Journal for ImmunoTherapy of Cancer 2017, **5**(Suppl 3):89
DOI 10.1186/s40425-017-0297-3

Journal for ImmunoTherapy of Cancer

MEETING ABSTRACTS Open Access

32nd Annual Meeting and Pre-Conference Programs of the Society for Immunotherapy of Cancer (SITC 2017): Late-Breaking Abstracts

National Harbor, MD, USA. 8-12 November 2017

Published: 7 December 2017

Oral presentations

Cellular Therapy Approaches

O35

The transcription factor Myb enhances CD8+ T cell stemness and polyfunctionality to promote curative antitumor immunity

Sanjivan Gautam[1], Yun Ji[1], Wei Zhu[2], Jessica Fioravanti[1], Jinhui Hu[1], Neal Lacey[1], James D Hocker[1], John Le Gall[1], Nga Voong[1], William G Telford[1], Philip Brohaun[2], Avinash Bhandoola[3], Hai-Hui Xue[4], Rahul Roychoudhuri[4], Nicholas P Restifo[1], Brandon Higgs[2], Timothy P Bender[5], Luca Gattinoni[1]
[1]National Cancer Institute, Bethesda, MD, USA; [2]Medimmune, Gaithersburg, MD, USA; [3]Carver College of Medicine, University of Iowa, Iowa City, IA, USA; [4]Cambridge University, Cambridge, UK; [5]Univeristy of Virginia, Charlottesville, VA, USA
Correspondence: Luca Gattinoni (Luca_Gattinoni@nih.gov)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**O35

Background
Following antigen encounter, $CD8^+$ T cells differentiate into effector and memory T cells to mediate pathogen clearance and provide life-long immunity. Although our understanding of the molecular mechanisms regulating $CD8^+$ T cell fate has expanded dramatically over recent years, the precise transcriptional programs underlying this process remains incompletely resolved. Myb is a transcription factor known to play a major role in stem cell and progenitor renewal and homeostasis, but its function in mature T cell differentiation is unknown. In this study, we demonstrate the role of Myb in $CD8^+$ T cell differentiation and antitumor function.
Methods
We employed $CD8^+$ T cells isolated from pmel-1 mice (which recognize the shared melanoma-melanocyte differentiation antigen gp100) carrying loxP-flanked Myb alleles and a fusion of Cre recombinase and the estrogen receptor T2 moiety, which retains Cre in the cytosol until tamoxifen is administered (pmel-1 Mybfl/flCre-ERT2 cells). Treating these mice with tamoxifen for several days immediately prior to $CD8^+$ T cell isolation ensured that pmel-1 $Myb^{-/-}$ T cells had undergone thymic development similar to their ERT2-Cre negative counterparts. pmel-1 $Myb^{-/-}$ or pmel-1 $Myb^{+/+}$ cells were adoptively transferred into wild-type mice infected with a recombinant strain of vaccinia virus encoding gp100 and antigen-specific $CD8^+$ T cell expansion and long-term persistence was monitored overtime. Evaluation of tumor treatment efficacy of $CD8^+$ T cells was performed in the pmel-1 model of adoptive cell therapy in the treatment of large established B16 melanomas.
Results
We demonstrate that Myb expression is progressively downregulated with T cell differentiation. We found that Myb deficient T cells were more prone to differentiate into short-lived $KLRG1^{hi}$ effector cells resulting in a severe impairment of $CD62L^{hi}$ stem cell-like memory cell formation, indicating that Myb is an essential regulator of T cell stemness. Conversely, enforced expression of Myb enhanced generation of $CD62L^{hi}$ memory cells, T cell polyfunctionality and recall responses, suggesting that these cells might be therapeutically superior for adoptive T cell therapy of tumors. Accordingly, Myb overexpressing T cells mediate enhanced antitumor immunity and promoted curative and long-lasting responses against large established vascularized tumors.
Conclusions
These findings identify Myb as a master regulator of $CD8^+$ T cell stemness and highlight the remarkable therapeutic potential of maneuvers aimed at increasing Myb activity in $CD8^+$ T cells.

Clinical Trials (Completed)

O36

First in human study with the CD40 agonistic monoclonal antibody APX005M in subjects with solid tumors

Melissa Johnson[1], Marwan Fakih[2], Johanna Bendell[1], David Bajor[3], Mihaela Cristea[2], Thomas Tremblay[4], Ovid Trifan[4], Robert Vonderheide[5]
[1]Sarah Cannon Research Institute/Tennessee Oncology, Nashville, TN, USA; [2]City of Hope, Duarte, CA, USA; [3]Case Western Reserve University School of Medicine, Cleveland, OH, USA; [4]Apexigen, Inc., San Carlos, CA, USA; [5]Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA
Correspondence: Ovid Trifan (otrifan@apexigen.com)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**O36

Background
Immune activating antibodies are being explored as the next generation of immuno-oncology therapeutics. Activation of CD40 can stimulate both innate and adaptive immune responses against cancer, making it an ideal target for the immune activating approach. CD40 engagement with its ligand CD154 leads to antigen presentation, maturation and expression of co-stimulatory molecules and cytokine production by antigen presenting cells (APC), which are requisite for optimal antigen-specific T-cell activation. Apexigen is developing APX005M – a humanized IgG_1 CD40 agonistic antibody that binds with high affinity to human CD40 (Kd=0.12nM) and carries an S267E mutation in the Fc region. APX005M recognizes a unique epitope that overlaps with the CD40 ligand binding sites and uses FcRyIIb to cluster and activate CD40 thus mimicking CD154 engagement.
Methods
In a "first in human" Phase 1 dose escalating clinical trial, APX005M was administered every 21 days at doses ranging from 0.0001 mg/kg to 1 mg/kg to 30 adult subjects with solid tumors. Primary objectives

were to evaluate the safety of APX005M, and to determine the maximum tolerated dose (MTD) and the recommended phase 2 dose (RP2D).

Results

APX005M demonstrated a dose-dependent APC activation (increases in expression of CD54, CD70, CD80, CD86, HLA-DR), dose dependent T cell activation and increases in circulating levels of IL-12, INF-γ, TNFα and IL-6. Five subjects had prolonged stable disease. Overall APX005M has been well tolerated; the majority of AEs were mild to moderate in severity, and the majority of serious AEs were considered unrelated to APX005M. The dose limiting toxicity of grade ≥ 3 cytokine release syndrome was observed in subjects receiving doses ≥ 0.6 mg/kg. The maximum administered dose of APX005M was 1 mg/kg. The dose of 0.3 mg/kg of APX005M was selected as the RP2D and represents the dose with maximum pharmacodynamic effects without grade > 2 toxicities. Increases in the dose of APX005M led to approximately dose-proportional increases in maximum serum concentration (Cmax) and area under the curve (AUC). No accumulation of APX005M was observed with every 21 day dosing.

Conclusions

APX005M produces dose-dependent activation of APCs and T cells at doses that are well tolerated. Toxicities generally associated with the on-target cytokine release are observed at doses above the doses that are required to activate APCs and T-cells. APX005M exhibits a highly differentiated and ideal profile for further clinical development as a single agent or in combination with other treatment modalities including immunomodulatory agents.

Clinical Trials (In Progress)

O37

Nivolumab in mismatch-repair deficient (MMR-d) cancers: NCI-MATCH Trial (Molecular Analysis for Therapy Choice) arm Z1D preliminary results

Nilofer Azad[1], Michael Overman[2], Robert Gray[3], Jonathan Schoenfeld[4], Carlos Arteaga[5], Brent Coffey[6], David Patton[7], Shuli Li[8], Lisa McShane[7], Larry Rubenstein[7], Lyndsay Harris[7], Robert Comis[9], Jeffrey Abrams[6], Paul M. Williams[6], Edith Mitchell[11], James Zweibel[6], Elad Sharon[7], Howard Streicher[7], Peter J. Dwyer[12], Stanley Hamilton[2], Barbara Conley[7], Alice P. Chen[13], Keith Flaherty[14]

[1]Johns Hopkins University, Baltimore, MD, USA; [2]MD Anderson, Houston, TX, USA; [3]Dana Farber Institute, Boston, MA, USA; [4]Brighan and Women's Cancer Center, Boston, MA, USA; [5]Vanderbilt, Nashville, TN, USA; [6]National Institute of Health, Rockville, MD, USA; [7]NCI, Rockville, MD, USA; [8]Harvard, Boston, MA, USA; [9]ECOG-ACRIN, Philadelphia, PA, USA; [11]Thomas Jefferson University, Philadelphia, PA, USA; [12]University of Pennsylvania, Philadelphia, PA, USA; [13]DTC National Cancer Institute, Bethesda, MD, USA; [14]Massachusetts General Hospital, Boston, MA, USA

Correspondence: Nilofer Azad; Stanley Hamilton (shamilto@mdanderson.org)

Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**O37

Background

The NCI-MATCH (Molecular Analysis for Therapy Choice) trial is the largest national study to date (1173 sites) for patients with relapsed/refractory solid tumors, lymphomas and myelomas, assigning rational targeted therapy based on individual tumor molecular alterations. Patients with mismatch repair-deficiency (MMR-d) may benefit from immune checkpoint inhibitor therapy secondary to increased mutational burden compared to MMR-proficient tumors. The anti-PD-1 inhibitor nivolumab has previously shown antitumor activity in MMR-d colorectal cancer; we hypothesized that nivolumab would have activity in patients with non-colorectal MMR-d cancers.

Methods

Eligibility for NCI-MATCH included relapsed/refractory cancers, good end-organ function, and ECOG performance status of ≤ 1. Patients enrolled were screened for molecular alterations by centralized testing on fresh biopsy tissue. MMR-d was defined by loss of nuclear expression of MLH1 or MSH2 by immunohistochemistry. Patients with MMR-d colorectal cancer were excluded. Patients received nivolumab 3 mg/kg q2weeks (28-day cycles) and 480 mg q4weeks past cycle 4. Disease reassessment was performed q2cycles. The primary endpoint of the study was RECIST 1.1 overall response rate (ORR). 35 enrolled patients were planned with the ORR compared against a null value of 5%. If the observed ORR was ≥5/31 (16%), the agent would be considered promising and worthy of further testing. The proposed design had power of 91.8% to find an agent promising assuming true OR rate was 0.25.

Results

4864 enrolled patients had interpretable results for MMR-d. 99 patients were MMR-d, 63 patients were assigned to nivolumab treatment, and 47 patients were treated (35:preplanned and 12:expansion). We report the preliminary results of the first 35 enrolled (70% MLH1 loss, 30% MSH2 loss). Minimum follow-up time for all patients was >6 months, median age was 60 y/o, and median prior therapies was 3. Common histologies included endometrioid endometrial (EEA: 10), prostate (6), and breast (3) cancer. 10 pts remain on treatment; 7 stopped treatment for AEs; 12 for progressive disease. The confirmed ORR was 24% (8/33 patients) with an additional 9/33 (27%) patients with stable disease. Three additional patients had unconfirmed responses [PD at next scan(1), off study prior to reassessment(1), and no follow-up scan yet(1)]. The disease histologies for the PR were prostate(3), EEA(2), breast(1), parathyroid(1), and gallbladder cancer(1). Estimated 6-month PFS was 43% and median OS has not been reached at this early time-point. Toxicity was predominantly low-grade.

Conclusions

We report the first results of a substudy of the NCI-MATCH trial. Nivolumab has promising activity in MMR-d, non-colorectal cancers.

