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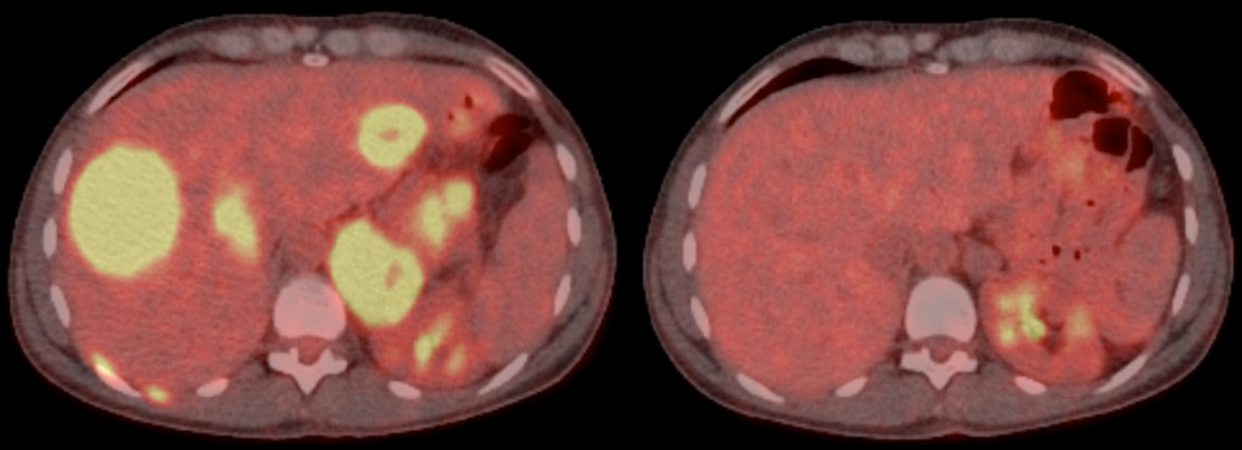

Cancer Immunology and Immunotherapy: Delivering the Promise

October 9–10, 2014

Masur and Lipsett Auditorium, Building 10
National Institutes of Health, Bethesda, MD

Sponsored by the **Center of Excellence in Immunology, Center for Cancer Research, National Cancer Institute**

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CANCER IMMUNOLOGY AND IMMUNOTHERAPY: DELIVERING THE PROMISE

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Program and Abstracts

Chair of the Organizing Committee:
Steven A. Rosenberg, MD, PhD

Sponsored by the **Center of Excellence in Immunology**, **Center for Cancer Research, National Cancer Institute**

The cover photograph represents before (left) and after (right) PET scans of a patient with Diffuse Large B cell Lymphoma treated with autologous lymphocytes genetically engineered to express a chimeric antigen receptor targeting CD19. This image is provided courtesy of the Surgery Branch, CCR, NCI.

Program

Thursday, October 9, 2014

Introductions

8:30 Robert Wiltrout
Steven Rosenberg

Session 1: Adoptive Cell Therapies
Chair: Steven Rosenberg

9:00 Steven Rosenberg, National Cancer Institute, Bethesda, MD
The Curative Potential of Cell Transfer Immunotherapy

9:30 Nicholas Restifo, National Cancer Institute, Bethesda, MD
Building a Better Anti-tumor T Cell

10:00 Dirk Busch, Technische Universität München, München, Germany
Clinical Purification of T-Cell Subsets Most Effective for Adoptive Immunotherapy

10:30 **BREAK**

11:00 Ton Schumacher, The Netherlands Cancer Institute, Amsterdam
Linking Cancer Exomes to Cancer Immunotherapy

11:30 Philip Greenberg, University of Washington, Seattle, WA
Engineering T Cells to Be Effective in Tumor Therapy

12:00 Carl June, University of Pennsylvania, Philadelphia, PA
CARs for Leukemia and Beyond

12:30 **LUNCH** and **POSTER VIEWING**

Thursday, October 9, 2014

Session 2: Genetic Engineering of the Immune Systems
Chair: Ron Gress

1:30 Malcolm Brenner, Baylor College of Medicine, Houston, TX
Extending Chimeric Antigen Receptor T-Cell Therapy to Solid Tumors

