictions were reported for a continuous scale of 1-100 and interpreted as low, neutral, or high. All samples were also included in a custom TMA and IHC was performed for known targets of various immunotherapies when available.

Results

At least one over expressed immunotherapeutic target was identified in more than 50% of the samples. By evaluating upstream and downstream mediators for each overexpressed target at least one high prediction score was identified in almost one-third of the samples. Not surprisingly the class of immune recognition, including checkpoint blockade, was the most frequently identified group of high prediction scores. High prediction scores were also identified for immunotherapy drugs for the classes of immune inhibition (eg IDO1), tumor targeting mAbs (eg CD56), and microbe-associated (TLR7).

Conclusions

Profiling by RNA-Seq allows for the identification of samples with over expression of multiple genes involved in the tumor immune repertoire. By applying a MAAA to these results in the context of a known database of results we can provide a prediction score that guides selection of various immune therapies, including more than just checkpoint blockade. As immune therapy moves from treatment of last resort to first and second line treatment an accurate scalable interrogation of the total tumor immune microenvironment will be required.

P99

PD-L1, PD-L2 and PD-1 expression in metastatic melanoma: correlation with tumor infiltrating immune cells and clinical outcome

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Background

The expression of PD-L1 in melanoma metastases limits immune control of tumor progression. Reliable biomarkers of patient survival, and response to treatment, are critical for patient care and selection. Yet the prognostic ability of PD-L1, or highly related PD-L2, remain controversial. We hypothesized that the expression of PD-L1 and PD-L2 by melanoma cells would correlate with both immune cell infiltration and patient survival, independent of checkpoint blockade therapy.

Methods

Tissue microarrays of metastatic melanoma samples from 147 patients were evaluated (median follow-up: 19 months). None had been
Background
PD-L1 expression is induced by IFNγ, providing a feedback mechanism to control inflammation locally. There are conflicting data concerning its prognostic implication in patients with NSCLC. We hypothesized that PD-L1 is a positive prognostic indicator in NSCLC and that tumors expressing PD-L1 also express other immune checkpoint molecules and are highly infiltrated by T cells.

Methods
Tissue microarrays (TMA) were constructed using two 1 mm cores from formalin-fixed paraffin-embedded surgical specimens of 151 NSCLC (90 adenocarcinomas (AdCA), 58 squamous cell, and 3 mixed histology). Patients were not treated with PD-1/PD-L1 antibodies, 93% had stage I/II disease (median follow-up: >27 months). TMAs were evaluated by immunohistochemistry for PD-L1 (clone SH1, membranous or cytoplasmic staining), GAL9, CD155, and PD-1 on tumor cells and CD8, FoxP3, PD-1 and LAG3 on immune cells. PD-L1 expression was recorded as the percent of staining tumor cells. GAL9, CD155, and PD-1 were assessed by intensity of staining on tumor cells (scale 0–4). Stained immune cells were enumerated per core. Overall survival (OS) was assessed as a function of PD-L1 expression using Cox-proportional hazard. Correlations between PD-L1 expression and other markers were assessed using Pearson correlation coefficients. The Cancer Genome Atlas (TCGA) data were explored for validation of co-expression profiles using Fisher exact tests.

Results
Increased percentages of PD-L1+ tumor cells were associated with longer OS (univariate: p=0.03 and multivariate accounting for stage, age, histology, CD8 counts and tumor grade: p=0.04). PD-L1 expression also correlated with increasing CD8 (p=0.002), FoxP3 (p=0.013), LAG3 (p=0.007) or PD-1 (p=0.049) expressing lymphocytes and with the expression of PD-1, GAL9 and CD155 on tumor cells (p≤0.02). Tumors with high PD-L1 and CD8 expression (21%) also highly expressed inhibitory markers (FoxP3, LAG3, CD155 and GAL9, Figure). Similar significant correlations with PD-L1 mRNA expression were found in the TCGA cohort of 517 AdCA patients with PD-1 and CD8, and with checkpoint molecules LAG3, TIM3, and PD-L2, as well as the TIM3 ligand Gal9 (Fig. 43).

Conclusions
Tumor cell expression of PD-L1 is a favorable prognostic indicator and correlates with CD8+ T cell infiltration and co-expression of other inhibitory checkpoint molecules and ligands. The simultaneous presence of additional tumor protective signals in these tumors may influence their response to PD-1/PD-L1 blockade. Patterns of co-expression of checkpoint molecules may guide selection of combination checkpoint blockade therapy.

Acknowledgements
Results presented are in part based upon data generated by the TCGA Research Network (http://cancergenome.nih.gov) (Cerami E, et al., PMID:22588877; Gao J, et al., PMID:23550210).

Fig. 42 (Abstract P100). The expression of different immune markers. Heatmap depicts protein expression intensities as determined by IHC in the 151 NSCLC tumors.

Treated with PD-1/PD-L1 blockade. Cancer vaccines had been administered to 49 of the patients (33%). Melanoma cells were assessed by immunohistochemistry for surface and cytoplasmic expression of PD-L1 (clone SH1) and PD-L2 (R&D Systems, AF1224). Immune cells were enumerated with stains for PD-L1, PD-1, CD8, CD45, CD4, CD3, CD163, CD20, CD138, and FoxP3. Relationships between the proportions of PD-L1 and PD-L2 expressing tumor cells with immune cell counts were assessed (by Spearman correlation), and with immune cell distribution (Immunotype, by Wilcoxon rank sum tests). Associations with patient survival were evaluated using Kaplan-Meier curves and Log-rank tests. P values less than 0.05 were considered significant.

Results
Surface expression of either PD-L1 or PD-L2 by melanoma cells correlated significantly with increasing intratumoral densities of immune cells expressing each of the following: CD45, CD3, CD4, CD8, PD-1 and FoxP3 (p≤0.001). PD-L1 expression was more common in tumors of patients who had previously participated in melanoma vaccine trials (p = 0.034). Diffuse infiltration (Immunotype C), as opposed to infiltration limited to the perivascular spaces (Immunotype B), was associated with increased PD-L1 (p = 0.058) and PD-L2 (p=0.033) expression by melanoma cells. Expression of PD-L2 on ≥5% of tumor cells was associated with improved overall survival (p=0.043), and the simultaneous positive expression of both PD-1 ligands was even more strongly associated with improved survival (p=0.005, Figure).

Conclusions
Both PD-L1 and PD-L2 are markers of immune infiltration. PD-L2 alone, or in combination with PD-L1, is a marker for prognosis in metastatic melanoma patients. The prognostic associations of the combination of PD-L1 and PD-L2 support future studies of the predictive value of these ligands in the setting of combination checkpoint blockade therapy.

P100
PD-L1 is expressed in inflamed non-small cell lung cancer (NSCLC) specimens and its expression predicts longer patient survival, despite co-expression of other checkpoint molecules

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of a single dose of ipilimumab resulted in robust augmentation of vaccination with a consistent immunodominance hierarchy. Addition specific T cell responses that typically peaked 2 to 3 weeks post-vaccination were longitudinally evaluated via flow cytometry, including intravenous administration of either saline as control or ipilimumab. Following vaccination, MCMs received intravenous administration of either saline as control or ipilimumab in NSCLC. Gene expression analysis in the 517 adenocarcinoma and 501 squamous cell carcinoma specimens from the TCGA provisional database.

**Methods**

Using this nonhuman primate PD model, we observed that the administration of ipilimumab enhanced the magnitude of vaccine-induced Ag-specific T cell responses. The ability to demonstrate such PD effects in monkeys allows for the testing of human clinical candidate antibodies in pharmacologically relevant models. This vaccine system can be used to provide early proof-of-concept data as well as aid in lead candidate selection by comparing antibodies targeting different epitopes or expressing different Fab domain formats (e.g., human IgG1 vs human IgG4). The described findings highlight the feasibility of this approach to investigate the impact of immunomodulatory antibodies/therapies on T cell immunity, particularly at an antigen-specific T cell level, and warrant the study of additional I-O agents.

**Background**

Currently, the preclinical assessment of immuno-oncology (I-O) agents relies heavily on murine tumor efficacy models, typically necessitating surrogate antibodies. Although nonhuman primate studies enable the use of human clinical candidate antibodies, such studies rely on limited pharmacodynamic (PD) markers. To more thoroughly evaluate the ability of I-O therapies to impact T cell immunity, we took advantage of the limited major histocompatibility complex (MHC) diversity in Mauritian cynomolgus macaques (MCMs) and utilized a highly immunogenic vaccine containing known MCM T cell epitopes. This design enabled longitudinal tracking of vaccine-elicited responses using antigen-specific T cell readouts. We then evaluated the effects of ipilimumab (anti-CTLA-4) in this nonhuman primate PD model.

**Methods**

Pre-selected MCMs with specific MHC class I alleles that restrict known CD8+ T cell epitopes were vaccinated intramuscularly with two non-replicating adenovirus serotype 5 (Ad5) viral constructs, one encoding simian immunodeficiency virus (SIV) Gag protein and the second encoding SIV Nef protein. Following vaccination, MCMs received intravenous administration of either saline as control or ipilimumab (anti-CTLA-4) at 10 mg/kg. Vaccine-induced SIV-specific T cell responses were longitudinally evaluated via flow cytometry, including the use of peptide loaded MHC class I tetramers and IFN-gamma ELISPOT assays.

**Results**

All vaccinated MCMs generated detectable levels of Gag- and Nef-specific T cell responses that typically peaked 2 to 3 weeks post-vaccination with a consistent immunodominance hierarchy. Addition of a single dose of ipilimumab resulted in robust augmentation of vaccine-induced CD8+ T cell responses both at the peak of the vaccine response and after the vaccine response waned (>6 weeks post-vaccination). Enhanced Ki-67 expression was also detected on bulk CD8+ and CD4+ T cells, signifying increased proliferative capacity with ipilimumab treatment.

**Conclusions**

Using this nonhuman primate PD model, we observed that the administration of ipilimumab enhanced the magnitude of vaccine-induced
Changes in uveal melanoma immune infiltrate in response to checkpoint blockade

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Background
Uveal melanomas are a subset of melanomas that contain few genomic mutations, thereby generating few neo-epitopes, and making them of low immunogenic potential. What is unknown is how systemic treatment for metastatic disease changes the immunogenicity of metastatic uveal melanomas. Previous work has demonstrated the immune infiltrate in cutaneous melanomas changes in response to treatment with checkpoint blockade. We undertook a pilot study to describe the changes in uveal melanoma immune infiltrate in response to treatment with systemic checkpoint blockade.

Methods
Twenty-two uveal melanoma patients who had received checkpoint blockade for metastatic disease, and who had archival pathologic tissue, were identified. Formalin-fixed paraffin-embedded tissue sections were obtained on available cases, and bleaching protocols were utilized to reduce heavy melanin pigmentation. Immunohistochemistry was performed using validated antibodies for CD3, CD8, CD68, FOXP3, PD-1, and PD-L1.

Results
Eleven uveal melanoma patients had at least one time point of tumor tissue collected and evaluated, most commonly before treatment initiation. Nine patients had at least two time points collected and evaluated, some while on treatment, others at progression of disease. In a representative case, a pre-treatment metastatic liver biopsy showed low level of CD8+ lymphocytes infiltrating the tumor. After 4 doses of ipilimumab, with best overall clinical response of disease progression, the patient had another liver biopsy. This tissue sample showed a notable increase in CD8+ lymphocyte infiltration via immunohistochemistry compared to the pre-treatment sample. This is consistent with similar data from cutaneous melanoma patients receiving checkpoint blockade, where CD8+ infiltrates at progression is lower than during treatment response, but higher than at baseline. Full details of all cases will be presented at the SITC Annual Meeting.

Conclusions
Immune infiltration does change in metastatic uveal melanoma in response to systemic checkpoint blockade. Some patients did experience an increase in infiltrating CD8+ T lymphocytes while on immunotherapy. The change in immune infiltrate did not, however, correlate with clinical response to treatment. Future analysis includes an evaluation for immune suppressive molecules, such as indoleamine 2,3-dioxygenase and transforming growth factor-beta, and evaluation of immune infiltration in response to combination checkpoint blockade as well as to targeted therapy.

Acknowledgements
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P104
Development of an automated brightfield duplex IHC for simultaneous detection of PD-L1 and CD8 on lung carcinoma and tonsil FFPE tissue sections
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Background
Behavior of various tumor-infiltrating immune cells in the tumor environment correlates with patient responses to therapies. Particularly, the localization of CD8+ T cells within and around a tumor mass containing immune and tumor cells expressing the programmed death-ligand 1 (PD-L1) checkpoint marker is positively correlated with response to immunotherapy targeting PD-L1 blockade. Simultaneous detection of CD8+ T cells and PD-L1+ cells could become a valuable diagnostic tool. We developed a fully automated duplex immunohistochemistry (IHC) assay to detect these two biomarkers on a single formalin-fixed, paraffin-embedded (FFPE) tissue section using VENTANA BenchMark ULTRA stainers and brightfield microscopy.
Methods
The duplex IHC assay uses anti-PD-L1 (SP263) and anti-CD8 (SP239) rabbit monoclonal primary antibodies and goat anti-rabbit secondary antibodies conjugated to either horseradish peroxidase (HRP) or alkaline phosphatase (AP). The detection and amplification of signal is achieved upon activation of tyramide- or quinone methide-conjugated chromogens by the corresponding enzymes and covalent binding of chromogens. The detection of PD-L1, with a purple chromogen and CD8 with either yellow or cyan is performed sequentially and fully automated on the VENTANA BenchMark ULTRA stainers and includes a heat deactivation (HD) step between the two rounds of detection to prevent cross-reactivity of same species antibodies. The assay was developed and tested using normal human tonsil tissue, lung carcinomas, and multi tissue arrays. The staining was assessed by trained pathologists or measured using commercially available imaging software.

Results
The PD-L1/CD8 duplex assay showed equivalent staining to the single PD-L1 or CD8 DAB staining (Fig. 46) on normal tonsil and lung carcinomas. The reproducibility test showed 100% agreement between three lots of reagents and 89% (blue detection) and 100% (yellow and purple detection) agreement between three testing laboratories. The staining precision is acceptable as the coefficient of variation of staining intensity was less than 2%. Effectiveness of heat deactivation was validated for the conditions of the duplex assay as we did not detect cross-reactivity or adverse effects on the epitopes and the chromogens.

Conclusions
We developed a fully automated and robust dual IHC staining assay for detection of PD-L1 and CD8 markers on the same slide. The chromogens used in this assay are compatible with regular counterstains and alcohol dehydration. The dual IHC assay could facilitate mining of spatial relationship between the two markers for evaluation of tumor microenvironment.

Fig. 46 (Abstract P104). Comparative single and dual staining of PD-L1 and CD8 in lung tumor tissue

P105
Application of a test developed for prediction of response to high dose interleukin-2 (HDIL-2) and the BDX008 test for prediction of outcomes following checkpoint inhibitors to cohorts of patients treated with HDIL-2 or nivolumab
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Background
With multiple approved immunotherapies for metastatic melanoma (MM), tests that enable optimal treatment selection are needed. BDX008 is a serum protein-based test that identifies patients with better (BDX008+) or worse (BDX008-) survival (OS) on nivolumab [1]. Using similar methods and samples from the IL-2 Select trial, a test for prediction of progression-free survival (PFS) after HDIL-2 treatment has been developed that divides patients into two groups, A and B (better and worse PFS, respectively). Performance of these tests is compared in two cohorts of MM patients treated with either HDIL-2 or nivolumab.

Methods
MALDI mass spectra were generated for pre-treatment samples from 114 pts in the IL-2 Select trial and 119 patients from a trial of nivolumab (MM), tests that enable optimal treatment selection are needed. BDX008 is a serum protein-based test that identifies patients with better (BDX008+) or worse (BDX008-) survival (OS) on nivolumab [1]. Using similar methods and samples from the IL-2 Select trial, a test for prediction of progression-free survival (PFS) after HDIL-2 treatment has been developed that divides patients into two groups, A and B (better and worse PFS, respectively). Performance of these tests is compared in two cohorts of MM patients treated with either HDIL-2 or nivolumab.

Results
Of the 112 patients treated with HDIL-2 (2 failed BDX008 QC), 39 (35%) were classified as group A, and 89 (79%) as BDX008+. All group A samples were BDX008+. PFS was strongly correlated with IL-2 test classification: IL-2 test group B had inferior PFS compared with
**P106**

A test identifying advanced melanoma patients with long survival outcomes on nivolumab shows potential for selection for benefit from combination checkpoint blockade

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**Background**

Multiple immunotherapeutic regimens for the treatment of metastatic melanoma (MM) are now approved and can provide long-term benefit to a proportion of patients. Blood-based assays that can assist treatment selection are of significant clinical relevance. Using serum mass spectrometry, a test has been developed to predict good outcomes on anti-PD-1 therapy. We present results on the development and validation of this test.

**Methods**

MALDI mass spectra were generated for pretreatment serum samples from 119 patients treated with nivolumab at Moffitt Cancer Center. Using a classifier development platform optimized for creation of multi-variate molecular diagnostic tests which can generalize well to independent datasets, a test was created to identify patients with particularly good outcomes. This test classifies samples into two groups: group 1 (very good outcome) and group 2 (inferior outcome). This test was validated in two independent cohorts of MM patients receiving anti-PD-1 agents: 30 patients from Yale University (YU) and 25 patients, most treated with pembrolizumab, from Massachusetts General Hospital (MGH). The test was also applied to pretreatment samples collected from 21 patients from YU receiving combination PD-1/CTLA-4 blockade. Difference in outcomes between patients classified as group 1 and group 2 within each cohort were assessed using log-rank p values and Cox proportional hazard ratios (HRs).

**Results**

Of the 119 patients used in test development, 34 (29%) were classified as group 1. Group 1 had better survival (OS) and time-to-progression (p=0.0001 and p=0.014, respectively), with two-year survival of 67%. In the YU cohort receiving an anti-PD-1 agent, 13 (43%) classified as Group 1, which had better OS than Group 2 (p<0.001) and two-year survival above 80%. Eleven (44%) patients from the MGH cohort were classified as Group 1. There was a trend to improved OS for Group 1 compared to Group 2 (p=0.062; HR=0.17). Within the cohort of patients receiving combination PD-1/CTLA-4 blockade, 13 (62%) classified as Group 1. Two-year survival was 83% in Group 1 and 63% in Group 2.

**Conclusions**

The test identifies a subgroup of patients with extremely good outcomes on anti-PD-1 therapy. High two-year survival in the Group 1 cohorts may indicate that the test has potential utility in identifying patients who derive significant benefit from anti-PD-1 monotherapy and might gain little benefit from the addition of an anti-CTLA-4 agent. As follow-up was only 2 years for OS, and the numbers are small, further validation is necessary.

**Trial Registration**

ClinicalTrials.gov identifier NCT01176461.
Methods
Previously, we demonstrated reproducible immunofluorescence labeling with a manual protocol utilizing microwave exposure to remove antibodies between each marker (Methods 70 (2014) 46–58). In the fully automated approach using the Bond Rx, we replace the microwave step with a 7-minute exposure to 5% SDS at 50°C. Tonsil and breast cancer sections were stained with a 6-plex, 7-color panel for PD-1, PD-L1, CD8, CD68, Foxp3, cytokeratin, plus DAPI counterstain. Serial sections were used to assess reproducibility, cross talk, interference, and signal-to-background ratio. Slides were scanned and analyzed using multispectral imaging (VectorialTM) to isolate fluorochrome signals for accurate measurement.

Results
Balanced specific staining was achieved with both methods. Crosstalk was below the limit of detection. Signal-to-background was above 10:1 for each label, as measured by looking at signal strength on and off cells positive for the marker of interest. Coefficient of variation of measured signals was generally less than 20% for the semi-automated approach and less than 15% for the fully automated approach.

Conclusions
This is the first demonstration of reproducible and independent fully-automated TSA-based automated multiplexed immunofluorescence using SDS to perform antibody denaturation. It is also a demonstration of a semi-automated approach that performs antibody stripping off of the autostaining instrument and is compatible with a broad range of mid-range and conventional autostaining platforms.

References

P108
Spatial distribution of CD8+ T cells predicts response to ipilimumab in malignant melanoma
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Background
Although checkpoint blockade immunotherapies are successful in some cancer patients, the majority of patients still do not benefit from these therapies. The advances of digital pathology and the availability of quantitative whole slide image analysis allows for a systematic search for novel tissue-based biomarkers to predict therapy response and overall survival. Here, we present a Tissue Phenomics case study to discover a potential companion diagnostic test for ipilimumab (IPI) in malignant melanoma.

Methods
The patient cohort is a subset of the MISIPI study [1], comprising 30 melanoma patients. Consecutive sections from FFPE tissue were immunohistochemically stained with CD3, CD8, and FoxP3. The sections were digitized and automatically aligned using an advanced staining-independent image registration algorithm. Tumor regions were manually annotated by a pathologist, excluding artifacts and necrotic regions. A novel parameter-free cell segmentation approach was used to automatically detect and classify cells with respect to their protein expression profile [2] and to the spatial distance to the annotated tumor border. We computed for each of the CD3+, CD8+ and FoxP3+ cell populations the average cell density in the tumor in a border region within the tumor and in a border region just outside the tumor. By computing a potentially predictive score for each ratio of the measured cell densities, we identified the most promising patient stratification algorithm in terms of predictive values for therapy response and the Kaplan-Meier statistics p-value for overall survival.

Results
We identified a promising scoring algorithm which is based on the ratio of the CD8+ cell density at the inner tumor border to the CD8+ cell density in the tumor. It enables patient stratification into IPI responders and non-responders with high predictive values (positive predictive value 68%, negative predictive value 79%, prevalence 39%) and at the same time, excellent prediction of overall survival (p-value < 0.0015).

Conclusions
A novel companion diagnostic algorithm for ipilimumab in malignant melanoma was discovered by Tissue Phenomics. It provides high predictive power and enables considerably improved treatment decisions. However, the results are still preliminary and need to be further validated.

References

P109
Tumor-associated macrophages (TAMs) as a prognostic marker for prostate cancer progression
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Background
Tumor-associated macrophages (TAMs) and tumor infiltrating T-cells (TILs) have been associated with tumor progression in various tumor entities. In this study, we applied the Tissue Phenomics technology to discover new TAM-related prognostic factors to predict prostate cancer progression. This technology correlates clinical outcome data with image analysis results from (virtually) multiplexed tissue slides. Multiplexing enables the co-analysis of multiple immunohistochemically (IHC) stained tissue sections. Using this method, we investigated the prognostic relevance of M1/M2 TAMs (CD68/CD163) and TILs (CD3/CD8) within prostate cancer (PCA) resection specimens from low-risk and intermediate-risk patients with respect to Prostate Specific Antigen (PSA) recurrence after radical prostatectomy (RP).

Methods
Analysis and quantification were performed on three consecutive duplex stained FFPE tissue sections from 89 patients (on which 40 with PSA recurrence) with low- to intermediate-risk PCA after RP with a Gleason-Score ≤ 7a. M1- and M2-type macrophages were characterized by CD68 and CD163, T-cells by CD3 and CD8, tumor and non-tumor epithelial tissue regions by CK18 and p63. CK18 positive glands without any p63-stained basal cell nuclei are considered as tumor glands. The three duplex stained slides were analyzed and converted into one virtual tissue slide through image co-registration. TAMs and TILs were quantified separately in the distinct tumor regions and the tumor microenvironment. Both, the overall number of TILs and TAMs and the ratio of M1- and M2-positive macrophages were analyzed using the Tissue Phenomics technology. The correlation between various TIL and TAM cell densities within the different regions of interest (tumor and tumor microenvironment), the tumor grading and tumor stage, and clinical parameters (PSA recurrence, overall and disease-free survival) were performed.

Results
Prostate cancer patients without PSA recurrence show a significantly higher ratio of CD8 to CD163 positive cell densities in the tumor microenvironment. The PSA recurrence prediction accuracy is 76.8%
with a Kaplan-Meier log-rank test p-value of 2.7e-5 for the disease free survival time.

Conclusions

These first results indicate a considerable prognostic potential of TAMs to predict PSA recurrence in PCa. The application shows that the Tissue Phenomics Technology enables the investigation and the evaluation of the prognostic relevance of certain immune cell populations. The analysis of the relation of TILs to TAMs is an excellent example for such kind of applications with a potentially high impact for prostate cancer patient treatment decisions.

P110

Co-expression of PD-L1 and other targetable protein and genomic markers: opportunities for combination therapy

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Background

Several immune checkpoint inhibitors have been approved to treat multiple solid tumor types. Immune responses could be modulated by targeted therapies as well as cytotoxic agents. The opportunity to combine immunotherapies with agents against targetable proteomic and genomic biomarkers may lead to improved outcomes and reduced toxicities; however, analysis of multiple biomarkers creates an increased demand for tissue. Using a multi-omic approach that incorporates mass spectrometry based proteomic analysis and NGS, we were able to objectively detect the expression of multiple therapeutically-relevant proteins and genomic alterations from a minimum two FFPE sections. Here, we report on patient samples that express the PD-L1 protein and either co-express therapeutically-relevant proteins and genomic alterations from a minimum two FFPE sections. For genomics analysis, genomic content was isolated from the FFPE tumor tissue sections as well as from corresponding normal samples and subjected to NGS for whole genome sequencing (tumor only) and whole transcriptome sequencing (tumor only) analysis.

Results

Quantitative proteomic analyses of 1710 cancer patient samples across multiple indications revealed a wide range (144 – 1025 amol/mg) of PD-L1 protein expression level. Several actionable protein targets were co-expressed with PD-L1; including targeted therapy markers (EGFR (80%), HER2 (29%), MET (56%), ROS1 (22%)) and chemotherapy markers (TOP1 (100%), etc.). PD-L1 protein expressing tumors were also found to harbor therapeutically-relevant genomic mutations, including MET (N375S and T1010I), JAK3 (V722I), KIT (M541L), BRAF (V600E), CDKN2A (D84H), etc.