Trial Registration

NCT02465060

O38

Nivolumab + Ipilimumab (N+I) vs Sunitinib (S) for treatment-naïve advanced or metastatic renal cell carcinoma (aRCC): results from CheckMate 214, including overall survival by subgroups

Robert J. Motzer[1], Nizar M. Tannir[2], David F. McDermott[3], Osvaldo Arén Frontera[4], Bohuslav Melichar[5], Elizabeth R. Plimack[6], Philippe Barthelemy[7], Saby George[8], Victoria Neiman[9], Camillo Porta[10], Toni K. Choueiri[11], Thomas Powles[12], Frede Donskov[13], Pamela Salman[14], Christian K. Kollmannsberger[15], Brian Rini[16], Sabeen Mekan[17], M. Brent McHenry[17], Megan Wind-Rotolo[17], Hans J. Hammers[18], Bernard Escudier[19]

[1]Memorial Sloan Kettering Cancer Center, New York, NY, USA; [2]University of Texas, MD Anderson Cancer Center Hospital, Houston, TX, USA; [3]Beth Israel Deaconess Medical Center, Dana-Farber/Harvard Cancer Center, Boston, MA, USA; [4]Centro Internacional de Estudios Clinicos, Santiago, Chile; [5]Palacky University, and University Hospital Olomouc, Olomouc, Czech Republic; [6]Fox Chase Cancer Center, Philadelphia, PA, USA; [7]Hôpitaux Universitaires de Strasbourg, Strasbourg, France; [8]Roswell Park Cancer Institute, Buffalo, NY, USA; [9]Davidoff Cancer Center, Rabin Medical Center, Petah Tikva, Israel, and Tel Aviv University, Tel Aviv, Israel; [10]IRCCS San Matteo University Hospital Foundation, Pavia, Italy; [11]Dana-Farber Cancer Institute, Brigham and Women's Hospital, and Harvard Medical School, Boston, MA, USA; [12]Barts Cancer Institute, Cancer Research UK Experimental Cancer Medicine Centre, Queen Mary University of London, Royal Free NHS Trust, London, UK; [13]Aarhus University Hospital, Aarhus, Denmark; [14]Fundación Arturo López Pérez, Santiago, Chile; [15]British Columbia Cancer Agency, Vancouver, British Columbia, Canada; [16]Cleveland Clinic Taussig Cancer Institute, Cleveland, OH, USA; [17]Bristol-Myers Squibb, Princeton, NJ, USA; [18]Sidney Kimmel Comprehensive Cancer Center, Johns Hopkins, Baltimore, MD, USA; [19]Gustave Roussy, Villejuif, France

Correspondence: Robert J. Motzer (motzerr@mskcc.org)

Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**O38

Background

We report results from the phase III CheckMate 214 study of N+I versus S for treatment-naïve aRCC.

Methods

Adults with clear-cell aRCC, measurable disease, Karnofsky performance status ≥70, and available tumor tissue were eligible. Patients were randomized 1:1 (stratified by IMDC score; region) to N 3 mg/kg + I 1mg/kg every 3 weeks for four doses followed by N 3 mg/kg every 2 weeks, or

S 50 mg daily orally for 4 weeks (6-week cycles). Co-primary endpoints were objective response rate (ORR), progression-free survival (PFS) per independent committee (IRRC), and overall survival (OS), all in intermediate- and poor-risk patients. Overall α for treatment effect was 0.05 (allocated as 0.001 ORR, 0.009 PFS, 0.04 OS).

Results

1,096 patients were randomized (N+I: n=550; S: n=546); 425 (N+I) and 422 (S) with intermediate/poor risk. With ~17.5 months minimum follow-up, confirmed ORR in intermediate/poor-risk patients was 41.6% (9.4% complete response [CR]) vs 26.5% (1.2% CR) for N+I vs S (P<0.0001); median duration of response was not reached (NR; 95% CI, 21.82-NR) vs 18.2 months (95% CI, 14.82-NR), respectively (Table 1). Median PFS with N+I vs S in intermediate/poor-risk patients was 11.6 vs 8.4 months (hazard ratio [HR] 0.82, P=0.0331, Table 1). At the first prespecified interim OS analysis, the Data Monitoring Committee recommended stopping the study early for statistically significant superiority in OS with N+I vs S (median not reached vs 26.0 months [HR 0.63], P<0.0001, Table 1). ORR favored N+I over S in intermediate/poor-risk patients irrespective of baseline tumor PD-L1 expression, while a PFS benefit with N+I vs S was seen only in patients with PD-L1 ≥1%. OS favored N+I over S across all prespecified subgroups (data to be presented), including baseline PD-L1 expression status. In all treated patients, drug-related AEs occurred in 509/547 (93% any grade, 46% grade 3-4) with N+I vs 521/535 (97% any grade, 63% grade 3-5) with S, including 22% vs 12% with AEs leading to discontinuation. Death occurred in 159 N+I arm patients (7 [1%] drug-related) and 202 S arm patients (4 [1%] drug-related).

Conclusions

This phase III study showed statistically significant OS benefit, significantly higher ORR, and numerically longer PFS for N+I vs S with a manageable safety profile in intermediate- and poor-risk patients with aRCC, supporting the use of N+I as a new first-line standard-of-care treatment option for these patients. OS benefit with N+I was seen irrespective of baseline PD-L1 status and was observed consistently across other subgroups.

Trial Registration

ClinicalTrials.gov Identifier: NCT02231749

Table 1 (abstract O38). See text for description

	Intermediate/poor risk					
	N+I	S	N+I PD-L1 <1%	S PD-L1 <1%	N+I PD-L1 ≥1%	S PD-L1 ≥1%
	N=425	N=422	N=284 (74%)[a]	N=278 (71%)[a]	N=100 (26%)[a]	N=114 (29%)[a]
ORR per IRRC, n (%) [95% CI]	177 (42) [37-47]	112 (27) [22-31]	106 (37) [32-43]	79 (28) [23-34]	58 (58) [48-68]	25 (22) [15-31]
	P<0.0001		Odds ratio (95% CI)=1.50 (1.04-2.17)[b]		Odds ratio (95% CI)=4.92 (2.61-9.34)[b]	
Median PFS per IRRC, months (95% CI)	11.6 (8.7-15.5)	8.4 (7.0-10.8)	11.0 (8.1-14.9)	10.4 (7.5-13.8)	22.8 (9.4-NR)	5.9 (4.4-7.1)
HR (CI)	0.82 (99.1% CI, 0.64-1.05)		1.00 (95% CI, 0.80-1.26)		0.46 (95% CI, 0.31-0.67)	
	P=0.0331		P=0.9672[b]		P<0.0001[b]	
Median OS, months (95% CI)	NR (28.2-NE)	26.0 (22.1-NE)	NE (28.2-NE)	NE (24.0-NE)	NE	19.6 (14.8-NE)
HR (CI)	0.63 (99.8% CI, 0.44-0.89)		0.73 (95% CI, 0.56-0.96)		0.45 (95% CI, 0.29-0.71)	
	P<0.0001		P=0.0249[b]		P=0.0006[b]	

[a]Percentage based on number of PD-L1 evaluable patients. [b]Exploratory unstratified analysis. NE, not estimable

O39

Phase I study of E7046, a novel PGE2 receptor type 4 inhibitor, in patients with advanced solid tumors with high myeloid infiltrate: effects on myeloid- and T-lymphoid cell-mediated immunosuppression

Aurelien Marabelle[1], Aparna Parikh[2], Geoffrey Shapiro[3], Andrea Vargas[1], Aung Naing[4], Funda Meric-Bernstam[4], Larisa Reyderman[5], Xingfeng Bao[5], Terri Binder[5], Min Ren[5], Amy Siu[5], Lucy Xu[5], Mingjie Liu[5], Satish Dayal[5], Vijay Bhagawati-Prasad[5], Ilian Tchakov[5], Takashi Owa[5], Chean Eng. Ooi[5], David Sanghyun Hong[4]

[1]Gustave Roussy Institute, Villejuif, France; [2]Massachusetts General Hospital, Boston, MA, USA; [3]Dana-Farber Cancer Institute, Boston, MA, USA; [4]The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [5]Eisai Inc., Woodcliff Lake, NJ, USA

Correspondence: Larisa Reyderman (larisa_reyderman@eisai.com)

Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**O39

Background

E7046 is a selective inhibitor of the prostaglandin E_2 (PGE_2) receptor-type-4, EP4, which transduces potent immunosuppressive activity of PGE_2 in both myeloid cells and T-lymphoid cells in the tumor microenvironment. In preclinical studies, E7046 reversed PGE_2-mediated inhibition of monocyte differentiation towards anti-tumorigenic antigen presenting cells and facilitated tumoral recruitment and activation of cytotoxic T-cells. Here, we present initial clinical, pharmacokinetic and pharmacodynamic results from a first-in-human study of single agent E7046 in patients with selected cancer types having high myeloid cell infiltration.

Methods

E7046 was administered orally, once-daily, in 21-day cycles in sequential dose-escalating cohorts of 6 pts each at 125, 250, 500 and 750mg. Tumor responses were evaluated by irRECIST and metabolic responses by ^{18}FDG-PET. Modulation of immune response was assessed in pre- and post-treatment tumor biopsies by immunohistochemistry, and in blood samples by TaqMan Low Density Array and Meso Scale Discovery assays. Blood samples were collected for PK analysis.

Results

Thirty patients were treated with no dose-limiting toxicities observed. The most common adverse events were fatigue (37%), diarrhea (33%), and nausea (30%). Grade 3/4 AEs in >1 patient were abdominal pain (3 patients, at 250 mg, 750 mg) and vomiting (2 patients, at 125 mg, 250 mg). Grade 3/4 treatment-related AEs occurred in 4 patients (rash in 2 patients, and diarrhea, allergic reaction, anaphylaxis, hypersensitivity, and hyperuricemia, in 1 patient each). Four patients discontinued treatment due to an AE (bowel obstruction, allergic reaction, abdominal pain, acute renal failure). No objective tumor responses were reported. Duration of treatment of ≥20 wks with best response of stable disease (SD) was observed in 5 patients, 3 of these had partial metabolic responses. E7046 exposure was dose-proportional up to the 500 mg dose with a plateau at 750 mg. Elimination half-life (11 hr) justified once-daily dosing. Treatment with E7046 significantly increased tumor $CD3^+$ and $CD8^+$ T-cell infiltration and expression of the T-effector cell-recruiting chemokine CXCL10 in blood. Gene expression analysis in blood showed modulation of EP4 signaling genes (including IDO1, EOMES, PD-L1). Longer duration of therapy with SD was associated with higher baseline tumor infiltrate of $CD8^+$ T-cells and $CD163^+$ macrophages.

Conclusions

E7046 demonstrated favorable tolerability profile with preliminary evidence of anti-tumor activity and immune modulation in tumor and peripheral blood. MTD was not reached. Further studies testing E7046 in combination with other agents are planned.

Trial Registration

NCT-02540291

O40

Interim safety analysis of Cancer Immunotherapy Trials Network – 12 (CITN-12): A phase 1 study of Pembrolizumab in patients with HIV and relapsed, refractory or disseminated malignancies

Thomas S. Uldrick[1], Priscila H. Gonçalves[1], Steven P. Fling[2], Karen Aleman[1], Brinda Emu[3], Marc S. Ernstoff[4], Ashley Jackson[4], Judith Kaiser[2], Holbrook E. Kohrt[5], Andreanne Lacroix[2], Matthew Lindsley[1], Lisa M. Lundgren[2], Kathryn Lurain[1], Matthew Madura[3], James S. Outland[6], Chris Parsons[6], Elad Sharon[1], Robert Yarchoan[1], Martin A. "Mac" Cheever[2]
[1]National Cancer Institute, Bethesda, MD, USA; [2]Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [3]Yale University, New Haven, CT, USA; [4]Roswell Park Cancer Institute, Buffalo, NY, USA; [5]Stanford University, Palo Alto, CA, USA; [6]Louisiana State University Health Science Center, New Orleans, LA, USA
Correspondence: Thomas S. Uldrick (uldrickts@mail.nih.gov)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**O40

Background
Anti-PD-1 and anti-PD-L1 antibodies are approved for multiple indications and are becoming mainstays of cancer therapy. However, patients with HIV have been excluded from clinical trials evaluating these agents largely due to safety concerns.

Methods
CITN-12 is a multicenter study of pembrolizumab in patients with HIV and advanced cancers not curable by standard therapies. Three parallel cohorts are accruing based on CD4+ counts; 1: 100-199, 2: 200-350, and 3: >350 cells/uL. Additional eligibility criteria: >4 weeks antiretroviral therapy (ART), HIV viral load <200 copies/mL, no uncontrolled infections including hepatitis B and C, ECOG performance status 0-1. Treatment: pembrolizumab 200mg intravenously every 3 weeks for up to 2 years. The primary objective is to assess safety and tolerability by summarizing adverse events (AEs) graded by CTCAEv4 and evaluating HIV viral load and CD4+ counts. Immune mediated adverse events are managed using standard guidelines. We performed an interim analysis of treatment emergent adverse events at least possibly related to pembrolizumab (rTEAEs), serious AEs, and HIV viral load and CD4 + counts on therapy.