2:00 James Kochenderfer, National Cancer Institute, Bethesda, MD
Chimeric Antigen Receptors for Treating Hematologic Malignancies

2:30 Michel Sadelain, Memorial Sloan Kettering Cancer Center, New York, NY
CARs, CCRs, and iCARS: A New Class of Drugs for Cancer Immunotherapy

3:00 Patrick Hwu, MD Anderson Cancer Center, Houston, TX
Understanding Immune Resistance at the Molecular Level

3:30 **BREAK**

4:00 Stanley Riddell, Fred Hutchinson Cancer Research Center, Seattle, WA
Novel CARs and Cell Compositions for Adoptive Cancer Therapy

4:30 Crystal Mackall, National Cancer Institute, Bethesda, MD
Chimeric Antigen Receptor–Based Therapies for Childhood Cancer

5:00 Laurence Cooper, MD Anderson Cancer Center, Houston, TX
Genetically Modifying with Transposons and Genetically Editing with Artificial Nucleases to Engineer T Cells for Human Application

Friday, October 10, 2014

Session 3: Checkpoint Modulators
Chair: Jay Berzofsky

8:30 Suzanne Topalian, Johns Hopkins University, Baltimore, MD
PD-1 Pathway Blockade in Cancer Therapy

9:00 Jedd Wolchok, Memorial Sloan Kettering Cancer Center, New York, NY
Anti-CTLA-4–Based Combinatorial Immunotherapeutic Strategies

9:30 Stephen Hodi, Dana Farber Cancer Institute, Boston, MA
Cytokines and Anti-angiogenesis Combinations with Immune Checkpoint Blockade

10:00 James Allison, MD Anderson Cancer Center, Houston, TX
Immune Checkpoint Blockade in Cancer Therapy: New Insights

10:30 **BREAK**

Session 4: Other Approaches to Cancer Immunotherapy
Chair: James Gulley

11:00 Dan Fowler, National Cancer Institute, Bethesda, MD
Allogeneic T-Rapamycin Cell Therapy of Refractory Lymphoma

11:30 Jeffrey Miller, University of Minnesota, Minneapolis, MN
Educating and Targeting NK Cells In Vivo to Exploit Their Activity for Cancer and Transplantation Therapy

12:00 Ronald Levy, Stanford University, Stanford, CA
Targeting the Tumor and Targeting the Host

12:30 **LUNCH** and **POSTER VIEWING**

Friday, October 10, 2014

2:00 Thomas Waldmann, National Cancer Institute, Bethesda, MD

The Evil Face of Janus Kinases: Disorders of Gamma Cytokines, JAKs, and STATS in T-Cell Malignancies

2:30 Jeffrey Schlom, National Cancer Institute, Bethesda, MD

Emerging Concepts in the Design and Development of Recombinant Cancer Vaccines

3:00 James Yang, National Cancer Institute, Bethesda, MD

Immunotherapy of Renal Cell Cancer

3:30–5:00 **POSTER VIEWING**

CANCER IMMUNOLOGY AND IMMUNOTHERAPY: DELIVERING THE PROMISE

Poster Abstracts

Poster #	First Author
1	Abid Azhar
2	Veronika Bachanova
3	Joseph Barchi Jr
4	Lewis H. Bender
5	Cristina Bergamaschi
6	Chelsea M. Black
7	Emily K. Bongiorno
8	John Bramley
9	Marco Bregni
10	Christine E. Brown
11	Brandon J. Burbach
12	Jian Li Campian
13	D. Chandra
14	Shilpak Chatterjee
15	Xin Chen
16	Yaya Chu
17	Christine A. Clarke
18	ABSTRACT WITHDRAWN
19	Chi-Ping Day
20	Kenneth DeSantes
21	Barna Dey
22	Gerald Dryden
23	Jonathan K. Fallon
24	Ana Carolina Franco Ferreira
25	Arundhati Ghosh
26	Matthias Gromeier
27	Andras Heczey
28	Lee Hong
29	Dewan Md Sakib Hossain
30	Arthee Jahangir
31	Yun Ji
32	Hong Jiang
33	Jamal A. Khan
34	Rhonda C. Kines
35	Chris E. Lawrence
36	Bai Liu
37	Zuqiang Liu
38	Jordana Lovett
39	Lily T. Lu
40	Danielle M. Lussier
41	Daniel H Madsen
42	Fazal Mahmood