Conclusions

Tumor molecular profiling by both proteomic and genomic analysis revealed co-expression of targetable proteins (EGFR, HER2, MET, TOP1, etc.) and genomic alterations (MET, KIT, BRAF, etc.) in patients that express the PD-L1 protein. From minimal amounts of tissue, we were able to assess multiple therapeutically-associated biomarkers using mass spectrometry based targeted quantitative proteomics and NGS based comprehensive genomic (DNA and RNA) analysis. Ongoing clinical trials involving immunotherapy and chemo/targeted therapies could benefit from molecularly stratifying patients by proteomic and genomic testing to increase efficacy and reduce toxicity.

P111

Immunomonitoring of patients with colorectal cancer

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Background

Colorectal cancer is the second leading cause of cancer death worldwide. This high mortality is because almost half of colorectal cancers are detected at an advanced stage of disease, and the currently used prognostic factors are not always accurate. Recently, the prognostic impact of the body’s immune response to cancer has been demonstrated. Immunoscore (in situ immune cell infiltrate in tumours) has been shown to be a very powerful prognostic indicator in patients with clinically localized colorectal cancer. These patients are usually treated with only surgical removal of the tumour; however, approximately 25% of these patients will have recurrence of their disease, indicating that occult metastases were already present at the time of curative surgery. No tumour-associated marker predicts the recurrence of this subgroup of patients that could benefit from adjuvant therapy. These days are investigated other parameters of the immune response – mostly cellular immunity and the production of immunosuppressive and neoangiogenic markers. VEGF is the factor responsible for neoangiogenesis and it is being considered as a possible prognostic marker of disease progression. Transforming growth factor-beta (TGF-beta) is also neoangiogenic and a highly immunosuppressive factor, as it suppresses the body's natural immunity against tumours. TGF-beta is also being considered as another possible prognostic marker of disease progression. The purpose of this study was to monitor the immune response in patients with stage II colorectal cancer, with a focus on cellular as well as humoral immunity. TGF-beta and VEGF levels were followed.

Methods

22 patients with stage II colon cancer included in the research project received routine cancer treatment. Basic parameters – histological type and grade, proliferative markers – were established at baseline. Patients were evaluated by a clinical immunooncologist to exclude any immune disorders of allergic or autoimmune origin. TGF-beta and VEGF were measured using ELISA, and anti-tumour cellular immunity (CD4, CD8, T-reg, B cells) were measured via flow cytometry.

Results

In patients with stage II colorectal cancer, predominantly a depression in cellular immunity was seen. Plasma levels of immunoglobulins were also reduced, particularly the IgG4 subtype. Most patients showed some clinical symptoms of immunodeficiency, such as frequent respiratory tract infections and/or herpetic infections. TGF-beta and VEGF plasma levels were increased.

Conclusions

The correlation of these neoangiogenic and immunosuppressive factors, as well as the state of anticancer immunity, could help in the future as a prognostic marker and contribute to the selection of targeted immune therapy in patients with colorectal cancer.
P112
Evaluation of immune cell infiltrates in non-small cell lung cancer as a potential comprehensive prognosticator
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Background
Therapeutic strategies in non-small cell lung cancer (NSCLC) are based on the histopathological evaluation of the tumor tissue which, in conjunction with the TNM staging system, remains the gold standard for prognostic assessment. Nevertheless, histopathology and stage-specific clinical outcomes vary significantly, indicating that there is a need for additional prognosticators. A previous study reported that intense lymphocytic infiltrates were found in 6-11% of NSCLC and were associated with a significant increase in disease-free and overall survival [1]. The aim of our study was to use multispectral imaging and digital quantification to assess relationships between T cells and FoxP3+ or PD-L1+ cells in tissue microarrays (TMAs) cored from “hot spots” of dense infiltrates at the invasive margin and center of the NSCLC.

Methods
Tissue microarrays (TMA) were generated from formalin-fixed paraffin-embedded tissue of 98 curatively resected patients with stage IA-IIIA NSCLC. TMAs included 3 cores for tumor center and 3 cores for invasive margin selected from the areas with the most dense (hot spot) lymphocytic infiltrate. TMAs were immunolabeled with mHIC technique for the following antibodies: PD-L1, CD8, CD3, FoxP3, CD163, cytokerin and DAPI and quantified with InForm software. Results were compared between groups of patients with squamous and non-squamous cell carcinoma and correlated with the course of the disease, overall-progression-free survival, metastases or relapse and clinicopathological parameters.

Results
In contrast to reports evaluating whole sections, analysis of “hot spots” in this cohort of patients failed to provide a prognostic biomarker for survival. Current studies are evaluating how an evaluation of the whole section correlates with results of this “hot-spot” analysis.

Our preliminary data suggests that the “hot-spot” analysis of CD8, FoxP3 and PD-L1 does not allow us to identify outcome in NSCLC.

Conclusions
Multispectral assessment of CD8, FoxP3, and PD-L1 performed on “hot-spots” of stage I-II NSCLC did not provide a prognostic biomarker. Given other reports that immune infiltrates are associated with improved outcome, this suggests it may be important to evaluate a larger percentage of the tumor. These findings appear to contrast with the colon immunoscore project which identified a correlation with an enumeration of CD3+ and CD8+ T cell numbers in hot-spots and disease-free and overall survival.

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References

P113
High NKG2A expression contributes to NK cell exhaustion and predicts a poor prognosis of patients with liver cancer
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Background
As the predominant lymphocyte subset in the liver, nature killer (NK) cells have been shown to be highly associated with the outcomes of patients with chronic hepatitis B virus infection (CHB) and hepatocellular carcinoma (HCC). Previously, we reported that NKG2A, a checkpoint candidate, mediates human and murine NK cell dysfunction in CHB. However, NK cell exhaustion and, particularly, the level of NKG2A expression within liver tumours have not been reported.

Methods
In this study, we analysed NKG2A expression and the related dysfunction of NK cells located in intra- or peritumour regions of liver tissue samples from 207 HCC patients, in addition to analysing disease outcomes.

Results
The expression of NKG2A in NK cells and the NKG2A ligand, HLA-E, in intratumour HCC tissues was observed to be increased. These NK cells, and particularly CD56dim NK cells, with higher NKG2A expression showed features of functional exhaustion and were associated with a poor prognosis. The increase in NKG2A expression might be induced by IL-10, which was present at a high level in the plasma of HCC patients. Blocking IL-10 could specifically inhibit NKG2A expression in NK cells.

Conclusions
These findings indicate that NKG2A expression is influenced by factors from cancer nests and contributes to NK cell exhaustion, suggesting that NKG2A blockade has the potential to restore immunity against liver tumours by reversing NK cell exhaustion.
Association of non-coding repeat RNA expression with immune infiltrates

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Background
Colorectal cancer (CRC) is the third most common type of cancer in the United States. There have been exciting responses to immunotherapy in CRC, but the ability to predict which patients would benefit from these drugs remains elusive. Recent work has demonstrated the aberrant expression of non-coding repeat RNAs across cancers including colon cancer [1, 2]. These repeats appear to trigger elements of the innate immune response that may have implications for response to immunotherapy [1]. To determine if repeat RNAs are linked to the tumor immune microenvironment, we developed a novel combined repeat RNA in situ hybridization (RNA-ISH) with T cell immunohistochemistry (IHC) on fixed formalin paraffin embedded (FFPE) tissues. We assessed two different repeat types (HSATII and HERV-H) that have been well documented in human cancers and reported to have an association with innate immune responses [2]. To investigate correlation of host immune T cells with these repeats a pilot study of seventy-five patients of CRC was performed.

Methods
FFPE tissue sections of 75 patients were stained by RNA-ISH and IHC on the Leica Bond RX. HSATII and HERV-H repeat RNA-ISH and CD8 (cytotoxic) and FOXP3 (regulatory) T cell marker IHC was performed on each case. Repeat RNA scores were determined as HIGH or LOW based on relative cancer cell expression to normal adjacent tissues. T cell density was determined by counting the number of positive cells in a 400 x 200 μm area. T cell density differences were calculated by t-test.

Results
We found HSATII was HIGH in 47 (63%) and HERV-H was HIGH in 36 (48%) of CRC cases with a concordance between repeats of 51%. Analysis with T cell subsets revealed lower CD8+ T cells in HSATII HIGH tumors (306 vs 686 CD8+ T cells/mm2; p=0.03).

Conclusions
Repeat RNAs are expressed across malignancies with varying levels that correlate with different immune infiltrates. Cancer expression of HSATII and HERV-H repeats offers a novel surrogate biomarker of cytotoxic and regulatory T cell tumor infiltration. The mechanistic effects of these repeats on immune cell function merits further investigation.

Acknowledgements
We thank Alex Forrest-Hay, Manoj Gandhi, Frank Witney, and Affymetrix, Inc. for sponsored research support.

References
Outcome disparities by sex in melanoma patients treated with anti-PD-1 therapy

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Background

PD-1 antibodies have shown significant clinical activity against advanced melanoma, but ORR with PD-1 monotherapy remains less than 50%. We previously reported and validated a clinical scoring model to predict response to anti-PD-1, and in our logistic regression analysis, female sex was found to be associated with lower response rate (OR 0.36, 95% CI 0.19-0.67). We report further on outcomes in immunotherapy-treated patients by sex and correlate this with results of T cell profiling of an exhausted, antigen-experienced phenotype (% PD1-high/CTLA-4-high CD8 cells) from pre-treatment biopsy samples.

Methods

336 patients (118 women, 218 men) with advanced cutaneous melanoma were treated with pembrolizumab or nivolumab at 4 academic cancer centers between December 2011 and October 2013. Baseline demographics and clinical characteristics were collected from electronic health records. Tumor response was assessed using RECIST v1.1 criteria. Pre-treatment flow cytometry data from 122 patients (45 women, 77 men) treated with both PD-1 monotherapy and PD-1/CTLA-4 combination therapy were analyzed by sex for correlative data.

Results

In the PD-1 monotherapy cohort, median ORR was 33.1% (95% CI 24.4-41.2) in females and 54.6% (95% CI 47.9-61.3) in males (p=0.0001). Median PFS was 5.5 months (95% CI 3.4-7.1) in females and 18 months (95% CI 11.3-23.8) in males (p=0.0004) (Fig. 51). Flow cytometry analysis of pre-treatment tumor biopsies revealed a statistically significant reduced proportion of exhausted PD1-high/CTLA-4-high CD8 T cells that persisted across age groups (Fig. 52).

Conclusions

There is a significant discrepancy between response to PD-1 monotherapy between men and women with advanced melanoma. The mechanisms of this may have immunologic basis given the difference in pre-treatment T cell profiles between men and women.

Trial Registration

ClinicalTrials.gov identifier NCT01295827, NCT01704287, NCT01721746, and NCT02156804.
**P116**

A flexible and versatile toolbox for parallel multiplex immunohistochemical detection using recombinant epitope-tagged antibodies and monoclonal anti-tag antibodies


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*Journal for ImmunoTherapy of Cancer* 2016, 4(Suppl 1), P116

**Background**

Contextual detection of multiple biomarkers on single formalin-fixed, paraffin-embedded (FFPE) slides for clinical applications remains an unmet need. Current multiplex immunohistochemistry (IHC) procedures entail successive rounds of antibody application and fluorophore attachment followed by antibody inactivation. We developed a parallel multiplex IHC approach using series of epitope-tagged antibodies and anti-epitope antibodies conjugated to fluorophores, haptens, or enzymes, and demonstrated feasibility by 5-plex fluorescence and duplex brightfield assays of markers relevant for immunoncology.

**Methods**

DNA sequences corresponding to peptide epitope tags were fused in-frame to rabbit monoclonal antibody cDNAs for expression in mammalian cells. Recombinant tagging bypasses the potential antibody inactivation associated with chemical-based tagging. Conjugation of fluorophores on haptens to antibodies was performed using NHS ester precursors. Horseradish peroxidase (HRP) and alkaline phosphatase (AP) were conjugated to reduced antibodies via NHS-maleimide linkers. Affinity of anti-epitope antibodies to peptides was assessed using biolayer interferometry. IHC of FFPE tissue sections was performed on VENTANA BenchMark ULTRA platform.

**Results**

Rabbit monoclonal antibodies against CD3, CD8, CD68, FoxP3, and PD-L1 were each engineered with a unique epitope tag. Epitope-tagged primary antibodies produced identical diamino benzidine (DAB) staining intensity and pattern as untagged native antibodies. Multiple clones of recombinant rabbit monoclonal antibodies against each of the five epitope tags were conjugated and screened for retention of affinity, stability, and appropriate staining intensity and pattern in validated tissue microarrays. At least one clone of each anti-epitope antibody met the functional requirements and these were used to stain FFPE lung and tonsil tissue sections in conjunction with cocktail epitope-tagged antibodies. Epitope-tagged antibodies were detected using one of three detection configurations in order of sensitivity: 1) fluor-conjugated anti-epitope antibodies, 2) hapten-conjugated anti-tag antibodies and fluor-conjugated anti-hapten antibodies, and 3) attachment of tyramide- or quinone methide-fluors to tissue specimens with HRP- or AP-conjugated anti-epitope antibodies. Titration of antibodies and assay optimization enabled pairings of particular biomarkers with detection configurations to generate specific fluorescence patterns and relative intensities similar to those produced by DAB stains using untagged antibodies and HRP-conjugated anti-species antibodies. Two-color brightfield stains were generated using enzyme-conjugated anti-tag antibodies and HRP- and AP-activated chromogens.

**Conclusions**

We demonstrated feasibility of parallel multiplex IHC using a series of epitope-tagged antibodies and fluor-, hapten-, or enzyme-conjugated anti-epitope antibodies. Applying tagged antibodies and anti-tag antibody probes as cocktails streamlines multiplex detection and avoids the inactivation of antibodies that is necessary in multiplex approaches based on serially applied antibodies.

**P117**

Multiplex immunohistochemistry and image cytometry analysis for phenotyping spatial immune characteristics

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**Background**

While immune therapies targeting select checkpoint molecules have revolutionized cancer medicine, the fact that many patients fail to respond underscores the need for improved patient stratification and application of multi-nodal biomarkers reflecting in situ status of the tumor immune microenvironment.

**Methods**

We developed a multiplexed immunohistochemical (IHC) imaging approach based on sequential IHC with iterative labeling, digital scanning and subsequent stripping of sections. To elucidate contextual information on leukocyte presence, complexity and functional phenotype in situ, we investigated these parameters in head and neck squamous cell carcinoma (HNSCC) utilizing multiplex IHC analyses in de-identified tissue microarray sections reflecting human papilloma virus (HPV)-positive and negative oropharyngeal HNSCC and normal pharynx. Quantitative multiparameter image cytometry was developed to enable quantification of cell density, composition and location of 16-immune cell lineages, along with monitoring of programmed cell death-1 (PD-1) receptor and ligand (PD-L1) expression.

**Results**

Intratumoral immune cell densities were comprehensively evaluated by multiplex IHC/image cytometry via unsupervised clustering analysis and revealed presence of lymphoid, myeloid, and hypo-inflamed profiles correlating with HPV-status. Myeloid-inflamed profiles with low ratios of CD163/CD163 of CD68* CSFIR* macrophages and CD8* T cell/CD68* ratios was significantly associated with poor prognosis in both HPV-positive and negative HNSCC. Neoplastic cell nest vs. stromal region analyses revealed distinct distribution patterns for T1, T2 lymphocytes, showing intratumoral T1,2 orientation in HNSCC. Intratumoral CD66b* granulocyte infiltration was associated with unfavorable prognosis. Further analysis for spatial characteristics of immune complexity revealed high T1,T1,T2 ratios and CD8* T cell infiltration within a distance of 20 μm to PD-L1* cells, indicating an association between PD-L1 expression and regional characteristics of immune complexity.

**Conclusions**

These results demonstrate the capability of multiplex IHC-based image cytometry analysis toward improved understanding of heterogeneous tumor microenvironments.

**Acknowledgements**

This project was supported by Oregon Clinical and Translational Research Institute (OCTRI), grant number (UL1TR000128) from the National Center for Advancing Translational Sciences (NCATS) at the National Institutes of Health (NIH), and P30 CA095357-13 OHSU Knight Cancer Institute. LMC acknowledges support from the NIH/NCI, DOD BCRP Era of Hope Scholar Expansion Award, Susan G. Komen Foundation, Stand Up To Cancer – Lustgarten Foundation Pancreatic Cancer Convergence Dream Team Translational Research Grant, Breast Cancer Research Foundation, and the Brenden-Colson Center for Pancreatic Health.

**P118**

Analysis of immunerelated prognostic markers in colon cancer patients randomized to surgery alone or surgery and adjuvant cytostatic treatment

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*Journal for ImmunoTherapy of Cancer* 2016, 4(Suppl 1), P118
Background
We have previously shown that human leukocyte antigen (HLA)-A*02, a common allele in the Scandinavian population, is a negative prognostic factor in epithelial ovarian cancer [1]. It is a strong predictor of patient outcome, only inferior to clinical staging. We have also shown that this prognostic trait in epithelial ovarian cancer is stronger by the presence of the gene compared with the expression of its protein, MHC class I [2]. Finally, we have shown that HLA-G expression in ovarian cancer is a negative prognostic marker [3]. Our aim was to analyse the prognostic markers HLA-A*02 genotype, MHC class I and HLA-G expression on tumour cells and the CD8+ lymphocyte infiltration in colon cancer.

Methods
Clinical information and primary tumours were collected from 520 colon cancer patients and followed for overall survival for 120 months. HLA-A*02 genotype was determined by conventional PCR. MHC class I expression and CD8+ lymphocyte infiltration were determined by immunohistochemistry.

Results
Patients with a stage III tumour and HLA-A*02 genotype had a better outcome if randomized to adjuvant chemotherapy versus surgery alone (P=0.03). There was an indication that patients with complete absence of MHC class I expression had a better overall survival compared to patients with a decreased or increased expression. Expression of HLA-G was a negative prognostic marker for the male patients (P=0.002). Also a high infiltration of CD8+ lymphocytes was important in the male patients, where a high frequency of infiltration correlated with a good prognosis (P=0.002). These factors were not, however, significant in the female population. The superior negative prognostic marker in the female patients was HLA-A*02 genotype.

Conclusions
HLA-A*02 positivity may be an important marker to determine which colon cancer patients should receive adjuvant chemotherapy. It also seems to determine the outcome of the female patients, which raises new questions as to why this gene, which most likely has the same function in both genders, can be devastating to one gender and not the other.

References

Table 8 (Abstract P118).

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Cohort description

![Fig. 53 (Abstract P118), HLA-genotype and treatment](image1)

![Fig. 54 (Abstract P118), MHC class I expression](image2)

![Fig. 55 (Abstract P118), HLA G expression](image3)
immune checkpoint proteins, transcription factors, chemokines, and methods to allow digital quantification of both cell surface and intracellular proteins from primary cells such as PBMCs). NanoString has developed content and transcripts plus 30 proteins from as few as 20,000 cells (up to 50,000 primary cells). Now the nCounter technology can simultaneously quantify 770 RNA targets from a single sample.

**Results**

As proof of concept, RNA and protein were measured simultaneously from just 50,000 PBMCs treated with phorbol 12-myristate 13-acetate (PMA) and ionomycin, TNFα, INFγ, or anti-CD3/CD28. NanoString’s multiplex RNA and protein detection provides a thorough evaluation of the immunological response in these experiments and demonstrates the value of multi-analyte profiling from the same sample by characterizing the breadth of the response (via 770 RNA measurements) and providing depth to the analysis (via tandem measurements of RNA and protein for key targets). As independent confirmation, the findings were validated with flow cytometry.

**Conclusions**

This advance in multi-analyte, multiplexed digital molecular profiling with low sample input will accelerate immuno-oncology research and may enable the discovery and development of novel immuno-therapies and their associated companion diagnostics.

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**P119**

**Profiling immune cell populations and functional state with simultaneous, multiplexed detection of RNA and protein on the nCounter® platform**

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**Background**

One of the biggest challenges facing the field of immuno-oncology is development of a comprehensive understanding of how the immune system responds to a tumor. Multi-analyte profiling (DNA, RNA, and protein) from limited sample is crucial to furthering our understanding of tumor immunity. NanoString’s nCounter® technology has become an important platform for quantification of transcriptional responses by enabling direct digital quantification of up to 800 RNA targets from a single sample.

**Methods**

Now the nCounter technology can simultaneously quantify 770 RNA transcripts plus 30 proteins from as few as 20,000 cells (up to 50,000 primary cells such as PBMCs). NanoString has developed content and methods to allow digital quantification of both cell surface and intracellular protein targets that are essential to immuno-oncology research. These key targets include immune cell population markers, immune checkpoint proteins, transcription factors, chemokines, and cytokines. Protein detection is enabled via primary antibodies, which are covalently linked to single stranded DNA indexing oligos. Cells are stained with a multiplexed cocktail of labeled antibodies, and DNA oligos are subsequently released via cell lysis for hybridization to optical barcodes in the standard NanoString workflow. This technique enables quantitative, multiplexed protein detection over 5 logs of dynamic range.

**Results**

We demonstrate validation of this technology by characterization of a panel of immune proteins on FFPE tissue biopsies and tissue microarrays. We extend this technology to spatially-resolved multiplexed detection of RNA on a number of important immune targets (e.g., CD3, CD45). We further demonstrate that this approach enables protein detection at single cell resolution and enables simultaneous characterization of the abundance, distribution, and colocalization of key immunoregulatory proteins within the tumor microenvironment that is necessary for a thorough understanding of tumor immune responses. Historically, immunohistochemistry and immunofluorescence have been used to assess spatial heterogeneity of proteins and nucleic acids in tissue slices, but these techniques are of limited utility due to the challenge of measuring multiple targets in parallel. We have developed a platform to enable spatially-resolved protein and RNA detection with the potential to simultaneously quantify up to 800 targets with greater than 5 logs of dynamic range from a single formalin-fixed paraffin-embedded (FFPE) slide.

**Conclusions**

This advance in multi-analyte, multiplexed digital molecular profiling with low sample input will accelerate immuno-oncology research and may enable the discovery and development of novel immuno-therapies and their associated companion diagnostics.
multiplexed detection of CD3, CD4, CD8, CD45, CD45R0, PD-1, PD-L1, 
Vista, TIM-3, B7-H3, Ki67 and additional key immuno-oncology (IO) 
targets. Finally, we demonstrate detection of key IO RNA targets 
using direct hybridization of oligo-labeled probes.

Conclusions
The ability to measure DNA, RNA, and protein at up-to 800-plex from 
single slices of FFPE tissue may enable the discovery of key immune 
biomarkers in tumors and accelerate the development immunother-
apy and their associated companion diagnostics.

P121
Immunological biomarkers correlate to survival in CAR19-treated 
patients
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Background
CD19-targeting chimeric antigen receptor (CAR) T cell therapy 
achieved remarkable results in patients with acute lymphoblastic leukemia. However, few studies have shown promising results in pa-
tients with other CD19+ B cell malignancies, including lymphomas. 
We have completed a trial treating 15 patients with various B cell 
malignancies with third generation CD19-targeting (CD28/4-1BB) CAR 
T cells with or without preconditioning using a moderate dose of 
cyclophosphamide (500mg/m2) and fludarabine (25mg/m2). Herein, 
we report the results of a biomarker screening pre and post CAR T 
cell treatment using the trial biobank.

Methods
A biobank consisting of plasma and peripheral blood mononuclear 
cells obtained from CAR T cell treated patients with relapsed or re-
fractory CD19+ B cell malignancies was analyzed for immunological 
biomarkers. Samples were analyzed for immunosuppressive cells by 
flow cytometry and for systemic biomarkers using a 233-analyte 
proteomic analysis (ProSeek). Of the 15 treated patients, 11 patients 
had lymphoma and 6 had leukemia. During CAR T cell manufacture, 
lymphoma patients received standard treatment to control tumor 
burden. All patients were treated with one infusion of CAR T cells with 
dose ranges from 2×10^5 to 2×10^7 cells/m2. The two last patients 
received anti-PD-1 antibody therapy post CAR infusion. Correlation of 
biomarkers with survival was assessed by Spearman correlation.

Results
The patients were grouped into initial complete responders (6/15) and 
long-term survivors (4/15). In patient blood, CAR mRNA peaked at 1 
week post infusion and could still be detected with varying levels over 
time up to 12 months post infusion. Baseline levels of monocytic 
myeloid-derived suppressor cells were negatively correlated with sur-
vival (P=0.0231) and M2-like macrophages tended to be increased at 
the time of relapse compared to the time of complete response (Mean 
%HLA-DR+CD80-CD163+ of CD11b+cells: 15.4% vs. 8.3%). The proteomic 
the time of relapse compared to the time of complete response (Mean 

Conclusions
The ability to measure DNA, RNA, and protein at up-to 800-plex from 
single slices of FFPE tissue may enable the discovery of key immune 
biomarkers in tumors and accelerate the development immunother-
apy and their associated companion diagnostics.

Bispecific Antibodies

P122
Multiple bispecific checkpoint combinations enhance T cell activity
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Seung Chu, Runama Rashid, Kendra N Avery, Umesh Muchhal, John 
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Background
Combination checkpoint blockade has demonstrated increased clinical 
responses relative to single checkpoint blockade. However, the combination of nivolumab plus ipilimumab has also exhibited an-
creased immune-related adverse events. We reasoned that dual 
checkpoint blockade could be accomplished using a bispecific anti-
body format, with the potential benefits of reduced cost and more 
selective targeting of tumor-reactive lymphocytes to improve safety.
Numerous checkpoint receptors exist, including PD-1, CTLA-4, LAG-3, 
BTLA, and others, encouraging the exploration of multiple bispecific 
combinations.