Results
17 patients; Cohort 1 (4), Cohort 2 (9), Cohort 3 (4); were accrued starting April 2016 and followed through May 2017. Characteristics: 1 woman, 16 men; median age 56 years (range 43-77); white (13), African American (3), Hispanic (1); HIV viral load <20 copies/mL (94%). Cancers: non-Hodgkin lymphoma (3), Kaposi sarcoma (1), anal cancer (5), head and neck (1), lung (2), bladder (1), hepatocellular (1), pancreatic (1), cholangiocarcinoma (1). Median number prior therapies 1 (range 0-4), prior radiation (71%). Safety observed over a total of 100 cycles, median 4 (range 1-20). 82 rTEAEs were observed and comparable between cohorts. 93% were grade 1-2. Ten primary serious AEs were observed, 2 possibly attributable to pembrolizumab, both occurring in the setting of progressive malignancy. Immune mediated AEs managed with levothyroxine or prednisone included subclinical hypothyroidism 6 (35%), pneumonitis (2) and liver test elevations (2). Median $CD4^+$ counts increased over time, changes did not reach statistical significance. HIV remained suppressed on ART in all patients.

Conclusions
Pembrolizumab has an acceptable safety profile to date in CITN-12. Standard therapy with anti-PD1 is appropriate for FDA-approved indications in patients with HIV. Patients with HIV who meet appropriate immune eligibility criteria for a given cancer should be included in immunotherapy studies. Further evaluation of checkpoint inhibitors in HIV-associated tumors is justified.

Trial Registration
clinicaltrials.gov NCT02595866

Combination Therapy (IO/IO, IO/Standard of Care, IO/Other)

O41

Preliminary antitumor and immunomodulatory activity of BMS-986205, an optimized indoleamine 2,3-dioxygenase 1 (IDO1) inhibitor, in combination with nivolumab in patients with advanced cancers

Jason J. Luke[1], Karen Gelmon[2], Russell K. Pachynski[3], Jayesh Desai[4], Victor Moreno[5], Josep M. Tabernero[6], Carlos A. Gomez-Roca[7], Quincy Chu[8], Paul Basciano[9], Penny Phillips[9], Li Zhu[9], Zhaohui Liu[9], Lillian L. Siu[10]
[1]University of Chicago Medical Center, Chicago, IL, USA; [2]University of British Columbia, BC Cancer Agency, Vancouver, British Columbia, Canada; [3]Washington University School of Medicine, St. Louis, MO, USA; [4]Peter MacCallum Cancer Centre and Royal Melbourne Hospital, Melbourne, Australia; [5]START Madrid-FJD, Hospital Universitario Fundación Jiménez Díaz, Madrid, Spain; [6]Vall d'Hebron University Hospital, Barcelona, Spain; [7]Institut Universitaire du Cancer, Oncopole, Toulouse, France; [8]Cross Cancer Institute, University of Alberta/Alberta Health Services, Edmonton, Alberta, Canada; [9]Bristol-Myers Squibb, Princeton, NJ, USA; [10]Princess Margaret Cancer Centre, Toronto, Ontario, Canada
Correspondence: Jason J. Luke (jluke@medicine.bsd.uchicago.edu)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**O41

Background
Checkpoint inhibitors have transformed cancer care, but extending those benefits to more patients requires additional approaches. IDO1 allows tumor escape through kynurenine production, which decreases immune cell tumor infiltration/function and increases regulatory T-cell numbers. Anti–PD-1 treatment upregulates IDO1, supporting a rationale for combining nivolumab (anti–PD-1) with an IDO1 inhibitor. BMS-986205 is a selective, potent, once-daily, oral IDO1 inhibitor with a potentially best-in-class pharmacokinetic/pharmacodynamic/safety profile in combination with nivolumab that was previously disclosed (NCT02658890) [1]. Here we present updated safety and preliminary efficacy and pharmacodynamic data.

Methods
Dose-escalation methods were previously described [1]. During cohort expansion in this phase 1/2a open-label study, patients with advanced cancers were treated with BMS-986205 100 or 200 mg orally once daily + nivolumab 240 mg IV Q2W or 480 mg IV Q4W. Objectives included safety, preliminary antitumor activity, and pharmacodynamics (including immunomodulatory assays).

Results
As of the July 20, 2017, data cutoff, safety data were available for 216 patients across the study. Maximum tolerated dose during escalation was 200 mg; at 400 mg, 2/4 patients experienced dose-limiting toxicities (grade 3 AST/ALT increased; grade 2 anemia, fatigue). Treatment-related AEs occurred in 47% of patients (11% grade 3/4), and 4 patients (2%) discontinued due to study drug toxicity; the safety profile was generally consistent with that previously reported for nivolumab monotherapy. In the bladder cancer cohort, among 15 heavily pretreated patients (39% received ≥2 prior regimens), 5 partial responses (PRs), 3 stable disease (SD), and 6 progressive disease (PD, including a patient with prior anti–PD-[L]1 therapy) were reported, with 1 death prior to assessment. In the cervical cancer cohort, among 17 heavily pretreated patients (52% received ≥2 prior regimens), 3 PRs, 5 SD, and 7 PD were reported, with 2 deaths prior to assessment. Within 39 paired pre- vs on-treatment tumor samples across various tumor types, BMS-986205 plus nivolumab decreased kynurenine and increased the percentage of proliferating $CD8^+$ T cells.

Conclusions
BMS-986205 plus nivolumab was well tolerated, increased proliferating $CD8^+$ T cells in tumors, and demonstrated preliminary antitumor

activity. Updated efficacy, safety, and pharmacodynamic data will be presented.

Trial Registration

ClinicalTrials.gov, NCT02658890

Consent

Not applicable

References

1. Siu L, et al. AACR 2017, abstract CT116.

O42

First-in-human phase 1 dose escalation and expansion of a novel combination, anti–CSF-1 receptor (cabiralizumab) plus anti–PD-1 (nivolumab), in patients with advanced solid tumors

Zev Wainberg[1], Sarina Piha-Paul[2], Jason Luke[3], Edward Kim[4], John Thompson[5], Nicklas Pfanzelter[6], Michael Gordon[7], Drew Rasco[8], Amy Weise[9], F. Stephen Hodi[10], Sandeep Inamdar[11], Serena Perna[12], Christy Ma[11], Janine Powers[11], Michael Carleton[12], Hong Xiang[11], Lei Zhou[11], Helen Collins[11], Yeonju Lee[11], James Lee[13], Jennifer Johnson[14], Carolyn Britten[15], Majid Ghoddusi[11]

[1]UCLA School of Medicine, Santa Monica, CA, USA; [2]The University of Texas MD Anderson Cancer Center, Houston, TX, USA; [3]The University of Chicago Medicine, Chicago, IL, USA; [4]UC Davis Comprehensive Cancer Center, Sacramento, CA, USA; [5]Seattle Cancer Center Alliance, Seattle, Washington, CA, USA; [6]Rush University Medical Center, Chicago, IL, USA; [7]HonorHealth Scottsdale Shea Medical Center, Scottsdale, AZ, USA; [8]South Texas Accelerated Research Therapeutics, San Antonio, TX, USA; [9]Karmanos Cancer Center, Detroit, MI, USA; [10]Dana-Farber Cancer Institute, Boston, MA, USA; [11]FivePrime Therapeutics, South San Francisco, CA, USA; [12]Bristol-Myers Squibb, Lawrenceville, NJ, USA; [13]University of Pittsburg Medical Center, Pittsburg, PA, USA; [14]Thomas Jefferson University Hospital, Philadelphia, PA, USA; [15]Medical University of South Carolina, Charleston, SC, USA

Correspondence: Sandeep Inamdar (sandeep.inamdar@fiveprime.com)

Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**O42

Background

Resistance to immunotherapy may be related to activity of several immunosuppressive cell types. Depletion of tumor-associated macrophages (TAMs) may promote a pro-inflammatory state, increasing antitumor T-cell responses. Cabiralizumab, a humanized IgG4 monoclonal antibody, binds to CSF-1 receptor and blocks cytokine signaling that is needed for TAM activation and survival, leading to TAM depletion. The combination of cabiralizumab plus anti–PD-1 may work synergistically by modifying the immunosuppressive tumor environment while simultaneously suppressing the PD-1 checkpoint pathway. This is the first clinical disclosure of safety, pharmacokinetics, and pharmacodynamics of this novel combination, along with preliminary evidence of antitumor activity in pancreatic cancer (NCT02526017).

Methods

In phase 1a dose escalation, patients with advanced solid tumors were treated with cabiralizumab 1, 2, 4, and 6 mg/kg alone or combined with nivolumab 3 mg/kg, both given IV Q2W, in a 3+3+3 design.

Results

As of August 1, 2017, 205 patients were treated with the combination. Most received cabiralizumab 4 mg/kg Q2W plus nivolumab 3 mg/kg Q2W. Cabiralizumab, alone or with nivolumab, demonstrated target-mediated clearance and dose-dependent increase in exposure, and pharmacodynamic activity as evidenced by reduced circulating $CD14^+$ $CD16^+$ nonclassical monocytes. Grade 3–5 treatment-related AEs (TRAEs) attributed to cabiralizumab occurred in 43% of patients, with 13% of patients discontinuing due to AEs. Elevations in creatinine phosphokinase (14%) and AST (5%) were among the most common grade 3 TRAEs but were secondary to cabiralizumab's depletion of macrophages, which would otherwise metabolize these enzymes, and were reversible without significant clinical sequelae. Among the cohort of prior chemotherapy–treated and immunotherapy-naive patients with pancreatic cancer, 31 were efficacy evaluable. There were 3 confirmed partial responses in microsatellite-stable patients (293, 275+, and 168+ days on study) and 1 prolonged stable disease (182 days); 1 patient treated beyond progressive disease experienced >40% reduction in baseline target lesions (247 days on study). The 6-month disease control rate was 13%, and objective response rate was 10%. Studies in a larger pancreatic cohort and other tumor types are ongoing, and preliminary translational biomarker data will be presented.

Conclusions

Cabiralizumab plus nivolumab, a mechanistically novel immunotherapy combination, demonstrated a tolerable safety profile across several cohorts and promising preliminary antitumor activity in pancreatic cancer. These results also show a potential immunotherapeutic strategy to treat patients with tumors resistant to anti–PD-1 blockade.

Trial Registration

NCT02526017

Tumor Microenvironment (Mechanisms and Therapies)

O43

Monotherapy dose escalation clinical and translational data from first-in-human study in advanced solid tumors of IPI-549, an oral, selective, PI3K-gamma inhibitor targeting tumor macrophages

David Hong[1], Anthony Tolcher[2], Ryan Sullivan[3], Geoffrey Shapiro[4], Bartosz Chmielowski[5], Antoni Ribas[5], Les Brail[6], Joseph Pearlberg[6], Suresh Mahabhashyam[6], Lucy Lee[6], Claudio Dansky Ullmann[6], Brenda O'Connell[6], Jeffery Kutok[6], Michael Postow[7], Jedd Wolchok[7]

[1]UT MD Anderson Cancer Center, Houston, TX, USA; [2]START, San Antonio, TX, USA; [3]Massachusetts General Hospital, Boston, MA, USA; [4]Dana Farber Cancer Institute, Boston, MA, USA; [5]University of California, Los Angeles, Santa Monica, CA, USA; [6]Infinity Pharmaceuticals, Inc., Cambridge, MA, USA; [7]Memorial Sloan Kettering Cancer Center, New York, NY, USA

Correspondence: Jeffery Kutok (jeff.kutok@infi.com)

Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**O43

Background

IPI-549 is a potential first-in-class, oral, selective PI3K-gamma inhibitor being developed as an immuno-oncology therapeutic in multiple cancer indications. Preclinical research demonstrated that IPI-549 results in transcriptional reprogramming M2, pro-tumor macrophages to the M1, anti-tumor phenotype. In preclinical tumor models, IPI-549 was active as a monotherapy and was able to overcome checkpoint inhibitor (CPI) resistance in CPI-insensitive models. These preclinical data provide a strong rationale for the ongoing Phase 1/1b study.