Poster #	First Author
43	Amani Makkouk
44	Warren D. Marcus
45	Thomas Marron
46	Junko Masuda
47	Braden C. McFarland
48	Thounaojam C. Menaka
49	Mikayel Mkrtichyan
50	Divya Nagarajan
51	Daniel Neitzke
52	Michelle H. Nelson
53	Douglas C. Palmer
54	Maria Parkhurst
55	Samuel Pellom
56	Mariana Pereira Pinho
57	Rodrigo Nalio Ramos
58	John L. Reagan
59	Cecilia Pessoa Rodrigues
60	Roberta Neves Gago Rodrigues
61	Maria Alejandra Clavijo Salomón
62	Andrea Schuessler
63	Nazila Shafagati
64	Gautam N. Shenoy
65	Haley M. Simpson
66	Kelly L Singel
67	David R. Soto-Pantoja
68	Katherine Stagliano
69	Tom Li Stephen
70	Wern Su
71	Emma Svensson
72	Amelia J. Tesone
73	Ana Textor
74	Krishnamurthy Thyagarajan
75	Kevin R. Trabbic
76	Katarzyna Urbanska
77	Raul Vizcardo
78	Enxiu Wang
79	Jennifer Wu
80	Tori N. Yamamoto
81	Hao Yu
82	Lijie Zhai
83	Yi-Fan Zhang
84	Guizhi Zhu

Poster 1

Human TP53 Mutations at Codon 36, 72 And 240 in Oral Squamous Cell Carcinoma: A Promising Diagnostic Tool for Future Immunotherapies

Abid Azhar[1], Saima Saleem[1], Abdul Hameed[2], Zubair Ahmad Abbasi[3], Mansoor Ahmed Khan[1], Navid Rashid Qureshi[4]

[1]The Karachi Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, Karachi, Pakistan. [2]Institute of Biomedical and Genetic Engineering (IB&GE), Islamabad, Pakistan. [3]Karachi Medical and Dental College (KMDC), Karachi, Pakistan . [4]Liaquat College of Medicine and Dentistry (LCMD), Karachi, Pakistan

Oral squamous cell carcinoma (OSCC) is the leading cause of death in the developing countries like Pakistan. This problem aggravates because of the excessive use of available chewing products. In spite of widespread information on their use and purported legislations against their use, the Pakistani markets are classical examples of selling chewable carcinogenic mutagens. Reported studies indicated that these products are rich in reactive oxygen species (ROS) and polyphenols. TP53 gene is involved in the suppression of tumor. It has been reported that somatic mutations caused by TP53 gene are the foundation of the cancer. This study aims to find the loss of TP53 functions due to mutation/polymorphism caused by genomic alteration and interaction with tobacco and its related ingredients. Total 260 tissue and blood specimens were collected from OSCC patients and compared with age and sex matched controls. Mutations in exons 2-11 of TP53 were examined by PCR-SSCP. Samples showing mobility shift were directly sequenced. Two mutations were found in exon 4 at nucleotide position 108 and 215 and one in exon 7 at nucleotide position 719 of the coding sequence in patient's tumor samples. These result in substitution of proline with arginine at codon 72 and serine with threonine at codon 240 of p53 protein. These polymorphic changes, found in tumor samples of OSCC, could be involved in loss of heterozygocity and apoptotic activity in the binding domain of TP53. The interpretations could be helpful in establishing the pathways responsible for tumor formation in OSCC patients.

The study was funded by the Higher Education Commission (HEC), Islamabad, Pakistan. Research Project No: 20-695.