Methods
Antibodies specific to PD-1, CTLA-4, LAG-3, and BTLA were assem-
bled into a bispecific antibody format in select combinations and op-
timized for stability and affinity. In vitro T cell stimulation activity of 
these bispecific antibodies was measured in a SEB stimulation assay.
IL-2 and IFNγ production was measured by ELISA. In vivo activity was 
evaluated by engrafting human PBMCs into NSG mice (huPBMC-NSG) 
and measuring the extent of T cell engraftment by flow cytometry 
after weekly treatment. An anti-tumor model was also developed 
with the huPBMC-NSG system, in which KG1a AML cells were first in-
oculated and allowed to establish tumors, followed by engraftment of 
huPBMC and treatment with antibody.

Results
We produced PD-1 x CTLA-4, PD-1 x LAG-3, CTLA-4 x LAG-3, and PD-1 x 
BTLA bispecific antibodies. All of the bispecifics enhanced IL-2 produc-
tion in an in vitro SEB stimulation assay relative to control, indicating 
functional checkpoint blockade. In most cases, IL-2 production was 
similar or superior to that of anti-PD-1 alone. Treatment of huPBMC-
NSG mice with checkpoint bispecifics promoted enhanced T cell en-
graftment relative to control. Engraftment levels promoted by bispeci-
fics were generally superior to those found for anti-PD-1 treatment 
alone, and in some cases comparable to a combination of anti-PD-1 
and anti-CTLA-4 antibodies. In one run of the model, while anti-PD-1 
treatment alone promoted a 7-fold increase in human CD45+ cells, a 
PD-1 x CTLA-4 bispecific antibody induced a 22-fold increase, and a 
CTLA-4 x LAG-3 bispecific promoted a 12-fold increase. Combining 
CTLA-4 x LAG-3 with anti-PD-1 to achieve triple checkpoint blockade 
promoted a 67-fold increase in human CD45+ cells, leading to more se-
vere graft versus host disease. The bispecific antibodies also exhibited 
compelling anti-tumor activity in a mouse AML model.

Conclusions
Dual checkpoint blockade with bispecific antibodies is feasible and 
promotes strong T cell activation in vitro and in vivo. Multiple combi-
nations display compelling activity, suggesting clinical development 
is warranted for the treatment of human malignancies.

P123
Antibody blockade of PD-1 and CTLA-4 with bispecific antibodies 
promotes human T cell activation and proliferation
Michael Hedvat, Matthew J Berrett, Gregory L Moore, Christine Bonzon, 
Seung Chu, Runama Rashid, Kendra N Avery, Umesh Muchhal, John 
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Background
Combination checkpoint blockade has demonstrated increased clinical 
responses relative to single checkpoint blockade. However, the combination of nivolumab plus ipilimumab has also exhibited an-
creased immune-related adverse events. We reasoned that dual 
checkpoint blockade could be accomplished using a bispecific anti-
body format, with the potential benefits of reduced cost and more 
selective targeting of tumor-reactive lymphocytes to improve safety.
Numerous checkpoint receptors exist, including PD-1, CTLA-4, LAG-3, 
BTLA, and others, encouraging the exploration of multiple bispecific 
combinations.

Methods
Antibodies specific to PD-1, CTLA-4, LAG-3, and BTLA were assem-
bled into a bispecific antibody format in select combinations and op-
timized for stability and affinity. In vitro T cell stimulation activity of 
these bispecific antibodies was measured in a SEB stimulation assay.
IL-2 and IFNγ production was measured by ELISA. In vivo activity was 
evaluated by engrafting human PBMCs into NSG mice (huPBMC-NSG) 
and measuring the extent of T cell engraftment by flow cytometry 
after weekly treatment. An anti-tumor model was also developed 
with the huPBMC-NSG system, in which KG1a AML cells were first in-
oculated and allowed to establish tumors, followed by engraftment of 
huPBMC and treatment with antibody.

Results
We produced PD-1 x CTLA-4, PD-1 x LAG-3, CTLA-4 x LAG-3, and PD-1 x 
BTLA bispecific antibodies. All of the bispecifics enhanced IL-2 produc-
tion in an in vitro SEB stimulation assay relative to control, indicating 
functional checkpoint blockade. In most cases, IL-2 production was 
similar or superior to that of anti-PD-1 alone. Treatment of huPBMC-
NSG mice with checkpoint bispecifics promoted enhanced T cell en-
graftment relative to control. Engraftment levels promoted by bispeci-
fics were generally superior to those found for anti-PD-1 treatment 
alone, and in some cases comparable to a combination of anti-PD-1 
and anti-CTLA-4 antibodies. In one run of the model, while anti-PD-1 
treatment alone promoted a 7-fold increase in human CD45+ cells, a 
PD-1 x CTLA-4 bispecific antibody induced a 22-fold increase, and a 
CTLA-4 x LAG-3 bispecific promoted a 12-fold increase. Combining 
CTLA-4 x LAG-3 with anti-PD-1 to achieve triple checkpoint blockade 
promoted a 67-fold increase in human CD45+ cells, leading to severe 
graff versus host disease. The bispecific antibodies also exhibited 
compelling anti-tumor activity in a mouse AML model.

Conclusions
Dual checkpoint blockade with bispecific antibodies is feasible and 
promotes strong T cell activation in vitro and in vivo. Multiple combi-
nations display compelling activity, suggesting clinical development 
is warranted for the treatment of human malignancies.
Background
Treatment of melanoma patients with nivolumab plus ipilimumab increases progression-free-survival compared to each monotherapy. The increase in efficacy of the combination regimen is accompanied by an increase in adverse events. Since PD-1+CTLA-4+ tumor-infiltrating lymphocytes are dysfunctional in the tumor microenvironment, we attempted to specifically target PD-1×CTLA-4 bivalent antibodies to PD-1×CTLA-4 bivalent antibodies in an attempt to repopulate effector function. PD-1×CTLA-4 bivalent antibodies were evaluated in vitro by measuring antibody binding and de-repression of super-antigen stimulated peripheral blood lymphocytes (PBMCs) and in vivo by monitoring the engraftment of human PBMCs in NSG mice (huPBMC-NSG) by flow cytometry. To evaluate anti-tumor efficacy we monitored the growth of established KG1a AML cancer cells in huPBMC-NSG following treatment.

Results
Optimized candidate single-chain Fvs were confirmed to bind PD-1 and functionally block PD-L1 and PD-L2 binding to PD-1. We also generated optimized anti-CTLA-4 Fabts. Anti-PD-1 and anti-CTLA-4 targeting components were assembled into a bispecific format and displayed favorable biophysical and manufacturing properties. PD-1×CTLA-4 bivalent antibodies enhanced IL-2 secretion 5-fold relative to control (p<0.001, n=18 donors) in response to antigenic challenge of previously stimulated T cells, with 2-fold superior activity compared to anti-PD-1 bivalent antibody (p<0.001, n=18 donors). PD-1×CTLA-4 bivalent antibodies significantly enhanced T cell engraftment in huPBMC-NSG mice compared to vehicle controls (p<0.001, n=10/group). PD-1×CTLA-4 bivalent antibodies promoted higher T cell engraftment than anti-PD-1 alone (p<0.01, n=10/group) and similar engraftment to a combination of anti-PD-1 and anti-CTLA-4 bivalent antibodies. PD-1×CTLA-4 bivalent antibodies also exhibited compelling anti-tumor activity in a mouse AML model.

Conclusions
Dual blockade of PD-1 and CTLA-4 with a bispecific antibody results in T cell activation that is comparable to a combination of bivalent antibodies targeting PD-1 and CTLA-4. Specific targeting of human lymphocytes positive for both PD-1 and CTLA-4 with a bispecific antibody may promote similar efficacy compared to a combination of bivalent antibodies while reducing adverse events. These data suggest that clinical development of a PD-1×CTLA-4 bivalent antibody is warranted for the treatment of human malignancies.

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References

P124
A LAG-3/PD-L1 bispecific antibody inhibits tumor growth in two syngeneic colon carcinoma models
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Background
Lymphocyte activation gene-3 (LAG-3) is a member of the immunoglobulin superfamily expressed on activated T cells, NK cells, pDCs, B cells, and γδ T cells, and participates in immune suppression, particularly through persistent strong expression in a percentage of regulatory T cells (Tregs). Programmed cell death receptor (PD-1) binds to its ligand PD-L1, expressed not only on activated immune cells to inhibit cellular immune responses but also on tumor cells. Expression of LAG-3 and PD-1 leads to T cell exhaustion, allowing tumor escape from immune surveillance. Combining inhibitory antibodies to PD-1 and LAG-3 reactivates T cells leading to efficacy in murine models over single treatment [1] and prompted interest in combining these two in the development of a LAG-3 and PD-L1 bispecific antibody for inhibiting tumor growth. This format may additionally result in novel mechanisms of action that are impossible to attain with combinations.

Methods
A murine-specific anti-LAG-3 and PD-L1 bispecific antibody was engineered and analyzed for binding, blocking, and preventing LAG-3 and PD-L1-mediated T cell suppression in vitro. In addition, the LAG-3/PD-L1 bispecific antibody was tested in 2 syngeneic murine tumor models for its ability to suppress tumor growth.

Results
Not only is the bispecific anti-LAG-3/PD-L1 antibody (mAb2™) able to bind both antigens simultaneously and with nanomolar affinities, but it also demonstrates functional activity against both targets in vitro. This potency translates into in vivo efficacy, where the anti-LAG-3/PD-L1 mAb2 decreased tumor burden in the MC38 colon carcinoma tumor model, both in early-established or well-established tumors. At the end of the study tumor-free animals were more numerous in the LAG-3/PD-L1 bispecific group than in the group given a combination of individual anti-LAG-3 and PD-L1 antibodies. The results were recapitulated in the CT26 murine colon cancer model, where the LAG-3/PD-L1 mAb2 showed an increase of anti-tumor activity as compared to the combination of anti-LAG-3 and anti-PD-L1 antibodies.

Conclusions
The promising preclinical results from this anti-mouse LAG-3/PD-L1 bispecific antibody supports continuing to progress with the development of an anti-human LAG-3/PD-L1 mAb2 for use in the treatment of cancer patients.

Acknowledgements
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P125
A fusion monoclonal antibody (FmAb2) to target tumors and the immune system with a unique mechanism of action: preclinical proof-of-concept
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Background
We describe a novel therapeutic molecule that simultaneously inhibits two important targets which control pathways that are hallmark of tumorigenesis. Epidermal growth factor receptor (EGFR) is important for the growth and survival of tumor cells, and transforming growth factor beta (TGFβ) is a pleiotropic cytokine expressed in the tumor microenvironment that regulates important processes such as epithelial-to-mesenchymal cell transition (EMT), migration, invasion, and tumor-specific immunosuppression. To date, therapies have targeted these pathways individually: monoclonal antibodies (mAbs) and small molecule inhibitors of EGFR are in clinical use, and modulators of TGFβ pathway are in late-stage clinical development.

Methods
FmAb2 is a recombinant fusion mAb consisting of the TGFβ receptor II extracellular domain (T-ECD) fused to cetuximab, an anti-EGFR mAb. We hypothesized that using FmAb2 to block the EGFR and TGFβ pathways simultaneously would have a synergistic effect on inhibition of tumor growth and EMT, and also induce a tumor-specific immune response. Furthermore, this agent would neutralize TGFβ preferentially in the tumor microenvironment; thereby improving efficacy while reducing systemic toxicity.

Results
FmAb2 was expressed in Chinese hamster ovary (CHO) cells, purified using standard methodology and tested using in vitro and in vivo models.
FmAb2 is secreted as an intact fusion protein and simultaneously binds both targets in vitro binding assays. In surface plasmn resonance kinetic binding assays (Biacore), FmAb2 binds EGFR with a $K_D$ of $\approx 2.5$ nM and TGF$eta_1$ with a $K_D$ of $\approx 60$ nM. FmAb2 binds EGFR on the cell surface, neutralizes tumor cell-secreted TGF$eta_1$, inhibits proliferation of sensitive tumor cell lines and activates NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC). Using fluorescently labeled FmAb2, we demonstrate that systemically administered FmAb2 in mice preferentially localizes to tumors and is 10-fold more effective in neutralizing tumor TGF$eta_1$ compared to T-ECF fused to IgG-Fc (T-ECF-Fc). Consistent with this, we also observe significantly better inhibition of tissue inhibitor of metalloproteinase-1 (TIMP-1) in FmAb2-treated tumors compared to T-ED at equivalent doses. In a head-and-neck mouse xenograft model, FmAb2 administration is significantly superior to cetuximab or T-ECF-Fc alone at equivalent doses. Furthermore, FmAb2 treatment is more efficacious than the co-administration of cetuximab + T-ECF-Fc at equivalent doses, most likely due to better neutralization of tumor TGF$eta_1$.

Conclusions
FmAb2 is currently in preclinical development and is expected to enter clinical testing soon.

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**P126**
MCLA-117, a CLEC12AxCD3 bispecific IgG targeting a leukemic stem cell antigen, induces T cell mediated AML blast lysis
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**Background**
Patients with acute myeloid leukemia (AML) have a dismal prognosis despite improvements in chemotherapy and supportive care. Novel, more effective therapies are needed for these patients. We report the characterization of MCLA-117, a novel T cell redirecting antibody for the treatment of AML that targets CLEC12A on leukemic cells and CD3 on T cells. CLEC12A is a myeloid differentiation antigen that is expressed on 90-95% of newly diagnosed and relapsed AML. Moreover, CLEC12A is selectively expressed on leukemia stem cells (LSCs) but not on normal early hematopoietic progenitors, including hematopoietic stem cells (HSCs).

**Methods**
MCLA-117 is a human common light chain CLEC12AxCD3 full length IgG1 bispecific antibody. Heterodimerization of the bispecific IgG is facilitated by amino acid residues introduced at the CH2 interface. MCLA-117 lacks Fc effector function following amino acid substitutions in the CH2 region.

**Results**
MCLA-117 specifically binds to CLEC12A expressing myeloid cells and CD3 expressing T cells, as evaluated using healthy donor samples. In line with the CLEC12A expression MCLA-117 did not bind the HSCs, nor the common myeloid progenitor cells. The potency of MCLA-117 to induce T cell mediated lysis of CLEC12A+ LSC cells was evaluated in cytotoxicity assays. In co-cultures with resting healthy donor T cells and AML tumor cells, MCLA-117 efficiently induced CLEC12A antigen dependent T cell activation (EC$_{50}$ of 44 ng/mL), T cell proliferation and tumor target cell lysis (EC$_{50}$ of 66±37 ng/mL). The efficacy of MCLA-117 to induce lysis of AML blasts by autologous T cells in primary AML samples with low T cell to AML blast ratios was examined in an in vivo culture system. We demonstrated robust eradication of AML blasts by MCLA-117, even at low E:T ratios (Fig. 58). In 9/11 AML patient samples taken at diagnosis, MCLA-117 induced expansion of autologous T cells (7.22±0.68 fold) and killing (31-99%) of AML blasts at low E:T ratios (E:T 1:3–1:97).

**Conclusions**
MCLA-117 efficiently induced CLEC12A-mediated lysis of AML blasts by T cells present in AML samples, even at very low E:T ratios, and it provoked robust T cell proliferation. Based on its binding profile within the hematopoietic compartment, MCLA-117 is expected to specifically target myeloid blasts and LSCs while sparing the normal HSCs. A phase I clinical study (MCLA-117-CLO1) is ongoing to evaluate the preliminary safety and efficacy of MCLA-117 in adult AML patients.

**Fig. 58 (Abstract P126).** MCLA-117 efficiently induced T cell expansion and redirected lysis of AML blast in primary AML samples even at a low E:T ratio. Primary AML blast sample taken at diagnosis was incubated with MCLA-117 and a control BiPlicons (binding CD3 and an irrelevant antigen). CLEC12A expression, T cell proliferation and primary AML blast lysis was quantified using flow cytometry.

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**Clinical Trials in Progress**

**P127**
KEYNOTE-361: randomized phase III study of pembrolizumab with or without chemotherapy versus chemotherapy alone in advanced urothelial carcinoma
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**Background**
Cisplatin-based combination chemotherapy is standard first-line treatment for patients with advanced bladder cancer. The median survival with these regimens is 13-15 months, and only 5-15% of patients attain long-term survival. Programmed death ligand 1 (PD-L1) is expressed in select urothelial cancer lesions. In the phase Ib KEYNOTE-012 study, monotherapy with the anti-programmed death 1 antibody pembrolizumab demonstrated antitumor activity (objective response rate, 28%; 95% CI, 13%-47%) and acceptable safety in patients with chemotherapy-refractory metastatic PD-L1-positive urothelial carcinoma, and higher tumor PD-L1 expression was associated with higher response rates. KEYNOTE-361 is a randomized, open-label, phase III study of pembrolizumab with or without chemotherapy versus chemotherapy alone in patients with advanced urothelial carcinoma.

**Methods**
Key eligibility criteria include age $\geq 18$ years; histologically or cytologically confirmed diagnosis of advanced/unresectable or metastatic urothelial carcinoma of the bladder, renal pelvis, ureter, or urethra; measurable disease based on RECIST v1.1 as determined by the local site investigator/radiology assessment; no prior systemic chemotherapy for advanced/metastatic urothelial carcinoma (with the exception of neoadjuvant platinum-based chemotherapy with recurrence $>12$ months after completion of therapy; or adjuvant platinum-based
chemotherapy following radical cystectomy with recurrence >12 months after completion of therapy; ECOG performance status 0-2; and provision of a fresh tissue sample for biomarker analysis. Approximately 990 patients will be randomized 1:1:1 to receive: pembrolizumab 200 mg on day 1 of each 3-week cycle (Q3W) alone or in combination with investigator’s choice of chemotherapy (gemcitabine [1000 mg/m² on days 1 and 8 of each 3-week cycle] plus cisplatin [70 mg/m² Q3W] or gemcitabine plus carboplatin [AUC 5 Q3W for cisplatin- ineligible patients), or investigator’s choice of chemotherapy alone. Patients will be stratified based on chemotherapy regimen (cisplatin or carboplatin, chosen before randomization) and tumor PD-L1 expression (positive or negative). Treatments will continue until progressive disease, unacceptable adverse events (AEs), investigator decision, or 35 doses of pembrolizumab (pembrolizumab arms only). Response will be assessed per RECIST v1.1 by blinded independent central review (BICR) every 9 weeks until week 54, then every 12 weeks thereafter. AEs will be evaluated throughout treatment and for 30 days thereafter (90 days for serious AEs) and graded per National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. The primary end points are PFS per RECIST v1.1 as assessed by BICR and OS; key secondary end points include objective response rate and safety and tolerability. Enrollment in KEYNOTE-361 is ongoing.

Trial Registration
ClinicalTrials.gov identifier NCT02853305.

P128 Phase II study of pembrolizumab in patients with metastatic castration-resistant prostate cancer previously treated with targeted endocrine therapy and taxane chemotherapy: KEYNOTE-199
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Background
Treatment for patients with metastatic castration-resistant prostate cancer (mCRPC) has focused on suppression of testosterone and androgen receptor signaling, palliative radiation therapy, and chemotherapy. As expression of the programmed death-1 (PD-1) receptor and its ligand PD-L1 is present in mCRPC lesions, particularly after initial treatment with androgen deprivation and/or chemotherapy, targeting this pathway may be an attractive treatment option. Pembrolizumab, an anti-PD-1 antibody that blocks interaction between PD-1 and its ligands, PD-L1 and PD-L2, produced durable responses in patients with heavily pretreated PD-L1-positive prostate cancer in the KEYNOTE-028 study. KEYNOTE-199 is a randomized, multinational, open-label phase II study to evaluate pembrolizumab monotherapy in patients with mCRPC previously treated with chemotherapy.

Methods
Eligible patients must be ≥18 years old with histologically or cytologically confirmed adenocarcinoma of the prostate without small-cell histology, measurable disease per RECIST v1.1 or detectable bone metastases by whole-body bone scintigraphy and no RECIST v1.1 measurable tumors, supplied tumor sample for PD-L1 expression (new or archived), progression of disease within 6 months before screening, and ECOG performance status 0-2. Patients must have been treated with ≥1 target endocrine therapy (abiraterone or enzalutamide) and ≤2 chemotherapy regimens, 1 of which must have contained docetaxel. Patients also must have ongoing androgen deprivation with serum testosterone <50 ng/dL. Patients will be enrolled into 1 of 3 cohorts based on PD-L1 status and RECIST v1.1 measurability: patients with PD-L1-positive, RECIST v1.1 measurable disease (cohort 1, n=100), patients with PD-L1-negative, RECIST v1.1 measurable disease (cohort 2, n=100), and patients with bone metastases and RECIST v1.1 nonmeasurable disease (cohort 3, n=50). Patients will receive pembrolizumab 200 mg every 3 weeks until documented confirmed disease progression, unacceptable adverse events (AEs), or illness that prevents further treatment. Imaging response will be assessed every 9 weeks for approximately 1 year and every 12 weeks thereafter, per central imaging vendor review using RECIST v1.1 criteria and the Prostate Cancer Clinical Trials Working Group 3 guidelines. AEs will be monitored throughout the study and graded per Common Terminology Criteria for Adverse Events, version 4.0. Primary end points are the objective response rate and duration of response for cohorts 1 and 2 combined and by each cohort. Key secondary end points include safety and tolerability, disease control rate, radiographic progression-free survival, and overall survival for each cohort and all 3 combined. Exploratory translational analyses and expression of other immune checkpoints will also be evaluated.

Trial Registration
ClinicalTrials.gov identifier NCT02787005.

P129 KEYNOTE-155: phase Ib study of pembrolizumab in combination with dinaciclib in patients with hematologic malignancies
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Background
Pembrolizumab, a PD-1 immune checkpoint inhibitor, and dinaciclib, a cyclin-dependent kinase inhibitor, have demonstrated anti-tumor activity as monotherapies in various tumor types, including hematologic malignancies. In a preclinical study using a solid-tumor syngeneic model (MC38), enhanced antitumor activity was observed with the combination of pembrolizumab and dinaciclib, with no significant toxicities. KEYNOTE-155 is a phase Ib study designed to evaluate the safety and efficacy of the combination of pembrolizumab and dinaciclib in patients with relapsed or refractory chronic lymphocytic leukemia (CLL), multiple myeloma (MM), or diffuse large B cell lymphoma (DLBCL).

Methods
Key eligibility criteria include ≥18 years, ECOG performance status 0-1, and confirmed diagnosis of one of the following: CLL as defined by 2008 iwCLL criteria with ≥1 prior therapy; active MM with measurable disease and ≥2 prior therapies including an IMID and proteasome inhibitor; or DLBCL with progression following ≥2 prior therapies including autologous stem cell transplantation (ASCT; or ineligible for ASCT). An initial cohort of 12 patients (≥3 with each disease type) will be enrolled in the dose-evaluation phase (two 21-day cycles) to determine dose-limiting toxicities (DLTs). In cycle 1, patients will receive pembrolizumab 200 mg on day 1, dinaciclib 7 mg/m² on day 1, and dinaciclib 10 mg/m² on day 2. Dinaciclib 10 mg/m² is continued on days 3-21. In cycle 2 and beyond, dinaciclib 10 mg/m² is continued on days 3-21. If ≤4 patients experience DLTs in the first 2 cycles, expansion cohorts (~30 patients each) will be opened for signal detection. If ≥5 DLTs occur in the first 2 cycles, a lower dose of dinaciclib will be studied in up to 24 patients. Treatment will continue until disease progression, unacceptable toxicity, or 35 cycles. Response will be assessed every 3 cycles for CLL and DLBCL, and every 6 cycles for MM. Primary end points are safety and tolerability in both phases and objective response rate within each disease type per investigator assessment in the signal-detection phase. Secondary end points include duration of response, progression-free survival, and overall survival within each disease type in the signal-detection phase. Exploratory
P130 Avelumab in combination with axitinib versus sunitinib as first-line treatment for patients with advanced renal cell carcinoma: the phase III JAVELIN Renal 101 trial
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Background
Checkpoint inhibitors and tyrosine kinase inhibitors may have complementary mechanisms of action in advanced renal cell carcinoma (aRCC), providing a rationale for investigating combination treatment. Inhibition of the programmed death-1 receptor ligand (PD-L1) pathway leads to reactivation of an effective antitumor immune response against multiple cancers, including RCC. Avelumab (MSB0010718C) is a fully human IgG1 anti-PD-L1 antibody that has shown clinical activity in patients with several tumor types. Axitinib is an anti-VEGF receptor tyrosine kinase inhibitor approved for treatment of aRCC after failure of 1 prior systemic therapy. In an ongoing phase Ib study, avelumab plus axitinib administered at standard monotherapy doses showed encouraging safety and antitumor activity in treatment-naïve patients with aRCC. JAVELIN Renal 101 is a global phase III trial comparing avelumab plus axitinib versus sunitinib as first-line treatment for patients with aRCC.

Methods
The primary objective of this randomized, multicenter trial is to demonstrate superiority of first-line avelumab plus axitinib versus sunitinib monotherapy in prolonging progression-free survival (PFS). Eligibility criteria include: aRCC with a clear cell component, availability of archival or fresh tumor biopsy, ECOG PS ≤1, no prior systemic therapy for advanced disease or prior immunotherapy, and measurable disease per RECIST v1.1. Approximately 583 patients will be randomized 1:1 to receive either: Arm A, avelumab intravenously over 1 hour every 2 weeks plus axitinib orally once daily continuously (cycle length 6 weeks); or Arm B, sunitinib orally once daily for 4 weeks followed by 2 weeks off. Stratification factors are: ECOG PS (0 vs 1) and region (US vs Canada/Europe vs rest of world). Treatment is discontinued for unacceptable toxicity or if any criteria for withdrawal are met. Patients may continue treatment beyond progression if investigator-assessed clinical benefit is achieved and treatment is well tolerated. The primary endpoint is PFS per RECIST v1.1 by blinded independent central review. Secondary endpoints include overall survival, PFS by investigator assessment, objective response, duration of response, time to response, safety, and patient-reported outcomes. Pharmacokinetics, immunogenicity, and tumor tissue biomarkers will also be assessed. Enrollment in this pivotal trial began in March 2016.