Methods

This study (NCT02637531) is being conducted to evaluate the safety, tolerability, pharmacodynamics and pharmacokinetics to determine the recommended dose and activity of IPI-549 as monotherapy and in combination with nivolumab in patients with advanced solid tumors. The study design includes four parts: 1) monotherapy dose escalation 2) combination dose escalation of IPI-549 with nivolumab 3) monotherapy expansion, and 4) combination expansion in specific tumor types with de novo or acquired resistance to checkpoint inhibitors. Pre- and on-treatment blood samples are being obtained in all patients to perform flow cytometry, gene expression, and serum cytokine and chemokine analysis to better understand the biological effect of IPI-549 on immune cells and to identify correlations with any clinical response. Pre- and on-treatment biopsies are being mandated in the expansion cohorts to evaluate the effect of IPI-549 on the tumor microenvironment.

Results
A total of 19 patients have been enrolled (18 evaluable) in the monotherapy dose escalation phase (10, 15, 20, 30, 40, 60 mg qd). No DLTs, or drug related SAEs have been observed. The majority of treatment-emergent adverse events were low grade (grade 1-2). The most common (≥2 patients) drug related treatment-emergent adverse events are alanine aminotransferase increase, rash maculo-papular, white blood cell count decrease, and headache. Durable clinical benefit has been observed, with 8 patients able to remain on treatment ≥16 weeks, including 2 patients on study for ≥52 weeks. The PK profile of IPI-549 has favorable characteristics including dose proportionality. PD analysis demonstrates full and sustained suppression of PI3K-γ at 60 mg qd. Translational studies performed on peripheral blood demonstrated increased activation of circulating myeloid cells in patient subsets, as well as, evidence of interferon-gamma mediated immune stimulation after IPI-549 treatment. Detailed PK, PD, translational, safety, and efficacy data will be presented.
Conclusions
The monotherapy dose escalation has completed enrollment, demonstrating favorable tolerability, evidence of immune modulation, and PK/PD defining 60 mg qd as the monotherapy expansion dose. The monotherapy expansion phase in solid tumors is actively enrolling.

Poster presentations

Biomarkers and Immune Monitoring

P509

Modelling oscillatory human immune system dynamics of point-of-care biomarkers for targeting/sequencing vaccine immuno-chemotherapy in advanced melanoma

Brendon Coventry, Carrie Cooper, Mohsen Dorraki, Andrew Allison, Azhar Iqbal, Derek Abbott
University of Adelaide, Adelaide, Australia
Correspondence: Brendon Coventry (brendon.coventry@adelaide.edu.au)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P509

Background
Vaccine immunotherapy for advanced melanoma has demonstrated long-term survival (>15 years) and Complete Responses (CR=17%)[1]. Recent clinical responses from 3-5 years using anti-PD-1, anti-CTLA4 and other immunotherapies represent major advances over standard cytotoxic chemotherapies with 1-2% ≥1 year survivals. However, even with the best approved immunotherapies most patients still do not obtain CR's or achieve long-term survival[2]. Toxicity remains a serious problem[2]. Pre-treatment identification of responders and non-responders remains truly enigmatic. Optimal coordination with the individual patient's dynamic immune response has been proposed to better target treatment[3].
Methods
Patients with advanced melanoma received vaccine immunotherapy alone, or immuno-chemotherapy, and daily immune point-of-care monitoring with serum C-reactive protein as an inflammatory biomarker. The null hypothesis was stability. Immune oscillatory behavior was tested mathematically. Advanced mathematical analysis was performed to determine the validity of the null hypothesis.
Results
Oscillatory biochemical inflammatory marker behavior was identified in most patients during therapy, and investigated for correlation with clinical outcome. Monitoring periods containing ≥5 measurements, and of at least 3 in number, are required for statistically defining oscillatory cyclical behavior in humans with cancer.
Conclusions
The implications of these findings are that immunomodulatory therapies (eg. pathway inhibitors, cytotoxics, radiation & perhaps surgery) may require individualized tailoring to coordinate with immune system phase dynamics at delivery to direct immune control and influence clinical efficacy. These findings of immune fluctuation might explain why predictive biomarker identification has been so elusively problematic, and why toxicity is often variable and unpredictable.
Trial Registration
Australian Clinical Trials Registry [ACTRN] 12605000425695

References
1. Coventry BJ, Lilly C, Hersey P, Michele A, Bright R, et al. Prolonged repeated vaccine immuno-chemotherapy induces long-term clinical responses and survival for advanced metastatic melanoma. J Immunother. Cancer 2014; 2: 9-17.
2. Coventry BJ, Baum D, Lilly CA, et al. Long-term survival in advanced melanoma patients using repeated therapies: successive immunomodulation improving the odds? Cancer Manag. Res. 2015 Apr 29; 7:93-103.
3. Coventry BJ, Ashdown ML, Quinn MA, Markovic SN, Yatomi-Clarke, Robinson AP, et al. CRP identifies homeostatic immune oscillations in cancer patients: potential treatment targeting tool? J Transl Med 2009; 7: 102. Review.

P510

Multitumor profiling of lymphocyte activation gene 3 (LAG-3) and association with immune cell phenotypes

Robin Edwards[1], Cyrus Hedvat[1], John Cogswell[1], Darren Locke[1], George Lee[1], Vipul Baxi[1], Patrik Vitazka[1], Peter Szabo[1], Chelsea Jin[1], Dimple Pandya[1], Keyur Desai[1], Roland Meier[1], Matt Maurer[1], Donald Jackson[1], Petra B Ross-MacDonald[1], Megan Wind-Rotolo[1], Abdel Saci[1], Parminder Mankoo[1], Jean-Marie Bruey[1], Christopher Harbison[1], Mark Selby[1], Alan Korman[1], Kent Thudium[1], Riyue Bao[2], Janis M. Taube[3], Jason Luke[2]
[1]Bristol-Myers Squibb, Princeton, NJ, USA; [2]University of Chicago Medical Center, Chicago, IL, USA; [3]Johns Hopkins University School of Medicine, Sidney Kimmel Comprehensive Cancer Center and Bloomberg-Kimmel Institute for Cancer Immunotherapy, Baltimore, MD, USA
Correspondence: Robin Edwards (robin.edwards2@bms.com); Peter Szabo (peter.szabo@bms.com)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P510

Background
LAG-3 negatively regulates T-cell activation, is expressed on exhausted T cells, and may promote regulatory T-cell activity. By limiting antitumor T-cell activation, LAG-3 may contribute to immunotherapy nonresponsiveness, as observed in patients with melanoma who progressed during prior anti-PD-(L)1 therapy [1]. Here we describe first results from comprehensive multitumor profiling using quantitative immunohistochemistry (IHC) to characterize expression of LAG-3 and its ligand, MHCII, in the context of inflammation markers, as well as a bioinformatic investigation of LAG-3 using The Cancer Genome Atlas (TCGA).
Methods
Urothelial, gastric, non-small cell lung cancer, renal cell carcinoma (RCC), squamous cell carcinoma of the head and neck, and melanoma tumor specimens (N=245) were stained by IHC for LAG-3, CD8, FOXP3, CD68, CD163, PD-L1, and MHCII. The proportion of total nucleated cells in the tumor microenvironment expressing a given marker was determined using image analysis, and unsupervised clustering was used to identify subgroups within tumor types. A 160-gene T-cell-inflamed signature was applied to TCGA RNA-sequencing data to assess correlations between LAG-3 and IFNγ-induced gene expression.
Results
Unsupervised clustering of IHC results revealed inflammation-high, -moderate, and -low subgroups, and LAG-3 expression generally correlated with the level of inflammation: CD8 (r=0.65); CD68, CD163, and FOXP3 (r=0.49-0.53). MHCII tumor-cell expression was observed in inflammation-high and -low tumors and did not correlate with PD-L1 positivity, whereas LAG-3 was significantly higher in tumors with MHCII expression ≥1% vs <1% (P=0.001). In 6 individual tumors with heterogeneous MHCII tumor-cell expression, LAG-3 was higher in $MHCII^{hi}$ (>70%) vs $MHCII^{lo}$ (<10%) regions (P range=0.001-0.070).

TCGA analysis was consistent with IHC analyses, demonstrating a strong correlation of LAG-3 mRNA expression with CD8, PD-1, and CTLA-4 (r=0.81; r=0.87; r=0.69), moderate correlation with PD-L1 and MHCII (r=0.47; r=0.58), and correlation of LAG-3, CD8, and PD-1 mRNA expression with T-cell-inflamed gene signatures across tumor types. Exploratory analyses of clinical trials in RCC and melanoma showed increased mean LAG-3 mRNA expression after nivolumab (anti-PD-1) treatment.

Conclusions

LAG-3 expression correlates with tumor inflammation and is enriched in tumors with MHCII^{hi} tumor cells. Preliminary data suggest that preferential localization of LAG-3-expressing leukocytes to MHCII^{hi} tumor regions potentially serves as a mechanism for LAG-3 checkpoint pathway activation. These findings, and the observation that nivolumab may induce LAG-3 expression, underscore the importance of studies to define predictive biomarker profiles for relatlimab (anti-LAG-3) therapy in PD-1-naive and -progressed patients.

References

1. Ascierto, et al. *J Clin Oncol*. 2017; 35(suppl) [abstract 9520].

P511

Evaluating immune responses of patients receiving the DPV-001 cancer vaccine

Christopher Paustian[1], Yoshinobu Koguchi[2], Adi Mehta[3], Fridtjof Lund-Johansen[3], Sam Bookhardt[1], Purvish Patel[4], Danielle Svancara[4], Tarsem Moudgil[2], Christopher Dubay[2], William Redmond[2], Carlo Bifulco[2], Kyle Happel[5], Brian Boulmay[5], Augusto Ochoa[5], Brenda Fisher[2], Eileen Mederos[5], Hong Ming Hu[1], Traci Hilton[1], Bernard Fox[1], Walter Urba[2], Rachel Sanborn[2]
[1]UbiVac, Portland, OR, USA; [2]Robert W. Franz Cancer Center, Earle A. Chiles Research Institute, Providence Cancer Center, Portland, OR, USA; [3]Oslo University Hospital Rikshospitalet, Oslo, Norway; [4]Quanterix, Lexington, MA, USA; [5]LSUHSC School of Medicine, New Orleans, LA, USA
Correspondence: Traci Hilton (traci.hilton@ubivac.com)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P511

Background

DPV-001 DRibble® is a dendritic cell-targeted microvesicle (proteasome blocked autophagosome) vaccine derived from adenocarcinoma and mixed histology cancer cell lines. It contains multiple TLR agonists and >130 potential NSCLC antigens, many as prospective altered-peptide ligands or neoantigens. We hypothesize that the efficacy of DRibbles' vaccination can be attributed to tumor-derived short-lived proteins (SLiPs) and defective ribosomal products (DRiPs). SLiPs and DRiPs are typically not processed and presented by professional antigen presenting cells therefor the host may be less tolerant. The large number of potential antigens in the vaccine necessitate new techniques to monitor responses.

Methods

Patients received induction cyclophosphamide, then 7 vaccines at 3-week intervals. First vaccine was given intranodally; subsequent vaccines intradermally. Patients were randomized to receive DRibble alone (A), or with imiquimod (B) or GM-CSF (C). PBMCs and serum were collected at baseline and at each vaccination to assess changes in antibodies (Protoarray, microsphere affinity proteomics (MAP)) and cytokines (Quanterix), peripheral lymphocytes populations (flow cytometry) and TCR repertoires (Adaptive immunoSEQ).

Results

13 pts were enrolled (Arm A: 5; B: 4; C: 4). Serum cytokines (IL1β, IL8, IFNα, IFNγ, IL6, IL17 and TNFα) were measured and normalized and the sum plotted against time. The slope of the resultant trend line was used as an indicator of either increased (positive slope) or decreased (negative slope) systemic inflammation. DPV-001 alone did not change net cytokine load while the addition of the adjuvant immiquimod increased, and the addition of GM-CSF significantly lessened the slope. Vaccination induced or increased IgG Ab responses against targets over-expressed by NSCLC, correlating with activated Th1 cells in whole blood samples. New or augmented Ab responses were observed with continued vaccination. Pts receiving DPV-001 had a significant ($p<0.04$) increase in total (CD4 + CD8) TCRs that increased 10 fold over baseline compared to normal controls (independent from trial, n=3) and the increase in CD4 clones was similar to that seen following Ipilimumab (melanoma pts, independent from trial, n=9). Patients receiving DPV-001 alone had the largest increase in CD8 T cell clones.