Poster 2

Unrelated donor *KIR BIX* genotype reduces relapse and improves progression-free survival after HLA-matched allogeneic transplantation for relapsed/ refractory Non Hodgkin Lymphoma

Veronika Bachanova, MD[1], Daniel J. Weisdorf, MD[1]. Tao Wang, PhD[2], Marsh G.E. Stephen, PhD[3] , Elizabeth Trachtenberg, PhD[4], Michael D Haagensen, MS[5'], Stephen R. Spellman, MBS[5'], Guethlein A. Lisbeth, PhD[6], Martha Ladner, PhD[4'], Peter Parham, PhD[6'], Jeffrey S. Miller, MD[1] and Sarah A. Cooley, MD, MS[1]

[1]Blood and Marrow Transplant Program, Department of Medicine, University of Minnesota, Minneapolis, MN; [2]Division of Biostatistics, Institute for Health & Society, Medical College of Wisconsin , Milwaukee, W I; [3]Anthony Nolan Research Institute, London, England; [4]Children's Hospital of Oakland Research Institute, Oakland, CA; [5]Center for International Blood and Marrow Transplant Research, Minneapolis, MN; [6]Structural Biology and Microbiology and Immunology , Stanford University, Stanford, CA

The importance of natural killer (NK) cell killer immunoglobulin-like receptors (*KIR*) genetics in unrelated donor (URD) hematopoietic cell transplantation (HCT) has been demonstrated in acute myeloid leukemia (AML) where donors with favorable *KIR* gene content had reduced the risk of relapse by 30%. We investigated whether the *KIR* genotype impacts the survival of patients with non-Hodgkin lymphoma (NHL).

We evaluated the outcomes of 614 adults with NHL who underwent T cell replete URD HCT between 1990-2009 as reported to the Center for International Blood and Marrow Transplant Research (CIBMTR). Donor samples from the NMDP/CIBMTR Repository were genotyped by MALDl-TOF mass spectroscopy. *KIR* haplotypes (*A/A* or *B/x*) were determined by presence/absence of 15 individual *KIR* genes.

The median patient age was 50 years (range 19-72), 62% had chemosensitive lymphoma. Most transplants were 10/ 10 matched for HLA alleles (n=396), the remainder 9/10 matched (n=158) or =8/ 10 matched (n=60). The frequencies of *KIR* genotypes in donors reflected general population: 70% were *KIR B/x* (n=428) and 30% *KIR A/A* (n=180). Patients receiving 10/10 HLA-matched grafts (65%) experienced significantly lower relapse at 5 years when donors were *KIR* Blx genotype (n=281; 26% [95%CI21-32%]) compared to *KIR A/A* genotype (n=115; 37% [95% Cl27-46%);p=0.05) leading to improved progression-free survival (35% (95% 26-44) vs. 22% [95%CI 11-35%]; p=0.02). After adjusting for significant clinical variables, *KIR* Blx donors conferred significant protection against relapse (RR 0.63 (95%CI0.43-0.92); p=0.02) and improved PFS (RR 0.71 (95% Cl 0.55-0.91); p=0.008) compared to *KIR A/A* donors. The relapse protection was not observed in the HLA-mismatched transplants.

Our results demonstrate that donors with *KIR* Blx genotypes, whose NK cells express more activating *KIR*, are associated with decreased relapse and improved survival after 10/10 HLA-matched URD HCT for NHL. These results suggest that NK cells may contribute to graft-versus-lymphoma effect after allo HCT.

Poster 3

Gold nanoparticles as a novel platform for glycopeptide-based cancer vaccine development

Joseph Barchi Jr[1], Raymond Brinas[1], Kate Rittenhouse Olsen[2]

[1]Chemical Biology Laboratory, CCR, NCI-Frederick; [2]Department of Microbiology and Immunology, University of Buffalo, Buffalo, New York

Tumor-associated carbohydrate antigens (TACAs) were discovered more than 30 years ago and have been used in a variety of studies as haptens for anticancer vaccine design. In addition, some of these small saccharides have been directly implicated in the metastatic process of different solid tumors. We have concentrated on the Thomsen-Friedenreich disaccharide as a basis for the synthesis of nanoparticles containing this antigen in a range of presentations. This talk will highlight our latest in vitro and in vivo results on raising immune responses to mucin-based glycopeptides bearing the TF antigen on gold nanoparticles. The utility of these constructs as important anti-tumor tools and potential therapeutic agents will be discussed.

Poster 4

Novel cell penetrating formulation of cytotoxic agents administered intratumorally causes complete regression of large tumors and stimulation of an adaptive immune response in BALB/c syngeneic mice inoculated subcutaneously with CT26 colon cancer.