Trial Registration
ClinicalTrials.gov identifier NCT02684617.
Background
B7-H3 is part of the B7 superfamily of immune checkpoint molecules. While its regulation and mechanism of action are not completely understood, B7-H3 expression correlates with adverse pathology and clinical outcomes in men with prostate cancer. B7-H3 expression appears to be associated with biochemical and clinical progression following treatment. The correlation between B7-H3 overexpression and poor prognosis suggests a role in tumor immune escape. In addition, B7-H3 levels remain high in the presence of androgen deprivation. These results suggest a potential role for B7-H3 in prostate cancer progression and support its use as a therapeutic target. Enoblituzumab is an anti-B7-H3 humanized monoclonal antibody with a proposed mechanism of action of antibody directed cellular cytotoxicity. We propose a neoadjuvant study to determine the antitumor, immunological and biological effects of enoblituzumab in men with localized prostate cancer. We hypothesize that administration of enoblituzumab will be safe and feasible in the neoadjuvant setting for men with localized intermediate and high-risk prostate cancer. We also hypothesize that enoblituzumab will produce measurable tumor cell death and antitumor immune responses in harvested prostate glands.

Methods
This is a single-center, single-arm, open-label pilot study of enoblituzumab 15mg/kg IV weekly for 6 doses beginning 30 days prior to radical prostatectomy. The target population (N=16) is men with localized, intermediate or high-risk prostate adenocarcinoma (Gleason sum ≥7), with adequate organ function, no adverse indications for standard radical prostatectomy, and no previous local treatment to the prostate, anti-androgen exposure, or immunotherapy. The primary endpoint will be frequency/severity of adverse events from time of first administration of enoblituzumab until the 90th post-op day. Prostate glands will be harvested at time of radical prostatectomy and prostate tissue will be examined for the secondary endpoints and compared to pre-treatment biopsies. The secondary biological endpoint is tumor cell death, which will be quantified by caspase 3 staining and post-treatment apoptosis. The correlation between B7-H3 overexpression and poor prognosis suggests a potential role for B7-H3 in prostate cancer. We also hypothesize that enoblituzumab will produce measurable tumor cell death and antitumor immune responses in harvested prostate glands.

Conclusions
RT and NK immunotherapy appear synergistic in dog models of sarcoma, and preliminary results from a canine clinical trial of palliative RT and autologous NK transfer for osteosarcoma are promising, including possible abscopal effects. Further evaluation of this novel radio-immunotherapy approach is warranted.

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P133
Radiotherapy enhances natural killer cell homing and function in canine bone and soft tissue sarcoma
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Background
We have previously shown that radiotherapy (RT) increases natural killer (NK) cytotoxicity and homing in diverse pre-clinical models of human solid malignancies, including sarcomas. Since sarcomas commonly afflict dogs, we hypothesized that dog PBMC-derived NK cells would be effective in canine models of sarcoma, including adoptive transfer in a canine RT/NK clinical trial.

Methods
Canine NK cells were isolated from 15 mls whole blood using Ficoll separation and CDS depletion. Isolated NK cells were expanded in co-culture with irradiated KS62x9LL21 for 2-3 weeks. Using 6-month metastasis-free survival as the primary endpoint, a canine clinical trial is underway evaluating RT and adoptive intratumoral NK immunotherapy. For this trial, treatment consists of palliative RT weekly for one month followed by two intra-lesional injections of autologous canine NK cells. In correlative studies, including dog patient-derived xenografts (PDX), we assessed NK homing using eFluor 670 cell proliferation dye and NK function by expression of activation markers including IFNγ, granzyme B, and perforin.

Results
We have treated 7 of planned 14 dogs with osteosarcoma on protocol. Of 3 evaluable dogs who have reached the 6-month primary endpoint, we have observed 1 partial response and 2 are metastasis-free, including 1 dog with complete resolution of a suspicious 3 mm pulmonary nodule. In dog patients on trial, phenotyping of expanded NK cells from all patients showed > 90% granzyme B and IFNγ expression prior to adoptive transfer. Tagging experiments 1 week after intratumoral injection revealed that 11 ~ 60% of CD45+ cells are eFluor 670 positive, confirming persistence of injected NK cells post injection. Analysis of unactivated circulating PBMCs post-injection demonstrated a significant increase in granzyme B expression (2.25X ± 0.42, P < 0.01). Dog PDX studies demonstrate that focal RT increases NK homing to sarcomas on average 3.8±X ± 0.3 (P < 0.001) compared to unirradiated controls. Immunohistochemical analysis of irradiated dog sarcomas (historical controls) shows a significant increase in CD3+ tumor-infiltrating lymphocytes post RT (P < 0.04, see figure). Co-culture experiments of dog PDX sarcomas ex vivo with allogeneic NK cells showed RT-induced sensitization to NK killing at doses of 10 - 20 Gy (P < 0.01).

Conclusions

Fig. 59 (Abstract P133). Canine Sarcomas Show Increased CD3+ Lymphocytes after Radiotherapy. a High power view of normal dog lymph node in the absence of mouse anti-dog CD3 antibody. b High power view of normal dog lymph node shows diffuse expression of CD3+ lymphocytes. c High power view shows absence of CD3+ lymphocytes following amputation of distal tibia dog osteosarcoma. d High power view positive increased CD3+ cells in dog osteosarcoma following palliative RT (scale bars 100 μm)
Interim results of a phase II trial of adoptive transfer of tumor infiltrating lymphocytes in patients with metastatic uveal melanoma

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Background
Uveal melanoma (UM) is a rare tumor variant with no established treatments once metastases develop. Although a variety of immune based therapies have demonstrated anti-tumor activity in metastatic cutaneous melanoma, their use in UM has been disappointing. Recently, adoptive T cell therapy has shown salvage responses in multiple refractory solid tumors. Based upon our identification from UM liver metastases of tumor infiltrating lymphocytes (TIL) with strong autologous reactivity, we sought to determine if clinical adoptive transfer of such TIL could mediate regression in patients with metastatic UM. Here we present an interim analysis of the ongoing clinical trial, NCT01814046.

Methods
In the main arm of this phase II study, patients with metastatic melanoma of ocular origin received nonmyeloablative lymphodepleting conditioning chemotherapy consisting of intravenous cyclophosphamide (60 mg/kg/daily) for 2 days and fludarabine (25 mg/m²/daily) for 5 days followed by an intravenous infusion of autologous TIL and high-dose interleukin-2 (720,000 IU/kg). Clinical responses were evaluated using Response to Evaluation Criteria in Solid Tumors (RECIST), version 1.0. The study was designed to initially enroll 19 patients and if 4 or more patients achieved an objective clinical response, accrual would expand in a second phase to a total of 33 patients.

Results
At the current time, 17 patients with metastatic UM have completed TIL treatment; 16 of these patients are evaluable for response assessment. Five of 16 (31%) UM patients demonstrated objective tumor regression. Among the responders, 4 patients demonstrated partial tumor regression lasting between 4 and 10 months. One patient achieved complete tumor regression, ongoing 15 months after TIL infusion. In the year prior to TIL therapy, this patient demonstrated marked progression of multiple liver metastases after anti-CTLA-4 and anti-PD-1 checkpoint therapy. However, after a single infusion of TIL, we observed rapid and complete tumor regression. Circulating serum tumor DNA specific for the patient’s GNAQ driver mutation demonstrated complete clearance by 10 days following cell therapy consistent with the patient’s clinical response. Overall in the trial, the frequency of tumor-reactive T cells within the administered infusion and the levels of IFN-y released after autologous tumor stimulation were positively associated with clinical response (P=0.04, respectively).

Conclusions
To our knowledge, these clinical findings represent the first demonstration that autologous T cell transfer is capable of inducing complete and durable tumor regression of metastatic UM. Refinement of this cell therapy is aimed at identifying the antigenic targets associated with clinical response.

A phase Ib study of intratumoral CAVATAK (coxsackievirus A21) and ipilimumab in patients with advanced melanoma

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Background
CAVATAK™ is a bio-selected oncolytic and immunotherapeutic strain of coxsackievirus A21 (CAV21). In a phase II study, intratumoral (i.t.) CAVA21 injection of advanced melanoma lesions resulted in regression in 28.1% of patients including non-injected lesions, increases in tumor immune-cell infiltration, up-regulation of IFNγ response and immune-checkpoint genes, including CD122 which may be a marker for enhanced anti-tumor activity by anti-CTLA-4 blockade. Preclinical models using B16 ICAM confirmed enhanced antitumor effects when CVA21 was combined with anti-CTLA-4 or anti-PD-1. The preliminary data are presented from the open-label, phase Ib MITIC (Melanoma Intra-Tumoral Cavatak and Ipilimumab) in patients (pts) with advanced melanoma.

Methods
Pts received up to 3 x 10^7 TCID50 CVA21 i.t. on study days 1, 3, 5, 8 and 22, and then q3w for up to 14 additional i.t. injections. Ipilimumab (3 mg/kg) was administered q3w for 4 doses starting at Day 22. The first response assessment (irRC) occurred on Day 106. The primary endpoint was to assess safety of CVA21 in combination with ipilimumab treatment (tx). Immune monitoring samples were obtained to analyze T cells with regulatory, memory, and effector phenotypes. Biopsies of injected lesions have been obtained in some patients.

Results
No DLT’s have been reported in the 18 patients enrolled. Combination tx has been well-tolerated with only one grade 3 or higher treatment-related AE being ipilimumab-related fatigue. Colitis or other immune related toxicities have been grade 1 or 2. The study met its primary statistical futility endpoint of achieving ≥ 4 confirmed objective responses (CR or PR) in the first 12 pts enrolled. Of the first 11 pts eligible for investigator response assessment, ORR for the ITT population is 57.1% (4/7), with the ORR for ipilimumab-naïve pts being 67% (4/6). The DCR (CR+PR+SD) on the ITT population is currently 86% (6/7). All responses were observed by 3.5 months with complete tumor responses being observed in individual injected and non-injected lesions. One three-fold increase in peripheral blood CD4+ and CD8+ T cells with central and effector memory phenotypes were observed comparing baseline to day 85 in most of the patients analyzed thus far.

Conclusions
CAVA21 + ipilimumab tx of pts with advanced melanoma has been well tolerated. This combination immunotherapy induced anti-tumor activity in local, visceral and non-visceral lesions in a number of patients that have failed previous immunotherapies. Increases in T cell effector and memory subsets were also observed.

Trial Registration
ClinicalTrial.gov identifier NCT01636882.
KEYNOTE-177: randomized phase III study of pembrolizumab versus investigator-choice chemotherapy for mismatch repair-deficient or microsatellite instability-high metastatic colorectal carcinoma

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Background
A subset of colorectal carcinomas (CRCs) have deficiencies in the mismatch repair (MMR) system, resulting in microsatellite instability (MSI). MSI-high tumors contain high levels of lymphocyte infiltrates and strong expression of the programmed death 1 (PD-1) immune checkpoint receptor and its ligand, PD-L1. Pembrolizumab is an anti–PD-1 monoclonal antibody that blocks the interaction between PD-1 and its ligands, thereby enabling an antitumor immune response. In the phase II KEYNOTE-016 study, pembrolizumab showed promising antitumor activity against MMR-deficient tumors in patients with treatment-refractory metastatic CRC, with an objective response rate of 47% (95% CI, 23%-72%). KEYNOTE-177 (ClinicalTrials.gov identifier NCT02563002) is an international, randomized, open-label phase III study designed to evaluate the efficacy and safety of pembrolizumab compared with standard-of-care chemotherapy as a first-line treatment for MMR-deficient or MSI-high metastatic CRC.

Methods
Key eligibility criteria include age ≥18 years, confirmed MSI-high or MMR-deficient stage IV CRC, measurable disease per RECIST v1.1 by local site assessment, Eastern Cooperative Oncology Group performance status 0-1, no active autoimmune disease or brain metastases, and no prior therapy for metastatic disease. Patients are to be randomized 1:1 to receive either pembrolizumab 200 mg every 3 weeks (Q3W) or investigator choice of standard-of-care chemotherapy. CHEMOTHERAPY must be chosen prior to randomization; options include mFOLFOX6 or FOLFIRI alone or in combination with bevacizumab or cetuximab. Treatment is to continue until progressive disease, unacceptable toxicity, patient/Investigator decision, or completion of 35 cycles (pembrolizumab only). Response is to be evaluated by CT or MRI every 9 weeks per RECIST v1.1 by central imaging vendor review and per RECIST adapted for immunotherapy response patterns. Eligible patients may continue pembrolizumab beyond initial RECIST-defined progression. Patients in the standard-of-care arm who have progressive disease and meet crossover criteria may be eligible to receive pembrolizumab for up to 17 treatment cycles. Adverse events (AEs) are to be assessed throughout treatment and for 30 days thereafter (90 days for serious AEs) and graded per NCI CTCAE v4.0. Patients are to be followed for survival every 9 weeks. The primary end point is progression-free survival per RECIST v1.1; secondary end points are overall survival, objective response rate per RECIST v1.1, and safety and tolerability. Exploratory end points include duration of response and health-related quality of life. Enrollment in KEYNOTE-177 is ongoing, with 270 patients planned to be enrolled.

Trial Registration
ClinicalTrials.gov identifier NCT02563002.

Phase Ia/ib trial investigating the CSF-1R inhibitor LY3022855 in combination with durvalumab (MEDI4736) or tremelimumab in patients with advanced solid malignancies

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Background
Checkpoint inhibitors of programmed cell death-1 protein (PD-1)/programmed cell death-ligand 1 (PD-L1) and cytotoxic T lymphocyte associated protein 4 (CTLA-4) pathways have demonstrated clinically meaningful improvement in survival for patients with various tumor types. Preclinical data demonstrate significant interplay between innate and adaptive immune systems. Targeting colony-stimulating factor 1 receptor (CSF-1R) may lead to disruption of the immunosuppressive effects of innate immune cells expressing CSF-1R. Treatment with an anti-CSF-1R monoclonal antibody (mAb) induces anti-tumor responses in murine tumor models when combined with CTLA-4 blockade [1], suggesting that combining a checkpoint inhibitor with a CSF-1 pathway inhibitor may potentiate the anti-tumor response. Study I5F-MC-JSCC will evaluate the effects of CSF-1R inhibition using LY3022855 (anti-CSF-1R mAb) in combination with durvalumab (MEDI4736; anti-PD-L1 mAb) or tremelimumab (anti-CTLA-4 mAb) in participants with advanced solid malignancies.

Methods
JSCC is a phase Ia/ib open-label, 3+3 dose-escalation (Part A), followed by dose-expansion (Part B) study of LY3022855 in combination with either durvalumab or tremelimumab. Eligible patients have confirmed solid malignancies (regardless of PD-L1 status) and ECOG PS 0-1. Patients must not have received small molecule therapy, chemotherapy, radiation therapy, monoclonal antibody treatment, or immunosuppressive medication within 14-28 days of start of study treatment, but prior immune checkpoint therapy is permitted. Pretreatment and on-treatment biopsies will be obtained (Part A all patients, Part B ovarian cohort). In the LY3022855+durvalumab regimen, LY3022855 (IV) will be administered at increasing dose levels as tolerated; durvalumab (IV) will be administered at a fixed dose. In the LY3022855+tremelimumab regimen, both LY3022855 and tremelimumab (IV) will be administered at increasing dose levels as tolerated. Once a maximum tolerated dose has been identified for each combination, enrollment to Part B (5 disease-specific expansion cohorts of 20 patients per cohort: LY3022855+durvalumab- non-small cell lung cancer, ovarian, melanoma; LY3022855+tremelimumab- mesothelioma, melanoma) will begin. The primary objective is to characterize the safety and tolerability of each combination in treatment of patients with advanced solid malignancies, as well as define a recommended phase II dose. Secondary objectives include assessment of antitumor activity of each combination, immunogenicity, and pharmacokinetics. Exploratory objectives are to assess immunomodulatory effects of the combinations. Approximately 178 patients are planned.

Trial Registration
ClinicalTrials.gov identifier NCT02718911.
Phase III study of carboplatin-paclitaxel/nab-paclitaxel chemotherapy with or without pembrolizumab for first-line metastatic, squamous non–small cell lung carcinoma:

KEYNOTE-407
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Background
Chemotherapy with a platinum doublet has traditionally been the standard of care for most patients with treatment-naive non–small cell lung carcinoma (NSCLC). Preliminary data from the phase I/II KEYNOTE-021 (ClinicalTrials.gov, NCT02039674) study suggest manageable toxicity and encouraging efficacy of carboplatin and paclitaxel chemotherapy plus the anti–PD-1 antibody pembrolizumab in treatment-naive NSCLC. KEYNOTE-407 (Clinical-Trials.gov, NCT02775435) is a randomized, double-blind, placebo-controlled phase III study to compare the efficacy and safety of paclitaxel or nab-paclitaxel plus carboplatin with or without pembrolizumab as first-line treatment in advanced squamous NSCLC.

Methods
Eligible patients are aged ≥18 years and have stage IV squamous NSCLC, an ECOG PS 0–1, and no prior systemic chemotherapy. Patients with mixed histology (e.g., adenosquamous) are allowed if there is a squamous component in the specimen; small cell histology is excluded. Approximately 560 patients will be randomly assigned (1:1) to pembrolizumab 200 mg every 3 weeks (Q3W) plus carboplatin area under the curve (AUC) 6 Q3W and paclitaxel 200 mg/m² Q3W or nab-paclitaxel 100 mg/m² day 1, day 8, and day 15 Q3W for 4 cycles followed by pembrolizumab 200 mg Q3W or to the same regimen in which pembrolizumab is replaced by a normal saline placebo. Patients are to be stratified by choice of taxane (paclitaxel vs nab-paclitaxel), PD-L1 status (tumor proportion score [TPS] ≥1% vs < 1%), and geographic region of the enrolling site (East Asia vs non–East Asia) before randomization. Pembrolizumab/placebo will continue for 35 cycles or until disease progression, intolerable toxicity, or investigator or patient decision to withdrawal. Patients who received placebo may be able to cross over to pembrolizumab at the time of documented progression. Adverse events (AEs) will be monitored throughout the study and for 30 days (90 days for serious AEs) after treatment completion and graded per NCI CTCAE v4.0. Response will be assessed by RECIST v1.1 by independent central radiologic review at weeks 6, 12, and 18, then every 9 weeks until week 45, and every 12 weeks thereafter. The primary end points are progression-free survival (PFS) by independent radiologic review and overall survival; secondary end points are objective response rate, duration of response, and safety. Exploratory objectives include PFS by PD-L1 TPS status (≥1% vs < 1%) and choice of taxane (paclitaxel or nab-paclitaxel), patient-reported outcomes (EORTC QLQ-C30 and LC13 and EuroQoL-5D), pharmacokinetics, and biomarkers.

Trial Registration
ClinicalTrials.gov identifier NCT02775435.

P139
Preliminary manufacturing, safety, and immune monitoring results of an allogeneic tumor lysate-pulsed dendritic cell vaccine for patients with newly diagnosed glioblastoma

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Background
Clinical responses to dendritic cell (DC) vaccines for brain tumors have been demonstrably inconsistent. Several factors, including tumor and treatment related immunosuppression, poor DC maturation, and suboptimal antigen sources, likely contribute to this phenomenon. We report preliminary data from a clinical trial for patients with newly diagnosed glioblastoma (GBM) that combines current standard of care treatment with an autologous DC vaccine using mature DCs pulsed with allogeneic cultured GBM lysates.

Methods
Twenty adult patients with resected newly diagnosed GBM who had completed radiation/concurrent temozolomide were enrolled. DCs were manufactured in vitro from CD14+ monocytes purified from patient leukopheresis products and cultured to produce highly pure CD83+ mature DCs. The mature autologous DCs were pulsed with allogeneic tumor lysates with defined tumor-associated antigen expression. Patients received temozolomide plus intradural injections of the vaccine (10–20 x10⁶ DCs) for up to 6 cycles followed by vaccine alone for up to 6 cycles. In some cases, patients received injections using a novel intradermal delivery device (3M human microstructured transdermal system).

Results
Allogeneic cultured GBM lysate pulsed DCs were manufactured successfully to produce 15 doses of at least 10x10⁶ DCs/injection in 100% (19/19) of patients to date. Vaccines were manufactured consistently as DCs collectively averaged 91.4% CD83+ and 98.1% CD80– positive for these maturation markers. Patients on trial have received a median of 8 injections and three patients have received all 15 doses. Of the toxicities potentially related to the vaccine, only grade 1 and 2 toxicities (fever, rash, fatigue) have been observed. Mean follow-up to date is 0.98 years (range 0.19 – 1.77 years); 15/20 patients are still alive. In the first 8 patients, median survival has not been reached with median follow-up 1.56 years. To assess immune responses to the vaccine, patients were monitored for over 120 immunophenotypes and circulating tumor antigen specific T cells by flow cytometry with up to 8 longitudinal samples for some patients.

Conclusions
The combination of adjuvant temozolomide with an autologous mature DC pulsed with allogeneic tumor lysate vaccine was shown to be safe, feasible, and able to produce tumor antigen-specific immune responses in newly diagnosed GBM patients. This successful strategy is well suited for continuing into later stage clinical trials.

Acknowledgements
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Trial Registration
ClinicalTrials.gov identifier NCT01957956.
Background

Patients with HRLACC experience a 50% chance of disease recurrence/death following cisplatin-based chemoradiation (CCRT) plus brachytherapy, and represent a group with a significant unmet need for new treatments. Persistent infection with oncogenic strains of human papillomavirus (HPV) is the most common cause of CC, and provides rationale for therapeutic targeting of HPV. Axalimogene filolisbac (AXAL/ADXS11-001) is an irreversibly attenuated Listeria monocytogenes—listeriolysin O immunotherapy that secretes a HPV E7 fusion protein that induces HPV-specific cytotoxic T cell generation and reduces immune tolerance in the tumor microenvironment. Previous studies demonstrated AXAL was well tolerated and associated with objective tumor response and survival benefits in patients with recurrent/metastatic CC. AXAL has received FDA Fast Track Designation for the treatment of HRLACC.

Methods

This double-blind, placebo-controlled, multinational, multicenter randomized phase III trial is being conducted under a Special Protocol Assessment agreement with the FDA. The study will evaluate adjuvant AXAL in patients with HRLACC, defined as histologically confirmed squamous cell, adenocarcinoma, or adenosquamous carcinoma of the cervix and ≥1 of the following: 1) FIGO stage IB2, IIA2, IIB with biopsy-proven pelvic nodes, or ≥2 positive nodes by MRI/CT ≥1.5-cm diameter, or ≥2 positive pelvic nodes by PET; 2) all FIGO stage IIIA, IIIB, IVA; 3) any FIGO stage with para-aortic lymph node metastases criteria, defined by biopsy-proven para-aortic node(s), or ≥1 positive para-aortic node(s) by MRI/CT ≥1.5-cm shortest dimension, or ≥1 positive para-aortic node(s) by PET with SUV >2.5. Eligible patients must be disease free per RECIST 1.1 following completion of CRTT with curative intent and aged ≥18 with GOG performance status 0–1. Patients will be randomized 2:1 to AXAL (1×10^9 colony-forming units) or placebo and receive a 60-minute infusion of treatment every 3 weeks for 3 doses (weeks 1, 4, and 7) for the first 3 months (Induction Phase). Thereafter, patients will receive treatment every 8 weeks for 5 doses or until disease recurrence (Maintenance Phase); patients will receive a 7-day course of oral antibiotics/placebo 72 hours after completion of each treatment in both phases. Primary objective is to compare disease-free survival (DFS) of AXAL with placebo; secondary objectives are safety and overall survival (OS). Exploratory objectives will determine if there is an association between HPV subtypes and DFS/OS, and patient-reported outcomes. The design provides 85% power for a sample size of 450 to demonstrate a reduction in the hazard of recurrence by 38%.

Trial Registration ClinicalTrials.gov identifier NCT02853604.
ClinicalTrials.gov identifier NCT02625961.

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KEYNOTE-013: an open-label, multicohort phase Ib trial of pembrolizumab in patients with advanced hematologic malignancies

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Background

Pembrolizumab, a humanized monoclonal antibody that blocks interaction between PD-1 and its ligands, has demonstrated robust antitumor activity and a manageable toxicity profile in advanced solid tumors. KEYNOTE-013 is a multicenter, open-label, multicohort phase Ib trial designed to assess the safety and efficacy of single-agent pembrolizumab in patients with hematologic malignancies. The KEYNOTE-013 trial design has been updated to include a cohort evaluating combination therapy of pembrolizumab and lenalidomide in patients with relapsed/refractory disease of either B cell lymphoma (DLBCL) who have failed, are ineligible for, or refused stem cell transplantation (SCT). Combination therapy of pembrolizumab, lenalidomide, and dexamethasone in R/R multiple myeloma (MM) in KEYNOTE-023 has demonstrated synergistic efficacy and manageable toxicity (1).