Conclusions

Vaccination with DPV-001 increased the number of strong antibody responses to antigens commonly over-expressed in NSCLC and expanded populations of T cells. DPV-001 alone provided the greatest increase in CD8 TCRs. Interval monitoring of PBMCs/serum identified the complexity of the immune response to this vaccine and suggests possibilities to boost or sustain immunity.

Trial Registration

NCT01909752.

P512

Deep immunoprofiling of rare T-cell populations from clinical samples

Mark Knappenberger[1], Sri Krishna[1], Kit Fuhrman[2], Cheryl Tan[2], Douglas Hinerfeld[2], Karen S. Anderson[1]
[1]The Biodesign Institute at Arizona State University, Tempe, AZ, USA; [2]Nanostring Technologies, Inc., Seattle, WA, USA
Correspondence: Karen S. Anderson (karen.anderson.1@asu.edu)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P512

Background

The complexity and heterogeneity of the immune system combined with its central role in tumor biology necessitates sophisticated analytical approaches to reveal molecular mechanisms, novel therapeutic targets and clinically relevant biomarkers. T-cells have significant functional variation in activation states. However, the rare frequency of antigen-specific CD8+ cells, for example, limits transcriptomic and proteomic analysis to identify biomarkers of exhaustion and activation.

Methods

Utilizing a novel integrated workflow, we performed both proteomic and transcriptomic analysis of very rare populations of T-cells. Negatively selected CD3+ cells were derived from whole PBMCs and stimulated *in vitro* with allogeneic, CD40L-activated, viral-antigen presenting B cells for 8 days. The stimulated cell population was stained with HLA-A02:01 MHC Pentamers specific for Influenza A $M1_{58\text{-}66}$ (GILGFVFTL). The cells were then simultaneously labeled with fluorescent markers and 30 different DNA barcoded antibodies. Using the fluorescent markers, antigen-specific and naïve CD8+ T-cells were sorted, lysed, and then the antibody-bound DNA barcodes and the released cellular RNA's were simultaneously measured using the NanoString nCounter® system and analyzed using the nSolver™ software.

Results

By integrating flow cytometry with downstream analysis on the NanoString nCounter system, 30 proteins and 770 RNAs were quantitatively measured on the nCounter from as few as 400 petamer-positive T-cells. Using the nSolver Advanced Analysis software, differences in gene and protein expression between Influenza A $M1_{58\text{-}66}$ specific CD8+ T cells and a pentamer-negative CD8+ T cell population were quantitatively measured. $M1_{58\text{-}66}$ specific cells showed upregulation of extracellular markers of exhaustion and activation consistent with similar proteomic studies including 4-1BB, CD27, CD45, and ICOS. Additionally, normalized

mRNA counts from each population revealed increased presence of transcripts coding for Granzyme B, CD225, Interleukin-32, STAT1, and TCF7 in the Influenza-specific CD8+ cells.

Conclusions

The rarity and functional importance of immune cell subsets in clinical samples has necessitated the development of new analytical methodologies that permit quantitative multiplexed immunoprofiling of RNA and protein expression. Using the nCounter platform downstream of cell sorting uniquely allows simultaneous high-plex analysis of protein and RNA from small numbers of targeted cells. In addition to the data we present on pentamer-positive antigen-specific T-cells, this method can be applied to any number of immune cell populations.

Consent

Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

Cancer Vaccines

P513

Sustained complete response to Nivolumab in a HPV16+ head and neck cancer patient after treatment with MEDI0457 (INO-3112), a DNA immunotherapy targeting HPV16/18

Charu Aggarwal[1], Roger Cohen[1], Matthew Morrow[2], Kimberly Kraynyak[2], Dawson Knoblock[2], Joshua Bauml[1], Gregory Weinstein[1], Jian Yan[2], Drishty Mangrolia[2], Sandra Oyola[2], Susan Duff[2], David Weiner[3], Ildi Csiki[2], Mark Bagarazzi[2]

[1]Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA; [2]Inovio Pharmaceuticals, Plymouth Meeting, PA, USA; [3]Wistar Institute, Philadelphia, PA, USA

Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P513

Background

We report outcomes of a patient (pt) with HPV16+ head and neck squamous cell cancer (HNSCCa) who underwent treatment with 4 doses of MEDI0457 as part of a pilot prospective clinical trial. Disease progression was noted approx. 7 months after completion of adjuvant chemoradiation (due to extracapsular extension) following definitive surgery. The pt was subsequently treated with nivolumab (nivo) and was noted to have a Complete Response (CR) at 6 weeks by RECIST. We performed correlative immune analysis for this pt to understand the mechanism underlying this response.

Methods

This Phase I/IIa trial included pts with p16+ locally advanced HNSCCa. MEDI0457 was delivered IM followed by electroporation with the CELLECTRA® device, Q3 weeks x 4 doses. Trial methods and results have been previously reported (Aggarwal C et al JImmunotherCancer. 2015;3(Suppl2):P426). Humoral and whole PBMC immune responses were assessed by ELISA and IFNg ELISpot, respectively. CD8+T cell activity and PD1 were assessed by flow cytometry (FC). Tissue immune responses were assessed by IHC.

Results

Pt is a 66 yr old Caucasian male with HPV16+ Stage IVA (T2N2b) tonsillar SCCa. He received one dose of MEDI0457 before definitive surgery, and three doses post-operatively. Tissue immune assessment showed decrease in both CD8+ and FoxP3+ infiltrates. Assessment of peak antibody and IFNg ELISpot responses showed titers of 1:150 and 0 for HPV16 E6 and E7 antigens, respectively, and an elevation of 7 SFU/10^6 PBMC for each antigen. However, analysis of HPV16 specific CD8+T cells prior to and post dosing with MEDI0457 showed *de novo* induction of CD8+ Tcells cells expressing PD-1 (1.8% of all CD8 + Tcells), as well as cells co-expressing PD-1, granzyme A, granzyme B and perforin (0.70% of all CD8+ Tcells). The pt was noted to have CR after 4 nivo doses, and remains in complete clinical remission 14 months after initiation of nivo.

Conclusions

The data above suggest that the pt responded immunologically to treatment with MEDI0457 as evidenced by the expansion of antigen specific CD8+T cells noted by FC. The expression of PD1 on the CD8 + Tcells cells may have allowed them to be subsequently inhibited by binding to tumor cells expressing PD-L1. Nivo may have relieved this inhibition, allowing for an outgrowth of functional HPV16-specific CTLs, contributing to the sustained CR. An ongoing trial with MEDI0457 and durvalumab in HPV+ HNSCCa is evaluating the clinical and immunologic efficacy of the combination treatment. Clinical trial information: NCT02163057.

Cellular Therapy Approaches

P514

Utilizing T-cell activation signals 1, 2 and 3 for tumor-infiltrating lymphocytes (TIL) expansion: the advantage over the sole use of interleukin-2 in cutaneous and uveal melanoma

Marie-Andree Forget, Rene J. Tavera, Young Uk Kim, Ankit Bhatta, Donastas Sakellariou-Thompson, Caitlin A. Creasy, Orenthial J. Fulbright, Renjith Ramachandran, Shawne T. Thorson, Esteban Flores, Arely Wahl, Sapna P. Patel, Patrick Hwu, Rodabe N. Amaria, Chantale Bernatchez, Cara Haymaker

MDACC, Houston, TX, USA

Correspondence: Marie-Andree Forget (mforget@mdanderson.org)

Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P514

Background

MDACC has been conducting clinical trials using adoptive transfer of autologous tumor-infiltrating lymphocytes (TIL) in the context of metastatic melanoma for more than a decade [1]. The art of TIL expansion lies in a two-phase process. The initial phase utilizes high-doses of IL-2 (signal 3) and results in a 62% successful TIL growth rate averaged over the past 11 years (n=1135) with 68% for the last 5 years. The second phase is the rapid expansion process (REP) and relies on TCR activation (signal 1) and co-stimulation (signal 2) followed by high-doses of IL-2 (signal 3). This leads to successful expansion in the vast majority of cases.

Methods

It was recently demonstrated that uveal melanoma is infiltrated with $CD8^+$ TIL. However, the initial TIL expansion (pre-REP) from uveal melanoma tumors does not lead to comparable TIL growth with IL-2 alone as seen from cutaneous melanoma [2]. Once cultures from both types of melanoma reach the REP phase, our anecdotal observations concluded that there was no difference in expansion. Therefore, we hypothesized that TCR activation in the 1^{st} phase of expansion combined with an agonistic stimulation of CD137/4-1BB (Urelumab) to protect the TIL from over differentiation in this initial TCR stimulation would favor reliable expansion of $CD8^+$ TIL.

Results

This novel 3-signal approach resulted in a faster and more consistent expansion of TIL, up to 100% for both types of melanoma. For cutaneous melanoma, numbers were comparable to or higher than the traditional high-dose IL-2 method and favored expansion of $CD8^+$ TIL. Importantly, this new method allowed for better/enhanced pre-REP expansion of TIL from uveal melanoma which, in turn, would allow for this patient population to have access to TIL therapy. Finally, providing the 3-signal attributed to T-cell activation led to expansion of TIL capable of recognizing their tumor counterpart in cutaneous as well as uveal melanoma as determined using IFNg ELISPOT.

Conclusions
This new methodology for the initial phase of TIL expansion addresses one of the major critiques for TIL therapy - the time needed for proper expansion of a suitable product. It brings consistency in successful growth as well as a new opportunity for challenging malignancies such as uveal melanoma.

References
1. Radvanyi, et al. Clinical Cancer Research. 2012
2. Qin, et al. Oncoimmunology. 2017

P515

Novel cryopreserved tumor infiltrating lymphocytes (LN-144) administered to patients with metastatic melanoma demonstrates efficacy and tolerability in a multicenter Phase 2 clinical trial

Amod Sarnaik[1], Jason Chesney[2], Harriet Kluger[3], Brendan Curti[4], Omid Hamid[5], Jose Lutzky[6], Maria Fardis[7], Igor Gorbatchevsky[7], Sam Suzuki[7], Bente Larsen[7], Nancy L. Samberg[7], John Kirkwood[8]
[1]Moffit Cancer Center, Tampa, FL, USA; [2]James Graham Brown Cancer Center, Louisville, KY, USA; [3]Yale Cancer Center, New Haven, CT USA; [4]Earle A. Chiles Research Institute, Providence Cancer Center, Portland, OR, USA; [5]The Angeles Clinic and Research Institute, Los Angeles, CA, USA; [6]Mount Sinai Comprehensive Cancer Center, Miami Beach, FL, USA; [7]Iovance Biotherapeutics, Inc., San Carlos, CA, USA; [8]University of Pittsburgh Medical Center, Hillman Cancer Center, Pittsburgh, PA, USA
Correspondence: Igor Gorbatchevsky (igor.gorbatchevsky@iovance.com)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P515

Background
The safety and efficacy of adoptive cell therapy (ACT) with non-cryopreserved tumor infiltrating lymphocytes (TIL) has been studied in hundreds of patients with metastatic melanoma. This multicenter clinical trial was initiated with centrally manufactured TILs (LN-144) as non-cryopreserved and cryopreserved infusion products. Our novel manufacturing process for the non-cryopreserved LN-144 is used in Cohort 1, and a shortened 3 weeks, cryopreserved LN-144 is used in Cohort 2. The Cohort 2 manufacturing offers a significantly shorter process, which allows for flexibility of patient scheduling and dosing. The shorter manufacturing process reduces the wait time for the patient to receive their LN-144 product and adds convenience to logistics and delivery to the clinical sites.
Methods
C-144-01 is a prospective, multicenter study evaluating metastatic melanoma patients who receive LN-144. Following a non-myeloablative lymphodepletion with Cy/Flu preconditioning regimen, patients receive a single infusion of LN-144 followed by the administration of IL-2 (600,000 IU/kg) up to 6 doses. Patients are evaluated for objective response as a primary endpoint for up to 24 months.
Results
We characterize the cryopreserved LN-144 administered to a second cohort of patients, Cohort 2 following the same pre- and post-TIL infusion treatment regimen as used for Cohort 1.
Cohort 2 patients were heavily pretreated with increased number of prior lines with all patients having anti-CTLA-4 and anti-PD-1 therapies, and larger tumor burden (mean SOD: 15.3, 10.9 cm for Cohorts 2, 1). Median number of prior systemic therapies were 4 and 3 for Cohorts 2 and 1, respectively. An initial analysis of safety data demonstrates comparable tolerability of cryopreserved LN-144. The safety profile for Cohort 1 patients receiving the non-cryopreserved LN-144 continues to be acceptable for this late stage patient population. The most common TEAEs observed in both cohorts by frequency are nausea, anaemia, febrile neutropenia, neutrophil count decreased, platelet count decreased. Early review of efficacy data indicates anti-tumor activity, including PRs, to the TIL therapy observed in patients treated in Cohort 2.
Conclusions
This represents the first clinical trial in a multicenter setting with centrally manufactured TIL assessing a novel process for cryopreserved product with a significantly shorter process (~3 weeks). Preliminary results indicate cryopreserved LN-144 as a novel, well tolerated therapeutic option for patients with metastatic melanoma who have failed multiple prior therapies, including checkpoint inhibitors. The cryopreserved LN-144 provides greater flexibility for patients and caregivers and allows for more immediate treatment for patients with such high unmet medical need.
Trial Registration
NCT02360579.