Lewis H. Bender [1], Jay A. Berzofsky[2], Masaki Terabe[3]

[1]Intensity Therapeutics, Inc., Westport Connecticut; [2] Vaccine Branch, Center for Cancer Research NCI, Bethesda, Maryland; [3]Vaccine Branch, Center for Cancer Research, NCI, Bethesda, Maryland.

Few good treatment options are available for patients with metastasized cancer. Here we describe a new methodology; *in situ* chemovaccination. In this approach a novel drug product - Intensity Therapeutics' INT230-6 - appears to have a local ablative effect on large tumors and to stimulate an adaptive immune response when dosed intratumorally (IT). INT230-6 contains 0.5 mg/mL of cisplatin, 0.1 mg/mL of vinblastine and 10 mg/mL of a cell-penetration excipient. We conducted a study in syngeneic female BALB/c mice inoculated subcutaneously (SC) with 1x106 CT26 cells. Tumors grew to mean volumes of 325mm^3 predose. The study comprised four groups: 1) no treatment, 2) INT 230-6 given IT, 3) the drugs given intravenously (IV) without excipient and 4) the drugs given IT without excipient. Animals in the IT treatment groups received 0.1 mL per 400mm3 of tumor volume for 5 days. IV doses were comparable. There was no adjustment for weight. Median overall survival (MOS) for no treatment was 16 days versus 77 days for INT230-6, ratio of 0.2078 with 95% CI of 0.074 to 0.637 with a hazard ratio using Mantel-Haenszel of 0.03501 (95% CI 0.00862 to 0.1422). The INT230-6 MOS survival exceeded that of the IT dosing without the excipient (51 days) and the IV dosing group (42 days) ($p<0.0002$). Twenty percent of animals had a complete response (CR) using INT230-6. CR animals along with naïve controls were re-inoculated first SC then IV with CT26. Ninety percent of the CR animals were fully protected against both SC and IV re-inoculation. The re-inoculations occurred months after the last drug dose. Thus we hypothesize the observed long-term protection is due to an adaptive immune response induced by the dying tumor cells. *In situ* chemo vaccination using INT230-6 thus appears to be a promising new strategy to treat cancer.

Poster 5

Heterodimeric IL-15 promotes tumor control through the regulation of the balance of effector and regulatory cells via an IL-2 deprivation mechanism

Cristina Bergamaschi[1], Sin-Man Ng[2], Stephanie Chen[2], Jenifer Bear[1], Candido Alicea[1], Rachel Kelly Beach[1,2], Raymond Sowder[3], Elena Chertova[3], Barbara K. Felber[1], and George N. Pavlakis[2].

[1]Human Retrovirus Pathogenesis Section and [2]Human Retrovirus Section, VB, CCR, NCI-Frederick; [3]Retroviral Protein Chemistry Core, AIDS and Cancer Virus Program, Leidos Biomedical Research, Inc., FNLCR, Frederick.

Interleukin-15 (IL-15) is a common γ-chain cytokine that plays a significant role in the activation and proliferation of T and NK cells and holds great potential in fighting infection and cancer. We have previously shown that bioactive IL-15 in vivo comprises a complex of the IL-15 chain with the soluble or cell-associated IL-15 receptor alpha chain that are together termed heterodimeric IL-15 (hetIL-15). HetIL-15 produced from engineered human cell lines showed favorable pharmacokinetics and bioactivity compared to monomeric IL-15 in both mice and macaques. We investigated the anti-tumor efficacy of hetIL-15, using the MC38 colon carcinoma mouse model. hetIL-15 resulted in a significant delay in tumor growth, when administered intraperitoneally every 2 days for 2 weeks. In addition, intratumoral delivery of hetIL-15 induced regression of established tumors and cured 50% of mice. Tumor-free mice were resistant to tumor challenge, demonstrating the development of specific anti-tumor immune responses. We further dissected the mechanism leading to tumor control in hetIL-15-treated mice, discovering a new interplay between IL-15 and the cognate cytokine interleukin-2 (IL-2). Repeated injections of hetIL-15 resulted in an increased CD8+/Treg cells ratio in spleen, lymph nodes and tumor microenvironment. Interestingly, hetIL-15 induced a transient accumulation of CD8+ T cells expressing the high affinity IL-2 receptor CD25, that compete with CD25+Treg for the available IL-2. In addition, IL-15 decreased the frequency of IL-2-producing CD4+ T cells, while increasing their ability to secrete IFNγ. These data suggest that hetIL-15 treatment limits Treg fitness through a concerted action of both CD8+ and CD4+ T cells, that results in an IL-2 deprived environment. Preclinical cancer studies support the use of hetIL-15 in tumor immunotherapy approaches to promote the development of anti-tumor responses by favoring effector over regulatory cells.