Methods

Cohorts include patients with: (1) intermediate 1, intermediate 2, or high-risk myelodysplastic syndrome (MDS) that failed ≥2 lines of prior therapy, including a hypomethylating agent and IMiD; (2) R/R MM that failed ≥2 lines of prior therapy, including a proteasome inhibitor and IMiD; (3) R/R nodular sclerosis or mixed cellularity Hodgkin lymphoma (HL) (cohort 3); (4) R/R non-Hodgkin lymphoma (NHL) who failed, were ineligible for, or refused SCT, including: (4a) R/R primary mediastinal large B cell lymphoma (PMBCL), (4b) any other R/R PD-L1-positive NHL, (4c) R/R follicular lymphoma (FL), (4d) R/R DLBCL; and (5) R/R DLBCL. Key eligibility criteria for all cohorts are age ≥18 years, ECOG performance status 0/1, measurable disease, and adequate hematologic, renal, and hepatic function. Patients in cohorts 1-4 enrolled under amendments 1-3 will be treated with pembrolizumab intravenously (IV) 10 mg/kg every 2 weeks; those enrolled under amendments 4-6 will be treated with pembrolizumab IV 200 mg every 3 weeks (Q3W) because of updated program-wide PD/PD information. Patients enrolled in cohort 5 will be treated with pembrolizumab IV 200 mg Q3W in combination with lenalidomide taken orally once daily for 21 days of each 28-day cycle. Treatment will continue until disease progression, intolerable toxicity, or up to 35 doses of pembrolizumab (~2 years). The primary end points are objective response rate and safety. Secondary objectives include duration of response, progression-free survival, overall survival, and association between PD-L1 expression and response. Enrollment is open for MM (cohort 2), PMBCL (cohort 4a), and FL (cohort 4c), evaluating single-agent pembrolizumab; and for DLBCL (cohort 5), evaluating pembrolizumab in combination with lenalidomide. Approximately 222 patients will be enrolled.

Trial Registration
ClinicalTrials.gov identifier NCT01953692.

References

P143

Trials in progress: a phase II study of in situ therapeutic vaccination against refractory solid cancers with intratumoral poly-I:LC

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Background

Poly-I:LC, a double-stranded RNA complex, can directly activate dendritic cells and trigger NK cells to kill tumor cells. It can be given intramuscularly (IM) to induce systemic inflammation and intratumorally (IT) to induce immune infiltration of tumors.

Methods

In this phase II study, eligible subjects are head and neck, skin (melanoma and non-melanoma), and sarcoma patients with recurrent or metastatic disease who have failed prior systemic therapy. In each treatment cycle, one accessible tumor site was targeted for IT injection of 1 mg of Poly-I:LC 3 times a week for 2 weeks followed by IM boosters biweekly for 6 weeks and then a 2 week rest period. This 10-week cycle was repeated in cycle 2, followed by a 6 week no-treatment period. Tumor biopsies were performed at baseline, week 3, and week 26. Pre- and post-vaccination tumors were evaluated by quantitative multiplex immunohistochemistry (IHC) and RNA sequencing. Blood samples were collected at baseline and prior to each cycle for immune response evaluations.

Results

A phase I pilot portion of this study showed 1 patient who achieved clinical benefit with stable disease (progression-free survival of 6 months). Poly-I:LC was well tolerated with principal side effects of fatigue and inflammation at injection site (< grade 2). One case of grade 3 tumor necrosis was observed. In the patient with clinical benefit, IHC analysis of tumor showed increased CD4(60x), CD8(10x), CD5(20x), and PD-L1(3x) compared to patients with progressive disease whose tumor biopsies showed unchanged/decreased CD4, CD8, PD-1, and PD-L1 levels over treatment period. Furthermore, RNAseq analysis of the same patient's tumor and peripheral blood mononuclear cells (PBMC) showed dramatic changes such as upregulation of interferon-stimulated genes, chemokines, and genes associated with T cell activation and antigen presentation indicative of local and systemic immune activation in response to Poly-I:LC treatment.

Conclusions

Preliminary findings show that Poly-I:LC is well tolerated in advanced solid cancer patients, and generates local immune response in tumor microenvironment and systemic immune response as evident in the patient achieving clinical benefit. These results warrant further investigation, and are currently being explored in this ongoing larger multicenter adaptive phase II clinical trial.

Trial Registration
ClinicalTrials.gov identifier NCT01984892.

References
P144
Phase I/ib multicenter trial of CPI-444, an adenosine A2a receptor (A2aR) antagonist as a single agent and in combination with atezolizumab (atezo) in patients with advanced solid tumors
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Background
Adenosine is elevated within the tumor microenvironment and signaling through the A2aR is immunosuppressive. CPI-444 is an oral, selective A2aR antagonist that inhibits A2aR and demonstrates anti-tumor efficacy in mouse models alone and combined with PD-1/PD-L1 blockade. CPI-444 was well-tolerated in previous clinical trials in the non-oncology setting. This is the first report of adenosine blockade in cancer patients (pts).

Methods
This phase I/ib, open label clinical trial uses a two-step adaptive design to study CPI-444 alone and combined with atezo in pts with selected advanced cancers. The objectives are to evaluate safety and efficacy and to optimize dose/schedule. Eligible pts have failed standard therapies with histologies: non-small cell lung (NSCLC), melanoma (MEL), triple-negative breast (TNBC), renal (RCC), prostate, head and neck (H&N), colorectal (CRC) or bladder cancers. In step 1 of the trial, pts are randomized to one of 4 cohorts (12 pts/ cohort) including 3 single-agent cohorts or combined with atezo. Step 2 contains multiple diseasespecific expansion cohorts to evaluate CPI-444 alone and combined with atezo.

Results
Step 1 has enrolled 19 pts to date; median age 67 years (range, 36–84); three pts each with NSCLC, TNBC, RCC and CRC, 2 pts each with MEL, prostate and bladder cancer and one pt with H&N. Median number of prior regimens was 3 (range, 1–6). Seven pts received prior anti PD-1/ PD-L1 therapy; 4 pts were resistant and 3 were refractory. Twelve pts remain on study (range 1–17 weeks) and 7 have discontinued due to disease progression. Of 8 pts evaluable for response at 2 months, 3 had stable disease (2 received CPI-444 alone); 11 pts have not reached the first response assessment timepoint. A prostate cancer pt in the combination cohort remains on treatment for >4 months with minimal regression of nodal disease and a transient decrease in serum PSA. A TNBC pt on CPI-444 alone showed 15% reduction in tumor burden. No grade 3 or 4 adverse events or DLTs related to CPI-444 alone or in combination with atezo were observed. Dose-dependent inhibition of A2aR signaling was demonstrated in peripheral blood mononuclear cells using a CREB phosphorylation assay. Evidence of immune activation was observed in some pts treated with CPI-444 alone as reflected by an increase in circulating activated PD-1+/CD8+ T cells.

Conclusions
Early data demonstrate that CPI-444 is well-tolerated alone and combined with atezo and demonstrates preliminary evidence of immune activation and clinical activity. Enrollment is ongoing.

Trial Registration
ClinicalTrials.gov identifier NCT02655822.

P145
SEA-CD40 is a CD40 agonist with early evidence of pharmacodynamic and antitumor activity: preliminary results from a phase I study in advanced solid malignancies
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Background
SEA-CD40 is a non-fucosylated, humanized IgG1 monoclonal antibody which binds CD40, an immune-activating TNF receptor. Binding to CD40 on antigen-presenting cells (APCs) and enhanced crosslinking via FcγRIa stimulates pro-inflammatory cytokine production and induction of immune co-stimulatory receptors, leading to T cell activation and antitumor activity. Further, when CD40 is expressed on malignant cells, SEA-CD40 induces antibody-dependent cellular cytotoxicity through enhanced NK cell binding.

Methods
This ongoing phase I dose-escalation study evaluates the safety, tolerability, pharmacodynamic biomarkers, and antitumor activity of SEA-CD40 in adult patients with advanced metastatic solid tumors (relapsed, refractory, or progressive disease after ≥1 prior systemic therapy). Antitumor activity is assessed after every 4 cycles of treatment by immune-related response criteria and RECIST v1.1.

Results
To date, 22 patients (median age 59 years; range, 28–76) with solid tumors have received a median of 2.5 cycles (range, 1–16) of SEA-CD40, 0.6–60 mcg/kg IV q3wk. Two dose-limiting toxicities occurred at 60 mcg/kg (1 G3 and 1 G4 infusion-related reaction [IRI]). AEs were primarily infusion-related toxicities. Treatment-emergent AEs in ≥25% of patients were: chills (77%), nausea (55%), fatigue (45%), vomiting (41%), dyspnea and headache (32% each), and flushing and lymphopenia (27% each). Changes in pharmacodynamic biomarkers consistent with CD40 and CD16 engagement were observed, including: dose-proportional increases in inflammatory cytokine levels; B cell depletion with partial recovery by next cycle; reduction in T regulatory cells; transient margination of monocytes and NK cells with recovery by Day 8; and upregulation of MHC class II on APCs. In 18 efficacy-evaluable patients, 4 had SD and 1 had PR by RECIST (28% disease control rate [DCR]). Two patients had durable SD (gastroesophageal junction tumor, 10 mcg/kg, PD after 12 cycles; mesothelioma, 10 mcg/kg, PD after 8 cycles). The patient with PR achieved PR after Cycle 4 (basal cell carcinoma, 60 mcg/kg, PD following Cycle 8). Four patients remain on treatment.

Conclusions
SEA-CD40 is a biologically and clinically potent molecule in heavily pre-treated patients with advanced solid tumors. Strategies to manage IRIs are being evaluated. Cytokine elevations correlate with preclinical models and the proposed mechanism of action through CD40 activation on APCs. Pharmacodynamic data and evidence of clinical activity (28% DCR), along with preclinical evidence for synergy when SEA-CD40 is combined with checkpoint inhibitors [1], presents a compelling opportunity to enhance immunotherapy for cancer.
P146

A randomized phase II study of epigenetic therapy with azacitidine and entinostat with concurrent nivolumab versus nivolumab alone in recurrent metastatic non-small cell lung cancer

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Background

While survival results with immune checkpoint blockade in the second-line treatment setting of non-small cell lung cancer (NSCLC) are impressive, many patients’ cancer progresses or becomes resistant to these therapies. Combinatorial approaches are being investigated to improve response and outcomes with these agents. Epigenetic therapy has been found to be effective component of cancer treatment. Hypermethylation of DNA promoter regions by DNA methyltransferases (DNMT) and histone deacetylation by histone deacetylases (HDAC) represent two of these critical epigenetic mechanisms of tumor-specific gene silencing. Interestingly, 5 of 5 patients treated with combination epigenetic therapy followed by PD-1/PD-L1 therapy achieved long-term clinical benefit at our institution while on clinical trial. Preclinical studies have also shown that combined HDAC/DNMT inhibition can induce susceptibility to immune checkpoint therapy and inhibit tumor growth [1].

Methods

This is a multi-institution, open-label, randomized phase II study of azacitidine and entinostat with concurrent nivolumab compared to nivolumab alone in patients with recurrent, metastatic NSCLC. Patients with metastatic NSCLC who have received 1-2 prior therapies and are Eastern Cooperative Group (ECOG) performance status (PS) 0-1 are stratified by histology and randomized to receive azacitidine (40 mg/m2 subcutaneous (SQ) days 1-5, 8-10), entinostat (4 mg orally days 3 and 10) and nivolumab (3 mg/kg intravenous (IV) days 1 and 15) for 6 28-day cycles, followed by nivolumab (3 mg/kg IV days 1 and 15) alone (Arm D) or nivolumab (3 mg/kg IV days 1 and 15) on a 28-day cycle alone (Arm C) until disease progression (Fig. 60). Statistics: The primary endpoint of this trial is the percentage of patients progression-free at 24 weeks. Using planned study enrollment of 60 patients on each Arm (n=120) and using the exact Mantel-Haenszel test for analysis, this will provide 90% power to detect an odds ratio of 3 for combination therapy over single-agent nivolumab with respect to being progression-free at 24 weeks. Secondary endpoints include objective response rate, progression-free survival, time to progression, overall survival and safety and tolerability. Correlative Study: All patients are required to have pre- and post- treatment biopsies; Arm D patients will also have biopsies while on combination therapy. Plasma and peripheral blood mononuclear cells will also be drawn for analysis.

References


P147

KEYNOTE-427: phase II study of pembrolizumab in patients with locally advanced/metastatic renal cell carcinoma (mRCC)

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Background

Targeting the programmed death 1 (PD-1) pathway has been found to be effective in patients with previously treated, advanced or clear cell mRCC. Pembrolizumab is an anti–PD-1 antibody that blocks the interaction between PD-1 and its ligands, enabling an increased anti-tumor immune response. Most patients with mRCC progress within 1 year following the initiation of standard first-line treatment with anti-angiogenic therapy, highlighting the need for therapies that provide more durable benefit in mRCC.

Methods

KEYNOTE-427 (ClinicalTrials.gov, NCT02853344) is a phase II, open-label study designed to evaluate the efficacy and safety of pembrolizumab monotherapy as a first-line treatment for advanced, recurrent or metastatic clear cell or non–clear cell mRCC. Target enrollment in KEYNOTE-427 is 105 patients in a clear cell cohort (cohort A) and 150 patients in a non–clear cell cohort (cohort B). Key eligibility criteria include locally advanced, metastatic or recurrent disease, measurable disease per RECIST v1.1 assessed by blinded independent central review (BICR), no prior systemic therapy for advanced RCC, provision of a tumor tissue sample for biomarker analysis, and Kamofsky performance status ≥70%. Patients are to receive pembrolizumab intravenously 200 mg once every 3 weeks until progressive disease, the occurrence of unacceptable adverse events (AEs), or for up to 35 doses in patients without progressive disease. Patients who stop pembrolizumab after 35 doses without progressive disease or who stop treatment after a complete response will be allowed treatment with an additional 17 doses of pembrolizumab upon progression. Response will be assessed per RECIST v1.1 by BICR using CT and/or MRI. Bone scans will be performed at baseline and throughout the study for patients with a positive bone scan at baseline. AEs will be graded per National Cancer Institute Common Terminology Criteria for
Adverse Events, version 4.0. The primary objective is estimation of the objective response rate in each cohort per RECIST v1.1. An interim analysis for response rate will be performed for the non-clear cell RCC cohort when the 30th patient in this cohort has completed the third scan. Secondary objectives include estimation of duration of response, disease control rate, progression-free/overall survival, and safety and tolerability of pembrolizumab treatment by cohort.

**Trial Registration**
ClinicalTrials.gov identifier NCT02853344.

**P148**

KEYNOTE-170: phase II study of pembrolizumab in patients with relapsed/refractory primary mediastinal large B cell lymphoma (rPMBCL) or relapsed or refractory Richter syndrome (rrRS)

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**Background**
The 9p24.1 locus is frequently amplified in rPMBCL, leading to overexpression of the PD-L1 and PD-L2 immune checkpoint ligands and providing a potential mechanism of immune evasion. Pembrolizumab is an anti–PD-1 monoclonal antibody that blocks the interaction between PD-1 and PD-L1 and PD-L2, thereby enabling an antitumor immune response. In the multicohort, phase IIb KEYNOTE-013 study, pembrolizumab was associated with a tolerable safety profile and promising antitumor activity (overall response rate [ORR] of 38% [6/16]) in patients with rPMBCL.

In the phase II MC1485 study, pembrolizumab demonstrated promising preliminary efficacy (ORR, 43% [3/7]) in patients with rrRS. The multicenter, phase II KEYNOTE-170 study was designed to further evaluate the safety and efficacy of pembrolizumab in patients with rPMBCL or rrRS.

**Methods**
Eligible patients must be at least 18 years of age and fit into 1 or 2 profiles: (1) diagnosis of rPMBCL according to World Health Organization 2008 criteria, failed to achieve a complete response (CR) or relapsed after autologous stem cell transplantation (auto-SCT), or are ineligible for auto-SCT and have failed to respond or relapsed after ≥2 lines of prior treatment; or (2) pathologic diagnosis per local institutional review of rrRS that transformed from underlying chronic lymphocytic leukemia (CLL), received at least 30 cycles of treatment, and that by blocking PD-1/PD-L1 disinhibitory signals, pembrolizumab may improve the function and durability of TILs.

**Conclusions**
We anticipate that treatment with pembrolizumab and vemurafenib will be safe and well tolerated. In addition to the primary efficacy endpoint of ORR and secondary endpoints of PFS and OS, this study includes extensive immune correlative analyses, including analysis of PD-1/PD-L1 and levels of Treg, MDSC, and inhibitory cytokines in the tumor parenchyma and peripheral blood. One patient on trial thus far is tolerating treatment well, with no significant toxicities to date.

**Trial Registration**
ClinicalTrials.gov identifier NCT02576990.

**P149**

Dose-seeking and efficacy study of anti-PD-1 therapy with pembrolizumab (P) combined with BRAF inhibitor (BRAFi) therapy with vemurafenib (V) for therapy of advanced melanoma

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**Background**
BRAFi inhibitors induce antitumor responses in >90% of patients (pts) with BRAF V600E/K mutant metastatic melanoma, but some patients do not respond and most responders have only partial responses, the median duration of which is 7-9 months. BRAFi markedly increase tumor infiltrating lymphocytes (TIL), although markers of T cell exhaustion and PD-L1 are increased. We hypothesize that BRAFi will drive TIL into the tumor parenchyma, and that by blocking PD-1/PD-L1 disinhibitory signals, pembrolizumab may improve the function and durability of TILs.

**Methods**
This study is an investigator-initiated phase I/II dose-seeking and efficacy trial of P and V for pts with unresectable/metastatic BRAF mutant melanoma. Primary objectives are to determine safety and maximum tolerated dose (MTD) of V combined with P in this population, and to assess ORR with the combination, in comparison to historical benchmarks. Secondary objectives are to evaluate PFS and OS. Exploratory objectives are to assess whether the combination favorably modulates the tumor microenvironment and decreases T cell exhaustion in sequential biopsies of tumor and blood samples. We aim to accrue up to 50 patients to this study. Using the modified toxicity probability interval (mTPI) will allow efficient identification of the MTD; we expect at least 30 patients to be enrolled at the recommended phase II dose. Pts will receive P 200 mg q3 wks, and V 480 mg, 720 mg, or 960 mg BiD, per mTPI. Treatment with both V & P will start on day 1. The DLT monitoring period is 3 wks. Pts will have CT scans at baseline and wk 9, then q12 wks. For pts with biopsiable disease, biopsies at baseline and wk 3 are mandatory. Blood for correlative studies will be drawn at baseline, wk 3, wk 9 and progression. Pts will be treated until DLT or PD for up to 2 years.

**Results**
One patient has been started on therapy and is in wk 2 of the 3 wk DLT monitoring period, with no significant toxicities thus far.

**Conclusions**
We anticipate that treatment with pembrolizumab and vemurafenib will be safe and well tolerated. In addition to the primary efficacy endpoint of ORR and secondary endpoints of PFS and OS, this study includes extensive immune correlative analyses, including analysis of PD-1/PD-L1 and levels of Treg, MDSC, and inhibitory cytokines in the tumor parenchyma and peripheral blood. One patient on trial thus far is tolerating treatment well, with no significant toxicities to date.

**Trial Registration**
ClinicalTrials.gov identifier NCT02818023.

**P150**

CX-1158-101: a first-in-human phase I study of a small molecule inhibitor of arginase (CB-1158) as monotherapy and in combination with an anti-PD-1 checkpoint inhibitor in patients with solid tumors

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**Background**

T cells and natural killer (NK) cells require L-arginine for proliferation and activation. Arginine depletion by arginase in the tumor microenvironment induces immunosuppression and is associated with tumor immune evasion, advanced disease stage and poor outcomes. Arginase is secreted by granulocytic myeloid-derived suppressor cells (G-MDSCs) and its pharmacological inhibition is expected to restore arginine levels and relieve immunosuppression. CB-1158 is a potent, selective, and orally bioavailable small molecule inhibitor of arginase (IC_{50}=98 nM). CB-1158 reverses G-MDSC-mediated immunosuppression by blocking arginine depletion in an ex vivo human model. T cells activated in the presence of G-MDSC-conditioned media demonstrate reduced proliferation and suppressed production of Th1 cytokines; this effect is reversed by the addition of CB-1158. In vivo, twice daily dosing of CB-1158 causes dose-dependent increases in plasma and tumor arginine levels and is associated with single agent anti-tumor efficacy in multiple syngeneic models. CB-1158 also enhances the antitumor efficacy of checkpoint inhibitors.

**Methods**

CX-1158-101 is a phase I first-in-human study of CB-1158 in patients with solid tumors. The primary objective is to evaluate the safety and tolerability of CB-1158, as monotherapy and in combination with nivolumab. Secondary objectives include selection of the recommended phase II dose (RP2D), determination of CB-1158 pharmacokinetics and evaluation of the anti-tumor effect, for monotherapy and nivolumab combination. Exploratory objectives include an evaluation of pharmacodynamic biomarkers, including plasma arginine, plasma arginase activity, and effects on immune function in the peripheral blood and tumor biopsies. In Part 1a, safety/tolerability of escalating doses of CB-1158 will be evaluated in patients with solid tumors of any type. In Part 2, three expansion cohorts will open at the monotherapy and RP2D. These cohorts include non-small cell lung cancer (NSCLC, Cohort A), colorectal cancer (CRC, Cohort B), and Cohort C, which will include patients with squamous cell carcinoma of the head and neck (SCCHN), renal cell cancer (RCC), gastric or gastro-esophageal junction (GEJ) cancer, urothelial cell cancer (UCC), and/or melanoma. Upon demonstration of sufficient PK and/or PD effect of CB-1158 in Part 1a, dose escalation of CB-1158 in combination with full dose nivolumab will open in Part 1b. The dose escalation will enroll patients with NSCLC, RCC, and melanoma. In Part 3, two expansion cohorts will enroll patients to the combination of CB-1158 and nivolumab at the RP2D. These cohorts will enroll NSCLC and melanoma patients that have received prior anti-PD-1/PD-L1 therapy and had progressive disease or prolonged (>24 weeks) stable disease.

**P151**

A first-in-human, first-in-class phase I trial of the anti-CD47 antibody HuSF9-F4 in patients with advanced cancers

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**Background**

HuSF9-F4 is a humanized monoclonal antibody that targets CD47, blocking its anti-phagocytic “don’t eat me” signal through macrophage receptor SIRPa, leading to tumor phagocytosis. CD47 is overexpressed on human cancers and also on red blood cells (RBCs). In preclinical toxicity studies, HuSF9-F4 caused a transient anemia that was improved with a single lower priming dose allowing higher maintenance doses.

**Methods**

Relapsed/refractory solid tumors and lymphomas were included. This dose escalation study included: Part A, to determine the priming dose and Part B, to determine the maintenance dose. The maximum tolerated dose (MTD) in part A was used for the single priming dose in part B (HuSF9-F4 dosed weekly). The primary objective is to determine safety and secondary objectives are to determine PK and PD. Preliminary data reported from data cutoff of July 22, 2016.

**Results**

21 patients have enrolled. Part A included 0.1 (N=1), 0.3 (N=2), 1 (N=6), and 3 mg/kg (N=2). There were 2 dose-limiting toxicities (DLTs) in Part A at the 3 mg/kg dose: grade (G) 3 abdominal pain and G3 hemagglutination (H) (protocol-specific scale of 1+H on peripheral blood smear (PBS) and G2 headache). 1 mg/kg was selected as the priming dose, with no >G2 anemia and almost 100% RBC receptor occupancy. Treatment-related adverse events (TRAE) in Part A included anemia (2 G1, 3 G2), hyperbilirubinemia (1 G1, 1 G3; unconjugated), headache (6 G1, 1 G2), H on PBS (8 G1), and nausea (3 G1). Part B included 3 (N=4), 10 (N=3), and 20 mg/kg (N=3, ongoing). There have been no DLTs in 3 patients on 10 mg/kg (last fully evaluable cohort). Most toxicity was associated with the single priming dose and reversible. TRAE in Part B at 3 mg/kg included: anemia (2 G1, 2 G2), hyperbilirubinemia (1 G1, 1 G3; unconjugated), headache (3 G1), H on PBS (1 G1), retinal toxicity (G2 protocol-specific scale, asymptomatic). TRAE at 10 mg/kg included: anemia (3 G1), headache (2 G1), and nausea (1 G1). Target trough levels associated with preclinical activity are being achieved at the 10 mg/kg dose, and half-life increases with repeated dosing. Two patients with adenocystic carcinoma in Part A had stable disease for 16 and 8 months.

**Conclusions**

HuSF9-F4 is well tolerated at 10 mg/kg weekly, with 1 mg/kg priming dose. Part B maintenance dose 20 mg/kg enrolling.

**Acknowledgements**

Stanford Clinical and Translational Research Unit; California Institute for Regenerative Medicine; Forty Seven, Inc.

**Trial Registration**

ClinicalTrials.gov identifier NCT02216409.

**P152**

A pilot/phase II study evaluating pembrolizumab plus standard-of-care chemotherapy in metastatic triple-negative breast cancer, with companion comprehensive immunological monitoring

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**Background**

While sustained cytotoxic chemotherapy may be associated with lymphopenia and immunosuppression, chemotherapy may also facilitate antigen release/presentation, adaptive expression of PD-L1, and relative depletion of suppressive immune cell populations. Metastatic triple-negative breast cancer (MTNBC) is an aggressive and incurable...
disease associated with high mutational load and tumor-infiltrating lymphocytes, and is treated conventionally with sequential, sustained cytotoxic chemotherapy. In heavily pre-treated patients, anti-PD-1/L1 monotherapy yielded modest response rates of 9-19% [1]. However, we hypothesize that up-front treatment with 1\textsuperscript{7/2} \textsuperscript{nd} line chemotherapy (either weekly paclitaxel or oral capcitabine) would be more effective. In preliminary cohort, anti-PD-L1 plus chemotherapy (nab-paclitaxel) was safe, with favorable response rates compared to historical controls [2].