P516

An evaluation of autologous tumor-reactive TIL generation from head and neck squamous cell cancers

Ashish Patel[1], Bryan Bell[2], Bernard Fox[2], Carlo Bifulco[2], Walter Urba[2], Tarsem Moudgil[2], Shawn Jenson[2], Christopher Paustian[3], Zipei Feng[2], Carmen-Ballesteros Merino[2], Rom Leidner[4], Christopher Dubay[2], Brenden Curti[2], Hong-Ming Hu[2]
[1]PPMC, Portland, OR, USA; [2]E.A.Chiles Research Institute, Portland, OR, USA; [3]UbiVac, Portland, OR, USA; [4]Providence Cancer Center, Portland, OR, USA
Correspondence: Bernard Fox (bernard.fox@providence.org)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P516

Background
Head and neck squamous cell carcinoma (HNSCC) remains a significant unmet medical need. While checkpoint blockade has provided improved outcomes for some patients, the majority of patients do not benefit. We hypothesize that the lack of clinical benefit is secondary to the absence of tumor-specific T cells that can expand and mediate tumor destruction. To begin to address this hypothesis we have investigated the percentage of HNSCC from which we can generate TIL reactive with the autologous tumor.
Methods
Over the past 5 years we have collected and processed more than 300 HNSCC specimens. When sufficient tumor material was available, tumor-infiltrating lymphocytes (TIL) and primary tumor cultures were initiated from enzyme digests (collagenase, thermolysin and DNAase) of freshly resected surgical samples. TIL cultures were assessed for growth and autologous tumor reactivity measured by IFN-g release. IFN-g was measured by ELISA. Once established, tumor cell lines were characterized for phenotypic markers by flow cytometry.
Results
For 242 tumor samples with sufficient tumor, 51 (21%) were identified as being contaminated. TIL were generated from 82 (42%) of the remaining 191 tumors. Of the 59 where testing is complete, 46 TIL (77.9%) were autologous tumor-reactive. Overall, we were able to generate TIL from 33% of tumors tested. We identified TIL cultures with a range of autologous tumor reactivity that ranged from very strong to no cytokine-release following stimulation with autologous tumor. At least 20 (10.2%) cell lines were established from the HNSCC samples. A majority of these tumor cell lines contain cells that express CD44, a marker of cancer stem cells.
Conclusions
Tumors from one-third (33%) of the 191 patients evaluated contained TIL that could be expanded and recognize autologous tumor cells. This may correspond to the patients that benefit from immunotherapy with checkpoint blockade or T cell agonists. The remaining patients may require vaccines or other therapies that will prime T cells that can recognize autologous cancer cells. Our group is preparing to undertake a clinical trial of vaccine plus anti-OX40 in patients with HNSCC. Current efforts are examining whether multiplex IHC will be useful in identifying tumors that contain tumor-reactive TIL.

P517

Regional intraventricular delivery of HER2-specific CAR T cells targets breast cancer metastasis to the brain

Saul Priceman[1], Dileshni Tilakawardane[1], Brook Jeang[1], John Murad[1], Anthony Park[1], Wen-Chung Chang[1], Julie Ostberg[1], Josh Neman[2], Rahul Jandial[1], Jana Portnow[1], Stephen Forman[1], Christine Brown[1]
[1]City of Hope National Medical Center, Duarte, CA, USA; [2]University of Southern California, Los Angeles, CA, USA
Correspondence: Christine Brown (cbrown@coh.org)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P517

Background
Metastasis to the brain from breast cancer remains a significant clinical challenge, and may be targeted with CAR-based immunotherapy. CAR design optimization for solid tumors is crucial due to the absence of truly restricted antigen expression and potential safety concerns with "on-target off-tumor" activity. Here, we have optimized human epidermal growth factor receptor-2 (HER2)-CAR T cells for the treatment of breast to brain metastases, and determined optimal second generation CAR design and route of administration for xenograft mouse models of breast metastatic brain tumors, including multifocal and leptomeningeal disease.
Methods
HER2-CAR constructs containing either CD28 or 4-1BB intracellular co-stimulatory signaling domains were compared for functional activity *in vitro* by measuring cytokine production, T cell proliferation, and tumor killing capacity. We also evaluated HER2-CAR T cells delivered by intravenous, local intratumoral, or regional intraventricular routes of administration using *in vivo* human xenograft brain metastatic breast cancer models.
Results
Here, we have shown HER2-CARs containing the 4-1BB intracellular co-stimulatory domain confer improved antigen-selective tumor targeting with reduced T cell exhaustion phenotype and enhanced antigen-dependent proliferative capacity compared to HER2-CARs containing the CD28 co-stimulatory domain. Local intracranial delivery of HER2-CARs showed *in vivo* anti-tumor efficacy in an orthotopic xenograft model using a tumor line generated from a breast cancer patient with brain metastasis. Importantly, we demonstrated robust anti-tumor activity following regional intraventricular delivery of HER2-CAR T cells for treatment of multifocal brain metastases and leptomeningeal disease.
Conclusions
Our study shows the importance of CAR design in defining an optimized CAR T cell, and highlights intraventricular delivery of HER2-CAR T cells for treating multifocal brain metastases.

Combination Therapy (IO/IO, IO/Standard of Care, IO/Other)

P518

Generation of non-reprogrammable, dysfunctional CD8$^+$ T-cells following anti-PD-1 therapy in the presence of low antigen priming is a cause of failure of the treatment

Vivek Verma[1], Rajeev Shrimali[1], Shamim Ahmad[1], Winjie Dai[1], Hua Wang[1], Sumin Lu[1], Pankaj Gaur[1], Scott A. Hammond[2], Mikayel Mkrtichyan[1], John E. Janik[1], Seema Gupta[1], Samir N. Khleif[1]
[1]Augusta University, Augusta, GA, USA; [2]MedImmune LLC, Gaithersburg, MD, USA
Correspondence: Samir N. Khleif (skhleif@augusta.edu)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P518

Background
Currently, several clinical trials utilizing anti-PD-1 for cancer therapy are ongoing either alone or in combination with other immune modulators [1]. However, despite a durable response in some patients, monotherapy with anti-PD-1 fails in significant number of patients. Hence, understanding of the mechanisms that lead to failure of anti-PD-1 as an anti-cancer agent would help to harness its full potential.
Methods
Effects of PD-1 blockade, prior or together with tumor-specific vaccine, on tumor growth and survival were evaluated in TC-1 and B16 tumor mouse models. Immune responses were determined in TC-1 tumors in a time-dependent manner. *In vitro* mechanistic studies were carried out in pMel-1 CD8$^+$ T-cells.
Results
We show that in the immunosuppressive microenvironment of the tumor, anti-PD-1 treatment leads to induction of PD1^{high}CD38^{high} non-reprogrammable CD8$^+$ T-cells [2]. These cells are generated early after first anti-PD-1 treatment and remain unresponsive to subsequent antigenic stimulation indicating their dysfunctional state. Using in *vitro* assays, we found that treatment with anti-PD-1 prior to T-cell priming abrogates the ability of T-cells to upregulate CD40L and increase IFNg, and leads to significant cell death by apoptosis and failure to generate memory cells. On the other hand, anti-PD-1 therapy given concomitantly with strong T-cell antigen priming by vaccine produces a robust anti-tumor immune response accompanied with the generation of reprogrammable (plastic) PD1^{high}CD38^{high} cells that get activated, generating effector functions and immune memory.
Conclusions
Our data provide a plausible explanation for the inability of anti-PD-1 therapy to generate effective anti-tumor effects in some patients especially with non-immunogenic tumors. This suggests that the development of combination therapies that would increase the immunogenicity of tumors might enhance the efficacy of anti-PD-1 therapy. These results also suggest that recurrent patients that have undergone anti-PD-1 therapy earlier may not respond to combination therapies that further activate/stimulate the TCR signaling. Therefore, these findings have important implications in the design of future trials employing combination therapy with anti-PD-1 to achieve clinical success.

References

1. Page, et al. Immune modulation in cancer with antibodies. Annu Rev Med. 2014; **65**:185-202.
2. Philip, M et al. Chromatin states define tumour-specific T cell dysfunction and reprogramming. Nature. 2017; **545**:452-6.

Emerging Models and Imaging

P519

Artificial intelligence augmented phenotypic screens rapidly reveal novel macrophage biology

Viswa Colluru (viswa.colluru@recursionpharma.com)
Recursion Pharmaceuticals, Salt Lake City, UT, USA
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P519

Background
Tumor Associated Macrophages have long been recognized as a key component of the tumor microenvironment and play a critical role in tumor progression. However, our ability to fully leverage macrophages as treatment targets is hampered by the

complexity of immune phenotypes and low-dimensional screening practices. At Recursion Pharmaceuticals, we combine target-agnostic phenotypic imaging experiments with artificial-intelligence and advanced data analytics to interrogate complex biology in high-dimensional screens. We describe the application of our platform to the study of macrophage polarization states to deliver novel and actionable insights for immuno-oncology drug discovery.

Methods

PMA activated THP1 cells were treated with the relevant polarizing cytokines for 48h prior to staining with the Cell Painting method, which fluorescently labels 9 cellular components to provide a morpho-functional snapshot of a cellular state in the form of ~1000 unique features. Cells were then imaged for fluorescence across 5 channels to generate ~200 images per perturbation and processed through a custom cloud-based software pipeline enabled to run CellProfiler and proprietary artificial intelligence algorithms at scale. Secretome and transcriptome profiling were performed using a standard 65-plex Procartaplex panel and a custom 50-plex Quantigene Plex assay kit respectively (ThermoFisher).

Results

Our data demonstrate unique, sensitive, and functionally meaningful high-dimensional phenotypes for each of the M0, M1, M2a, M2c, and M2-like (M-CSF) macrophage polarizations (Figs. 1 and 2). Principal component analysis on the phenotypic features further suggests that the M1-M2-like-M2a-M2c macrophage states are distinct polarizations and not part of an axis, as current dogma holds. All polarizations were validated in orthogonal tumor relevant functional assays. Importantly, when compared to transcriptome and secretome analyses, our phenotypic approach is able to better differentiate between the different polarization states. We demonstrate near perfect distinguishability of the different macrophage classes by a Machine Learning classifier trained on phenotypic features (Fig. 3). A phenotypic screen of ~2000 diverse small molecules rapidly uncovered at least 1 novel drug class that modulates M-CSF induced polarization, and at least 3 drug classes that outperform CSF-1R inhibition alone for prevention of a M2-like phenotype. We also identified, in the same screen, putative compounds that could cause a M1-like re-polarization in M-CSF treated macrophages. Our entire screening campaign described here was executed in 3 weeks, highlighting the efficiency with which imaging and artificial intelligence can be used in drug discovery.

Conclusions

In conclusion, artificial intelligence augmented phenotypic approaches allow facile interrogation of complex phenotypic states at scale to drive rapid discovery.