Methods
In a pilot/phase II investigator-initiated trial, we will evaluate the safety and tolerability of anti-PD-1 (pembrolizumab 200mg IV every three weeks) plus investigator-selected 1\textsuperscript{st}/2\textsuperscript{nd} line standard-of-care chemotherapy. In a preliminary cohort, anti-PD-L1 plus chemotherapy (nab-paclitaxel) was safe, with favorable response rates compared to historical controls [2].

Results
As of 8/7/2016, five subjects have been registered for enrollment.

Conclusions
This investigator-initiated trial will provide important data, evaluating the efficacy of commonly used chemotherapies (paclitaxel or capcitabine) and anti-PD-1, as well as evaluating the effect of these regimens on general immune status and T cell activation, and tumor infiltrating lymphocytes. A registration trial of nab-paclitaxel +/- anti-PDL1 is ongoing.

Trial Registration
ClinicalTrials.gov identifier NCT02734290.

References
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Preliminary safety data from a randomized multicenter phase Ib/II study of neoadjuvant chemoradiation therapy (CRT) alone or in combination with pembrolizumab in patients with resectable or borderline resectable pancreatic cancer

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Background

Tumor-infiltrating lymphocytes (TILs) do not reach the pancreatic cancer (PC) cells in significant numbers due to the presence of stroma and a suppressive microenvironment [1]. Neoadjuvant chemoradiation (CRT) can increase the presence of TILs in the PC microenvironment (PCME) [2]. We tested the combination of CRT and pembrolizumab for the first time in patients with pancreatic cancer in the neoadjuvant setting.

Methods

Patients with resectable or borderline resectable pancreatic cancer are randomized 2:1 to receive pembrolizumab interwovenly every 3 weeks during concurrent CRT with capecitabine (825 mg/m2 orally twice daily, 5 days/week, on days of radiation only) and radiation (50.4 Gy in 28 fractions over 28 days) or Arm B to receive only concurrent CRT with capecitabine.

Results

As of July 5, 2016 a total of 13 patients have been enrolled on the study (9 on Arm A and 4 on Arm B). Six patients had resectable disease (4 on arm A and 2 on arm B) while 7 patients had borderline resectable disease (5 on Arm A and 2 on arm B). Post-neoadjuvant therapy, 2 patients were determined to have unresectable disease (both on arm B) and 4 patients underwent surgery (2 on each arm). Seven patients remain on neoadjuvant treatment. All grade toxicities related to treatment are summarized in Fig. 62. There were five grade 3 toxicities reported in Arm A: 2 patients had diarrhea attributed to CRT and resolved after holding the treatment; 3 patients had lymphopenia attributed to pembrolizumab or the combination; and one patient had elevated alkaline phosphatase related to the combination that met the definition of DLT and resolved after holding the treatment and receiving steroids. There was only one grade 3 toxicity reported on Arm B: lymphopenia attributed to CRT. There were no surgical complications reported within 30 days post-surgery in all patients who underwent surgery.

Conclusions

The study safety stopping boundaries have not been met and the study will continue enrolling patients.

Trial Registration

ClinicalTrials.gov identifier NCT02305186.

References

Background
Standard first-line treatments for mRCC include antiangiogenic agents sunitinib, pazopanib, and bevacizumab plus interferon, which yield a median progression-free survival (PFS) of approximately 10-11 months and median survival of 23-29 months. 5-year survival in this setting is approximately 20%, with a median progression-free survival (PFS) of approximately 10-11 months and median survival of 23-29 months. Pembrolizumab, a programmed death 1 immune checkpoint inhibitor, has been tested in combination with anti-angiogenic therapy in treatment-naive advanced/metastatic renal cell carcinoma with clear cell histology, standard first-line treatments for mRCC include antiangiogenic agents sunitinib, pazopanib, and bevacizumab plus interferon, which yield a median progression-free survival (PFS) of approximately 10-11 months and median survival of 23-29 months. 5-year survival in this setting is approximately 20%, with a median progression-free survival (PFS) of approximately 10-11 months and median survival of 23-29 months. Pembrolizumab, a programmed death 1 immune checkpoint inhibitor, has been tested in combination with anti-angiogenic therapy in treatment-naive advanced/metastatic renal cell carcinoma. This combination has demonstrated manageable toxicity and promising clinical efficacy. The combination of pembrolizumab plus axitinib is being further tested in a randomized, placebo-controlled trial comparing pembrolizumab plus axitinib to sunitinib monotherapy. Key eligibility criteria include age ≥18 years, treatment-naive advanced/metastatic renal cell carcinoma with clear cell histology, measurable disease per RECIST v1.1, adequate organ function, and Karnofsky performance status ≥70%. Patients will be stratified per International Metastatic RCC Database Consortium risk category and geographic region, and then randomly assigned 1:1 to receive pembrolizumab 200 mg once every 3 weeks plus axitinib 5 mg twice daily or sunitinib 50 mg once daily for 4 weeks, then 2 weeks off. Treatment will continue until progressive disease, unacceptable adverse events, death, or withdrawal of consent. The primary end points are PFS per RECIST v1.1, as assessed by blinded independent central review, and overall survival. Secondary end points include objective response rate, duration of response, disease control rate, safety, and patient-reported outcomes. Approximately 840 patients will be enrolled globally.

Trial Registration
ClinicalTrials.gov identifier NCT02853331.
Conclusions
These results support development of JTX-2011 in HNSCC patients expressing high levels of ICOS and suggest that combination of JTX-2011 with an anti-PD-1 agent may be an effective treatment paradigm.

P158
KEYNOTE-183: randomized, open-label, phase III study of pembrolizumab in combination with pomalidomide and low-dose dexamethasone in patients with refractory or relapsed/refractory multiple myeloma
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Background
Response rates remain low for refractory or relapsed/refractory multiple myeloma (rMM) treated with currently approved therapies, including proteasome inhibitors and immunomodulatory drugs (IMiDs). Lenalidomide reduces PD-L1 and PD-1 expression on MM cells and enhances checkpoint blockade–induced cytotoxicity; PD-1 blockade with pembrolizumab may thus act synergistically with IMiDs to enhance tumor suppression. In the phase I KEYNOTE-023 study, the combination of pembrolizumab + lenalidomide and low-dose dexamethasone was associated with an acceptable safety profile and a promising overall response rate (ORR) of 50% (20/40) in patients with rMM, supporting further evaluation of pembrolizumab in combination with immunomodulatory agents. The randomized, open-label, multicenter, phase III KEYNOTE-183 study (ClinicalTrials.gov identifier NCT02576977) was designed to compare the efficacy and safety of pomalidomide and low-dose dexamethasone with or without pembrolizumab in patients with rMM.

Methods
Key eligibility criteria include age ≥18 years, Eastern Cooperative Oncology Group performance status 0-1, confirmed diagnosis of rMM with measurable disease, ≥2 lines of prior treatment, and documented progression on the last line of therapy. Prior therapies must have included an IMiD, such as lenalidomide or thalidomide, and a proteasome inhibitor, such as bortezomib, ixazomib, or carfilzomib. Patients are randomly assigned 1:1 to receive pomalidomide 40 mg daily on days 1-21 and low-dose dexamethasone 40 mg (20 mg for patients aged ≥75 years) daily on days 1, 8, 15, and 22 of repeated 28-day cycles, with or without pembrolizumab 200 mg every 3 weeks. Stratification is based on prior lines of treatment (2 vs ≥3) and disease status (refractory vs. sensitive to lenalidomide). Patients receiving pomalidomide must receive appropriate anticoagulation prophylaxis therapy. Treatment will continue until disease progression or unacceptable toxicity. Response will be assessed every 28 days by Clinical Adjudication Committee blinded central review and investigator review based on International Myeloma Working Group (IMWG) criteria and overall survival; secondary endpoints are ORR, safety and tolerability, disease control rate, duration of response, and second PFS. Enrollment is ongoing and will continue until approximately 300 patients are enrolled.

Trial Registration
ClinicalTrials.gov identifier NCT02576977.

P159
KEYNOTE-185: randomized, open-label, phase III study of pembrolizumab in combination with lenalidomide and low-dose dexamethasone in patients with newly diagnosed and treatment-naive multiple myeloma (MM)
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Background
Lenalidomide in combination with low-dose dexamethasone is one of the standard-of-care treatments for MM. Preclinical and experimental data has shown that lenalidomide reduces PD-L1 and PD-1 expression on MM cells and enhances checkpoint blockade-induced cytotoxicity; thus, PD-1 blockade with pembrolizumab may act synergistically with lenalidomide to enhance tumor suppression. In the phase I KEYNOTE-023 study, the combination of pembrolizumab + lenalidomide and low-dose dexamethasone was associated with an acceptable safety profile and a promising overall response rate (ORR) of 50% (20/40) in patients with relapsed/refractory MM, supporting further evaluation of pembrolizumab in combination with lenalidomide. The randomized, open-label, phase III KEYNOTE-185 study was designed to compare the efficacy and safety of lenalidomide and low-dose dexamethasone with or without pembrolizumab in patients with newly diagnosed and treatment-naive MM not eligible for transplantation.

Methods
Key eligibility criteria include age ≥18 years; newly diagnosed, treatment-naïve, active MM with measurable disease; ineligibility for autologous stem cell transplantation; and Eastern Cooperative Oncology Group performance status 0-1. Patients are randomized 1:1 to receive lenalidomide 25 mg daily on days 1-21 and low-dose dexamethasone 40 mg (20 mg for patients aged ≥75 years) on days 1, 8, 15, and 22 of repeated 28-day cycles, with or without pembrolizumab 200 mg every 3 weeks. Stratification is based on age (<75 vs ≥75 years) and International Staging System (ISS) stage (ISS I or II vs ISS III). Treatment will continue until disease progression or unacceptable toxicity. Response will be assessed every 28 days by Clinical Adjudication Committee blinded central review and by investigator review based on International Myeloma Working Group (IMWG) 2011 response criteria. Adverse events will be assessed throughout treatment and for 30 days thereafter (90 days for events of clinical interest) and graded per NCI CTCAE v4.0. Patients will be followed for survival every 12 weeks. The primary endpoint is progression-free survival (PFS) as assessed by central review according to IMWG criteria; secondary end points are overall survival, overall response rate, duration of response, second PFS, and safety and tolerability. Enrollment is ongoing and will continue until approximately 640 patients are enrolled.

Trial Registration
ClinicalTrials.gov identifier NCT02579863.
P160
KEYNOTE-122: phase II study of pembrolizumab versus standard-of-care chemotherapy in platinum-pretreated, recurrent or metastatic nasopharyngeal carcinoma
Anna Spreatifico, Victor Lee, Roger K C Ngan, Ka Fai To, Myung Ju Ahn, Quan Sing Ng, Ruey-Long Hong, Jin-Ching Lin, Ramona F Swaby, Christine Gause, Sanatan Saraf, Anthony T C Chan.
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Correspondence: Anna Spreatifico (anna.spreatifico@uhn.ca) Journal for ImmunoTherapy of Cancer 2016; 4(Suppl 1):P160

Background
Current treatment for recurrent/metastatic nasopharyngeal carcinoma (NPC) that progresses on a platinum-based regimen includes monotherapy with gemcitabine, capecitabine, or docetaxel. Although these treatments are used in clinical practice, they have not been formally approved for second-line NPC. Prolonged exposure to Epstein-Barr virus (EBV) in NPC leads to increased expression of programmed death-1 (PD-1) and programmed death-ligand 1 (PD-L1) in NPC cell lines patients with NPC. KEYNOTE-122 is a multicenter, open-label, randomized phase II study designed to evaluate the efficacy and safety of pembrolizumab monotherapy versus chemotherapy in patients with platinum-pretreated, recurrent or metastatic NPC.

Methods
Key eligibility criteria include age ≥18 years, histologically confirmed nonkeratinizing differentiated NPC (WHO Class II) or undifferentiated NPC (WHO Class III), metastatic or recurrent disease, ECOG performance status 0-1, and measurable disease by RECIST v1.1. Patients will be randomly assigned 1:1 to receive pembrolizumab 200 mg every 3 weeks (Q3W) or investigator choice of chemotherapy (capcitabine 1000 mg/m² twice daily on days 1-14 of each 3-week cycle, gemcitabine 1250 mg/m² once per week on days 1 and 8 of each 3-week cycle, or docetaxel 75 mg/m² Q3W). Treatment will continue until disease progression, unacceptable toxicity, investigator decision, or 35 cycles of pembrolizumab. Patients who complete 35 pembrolizumab cycles or discontinue pembrolizumab following a complete response may receive an additional 17 pembrolizumab cycles upon disease progression. Response will be evaluated every 6 weeks for the first year of treatment and every 9 weeks thereafter per RECIST v1.1 by central imaging assessment. Adverse events (AEs) will be assessed throughout treatment and for 30 days thereafter (90 days for serious AEs) and graded per National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0. Upon disease progression, patients will be followed for survival every 12 weeks. Primary end points are overall survival and progression-free survival per RECIST v1.1 by central imaging assessment; secondary end points include objective response rate and duration of response per RECIST v1.1 by central imaging assessment. Enrollment is ongoing and will continue until approximately 160 patients have enrolled.

P161
CX-839-004: a phase I/II study of the safety, pharmacokinetics, and pharmacodynamics of the glutaminase inhibitor CB-839 combined with nivolumab in patients with renal cell carcinoma, melanoma, and non-small cell lung cancer
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Background
T cells require glucose and glutamine for activation and proliferation. Tumor consumption of nutrients within the tumor microenvironment may contribute to localized immune suppression, termed a “metabolic checkpoint,” and selective inhibition of tumor metabolism may reverse this immunosuppression. CB-839 is a potent, first-in-class, oral inhibitor of glutaminase 1 that inhibits the tumor TCA cycle by blocking glutaminase-mediated conversion of glutamine to glutamate. In vitro studies of CB-839 demonstrated that blocking glutaminolysis in tumor cells increased extracellular glutamine and stimulated proliferation of T cells. In the CT26 syngeneic colon carcinoma model, combining CB-839 with a PD-1/PD-L1 inhibitor doubled the rate of complete regressions over the checkpoint inhibitor alone. Safety and preliminary evidence of activity of CB-839 as monotherapy and in combination with everolimus and paclitaxel were reported previously.

Methods
CX-839-004 is a phase I/II trial evaluating CB-839 + nivolumab in patients with clear cell RCC (ccRCC), melanoma (mel), and NSCLC. The primary objectives are to evaluate (i) safety and tolerability and (ii) anti-tumor effect of the combination. Secondary objectives include determining the MTD/RP2D of CB-839 in combination with standard dose nivolumab. Exploratory objectives include characterization of the pharmacodynamics of the combination and evaluation of biomarkers that may predict anti-tumor effect. Eligibility criteria include incurable metastatic or locally advanced ccRCC, mel or NSCLC previously treated with standard of care therapy, ECOG 0-1, and measurable disease by RECIST 1.1. In the phase I portion, a minimum of 6-9 patients will receive escalating doses of CB-839 orally BID and nivolumab 3 mg/kg IV on Days 1 and 15 every 28 days. Subsequent to determining the MTD or RP2D, patients will be enrolled into phase II cohorts as follows: Cohort 1 ccRCC checkpoint inhibitor naive; Cohort 2 ccRCC with documented progressive disease (PD) or stable disease (SD) ≥ 6 mo on nivolumab in most recent line of therapy; Cohort 3 ccRCC with no better than SD before documented PD on any checkpoint inhibitor in any prior line of therapy; Cohort 4 melanoma with documented PD on a PD-1/PD-L1 inhibitor in most recent line of therapy; Cohort 5 NSCLC with documented PD or SD > 6 mo on PD-1/PD-L1 inhibitor in most recent line of therapy. Tumor burden will be assessed approximately every 8 weeks by RECIST 1.1 and irRECIST. Adverse events will be graded per NCI CTCAE. Samples obtained for pharmacodynamic and biomarker analyses will include pre-treatment and on-treatment tumor biopsies, blood and plasma.

Trial Registration
ClinicalTrials.gov identifier NCT02771626.
P162
KEYNOTE-365: multicohort, phase Ib/II study of pembrolizumab combination therapy in patients with metastatic castration-resistant prostate cancer (mCRPC)
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Background
Approved treatments for mCRPC include second-generation endocrine therapies (abiraterone and enzalutamide) and taxanes (docetaxel and cabazitaxel). These agents may increase programmed death ligand 1 (PD-L1) expression and/or facilitate release of neoantigens. Additionally, the PARP inhibitor, olaparib, has activity in patients with mCRPC who have DNA-repair defects. Pembrolizumab is an anti–programmed death 1 (PD-1) antibody that blocks the interaction between the immune checkpoint receptor PD-1 and its ligands. KEYNOTE-365 (ClinicalTrials.gov, NCT02861573) is an international, nonrandomized, multicohort, open-label phase Ib/II study evaluating the safety and response rate of pembrolizumab in combination with olaparib (cohort A), docetaxel + prednisone (cohort B), or enzalutamide (cohort C) in patients with mCRPC.

Methods
Key eligibility criteria include: histologically or cytologically confirmed adenocarcinoma of the prostate without small-cell histology, documented prostate cancer progression within 6 months preceding screening, and ongoing androgen deprivation with serum testosterone < 50 ng/dL. Cohort A will include patients previously treated with docetaxel (prior treatment with 1 other chemotherapy is allowed, as well as ≤2 second-generation hormonal manipulations); cohort B will include patients previously treated with either abiraterone acetate or enzalutamide (but not both) before chemotherapy; and cohort C will include patients previously treated with abiraterone acetate before chemotherapy. Patients will receive pembrolizumab 200 mg once every 3 weeks (Q3W) (all cohorts) and either olaparib 400 mg twice daily (cohort A), docetaxel 75 mg/m² Q3W + prednisone 5 mg twice daily (cohort B), or enzalutamide 160 mg every day (cohort C). Treatments will continue until disease progression or unacceptable adverse events (AEs). There will be a maximum of 35 cycles of pembrolizumab (all cohorts) and 10 cycles of docetaxel + prednisone (cohort B). Patients who must discontinue a combination component may continue with the other drug until progression or unacceptable AEs. Response will be assessed by evaluating prostate-specific antigen (PSA) levels Q3W, and with radiologic imaging every 9 weeks for the first year and every 12 weeks thereafter. Primary endpoints are safety/tolerability and PSA response rate (decline of ≥50% from baseline measured twice at least 3 weeks apart). Secondary end points include time to PSA progression, radiographic progression-free survival based on investigator-assessed RECIST v1.1 per the Prostate Cancer Working Group 3 guidelines, and overall survival. Approximately 70 patients will be enrolled in each cohort.

Trial Registration
ClinicalTrials.gov identifier NCT02861573.

Clinical Trials: Cutting-Edge (Completed Trials)
P163
Final statistical analysis of a pilot trial of hu14.18-IL2 in patients with completely resectable recurrent stage III or stage IV melanoma
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Background
Phase I testing of the hu14.18-IL2 immunocytokine (IC), a mAb reactive with GD2 disialoganglioside, linked to IL2, in patients (pts) with melanoma showed immune activation, reversible toxicities, and a maximal tolerated dose of 7.5 mg/m²/day. Predclinical data in IC-treated tumor bearing mice with low tumor burden documented striking antitumor effects. The main goal of this study was to obtain pilot data with hu14.18-IL2 in subjects with advanced melanoma who achieved a complete response (CR) through surgery.

Methods
Pts with completely resectable recurrent stage III or stage IV melanoma were scheduled to receive 3 cycles of IC at 6 mg/m²/d IV over 4 hours on days 1, 2 and 3 of each 28-day cycle. Pts were randomized to surgical resection of all sites of disease either following (Group A) or prior to (Group B) IC cycle 1. Primary objectives were 1) to evaluate histological evidence of anti-tumor activity in terms of apoptosis/necrosis and immune-mediated destruction of tumor cells and 2) to evaluate recurrence-free survival (RFS) and overall survival (OS). The secondary objectives were 1) to evaluate adverse events associated with treatment and 2) to evaluate biological endpoints.

Results
Twenty melanoma pts (11 recurrent stage III, 8 stage IV, one unknown primary) were randomized to Group A (11 pts) or B (9 pts). Two Group B pts did not receive IC due to persistent disease following surgery. Six of 18 IC-treated patients remained free of recurrence, with a median recurrence-free survival of 5.7 months (95% CI: 1.8-not reached). The 24-month RFS rate was 38.9% (95% CI: 17.5-60.0%). The median follow-up of surviving patients was 50.0 months (range: 31.8-70.4). The 24-month OS rate was 65.0% (95% CI: 40.3-81.5%). Twelve pts had evaluable tumor samples for GD2 analysis and 6/12 showed expression of GD2 on melanoma cells. There was no significant difference in RFS (p=0.791) or OS (p=0.567) based on GD2 expression. There was a significant
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mune response. The development of PD-L1 expression likely reflects an induced im-

DCVax treatment may be mediated through direct cytotoxic effects, as study outcomes such as stabilization of disease and survival correlated post treatment, with a trend towards improved survival (p=0.1).

Conclusions
Prolonged tumor-free survival was seen in some melanoma pts at high risk for recurrence who were treated with IC.

Trial Registration
ClinicalTrials.gov identifier NCT00590824.

P164
Cytokine production by intratumorally administered activated dendritic cells correlates with survival in a phase I clinical trial
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Background
Activated, autologous dendritic cells (aADC) can be used to induce anti-tumor immune responses. A unique method of applying aADC is through intratumoral injection, where the tumor cells serve as the source of antigen required for an adaptive anti-tumor response. A local effect may also occur as a result of cytokine production by the injected DC which makes the tumor more susceptible to a pre-existing or an induced immune attack.

Methods
Forty patients with locally advanced or metastatic solid tissue can-
cers were treated in a dose escalation trial in which aADC were injected percutaneously under image guidance into a single tumor. Subjects had a median of 3 tumors (range 1 – 5) and had received an average of 3.1 prior treatments. To generate the aADC, autolo-
gous monocytes were converted ex vivo into DC which were then activated. All batches of DC were released based on pre-specified criteria which included immunophenotyping and a T cell-stimulation assay, as well as sterility and endotoxin levels. Cytokine levels produced by the activated DC during manufacturing were measured and patient outcomes were correlated to these expression levels.

Results
All three doses levels were well tolerated. The main adverse events related to treatment were grade 1 and 2 fevers. Twenty-one patients achieved stable disease (SD) 8 weeks after initiating treatment, and this was found to correlate with survival (p=0.01). Levels of certain cytokines, such as such IL-8 and IL-12 p40, and TNFα were sub-
teriorly elevated in vitro and IL-8 and IL-12 p40 production were pre-
dictive of survival (p=0.001 and p=0.008, respectively). TNFα levels also correlated with SD at week 8 (p=0.01). More than 70% of pa-
tients tested were found to have significant T cell responses, and/or de novo or significantly enhanced PD-L1 expression in the tumor post treatment, with a trend towards improved survival (p=0.1).

Conclusions
Study outcomes such as stabilization of disease and survival correlated with high DC cytokine levels, in the absence of meaningful toxicity. The DCVax treatment may be mediated through direct cytotoxic effects, as well as modulation of the tumor microenvironment to increase tumor infiltration by T cells, and attraction of inflammatory cells to the tumor. The development of PD-L1 expression likely reflects an induced im-

P165
Augmentation of tumor infiltrating CD8+ T cells and specific response to autologous tumor antigens in a phase I trial of in situ vaccination with CCL21 gene-modified dendritic cells
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Background
Intratumoral (IT) infiltration by activated immune effector cells is associated with a significantly better prognosis, however, tumor-associated immune suppression commonly occurs in non-small cell lung cancer (NSCLC). CD8+ T cell or dendritic cell (DC) infiltration is an inde-
pendent favorable prognostic indicator. CCL21 is a lymphoid chemokine that chemoattracts both lymphocytes and DC. Our preclinical studies re-
vealed potent systemic antitumor immune responses following IT in situ vaccination with DC overexpressing CCL21. In clinical evaluation of this strategy, we investigated anti-tumor specific systemic immune responses and tumor-infiltrating CD8+ T cells (CD8+ TIL) in NSCLC patients in re-
response to in situ vaccination via IT administration of autologous DC transduced with a replication-deficient adenoviral (Ad) vector expressing the secondary lymphoid chemokine (SLC/CCL21) gene.

Methods
In a phase I trial, sixteen stage IIIB/IV NSCLC subjects received two vac-
cinations (1 x 10⁶, 5x 10⁶, 1x 10⁷, or 3x 10⁷ dendritic cells/injection) by CT- or bronchoscopic-guided IT injection (days 0 and 7). Immune re-
sponses were assessed by tumor antigen-specific peripheral blood lymphocyte induction of IFN-γ in ELISPOT assays. Tumor biopsies were evaluated for CD8+ T cells and tumor PD-L1 by immunohistochemistry.

Results
Twenty-five percent (4/16) of patients had stable disease at day 56 follow-up by RECIST criteria. Four possible vaccine-related grade 1 adverse events (AE) occurred in 3 patients with no clear association to dose or schedule; the AE included flu-like symptoms, blood-tinged spu-

mum after each injection, nausea, and fatigue. ELISPOT assays revealed 38% (6/16) of patients had systemic responses against autologous tumor associated antigens (TAA). Tumor CD8+ T cell infiltration was in-
duced in 54% of subjects (7/13; 3.4 fold average increase in the number of CD8+ T cells per mm²). Patients with increased intratumoral CD8+ T cells following vaccination showed significantly increased PD-L1 mRNA expression (p=0.02).

Conclusions
Intratumoral vaccination with Ad-CCL21-DC was well-tolerated and resulted in 1) induction of systemic tumor antigen-specific immune responses and 2) enhanced tumor CD8+ T cell infiltration accompanied by increased PD-L1 expression. DC-CCL21 in situ vaccination may be an effective approach to induce tumor CD8+ T cell infiltration in combination with checkpoint inhibitor therapy. This will be assessed in combination with PD-1/PD-L1 checkpoint inhibition.

Acknowledgements
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P166
Increased immune responses in melanoma patients pre-treated with CDX-301, a recombinant human Flt3 ligand, prior to vaccination with CDX-1401, a dendritic cell targeting NY-ESO-1 vaccine, in a phase II study
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Background
Patients with high-risk melanoma have a 20-60% recurrence rate with 5-year OS between 45% and 70%. We evaluated vaccination with CDX-1401, a fusion protein consisting of human monoclonal IgG1 antibody targeting the dendritic cell (DC) receptor DEC-205 linked to the full-length NY-ESO-1 tumor antigen, with or without pretreatment with CDX-301, a recombinant human Flt3 ligand (Flt3L), in a phase II, randomized study of subjects with resected Stage IIIb-IV melanoma. The primary objective was to determine whether the immune response to NY-ESO-1 elicited by vaccination with CDX-1401 and Hiltonol® (Poly-ICLC, from Oncovir) is substantially increased by prior expansion of circulating dendritic cells (DC) with CDX-301 therapy. Prevention of disease recurrence was not a clinical endpoint for this study.

Methods
60 pts with resected melanoma were randomized to two cohorts. Cohort 1 (CH1) received CDX-301 pretreatment (25 mcg/kg SQ daily x 10 days) in the first 2 of 4 cycles of vaccination with CDX-1401 (1 mg IC day 1) + poly-ICLC (2 mg SQ, days 1 and 2). Cohort 2 (CH2) received 4 monthly cycles of vaccine with CDX-1401 and poly-ICLC w/o prior CDX-301. Antigen-specific immune responses were measured by Eilspot and ELISA; flow cytometry and T cell assays were performed to evaluate the effects on immune cell subsets.

Results
The combination regimens of Flt3L, DC targeted NY-ESO-1 and poly-ICLC were well tolerated. Substantial increases in innate immune cells (DCs, NK cells and monocytes) were elicited in Flt3L treated (CH1), patients and w Flt3L treatment was associated with significant increases in activated T cells. T cell responses were elicited in both cohorts but were elicited earlier in CH1 and the number of individual responders to NY-ESO-1 in CH1 was significantly greater than in CH2. In addition, anti-NY-ESO-1-specific T cell responses in CH1 were significantly more robust. Significant increases in antibody titer were noted in both co-
horts, but the average peak titer was significantly higher in CH1 vs CH2. Additional flow analyses, gene expression profiling and functional phenotyping of antigen-specific T cells are in progress.

Conclusions
In melanoma patients, DC mobilization with Flt3L is safe and leads to substantially enhanced B and T cell responses to DC targeted vaccines.

Trial Registration
ClinicalTrials.gov Identifier NCT02129075.

P167
A first-in-human phase I dose-escalation study of NHS-IL12 in solid tumors
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Background
NHS-IL12 (MSB0010360N; M9241), a tumor-targeting immunocyto-
kine comprising 2 heterodimers of IL-12 fused to the H-chain of the NHS76 antibody, has affinity for single- and double-stranded DNA and is designed to target necrotic tissue and deliver IL-12 to tumor. In previous trials with human recombinant IL-12, clinical activity was limited by toxicity. The goal is to reduce toxicity associated with systemic IL-12 through highly specific delivery to the tumor.

Methods
A 3+3 dose-escalation study in patients with advanced solid tumors was designed to demonstrate the safety of NHS-IL12 while evaluating pharmacokinetic (PK) and pharmacodynamic (PD) effects. Dosing started at 0.1 mcg/kg, with a planned maximum DL9 (21.8 mcg/kg). Patients were admitted for PK/PD draws and 48-hour observation after the first 2 doses. Restaging evaluations were performed every 8 weeks using standard RECIST 1.1 and immune-related response criteria. An expansion cohort (n=10) was enrolled at the maximum-tolerated dose (MTD) for further PK/PD analysis.
Results
From 12/2011–05/2016, 58 patients enrolled in DLs 1–9. 22 patients enrolled in single-dose cohorts (SDC); 36 patients in multiple-dose cohorts (MDC; q4wk). In SDC, all patients completed treatment; there was 1 death unrelated to study treatment. In MDC, 52 patients discontinued due to progression (20), adverse events (AEs, 4), alternative treatment (3), treatment-schedule conflict or withdrawal (4), and 3 death unrelated to study treatment. Median administrations in the MDC was 2.5 doses (range 1–22). Treatment-related AEs occurred in 47/58 patients (81%). Dose-limiting toxicities were observed in 1/6 patients on DL8 (16.8 mcg/kg, grade 3 alanine aminotransferase (ALT) elevation) and 2/6 patients in DL9 (21.8 mcg/kg, grade 3 aspartate aminotransferase (AST) and ALT elevation and lipase elevation), making the MTD 16.8 mcg/kg. 10 patients were enrolled in expanded DL8 at 16.8 mcg/kg. Most common AEs in DL8 (n=16) were decreased lymphocyte count (81.3%), increased AST (81.3%), decreased white blood cell count (75%), increased ALT (75%), and fever (62.5%). Most AEs were transient and resolved within 10 days. Most symptoms were controlled with NSAIDs or acetaminophen. Serum cytokines showed time- and dose-dependent changes for IFN-gamma, IL-10, IL-12p70, and TNF-a. Best overall response was stable disease in 15/30 evaluable subjects. Five patients stayed on study treatment ≥182 days.

Conclusions
NHS-IL12 administered s.c. q4wk was safe and well-tolerated with predictable adverse events. Analysis of paired tumor biopsies to determine intratumoral effects are ongoing. Updated data will be presented at the conference.

Trial Registration
ClinicalTrials.gov identifier NCT01417546.

P168
Salvage intravesical Mycobacterium phlei cell wall-nucleic acid complex (MCNA) for BCG-unresponsive patients
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Background
A standardized definition of BCG-unresponsive disease has been recently agreed upon to assess the efficacy of salvage treatments for patients with bladder cancer recurrence despite intravesical BCG therapy. Previously, we have reported the results from a single-arm trial of intravesical MCNA treatment in patients who have previously failed BCG [1]. We now report the results of a retrospective analysis, looking at efficacy with attention paid to the specific category of BCG-unresponsive patients.

Methods
High-risk NMIBC patients with either papillary and/or carcinoma in situ (CIS) having failed intravesical BCG treatment were enrolled. BCG treatment failure was defined as cancer recurrence after at least 2 courses of BCG (at least 5 of 6 induction instillations, or 2 induction courses). Patients received induction treatment with 6 weekly doses of 8 mg MCNA intravesically, followed up by maintenance treatment of 3 weekly instillations at months 3, 6, 12, 18 and 24. Treatment efficacy was evaluated by cystoscopy, urine cytology and biopsy, with disease-free status achieved when central review of biopsy reveals absent high grade disease.

Results
Of the 129 patients originally enrolled, 94 fit the newly defined BCG unresponsive criteria. The cohort consisted of 68 (72.3%) with CIS, with/ without papillary tumor, and 26 (27.7%) with exclusively papillary tumor upon recurrence after BCG. In the CIS-containing group, the complete response rate measured at months 6, 12, and 24 were 39.7% (28-52.3%), 23.5% (14.1-35.4%), and 13.2% (6.2-23.6%), respectively. In the group with only papillary tumors, the corresponding disease-free survival rates were 61.2% (38.2-78.8%), 61.2 (38.2-77.8%), and 50.1% (27.5-69%).

Conclusions
Intravesical MCNA therapy is an alternative therapy to radical cystectomy in the patients with recurring disease after intravesical BCG treatment. It has the potential to offer 24% of patients with CIS and 60% of patients with papillary tumors a chance to safely preserve their bladder.

References

P169
DNA methylation changes in CD8+ T cells following 4-1BB costimulation
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Background
4-1BB costimulation imprints long term changes in the behavior of costimulated T cells [1]. There is not a satisfactory mechanistic explanation yet.
Methods
To determine the specific DNA methylation changes occurring upon 4-1BB costimulation, purified human CD8+ T cells from three healthy donors were activated in vitro for 5 days with anti-CD3 monoclonal antibody (OKT3) and either with anti-4-1BB monoclonal antibody urelumab or its corresponding isotype (hulg4). Activated lymphocytes were left 5 days in culture with hulg7 without further stimulation. Such back-to-resting CD8+ lymphocytes were restimulated with OKT3 for 12, 24 and 36h to validate the expression of the genes differentially methylated upon primary stimulation at mRNA and protein levels. DNA methylation profiles of both activated and resting cell subsets were characterized with Infinium 450K DNA methylation array (Illumina). To further confirm our observations, identical experimental procedure was performed with anti-4-1BB 6B4 agonist antibody in a CD8+ T cell donor. Differentially methylated genes between OKT3-anti-4-1BB versus their corresponding control counterparts were validated by pyrosequencing on activated and resting CD8+ from independent group of healthy donors (n=8 for Urelumab and n=11 for 6B4). Expression changes were confirmed by qRT-PCR and flow cytometry in activated, rested and restimulated CD8+.

Results
853 genes were differentially methylated in urelumab-treated CD8+ T cells compared with their controls, 52 of which were shared with 6B4-activated CD8+ T lymphocytes. A number of differentially methylated genes are involved in i) T cell migration, ii) T cell activation, survival and homeostasis and iii) regulation of gene expression including key T cell transcription factors.

Conclusions
4-1BB costimulation induces CD8+ T lymphocytes that are poised to respond more effectively to a secondary antigen exposure. These acquired functions are imprinted in the genomic DNA of the CD8+ T cells by DNA methylation during 4-1BB co-stimulation, and involve key genes for CD8+ T cells.

References

P170
Novel tetravalent anti-GITR antibody is a potent anti-tumor agent in vivo
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Background
Glucocorticoid-induced TNFR-related (GITR, TNFRSF18) is a member of the TNFR superfamily with pleiotropic T cell modulatory activity. In the mouse, GITR is expressed at high levels on regulatory T cells (Treg) and has been reported to antagonize their suppressive capacity, whereas it is expressed at moderate levels on conventional effector T cells (both CD4+ and CD8+) for which it exerts stimulatory activity. Studies have indicated that GITR-targeting agents mediate anti-tumor control via two mechanisms: depletion and possibly suppression of Treg and/or direct agonism of effector T cells.

Methods
We have developed a novel anti-GITR antibody with enhanced agonist activity using single-domain antibodies (sdAbs) in a multivalent format. A tetravalent anti-GITR agonist antibody induces NF-kB activation and T cell stimulation in vitro that is superior to a conventional bivalent antibody; multivalency confers agonist activity in the absence of Fc-mediated crosslinking. Treg-depleting activity is obtained with an Fc effector-competent format.

Results
The tetravalent antibody potently controls tumor growth in vivo following a single dose with activity as low as 0.08 mg/kg. Treatment reduces Treg frequency, thereby altering the ratio to effector T cells within the tumor to create a favorable environment for an effective anti-tumor immune response. CD8+ T cell activation and proliferation is observed in vivo, and treatment confers long-term immunity to tumor re-challenge.

Conclusions
In summary, multivalent GITR agonist antibodies are a promising modality for the treatment of cancer and we are exploring candidates for clinical development.
### P172

**Activation of liver-resident myeloid cells to produce IL-27 initiates 4-1BB hepatotoxicity**

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#### Background

4-1BB agonist antibodies were the second T cell co-stimulatory agents to enter the clinic after αPD-1. Despite impressive efficacy against both hematologic and solid tumors and an ability to suppress adverse events associated with checkpoint blockade, their development has been stymied by poor understanding of their underlying mechanism and the resulting inability to separate off-target liver toxicity from on-target anti-tumor immunity. We sought to uncover the mechanisms by which 4-1BB agonist antibodies trigger hepatotoxicity in hopes of discovering approaches by which the anti-tumor and hepatotoxic effects could be separated.

#### Methods

C57BL6/J mice (Taconic) were treated every 3 days for 3 doses with the 4-1BB antibody 3H3 (250ug), anti-CD40 (FGK4.5 100ug), anti-CD80 (4S4), and panobinostat (LBH589), and its interaction with a PD-1 antibody, in preclinical settings.

#### Results

We find that α4-1BB mediated liver damage initiates through stimulation of myeloid cells, followed by subsequent recruitment and activation of CD8 and CD4 T cells in the liver. Moreover, we show that the inflammatory cytokine IL-27 is essential in myeloid conversion of T cells into mediators of liver damage. Conversely, FoxP3+ regulatory T cells (Treg) act to suppress 4-1BB agonist induced liver inflammation, and liver pathology worsens significantly when they are ablated. Further, we show that concomitant CTLA-4 blockade ameliorates 4-1BB hepatotoxicity by expanding peripheral Tregs, but that this effect is lost with addition of αPD-1. Additional differences exist between the tumor and liver microenvironments, which may be exploited to selectively promote on target activation of 4-1BB by agonist antibodies in the future.

#### Conclusions

4-1BB activation of liver-resident myeloid cells promotes the subsequent recruitment and activation of T cells in an IL-27 dependent manner. These T cells mediate the liver pathology associated with 4-1BB antibodies unless their activity is suppressed by Tregs. Our results support the use of 4-1BB agonists in rational combinations, in particular with CTLA-4 blockade, which may expand Tregs in the liver to ameliorate α4-1BB mediated toxicities.

#### Acknowledgements

We thank the MD Anderson IRG program and DOD PRCRP grant CA140792 for funding. Dr. Robert Mittler kindly provided 4-1BB knockout mice and Drs. Aymen Al-Shamkhani, Martin Glennie, and Stephen Beers kindly provided LOB12 antibody.

### P173

**Restoration of tumor-infiltrating lymphocyte function by panobinostat and tumor eradication with the combination of panobinostat and PD-1 blockade**

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#### Background

Tumor immunotherapy is a unique therapeutic modality in our fight against human cancers. The recent success of immune checkpoint therapies highlights the value and potential of this approach. Epigenetic regulation of tumor immunology is becoming a key area of investigation. Experimental data have linked HDAC-inhibition to the enhancement of tumor antigen expression and presentation, the activation of effector T and NK cells, and the suppression of T regulatory cells. These observations are confounded by the potential immune-inhibitory effects by HDAC-inhibitors on DC and T cell activation. Here, we have examined the immune modulatory effects of pan-HDAC-inhibitor, panobinostat (LBH589), and its interaction with a PD-1 antibody, in preclinical settings.

#### Methods

Panobinostat was tested *in vitro* in human peripheral blood mononuclear cell (hPBMC) cultures at concentrations from 5 nM to 500 nM. Next, panobinostat was examined *in vivo* as single agent and in combination with the PD-1 antibody, 1D2, in MC38, a murine syngeneic tumor model.

#### Results

Panobinostat restored IL-2, IFNγ, and TNFa expression that was inhibited during T cell exhaustion. Elevation of tumor-infiltrating lymphocytes (TIL) was observed by both flow cytometry and immunohistochemistry. Of note, the proportion of CD8+ effector-memory cells was increased by panobinostat. Proteomic analysis of the treated MC38 tumors revealed increases in IFNγ levels under panobinostat treatment. Molecular profiling of tumor samples by NanoString indicated that panobinostat treatment led to increases of MHC class I, II and invariant chain expression. This is coupled with inductions of chemokine and cytokine expression and increases in effector T cell functions as measured by Granzyme A and B expression. Finally, while panobinostat and PD-1 antibody each achieved some level of anti-tumor efficacy, the combination of panobinostat and PD-1 antibody achieved complete responses in 10 out of 10 mice tested. The tumor regression was durable as none of the treated mice had any recurrence of tumors more than 60 days after the cessation of treatment.

#### Conclusions

Our preclinical data point to a dichotomy of immune modulation by panobinostat. While it may be immune-suppressive during priming, panobinostat is immune-stimulatory under antigen-experienced, immune-exhaustive environment. With the latter more reflective of the tumor microenvironment, we saw evidence of panobinostat being immune-stimulatory on TIL in a preclinical setting, with induction of both TIL percentages as well as activity. The positive effects on TIL...
and the promising combination efficacy with PD-1 antibody in vivo support further testing of panobinostat as a possible immunoncology agent both in preclinical and clinical settings.

**P174**

Imprime PGG, a soluble yeast β-glucan, is a systemically administered PAMP that activates DCs and supports T cell priming, showing synergy with cancer immunotherapies

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**Background**

Pathogen-associated molecular patterns (PAMPs) provide crucial activating signals to the immune system. Importantly, due to their potential ability to induce pro-inflammatory cytokines that can cause systemic toxicity, most PAMPs are limited to local administration such as subcutaneous or intratumoral injection. Imprime PGG (Imprime) is a soluble yeast β-1,3/1,6 glucan that has been administered intravenously (i.v.) to >400 healthy volunteers and cancer patients with minimal adverse effects. Imprime has shown promising efficacy when combined with other therapeutic antibodies in multiple clinical trials. Imprime has been previously shown to have promising efficacy in combination with immune checkpoint inhibitors in pre-clinical mouse tumor models and is currently being explored clinically in combination with anti-PD-1 therapy. In data presented here, we sought to further understand how Imprime functions to link the innate and adaptive immune systems via dendritic cells (DCs) to induce T cell priming providing benefit to checkpoint inhibitor therapy.

**Methods**

To examine in vivo effects of Imprime, C57BL/6 mice were injected i.v. with 1.2 mg of Imprime. 16hr post treatment, lymph nodes (LNs) were harvested and mRNA levels were determined using the QuantiGene Plex platform (Affymetrix). To study Imprime’s effect on CD8 T cell priming, 1x10⁵ OT-I CD8 T cells were transferred into congenic mouse recipients that were also increased in the blood of healthy volunteers, as was detection of Imprime binding to DCs. In congenic mouse recipients that were immunized with peptide +/- Imprime after transfer of OVA-specific OT-I CD8 T cells, those primed in the presence of Imprime demonstrated greater overall expansion and acquisition of effector functions than peptide alone. Imprime’s transcriptional profile and ability to enhance T cell priming was dependent on the C-type lectin receptor Dectin-1.

**Conclusions**

Together, these data demonstrate that Imprime acts as a unique i.v.-administered PAMP that primes the immune system and inspires a coordinated adaptive immune response. These qualities make Imprime an attractive candidate to synergize with cancer immunotherapies.

**P175**

Functional characterization of CDX-1140, a novel CD40 antibody agonist for cancer immunotherapy

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**Background**

For the development of agonist antibodies that stimulate immune responses, a balance is required between sufficiently strong immune stimulation and the untoward effects of systemic immune activation. This is particularly true for CD40, a molecule expressed by antigen presenting cells, which is critical in the regulation of immune responses. Agonist CD40 antibodies are highly effective in preclinical tumor models either through direct interaction with CD40-expressing lymphomas, or indirectly through the activation of adaptive anti-tumor immunity. There are several agonist CD40 antibodies in clinical development, but limited data are available with regard to their clinical activity and safety profile. Data reported for the agonist anti-CD40 mAb CP-870,893 (Roche/Pfizer) [1] demonstrated clinical activity in cancer patients, but its low maximum tolerated dose (0.2 mg/kg) may impede the full potential of activating this pathway. We set out to develop a CD40 agonist antibody with strong immune stimulating properties and a safety profile that allows for systemic dosing.

**Methods**

Anti-CD40 monoclonal antibodies (mAbs) were generated by immunization of human Ig transgenic mice with recombinant and cell surface expressed human CD40. We selected hybridomas that produced human antibodies with an assay using a reporter cell line engineered to express CD40 and NFκB-responsive luciferase. The variable regions of lead antibodies that displayed differential activity were cloned in human IgG1 or IgG2 constant domains and expressed in CHO cells.

**Results**

Following i.v. administration of Imprime in non-tumor bearing mice, Imprime rapidly bound resident and migratory DC subsets, caused DC maturation, and increased DC recruitment into LNs. Transcriptional profiling in LNs showed increased mRNA levels of chemokines important in immune cell trafficking, pro-inflammatory cytokines, and a strong type I interferon signature. Many of these chemokines were also increased in the blood of healthy volunteers, as was detection of Imprime binding to DCs. In congenic mouse recipients that were immunized with peptide +/- Imprime after transfer of OVA-specific OT-I CD8 T cells, those primed in the presence of Imprime demonstrated greater overall expansion and acquisition of effector functions than peptide alone. Imprime’s transcriptional profile and ability to enhance T cell priming was dependent on the C-type lectin receptor Dectin-1.

**Conclusions**

These data support CDX-1140 as a novel CD40 agonist with potential for immunotherapy of cancer patients.

**References**

P176
Releasing the breaks: quantitative cell-based bioassays to advance individual or combination immune checkpoint immunotherapy
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Background
Blockade of immune checkpoint receptors (e.g., PD-1, CTLA-4) has emerged as a promising new approach to enhance anti-tumor responses. While immunotherapies directed against PD-1 and CTLA-4 are showing unprecedented efficacy in the treatment of cancer, some patients and tumor types remain refractory to these therapies. This has resulted in a broadening of immunotherapy research and development to include additional inhibitory receptors (e.g., LAG-3, TIM-3) targeted individually or in combination with other immune checkpoint receptors. A major challenge in the development of biologics is access to quantitative and reproducible functional bioassays. Existing methods rely on primary cells and measurement of complex functional endpoints. These assays are cumbersome, highly variable, and fail to yield the data quality required for drug development. To address this need, we have developed a suite of immune cell line-based bioassays for individual and combination immune checkpoint immunotherapy targets including PD-1 (PD-L1 or PD-L2), CTLA-4, LAG-3, TIM-3, PD-1+TIM3 and more.

Methods
These reporter-based bioassays were rationally designed to reflect the mechanism of action (MOA) of drug candidates targeting individual and combination immune checkpoint receptors. Each assay consists of an effector cell and an artificial antigen presenting cell (aAPC). Effector cell lines were engineered on a T cell background to express inhibitory receptor(s) and a luciferase reporter driven by specific response elements under the precise control of intracellular signals mediated by the T cell receptor (TCR) and inhibitory receptor target(s). The aAPCs were specially engineered to be able to activate the TCR in an antigen-independent manner and also stably express the ligand(s) of checkpoint receptor(s). These cell lines were developed in Thaw-and-Use format and can be used in assays without cell culture.

Results
Upon co-culture of effector cells with aAPCs, TCR activation in effector cells is suppressed by signaling from immune checkpoint receptor(s), which can then be specifically reversed by blocking antibodies targeting the inhibitory receptor(s) and/or their ligand(s). The bioassays were demonstrated to be specific to research-grade antibodies with known blocking activity as well as FDA-approved drugs (e.g., nivolumab, ipilimumab). In addition, the bioassays are pre-qualified according to ICH guidelines and demonstrate the performance required for use in antibody screening, potency testing and stability studies.

Conclusions
We have developed a suite of MOA-based bioassays for immune checkpoint receptors. These assays are easy to use and demonstrate high assay specificity, sensitivity and reproducibility. They are suitable for drug development in a quality-controlled environment.

Background
CD137 (4-1BB) is a key costimulatory immunoreceptor and a highly promising therapeutic target in cancer. To overcome limitations of current mAb-based approaches which monospecifically target CD137, we generated PRS-343, a CD137/HER2 bispecific designed to promote CD137 clustering by bridging CD137-positive T cells with HER2-positive tumor cells, thereby providing a potent costimulatory signal to tumor antigen-specific T cells.

Methods
We have previously described the generation of PRS-343 as a fusion of a CD137-specific Anticalin® protein to a variant of the HER2-targeting monoclonal antibody trastuzumab with an engineered IgG4 backbone. PRS-343 was found to efficiently activate T cells ex vivo in the presence of HER2-positive cells. Here, in vivo proof of concept data is presented utilizing a humanized mouse model in immunocompromised mice and the SK-OV-3 cell line as a HER2-positive xenograft. Tumor-bearing mice received human PBMC and weekly injections of PRS-343 for three weeks. An IgG4 isotype antibody served as the negative control, while a CD137-targeting benchmark antibody and trastuzumab with an engineered IgG4 backbone (“tras-IgG4”) served as controls for monospecific targeting of CD137 and HER2, respectively.

Results
PRS-343 activity was investigated at four different weekly doses, ranging from 4μg to 200μg. We found that PRS-343 dose-dependently led to strong tumor growth inhibition compared to treatment with the isotype control. Tumor response was accompanied by a significantly higher frequency of hCD45(+) tumor infiltrating lymphocytes (TIL) as determined by immunohistochemistry (IHC). Single IHC stainings against the T cell markers hCD3, hCD4 and hCD8 indicated that the rise in TIL frequency was due to an expansion of CD3(+)/CD8(+)-T cells, while CD4+ lymphocytes remained at a low frequency both in the treatment and control groups. Interestingly, we observed neither tumor growth inhibition nor an increase in human TIL frequency with the anti-CD137 benchmark. The tras-IgG4 control was also devoid of lymphocyte infiltration into the tumor, but displayed a tumor growth inhibition comparable to PRS-343.

Conclusions
We report potent costimulatory T cell engagement of the immune receptor CD137 in a HER2-dependent manner, utilizing the CD137/HER2 bispecific PRS-343. PRS-343 displays dual activity in vivo based on monospecific HER2-targeting and bispecific, tumor-localized costimulation of CD137. Compared to known CD137-targeting antibodies in clinical development, PRS-343 has the potential to provide a more localized activation of the immune system with higher efficacy and reduced peripheral toxicity. The positive functional data of PRS-343 support investigation of its anti-cancer activity in clinical trials.