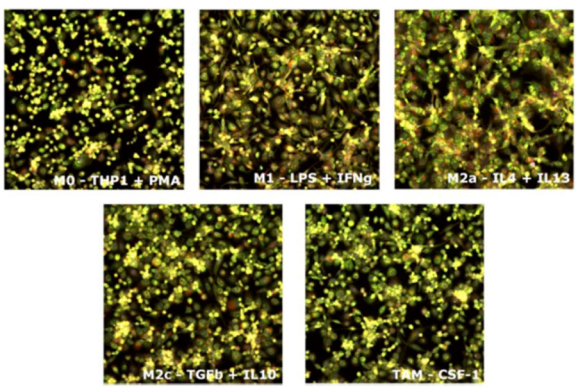

Fig. 1 (abstract P519). Cell painting reveals rich morphological profiles of macrophage polarizations

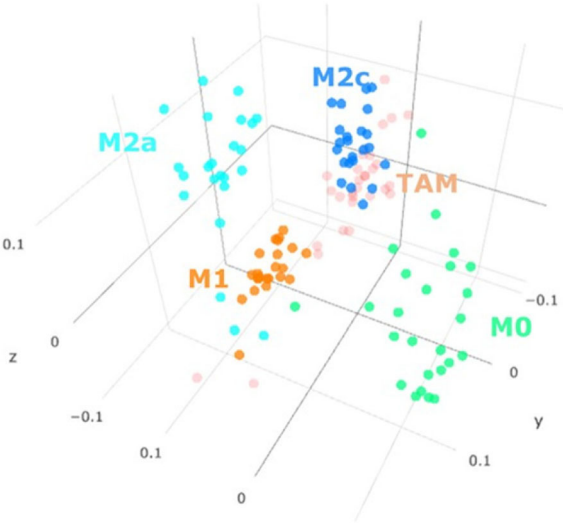

Fig. 2 (abstract P519). Principal component analysis of phenotypic features reveals meaningful clustering and novel relationships between polarizations

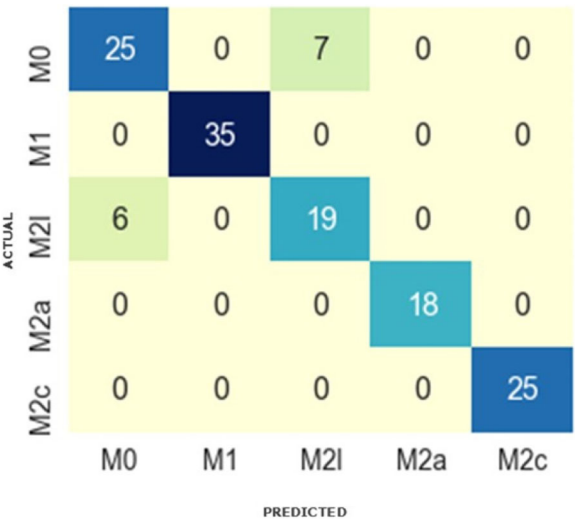

Fig. 3 (abstract P519). Machine learning can be leveraged to classify macrophage phenotypes with high confidence

Mechanisms of Efficacy or Toxicity

P520

Tuberculosis following PD-1 blockade in a patient with Merkel cell carcinoma (MCC): coincidence or causality?

Elad Sharon[1], Daniel Barber[2], Ragini Kudchadkar[3], Steven Fling[4], Tracey Day[5], David Ashkin[6], Lisa Lundgren[4], Martin Cheever[4], Paul Nghiem[7], Shunsuke Sakai[2]

[1]National Cancer Institute, Bethesda, MD, USA; [2]National Institute of Allergy and Infectious Diseases, Bethesda, MD, USA; [3]Emory University School of Medicine, Atlanta, GA, USA; [4]Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [5]Infectious Disease Research Institute, Seattle, WA, USA; [6]University of Florida College of Medicine, Gainsville, FL, USA; [7]University of Washington, Seattle, WA, USA

Correspondence: Elad Sharon (sharone@mail.nih.gov)

Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P520

Background
Preclinical data suggest that PD1 blockade may assist in eradicating a variety of infections. In contrast, PD1 knockout mice are hypersusceptible to mycobacterium tuberculosis (Mtb) infection, developing necrotic pulmonary lesions with high bacterial loads. We report tuberculosis in a patient treated with pembrolizumab for Merkel cell carcinoma (MCC). We also characterize Mtb-specific immunity and bacteria isolated from a pulmonary lesion that arose after initiating PD1 therapy.
Methods
An 83-year old man began a clinical trial of pembrolizumab in June 2015 for advanced MCC. The patient had no risk factors and no testing for latent tuberculosis was performed. CT scan after 12 cycles revealed known sites of MCC decreasing in size or remaining stable, and a new right lower lobe pulmonary nodule was noted (1.1 x 1.6 cm). The patient underwent excision of the nodule in January 2016. Pathology revealed necrotizing granulomas staining for acid-fast bacilli. Cryopreserved PBMC obtained immediately prior to pembrolizumab and at cycles 5, 8, 11, and 14 were analyzed for antigen-specific CD4 and CD8 T cell responses by intracellular cytokine staining after stimulation with PPD. Serum samples were also analyzed for IgG responses to a panel of different Mtb antigens. Mtb genotyping was performed.
Results
PD1 blockade in this individual was associated with significantly increased circulating Mtb-specific Th1 responses prior to development of the necrotic pulmonary tuberculoma. However, neither Th17 cells nor CD8 T cells specific to Mtb were detectable in PBMC at any time. Circulating Foxp3+ Tregs did not change in number during pembrolizumab treatment in this individual. Mtb-specific IgG levels, although detectable, did not display significant changes before the development of the necrotic granuloma. TB genotyping also did not correlate with any known new clusters of TB in North America in the recent past. Collectively, these data show that the development of tuberculosis following PD1 blockade in this individual was selectively associated with increases in Mtb-specific Th1 responses.
Conclusions
Four previous cases of TB have been reported following PD-1 blockade. In this case, Mtb-specific Th1 responses increased after PD1 blockade was initiated, and clinical tuberculosis arose subsequently. Importantly, this nodule was assumed to be an MCC metastasis, and would not have been recognized as due to Mtb if an excisional biopsy had not been performed. In conjunction with animal model data suggesting a plausible mechanism, and the prior reported cases, these findings suggest that Mtb is possibly a concern following PD1 blockade.
Trial Registration
NCT02267603

Mechanisms of Resistance to Immunotherapy

P521

Imprime PGG, a novel phase 2 immunotherapeutic, enhances the anti-tumor activity of checkpoint inhibitors (CPI) and suppresses CPI-induced Indoleamine 2, 3-dioxygenase (IDO) expression

Xiaohong Qiu, Anissa SH Chan, Adria B. Jonas, Michael E. Danielson, Nadine R. Ottoson, Kyle S. Michel, Steven M. Leonardo, Ross B. Fulton, Kathryn Fraser, Takashi O. Kangas, Mark Uhlik, Jeremy R. Graff, Nandita Bose
Biothera Pharmaceuticals, Inc., Eagan, MN, USA
Correspondence: Nandita Bose (nbose@biothera.com)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P521

Background
Although checkpoint inhibitors (CPI) have shown unprecedented efficacy in cancer treatment, a significant fraction of patients eventually develop resistance to CPI. Therefore, there is a growing need to identify resistance mechanisms as well as rational combination strategies to combat this resistance. Imprime PGG (Imprime), a novel yeast derived β-glucan pathogen-associated molecular pattern (PAMP), is being developed as a combination agent with CPI in patient populations who have failed single- agent CPI therapy. In pre-clinical mechanistic studies, Imprime has been shown to reprogram the immuno-suppressive myeloid cells in the microenvironment and enhance the effector functions of tumor infiltrating T cells. The objective of this study was to focus on IDO1, one of the critical resistance mechanisms in the microenvironment that hinders T cell anti-tumor immunity.
Methods
The anti-tumor efficacy of Imprime in combination with anti-PD-1 was evaluated in the murine colon cancer model MC38. Transcriptional changes in the tumor were assessed by QuantiGene Multiplex platform. IDO1 gene expression in IFN-γ-stimulated human whole blood post Imprime treatment was assessed by qRT-PCR. Tryptophan and kyneurinine levels were measured in the serum by LC/MS.
Results
In the MC38 model, Imprime in combination with anti-PD-1 resulted in significantly reduced tumor growth as compared to anti-PD-1 monotherapy. Consistent with our previous results, transcriptional analyses of tumor tissues showed that Imprime alone induced a M1 skewing gene expression profile by modulating several genes including iNOS, TNF, CXCL10, Arg1, and CCL17. Anti-PD-1 treatment alone up-regulated several genes affecting T cell functionality, such as IFN-γ, PD-L1 and GzmB. Interestingly, anti-PD-1 treatment also resulted in increased expression of several immuno-suppressive genes, such as IL10, Arg1, and most notably, IDO1. Furthermore, IDO1 expression was inversely correlated with tumor volume, suggesting IDO1 up-regulation is a counter-regulatory mechanism induced in the tumor and/or myeloid cells in response to enhanced IFN-γ production by anti-PD-1-treated tumor-infiltrating T-cells. Interestingly, this anti-PD-1 mediated IDO1 induction was dampened significantly by the addition of Imprime to anti-PD-1. Flow cytometry showed that Imprime treatment affected IDO expression in the Ly6C^{hi} monocytes and macrophages but not in tumor cells. In human whole blood, IFN-γ- treatment increased the transcriptional level of IDO1 and the ratio of tryptophan to kyneurinine, but Imprime treatment significantly inhibited this IFN-γ-induced IDO1 increase.
Conclusions
These results collectively demonstrate that Imprime treatment can enhance efficacy of anti-PD-1 treatment and may do so by restricting compensatory immuno-suppressive mechanisms mediated by myeloid cells.

Microbiome

P522

Clostridium species control primary liver cancer and liver metastasis via bile acids/CXCL16/CXCR6 mediated NKT cell immunity

Chi Ma, Miaojun Han, Bernd Heinrich, Qiong Fu, Qianfei Zhang, Masaki Terabe, Jay Berzofsky, Xin Wei Wang, Giorgio Trinchieri, Tim Greten
NCI, Bethesda, MD, USA
Correspondence: Tim Greten (tim.greten@nih.gov)
Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P522

Background
Gut commensal bacteria have been described as important regulators of anti-tumor immunity. Primary liver tumors and liver metastasis represent the leading cause of cancer-related death [1]. The liver is exposed to gut bacteria, and gut sterilization has profound effects on hepatocellular carcinoma (HCC) development [2, 3]. However, the role of gut bacteria in anti-tumor surveillance in the liver is poorly understood.
Methods
Gut commensal bacteria were depleted by feeding mice with antibiotic cocktail in drinking water, or using Germ-free mice. Primary HCC was induced in liver-specific MYC transgenic mice. Liver metastasis was induced by intrasplenic injection of B16 melanoma tumors cells or *i.v.* injection of A20 lymphoma or EL4 thymoma tumor cells into C57BL/6 or BALB/c mice. Immune cell monitoring was performed by flow cytometry analysis. Stool bacteria was analyzed by 16S rRNA sequencing. Patient liver bile acids were measured by Metabolon's Discover HD4 Platform.
Results
Depleting gut commensal bacteria induced a liver-selective anti-tumor effect using both primary MYC-HCC model or A20 or EL4 liver

metastasis models. An increase of hepatic CXCR6$^+$ NKT cell number and function was observed, independent of mouse strain, gender or presence of liver tumors. *In vivo* functional studies confirmed that NKT cells mediated a tumor inhibition in the liver. Further investigation showed that NKT cell accumulation was regulated by CXCL16 expression of liver sinusoidal endothelial cells, which was controlled by *Clostridium* species-mediated primary-to-secondary bile acid conversion. Feeding mice with secondary bile acid w-MCA reversed both the NKT accumulation and the inhibition of liver tumor growth caused by depleting gut microbiome. In human livers, primary bile acid CDCA levels correlated with *CXCL16* expression, and the opposite was found with the secondary bile acid glycolithocholate (GLCA).

Conclusions

Gut bacteria such as *Clostridium* species control liver anti-tumor immunesurveillance by altering bile acid composition, which regulates CXCL16 expression in LSECs, thus affects NKT cell level in the liver. This study shows that the gut microbiome utilizes bile acid metabolism to control anti-tumor immunity in the liver and opens novel opportunities to treat primary liver cancer as well as liver metastasis.