P178
IL-2 signaling on tumor-infiltrating CD8+ T cells is not required for successful 4-1BB combination immunotherapy
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Background
Antibodies against the PD-1/PD-L1 pathway have yielded impressive results, however, not all patients benefit from these treatments, and many experience only partial responses. There is therefore a continued interest in developing new strategies to further boost anti-tumor immune responses and maximize therapeutic efficacy. CD8+ Tumor infiltrating lymphocytes (TIL) in both human and mouse tumors have been shown to express the co-stimulatory molecule 4-1BB. In addition to PD-1, 4-1BB agonists can induce tumor regression in pre-clinical models that is further boosted by anti-PD-L1 mAbs, but the detailed mechanism remains unclear.
Methods
We utilized the murine melanoma cell line B16, expressing the model antigen SIY, implanted subcutaneously into syngeneic C57BL/6 mice for these experiments.

Results
To determine if TIL already present in the tumor were sufficient for tumor regression, we utilized the S1P inhibitor FY720. Therapeutic efficacy was preserved when anti-41BB combination immunotherapy was administered with FY720, arguing it works directly on T cells in the tumor microenvironment. A markedly augmented number of antigen-specific CD8+ TIL occurred after combination immunotherapy even with FY720 administration, arguing that antigen-specific TIL were expanded directly in the tumor microenvironment. To assess the mechanism of TIL accumulation, we performed BrdU labeling to measure proliferation, and detection of active caspase-3 to measure apoptosis. Unexpectedly, we found that proliferation of CD8+ TIL was not affected by combination immunotherapy. Instead, a significant decrease of active caspase-3 levels occurred in CD8+ TIL after immunotherapy, arguing that the accumulation of antigen specific TIL was due to decreased apoptosis. To further study the molecular mechanism of intratumoral TIL accumulation, we performed a gene expression profiling on untreated and immunotherapy-treated CD8+ TIL. Pathway analysis revealed that IL-2 and NF-kB were major hubs of differentially regulated genes. Consistently, we found increased IL-2 production in CD8+ TIL after immunotherapy. Therefore, we tested if T cell-intrinsic IL-2 signaling within the tumor site was required for successful immunotherapy. Intratumoral antibody-mediated blockade of IL-2 did not decrease the efficacy of combination immunotherapy. Mixed bone marrow chimeras of wild type (WT) and CD25- bone marrow confirmed that there was no defect in CD25+ CD8+ TIL accumulation after immunotherapy.

Conclusions
These results suggest that restored IL-2 production by TIL is a marker of successful immunotherapy but is not required for therapeutic efficacy. Current studies are focusing on the role of T cell-intrinsic NF-kB signaling in successful combination immunotherapies that utilize agonist 4-1BB antibodies.

P179
Dual function STAT3 inhibitor (CpG-STAT3ASO) generates systemic antitumor immune responses resulting in eradication of bona-located prostate tumors in mice
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Background
STAT3 transcription factor promotes prostate cancer progression and sustains potently immunosuppressive tumor microenvironment. STAT3 activity in both cancer cells and in tumor-associated immune cells is likely responsible for resistance of metastatic prostate cancers to current treatments, including immunotherapy.

Methods
We previously demonstrated that ligands for endosomal Toll-like Receptor 9 (TLR9), CpG oligonucleotides, allow for cell-selective delivery of therapeutics to TLR9+ myeloid immune cells and tumor-propagating cells in prostate cancers. Here, we describe new CpG-conjugated STAT3 antisense oligonucleotides (ASO), chemically modified for improved nuclease resistance (T1/2 = 106 h in human serum).

Results
CpG-STAT3ASOs are quickly and efficiently internalized by human (DU145, LN-TLR9) and mouse (Myc-CaP, Ras/Myc-driven RM9) prostate cancer cells as well as by TLR9+ immune cells, including polymorphonuclear myeloid-derived suppressor cells (PMN-MDCs) derived from blood of prostate cancer patients. In contrast to STAT3ASO alone, the naked CpG-STAT3ASO was internalized by rapid scavenger receptor-mediated mechanism even at low concentrations. Correspondingly, CpG-STAT3ASOs showed improved potency of STAT3 knockdown at mRNA and protein levels in target cells. As assessed by biodistribution studies in mice, the intravenous (IV) injections of fluorescently-labeled CpG-STAT3ASO effectively targeted TLR9+ myeloid cells and cancer cells in organs, such as spleen and bone marrow. For efficacy studies, we used syngeneic (RM9) and xenotransplanted (LN-TLR9, DU145), castration-resistant prostate tumors implanted intratibially in mice. Repeated IV injections of 5 mg/kg CpG-STAT3ASO resulted in regression of bone-localized RM9 tumors while treatments using STAT3ASO alone or CpG-scrambled ODN failed to control tumor progression. Antitumor effects of CpG-STAT3ASO depended on combination of direct and immune-mediated cancer cell killing as suggested by reduced antitumor efficacy in xenotransplanted tumor models in immunodeficient mice. Both STAT3ASO and CpG-STAT3ASO reduced STAT3 activity in tumor and tumor-associated immune cells, but only the latter resulted in tumor infiltration by neutrophils and T cells. These effects were associated with reduced PD-L1 expression on MDSCs and the reduced percentage of regulatory T cells. Our preliminary results using blood samples from prostate cancer patients suggest that CpG-STAT3ASO effectively alleviates tolerogenic effects of human PMN-MSCs on T cell activity.

Conclusions
Overall, our strategy allows for two-pronged targeting of metastatic, castration-resistant prostate cancers using safer and more efficient reagents based on TLR9-targeted oligonucleotide delivery.

P180
A nanoparticle based approach for optimizing T cell activation, signaling, and proliferation for adoptive immunotherapy
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Background
Artificial antigen presenting cells (aAPCs) are platforms that present the two necessary signals for T cell activation. Traditionally, aAPCs have signaling molecules randomly dispersed on their surface, although data has shown the importance of nanoscale signal organization and co-stimulatory molecule choice on stimulation [1]. Traditional aAPCs are thus inefficient for studying the dynamics of T cell activation. Here, we have developed a novel platform where signal 1 and 2 molecules are on distinct nanoparticles that are co-clustered on the T cell surface within a magnetic field. By manipulating nanoparticle properties and the types of co-stimulatory molecules, we show the benefit of this platform as a tool to study T cell stimulation.

Methods
Iron-dextran or polystyrene particles where conjugated with signal 1 peptide-MHC and signal 2 anti-CD28 mAb either on the same or separate 30-4500nm nanoparticles. Murine CD8+ cells were stimulated with nanoparticles and a 0.2T magnetic field. Activation was measured by cell proliferation, cytokine secretion, and cytotoxicity.

Results
We first showed that signal 1 and 2 can be separated onto distinct particles when these particles are clustered within a magnetic field (Fig. 66a). The resultant CD8+ T cells have equivalent cytotoxicity as compared to cells stimulated with traditional aAPCs (Fig. 66b). T cell activation is dependent on the co-clustering of both signaling molecules – when either is presented on a non-paramagnetic polystyrene particle, proliferation is decreased (Fig. 67a). The two signaling molecules also must be clustered sufficiently close, at the scale of tens of nanometers, as there was an inverse correlation between particle size and T cell proliferation (Fig. 67b). Finally, signal separation allows for greater enrichment of the clonal T cell subsets using signal 1 only
particles, and thus greater activation over the same time period after the addition of signal 2 particles and a magnetic field. Signal separation also allows for easy optimization of signal 2 type and ratio (preliminary data not shown).

Conclusions
Here, we have demonstrated a novel T cell activation platform that involves simpler aAPC synthesis without sacrificing activation potential. This platform enables the study of the clustering of signaling molecules as well as easy manipulation of the types and ratios of different co-stimulatory signals.

Acknowledgements
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References

Fig. 66 (Abstract P180). a TCR transgenic PMEL CD8+ T cells were stimulated for 7 days with Db-gp100 signal 1 only particles with increasing molar doses of anti-CD28 signal 2 (s2) only particles (i.e. 2x = 2:1 molar excess signal 2). aAPC refers to traditional signal 1 and 2 aAPCs. Cells were incubated in the presence or absence of a magnetic field for the first three days, and cell counts were taken on day 7. b PMEL CD8+ T cells were stimulated for 7 days with signal 1 (s1) only particles, signal 1 and 2 on separate particles (s1 + s2), or signal 1 and 2 on the same particle (s1/2 aAPC). After 7 days, T cell mediated killing of target B16-F10 cells was measured (*p<0.05 by two-way ANOVA)

Fig. 67 (Abstract P180). a Db-gp100 signal 1 and anti-CD28 signal 2 mAbs were conjugated to the surface of 100 nm iron dextran or polystyrene nanoparticles. PMEL CD8+ T cells were stimulated with different combinations of these particles in the presence or absence of a magnetic field, and cell counts were taken after 7 days (*p<0.05 by two-way ANOVA). b PMEL CD8+ T cells were stimulated with signal 1 and 2 on separate iron-dextran particles of increasing size within a magnetic field. Cell counts were taken after 7 days (*p<0.05 by one-way ANOVA)

P181
A secreted PD-L1 splice variant expressed across tumor types inhibits lymphocyte function
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Background
Targeting immune checkpoint pathways, such as programmed death ligand-1 (PD-L1, also known as CD274 or B7-H1) or its receptor programmed cell death-1 (PD-1) have shown clinical benefit in patients with many different types of tumors. While PD-L1 expression is not a reliable biomarker for predicting response to therapy, the expression of the coinhibitory molecule PD-L1 on the surface of tumor cells is associated with worse prognosis in many tumors. This negative effect of PD-L1 expression is seen even when only a small fraction of the tumor expressed PD-L1 on the tumor cell surface. Here we describe a splice variant (secPD-L1) that does not splice into the transmembrane domain, producing a secreted form of PD-L1.

Methods
Lymphoma, kidney and breast cancer cell lines were analyzed for full length and secPD-L1 mRNA expression by qRT-PCR. RNAseq analysis in the Cancer Cell Line Encyclopedia confirmed expression of secPD-L1 in human tumor cell lines, RNAseq analysis of full-length PD-L1 and secPD-L1 was performed on The Cancer Genome Atlas, the GTEx database of non-malignant human tissue and sorted immune cells from healthy donors. Monocyte-derived dendritic cells were generated from healthy donors, stimulated with TNFα/PGE, polyIC, or LPS and assayed for expression of full-length PD-L1 and secPD-L1. Recombinant His-tagged secPD-L1 was produced to test whether it functioned as an inhibitor of proliferation and IFNγ production in coactivation assays with T lymphocytes isolated from healthy donors.

Results
The secPD-L1 variant is expressed by cancer cell lines that also express the full-length PD-L1, as well as non-malignant immune cells particularly activated monocyte-derived dendritic cells. In The Cancer Genome Atlas, expression of secPD-L1 is found in primary tumors with higher expression of full-length PD-L1. Furthermore using recombinant secPD-L1 we found that secPD-L1 contains a unique 18 amino acid tail containing a cysteine and can dimerize, and inhibit T cell proliferation and IFN-γ production in vitro.

Conclusions
This is the first report that a secreted splice variant of PD-L1 does not splice into the transmembrane domain, producing a secreted form of PD-L1.

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P182

Combined targeting of OX40 and PD-L1 metabolically reprograms T cells to promote regression of large established tumors
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Background
The protective capability of tumor antigen specific T cells is regulated by both co-stimulatory and inhibitory signals in the tumor microenvironment. Current approaches in cancer immunotherapy seek to restore the function of unresponsive T cells by blocking inhibitory pathways. However, providing exogenous co-stimulatory signals are showing clinical promise and provide novel approaches for combination therapy.

Methods
Multiple solid tumor mouse models in multiple different mouse strains were used (MCA205/B6, CT26/Balb/C, 424m1T3/129) to determine the synergy of aOX40 (clone OX86) with aPD-L1 (clone 10F.9G2) in large, established tumors. Tumors were allowed to grow until they were ~50-70 mm² before treatment began. Animals were treated either alone or concurrently with 250 µg aOX40 or 200µg aPD-L1 for a total of 500µg aOX40 and 600µg aPD-L1. Animals were euthanized and T cells isolated from secondary lymphoid organs and tumors. T cells were sorted for TCRβ chain sequencing based on Nur77GFP expression and/or Foxp3RFP expression. TCRβ chain sequencing was performed by Adaptive Biotechnologies. T cell metabolic function was assessed using 2NBDG and TMRE as well as a Seahorse Analyzer. Phenotyping and functional assays were performed using traditional methods of flow cytometry.

Results
We demonstrate for the first time that agonist OX40 monoclonal antibodies metabolically reprogram CD4 Th1 skewed and CD8 effector T cells. In addition, using tetramers to track tumor-antigen specific CD8 T cells, we observe a significant expansion of effector memory CD8 T cells with enhanced cytotoxic function. Moreover, despite increased regulatory T cell (Treg) proliferation in the periphery, the CD8/Treg and CD4/Treg ratio is significantly increased in the blood and tumor of combination therapy treated animals. Furthermore, using a TCR signal strength reporter system, we observed the clonal expansion of a number of tumor infiltrating CD8 T cells that were actively receiving strong TCR signals in situ. Unlike aOX40 or aPD-L1 alone, combination immunotherapy focused the CD8 T cell repertoire response such that the 10 most frequent clones within the tumor made up almost 40% of the total CD8 T cell response.

Conclusions
Our findings suggest that concurrently targeting both positive (OX40) and negative (the PD-1/PD-L1 axis) T cell pathways can re-activate the function of failed tumor antigen specific T cells and promote tumor regression of well established tumors.

P183

Characterization of infiltrating lymphocytes in benign, malignant, and healthy prostate tissue
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Background
Prostate cancer (PC) is the second most common cancer type among men worldwide. Other non-malignant conditions of the prostate, such as benign prostatic hyperplasia (BPH), affect a majority of the older male population. Furthermore, chronic prostatic inflammation is common and is discussed to be a potential driver of benign and malignant prostate conditions. We aimed to compare the phenotype of lymphocytes present in prostates without pathologic changes with those infiltrating PC or BPH lesions, focusing on characterization of co-inhibitory receptor expression.

Methods
Lymphocytes were isolated from prostates of patients with PC (n=5), BPH (n=5), and deceased transplantation donors (n=5). Two tissue samples per PC patient were processed, one primary malignant and one adjacent non-malignant. Prostate-infiltrating lymphocytes were isolated using mechanical dissociation followed by density gradient centrifugation and flow cytometric analysis.

Results
Analysis showed that the majority of prostate-infiltrating T cells in all prostate conditions were CD8+ and had a CD45ROCCR7+ effector memory phenotype. The ratio of CD4+/CD8+ T cells was comparable in all conditions, except BPH, where it was slightly higher than in healthy prostate. T cells expressing CD25 were more abundant in prostate tissue of PC patients (median 13.7% at malignant site, 15.1% at non-malignant site) and BPH (13.6%) compared to healthy controls (3.3%). Analysis of co-inhibitory receptor expression revealed that PD-1 was expressed by a larger proportion of T cells in PC specimens (71.0%, 60.0%) than in BPH (34.6%) and control prostates (47.2%). The opposite was found for LAG-3, which was expressed by a larger proportion of T cells in BPH (18.1%) than in PC (8.6%, 2.6%). Median frequency in control prostates was 13.1%. There were no differences between the sample types regarding frequencies of T cells expressing TIM-3 or CTLA-4, nor in quantity of Tregs or Tδ T cells. Compared to peripheral blood, the frequency of Tregs was significantly higher in all prostate tissue types; 17.0%, 16.6%, 16.5% and 20.5% in control, BPH, malignant and non-malignant site compared to 7.2% and 5.4% in blood of BPH and PC patients.

Conclusions
Many prostate-infiltrating T cells seem to express co-inhibitory receptors LAG-3 and PD-1, regardless of prostate condition. Furthermore, presence of Tregs does not seem to be unique to the PC environment. However, we did find an increased frequency of T cells expressing PD-1 in PC lesions. It is of great importance to elucidate expression of co-inhibitory receptors for different solid cancers to narrow down patient groups that might benefit from different immunotherapies.

P184

Understanding the therapeutic effectiveness of allogeneic immune responses in both murine and human acute myeloid leukemia during nongraftment cellular therapy
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Background
In stem cell transplant, few have studied the recipient lymphocytes’ recognition of mismatched donor antigens and MHC molecules, and how these primed host T cells may then recognize and kill leukemic cells. Previously, our group infused haploidentical, G-CSF mobilized, donor T cells into patients with refractory hematological malignancies preconditioned with 100 cGy total body irradiation. Strong recipient immune responses, with some durable remissions, were seen in hematologic malignancies. In a second trial without G-CSF mobilization or recipient radiation, patients exhibited weaker inflammatory and anti-leukemic responses [1]. We therefore decided to characterize the allogeneic antileukemic response in a murine preclinical model with the AML leukemic cell line, C1498.

Methods
B6D2F1 mice were injected with C1498 leukemic cells and subsequently on day 7 were injected with haploidentical spleen cells from C57BL/6 mice. They were then monitored for signs of tumor progression and euthanized when they became moribund. T lymphocytes
obtained from these euthanized mice were also tested for their ability to generate anti-C1498 responses when stimulated with haploidentical stimulator cells. We performed vitro experiments whereby healthy BD2F1 splenocytes were stimulated with mitomycin-treated haploidentical spleen cells from a C57BL/6 mouse, and grown in mixed lymphocyte culture and tested on day 5 for their ability to lyse Cr51-labeled syngeneic blasts and C1498 leukemic target cells.

Results
Results of the murine model demonstrated the ability of alloreactive cytolytic T lymphocytes to lyse C1498 leukemic cells but not syngeneic blast cells. In addition, isolated CD3+ cells obtained from C1498 bearing mice were able to generate anti-C1498 lytic activity when stimulated with haploidentical cells in vitro. These responses are being explored further in vivo.

Conclusions
In previous trials, increased PD-1 expression in recipients following initial markers of T cell activation suggest that T cell exhaustion mediates leukemic cell survival. Increased PD-L1 expression on leukemic cells provides one explanation for their eluding the host inflammatory response. Total body irradiation may be disrupting the equilibrium of host-tumor tolerance. G-CSF mobilization of donor lymphocytes increases donor antigens for host recognition and subsequent alloreactivity. The murine model shows that alloreactive T cells can generate lysis specific to tumor but not self antigens. This model of alloreactive tumor response can be explored as an adjuvant therapy to other therapeutic approaches, including checkpoint inhibition.

Acknowledgements
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References

P185
First-in-class orally bioavailable checkpoint inhibitors targeting single or multiple immune inhibitory pathways
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Background
Immune checkpoint inhibitors have changed the landscape of cancer therapy with the general acceptance that they will be the mainstay of future therapies. This is evident from a large number of ongoing clinical trials evaluating checkpoint inhibitors as a single agent or in combination with other therapeutic modalities. While these antibody-based therapies show impressive clinical activity, they suffer from the shortcomings including the failure to show responses in a majority of patients, immune-related adverse events (irAEs) due to the breaking of immune self-tolerance and need to administer by intravenous injection. The recent reports on severe demyelinating polyradiculoneuropathy leading to death observed in two patients with the anti-PD-1 immunotherapy also point to the need for shortacting agents for the better management of irAEs. Towards addressing these shortcomings, we are developing small molecule agents targeting one or more immune checkpoint pathways to increase the response rate and dosing by oral route with relatively shorter pharmacokinetic exposure.

Methods
We at Aurigene have devised a strategy to identify agents targeting single or multiple immune checkpoint proteins by taking advantage of the sequence/structural similarities among immune checkpoint ligands and receptors. In this strategy, high affinity shortest pharmacophore derived from the extracellular domain of checkpoint proteins are first identified and transformed into therapeutic agents with optimized drug-like properties. Our strategy has resulted in the identification of agents targeting PD-L1 alone, VISTA alone, PD-L1 and VISTA, and PD-L1 and TIM-3. The first compound from this approach AUPM-170/CA-170, a first-in-class dual antagonist targeting PD-L1 and VISTA, is undergoing clinical trials.

Results
Herein we report the pharmacological evaluation of another novel small molecule antagonist dually antagonizing PD-L1 and TIM-3 pathways. Potent functional activity comparable to that obtained with an anti-PD-1 or anti-TIM-3 antibody in rescuing T cell proliferation and effector functions was observed with the lead compound, AUPM-327. AUPM-327 showed selectivity against other immune checkpoint proteins as well as in a broad panel of receptors and enzymes. In preclinical models of melanoma, breast and colon cancers, AUPM-327 showed significant efficacy in inhibition of tumor growth upon oral dosing with excellent tolerability.

Conclusions
These findings support further development of AUPM-327 in the clinic.


Fig. 68 (Abstract P186). Clinicopathologic characteristics of patients by HHLA2 staining status

Fig. 69 (Abstract P186). Example of a human urothelial tumor that expresses HHLA2

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**P187**

**Therapeutic T cell activation using engineered variant IgSF domains**

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**Background**

The large number of immunoglobulin super family (IgSF) receptors on immune cells and tumors are attractive targets for the development of cancer immunotherapies. While nearly all commercial strategies targeting this family are focused on antibody-based biologics, we have developed a novel affinity ligand platform based upon yeast affinity maturation of human IgSF family extracellular domains (ECDs), which we term variant Ig Domains (vIgDs). vIgDs unique biochemical properties, including small size, monomeric/single domain structure, and capacity to interact with multiple counterstructures, combined with the near universal expression of IgSF family members and their counterstructures on immune cells and tumors, position the vIgD platform as an exciting new option for development of immuno-oncology biologics with first-in-class mechanisms of action.

**Methods**

IgSF domains of interest were mutagenized and cloned as yeast display libraries. Soluble counterstructure ligands were used to stain the display libraries, and yeast clones exhibiting the highest binding were isolated by flow cytometry sorting. vIgDs from the sorting outputs were subcloned into a mammalian Fc fusion expression vector, and individual vIgD-Fc fusions were recombinantly expressed in HEK293 cells. Purified preparations of vIgD-Fc fusions were used to stain eukaryotic cells transfected to surface express the target counterstructure. vIgD-Fc fusion proteins possessing desirable binding properties were tested for functional activity using in vitro assays with human primary T cells.

**Results**

Random and rationally designed mutant vIgD libraries were stained with counterstructure ligands and successfully sorted to isolate vIgDs with increased binding to the target counterstructures as demonstrated by elevated binding curves of successive selection outputs. vIgDs were expressed as recombinant Fc fusion protein constructs and demonstrated superior binding of transfecants expressing counterstructures compared to their parental wild-type IgSF domain Fc fusion protein. The resulting variants also demonstrated superior costimulatory activity when co-immobilized with anti-CD3 in human primary T cells.

**Conclusions**

Receptors built from IgSF domains are critical orchestrators of cellular communication in the immune system. However, IgSF receptors typically exhibit relatively low affinities for their target counterstructures, prohibiting their use as oncology therapeutics. Engineering of IgSF domains using yeast display affinity maturation allows the generation of variant proteins possessing superior binding to single or multiple native counterstructures. These binding improvements translate into beneficial alterations of functional activity, including improved costimulatory activity. The vIgD therapeutic platform is a new option for both development of soluble recombinant therapeutics or improvement of engineered cellular therapies.

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**P188**

**Impact of BTK/ITK inhibition with ibrutinib on neuroblastoma and osteosarcoma syngeneic solid tumor models**

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**Background**

Ibrutinib, a covalent inhibitor of BTK, is currently revolutionizing B cell lymphoma treatment. Most solid tumors do not express BTK. However, BTK has been identified as a critical pathway in synthetic lethality assays in MYC dependent tumor cell lines. Interestingly, BTK can also be expressed in myeloid cells downstream to Fcg receptors. Also, ibrutinib inhibits interleukin-2-inducible T cell kinase (ITK), with potential ability to shift the balance from Th2 to Th1 T cells. Therefore, ibrutinib might have immunomodulatory properties.
effects in addition to its direct effects on cancer cells. Interestingly, aggressive MYCN amplified neuroblastoma (NB) are also highly infiltrated by myeloid cells. In osteosarcoma (OS), osteoclasts are derived from myeloid lineage. Thus, BTK blockade could be of double interest in NB and OS through both tumor- and immune-targeted effects.

Methods
We analyzed BTK expression by quantitative RT-QPCR and FACS analysis in different NB or OS cell lines and tumors from patients. We assessed the impact of ibrutinib on macrophage differentiation assays. In vivo anti-tumoral ibrutinib efficacy was evaluated in a mouse transplantable NB and rat orthotopic OS syngeneic models.

Results
High levels of BTK expression in some neuroblastoma tumor samples and variable levels of expression of BTK in patient tumor cell lines were observed. Interestingly, ibrutinib therapy had a positive impact on Neuro2A syngeneic NB models. No synergic effect of ibrutinib and anti-PD-L1 therapy could be obtained. In the rat OS model, ibrutinib had also therapeutic activity with a positive impact on growth of orthotopic bone tumors. However, we observed a negative impact of ibrutinib therapy on the number of spontaneous OS lung metastasis. Ibrutinib had no impact on the viability and differentiation of M1, M2 or Mo-DCs. However, it impaired human M1 macrophages differentiation towards a more immunosuppressive M2 phenotype with lower CD86 and CD163 expression, but no variation of HLA-DR and PD-L1. This negative effect was reversed by a TLR4 agonist.

Conclusions
Ibrutinib therapy has shown a positive therapeutic efficacy on the growth of primary tumors of a mouse NB and rat OS syngeneic models. However, in the OS model, ibrutinib therapy resulted in a higher number of spontaneous lung metastasis. This effect could be due to a negative impact of ibrutinib on anti-tumoral M1 macrophage differentiation. However this effect can be blocked by TLR4 stimulation suggesting that combinations of ibrutinib with TLR agonists might be of interested in solid tumors.

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