References

1. Disibio, et al. Metastatic patterns of cancers: results from a large autopsy study Arch Pathol Lab Med. 2008; 132:931-9.
2. Dapito, et al. Promotion of hepatocellular carcinoma by the intestinal microbiota and TLR4. Cancer Cell. 2012; 21:504-16.
3. Yoshimoto, et al. Obesity-induced gut microbial metabolite promotes liver cancer through senescence secretome. Nature. 2013; 499:97–101

Oncogenetics and Immunogenomics

P523

Gene expression profiling of dermatologic toxicities from immune checkpoint therapy

Jonathan Curry, Michael Tetzlaff, Alexandre Reuben, Robert Szczepaniak, Saira George, Carlos Torres-Cabala, Daniel Johnson, Victor Prieto, Adi Diab

MD Anderson Cancer Center, Houston, TX, USA

Correspondence: Jonathan Curry (jlcurry@mdanderson.org)

Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P523

Background

Cancer patients receiving antibodies abrogating immune checkpoints antibodies may develop a diverse array of histologic skin reactions to these therapies that can include immunobullous, spongiotic, and lichenoid dermatitis (LD), and infrequently Steven Johnson Syndrome/Toxic Epidermal Necrolysis. The development of any type of these adverse immune-related cutaneous reactions may be sufficiently severe to warrant cessation of potentially efficacious treatment regimen. There is therefore a critical need to understand the pathogenesis of dermatologic toxicities in order to devise rational therapies to manage them more effectively.

Methods

Total RNA from formalin-fixed paraffin, embedded tissue from patients who developed LD skin toxicity [n=3; mean age (range) = 60.7 years] while receiving immune checkpoint therapy (nivolumab =1, pembrolizumab = 1, nivolumab + ipilimumab = 1) for metastatic melanoma and from benign lichenoid keratosis (BLK) in control patients [n=3; mean age (range) = 48.7 (37-56) years] were profiled with the NanoString nCounter PanCancer Immune Profiling Panel interrogating the mRNA levels of 770 genes. Fold differences in mRNA transcript levels were compared between the two groups using two-sample tests and p-value < 0.05 were considered significant.

Results

Of the 770 genes, significant log fold difference in gene expression was observed between the two groups in 167 genes. Compared to the BLK control group (Fig. 2), the LD skin toxicity group (Fig. 3) showed down-regulation of 93 mRNAs ($p < 0.05$) and up-regulation of 74 mRNAs ($p < 0.05$). The ten most significantly down- and up-regulated transcripts in the LD skin toxicity group are listed (Fig. 1). Among the down-regulated genes are CCL27, CCL18, CD83, ILIRN, and IL2RA (Log_2 fold change range: -1.81 to -1.17; all p values < 0.05). Among the up-regulated genes were CD14, CXCL12, and CCL14 (Log_2 fold change range: 1.28-2.52, all p values < 0.04).

Conclusions

LD skin toxicity from immune checkpoint therapy exhibits an mRNA gene expression profile distinct from BLK. Despite showing histopathologically identical reaction patterns between the LD skin toxicity from immune checkpoint therapy and BLK, we observed differences in the mRNA transcript levels of 167 genes. Down-regulation of CCL27, CCL18, XCL2, CD83, ILIRN, and IL2RA genes implies dysregulation of normal homeostasis and immune regulation in the skin. Up-regulation of genes that encode chemotactic molecules (e.g. CXCL12 and CCL14) functions to recruit distinct subsets of skin immunocytes specific to immune checkpoint mediated LD and further suggest that abrogation of these signaling pathways may spare patients from developing this type of skin toxicity.

Gene	Log2 fold change	P-value	FDR
CCL27 (C-C motif ligand 27)	-1.81	0.05	1
GNLY (granulysin)	-1.66	< 0.01	0.347
CCL18 (C-C motif ligand 18)	-1.55	< 0.01	0.347
XCL2 C motif ligand 2)	-1.49	< 0.01	0.347
CD83	-1.3	< 0.01	0.449
CD1B	-1.27	0.02	0.959
CD1C	-1.26	< 0.01	0.621
KLRC2 (killer cell lectin-like receptor subfamily C member 2)	-1.24	< 0.01	0.242
ILIRN (interleukin 1 receptor antagonist)	-1.22	< 0.01	0.761
IL2RA (IL2 receptor alpha)	-1.17	< 0.01	0.689
CXCL12 (C-X-C motif ligand 12)	2.52	< 0.01	0.585
THBS1 (thrombospondin 1)	2.17	0.05	1
CFD (complement factor D)	2.01	< 0.01	0.621
CCL14 (C-C motif ligand 14)	1.68	< 0.01	0.242
CTSG (cathepsin G)	1.32	0.01	0.85
CD14	1.28	0.04	1
PDGFB (platelet-derived growth factor beta)	1.28	< 0.01	0.6
CMA1 (chymase 1 mast cell)	1.27	0.02	1
LRP1 (low density lipoprotein receptor-related protein 1)	1.26	< 0.01	0.6
CCL21(C-C motif ligand 21)	1.24	< 0.01	0.736

Fig. 1 (abstract P523). Most highly down-regulated and up-regulated genes in LD skin toxicity from immune checkpoint therapy.

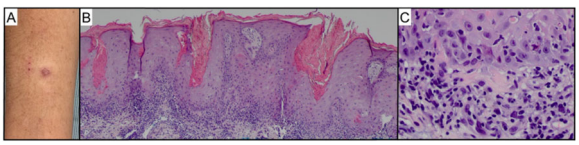

Fig. 2 (abstract P523). Representative case of lichenoid dermatitis (LD) skin toxicity to immune checkpoint therapy

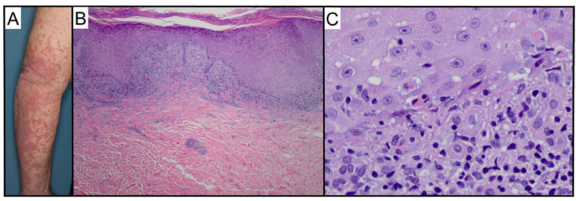

Fig. 3 (abstract P523). Representative case of lichenoid dermatitis (LD) skin toxicity to immune checkpoint therapy.

Oncolytic Viruses and Intratumoral Therapies

P524

Clinical and biomarker analyses of a phase II study of intratumoral tavokinogene telseplasmid (pIL-12) plus pembrolizumab in stage III/IV melanoma patients predicted to not respond to anti-PD-1

Alain P. Algazi[1], Katy K. Tsai[1], Michael D. Rosenblum[1], Robert Andtbacka[2], Carmen Ballesteros-Merino[3], Shawn Jensen[3], Carlo B. Bifulco[3], Bernard A. Fox[3], SuFey Ong[4], Alessandra Cesano[4], Joseph Beechem[4], Chris Twitty[5], Jean S. Campbell[5], Erica Browning[5], Reneta Talia[5], Shawna A. Shirley[5], Mai H. Le[5], Robert H. Pierce[5], Sharron Gargosky[5], Adil I. Daud[1]

[1]University of California, Oakland, CA, USA; [2]University of Utah, Salt Lake City, UT, USA; [3]Providence Portland Medical Center, Portland, OR, USA; [4]NanoString Technologies, Inc., Seattle, WA, USA; [5]OncoSec Medical Incorporated, San Diego, CA, USA

Correspondence: Chris Twitty (ctwitty@oncosec.com)

Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P524

Background

Melanoma patients with a low frequency of PD-1^{hi}CTLA-4^{hi} TIL are predicted to not respond to pembrolizumab, yet our previous interim analysis demonstrated that the combination of intratumoral (IT) plasmid IL-12 (tavokinogene telseplasmid; TAVO) and pembrolizumab yields robust clinical responses with an excellent safety profile. Updated clinical analyses (locked August 2017) from this multicenter, phase II, open-label trial including 2-year progression free survival (PFS) and DOR are presented. New biomarker data reveals coordinated anti-tumor immunological mechanisms in both the tumor microenvironment (TME) and in the peripheral blood.

Methods

Melanoma stage III/IV patients with a low CD8$^+$ TIL status (<25% PD-1^{hi}CTLA-4^{hi}) were treated with pembrolizumab (200mg every 3 weeks) concurrently with electroporation of IT-TAVO on days 1, 5 and 8 (6 week cycles). Tumor samples were profiled with multispectral immunohistochemistry (mIHC) and NanoString's Human Immunology and the PanCancer IO360 Beta Version panels. PBMC were analyzed for immune phenotype (flow cytometry) as well as NanoString's PanCancer Immune Profiling RNA and Protein panels.

Results

Progression free survival (PFS) rates for this treatment were 62% at 6 months and 57% at 18 months (median PFS not reached at 24 months) with a 48% BORR. DOR was not assessable as no responders have progressed and no safety signals were observed with only 2/22 grade 3 treatment-emergent adverse events. In responding patients, significant post-treatment increases were observed in both the Th1-associated gene expression of *STAT4* and *IL-12RB* in biopsies, and frequencies of CD8$^+$PD-1$^+$TIGIT$^+$ and proliferating CD8$^+$PD-1$^+$ peripherally. Additionally, responding patients had a significant increase of TCR clonality in the tumors compared to PBMCs with a reversed relationship in non-responding patients. Spatial analysis by mIHC revealed a significant increase of both PD-L1$^+$ and FoxP3$^+$ cells <15um from CD8$^+$ T cells in non-responders. Exploratory analysis with Nanostring's IO360 Beta Version panels highlighted underexpression of *WNT2B* and overexpression of *MICB* in the pretreatment responder biopsies.

Conclusions

Durable responses and favorable PFS rates in likely PD-1 non responders continues to suggest that combination IT-TAVO-EP with pembrolizumab is an effective therapeutic modality with an excellent safety profile. Associated biomarker data highlights connected immunological mechanisms, whereby intratumoral Th1-polarization, associated TCR clonality and limited suppressive cell types can drive robust anti-tumor responses (intratumoral and systemic) that positively impact this difficult to treat patient population.

Trial Registration

NCT02493361

Tumor Microenvironment (Mechanisms and Therapies)

P525

Exploring tumor microenvironment and human bone marrow stromal cells by single cell sequencing

Shutong Liu, Ping Jin, Yindong Zhao, Jiaqiang Ren, Steven Highfill, Jinguo Chen, Rongye Shi, Hui Liu, David Stroncek

National Institutes of Health, Rockville, MD, USA

Correspondence: David Stroncek (dstroncek@mail.cc.nih.gov)

Journal for ImmunoTherapy of Cancer 2017, **5(Suppl 3):**P525

Background

Proinflammatory stimulation can lead to phenotype changes in marrow mesenchymal stromal cells (MSCs). We have been using MSCs to study the role of stromal cells in the tumor microenvironment. Our previous study showed that IFN-γ (Interferon Gamma) and TNF-α (Tumor Necrosis Factor Alpha) together result in the synergist uniform polarization of MSCs toward primarily Th1 phenotype, suggesting that tumor associated stromal cells may contribute to immune-mediated tumor killing. MSCs are heterogeneous and contain both stromal cells and skeletal stem cells that are responsible for osteogenesis. Prolonged passages of MSC can result in a change in phenotype associated with the loss of the skeletal stem cells. In this study, we used IFN-γ and TNF-α to stimulate different passages MSCSs. We hypothesize that if stromal cells remain in late passage and if late passage Th1 polarization is similar to that of early passage, Th1 polarization is likely an intrinsic property of MSCs.

Methods

MSCs from bone marrow of one healthy donor were cultured and treated by IFN-γ (6.5ng/ml) and TNF-α (1.5ng/ml) at passages 2 (Bulk only), 3 (Single cell only), 4, 6, 8, 9 and 10(Bulk only) for 24 hours. RNA or cells were harvested from treated and control MSCs for mRNA Next Generation Sequencing (NGS) respectively.

Results

Control and stimulated MSCs NGS analysis found that the transcriptome of all passages of stimulated MSCs changed. Differences in the transcriptome of stimulated MSCs among different passages were observed, however, the expression by stimulated MSCs of important immune modulatory genes such as CXCL9, HLA-DRA, IL15 and IDO1 were up-regulated in all passages but their expression levels varied among passages.

Conclusions

Results show that after stimulation, different passages have similar but not identical gene expression changes. This suggests that immune modulation is an intrinsic property of MSCs, immune modulation may vary among MSCs types and some variability may exist among MSCs in different cancer types.

Consent

Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.