Poster 6

Ex vivo stimulation assays have the potential to identify patients more likely to respond to cancer immunotherapy

Chelsea M. Black, Paul B. Robbins

MedImmune, Gaithersburg, Maryland

Cancer immunotherapy has recently shown great promise in successfully treating otherwise refractory patient populations. However, although some patients have complete tumor regression, many have no response at all. The ability to identify patients more likely to respond to these drugs could have a large impact by matching individual patients to the treatments most likely to be effective. One approach for identifying individuals who may be more likely to respond relies on ex vivo T cell stimulation assays. These assays measure the ability of immune cells to respond to stimulation outside of the patient. Because immunotherapies allow the immune system to better recognize and eliminate tumors, it is reasonable to infer that immune cells capable of being activated ex vivo in the presence of immunotherapy would be more likely to be functionally active in recognizing and eliminating tumors within the patient. To evaluate methodologies of use in testing this hypothesis, multiple types of stimulation were used to activate healthy donor PBMCs in the presence of αCTLA4 and αPD-L1 blocking antibodies. Intracellular cytokine staining was used to measure activation. Responses to immunotherapy for each individual were determined by comparing them to baseline responses of that individual to stimulation alone. This technique distinguishes individuals who have increased T cell responses to immunotherapy ex vivo from individuals that do not, and may aid in identifying individuals able to respond to one drug but not another by testing individual responses to multiple types of immunotherapy. In conclusion, ex vivo stimulation assays may be a viable approach for determining which patients would be ideal candidates for immunotherapy.

Poster 7

Attenuated Rabies Virus Promotes a Th1 Anti-tumor Response and CNS Immune Infiltration in a Mouse Model of Glioblastoma Multiforme

Emily K. Bongiorno[1], Sami Sauma[2], and D. Craig Hooper[1,2]

[1]Department of Cancer Biology, Thomas Jefferson University, Philadelphia, PA; [2]Department of Neurological Surgery, Thomas Jefferson University, Philadelphia, PA

Glioblastoma multiforme (GBM), an aggressive grade IV astrocytoma, is the most deadly form of cancer. Even with standard surgical resection, chemotherapy and radiation, the 5 year survival rate remains less than 5%. This poor prognosis has led to interest in immunotherapy, yet thus far several immunotherapies utilizing the mouse GL261 model for GBM have been more effective at treating flank tumors compared to intracranial (IC) tumors. This indicates that the blood-brain barrier (BBB) plays a role in the ability of the immune system to eliminate CNS tumors. Evidence also suggests that while certain tumor vaccination strategies promote a Th2 response that can prevent peripheral tumors, a Th1 response is required for immune cell trafficking to the CNS. To address these concerns we have utilized an attenuated rabies virus (RABV) SPBN-GAS that causes BBB modulation, allowing immune effectors to reach the CNS without inducing rabies immunopathology. SPBN-GAS, delivered intranasally after establishment of an IC GL261 tumor, resulted in significantly prolonged survival in mice. RABV-infected tumor-bearing mice exhibited increased mRNA levels of T and B cell, as well as NK markers and IFNγ in CNS tissue. Additionally, infected mice had increased levels of GL261-specific Th1 antibodies, indicating a stronger inflammatory response, and histology revealed increased necrosis in the tumors of infected mice. Here we further examine infected tumor-bearing mice to determine whether the increased survival and distinct tumor phenotype is a result of a non antigen-specific antiviral effect or due to BBB modifications that facilitate contact between the tumor cells and immune system. Additionally we investigate whether using SPBN-GAS in conjunction with GL261 exosome vaccination will produce an increased Th1 response to tumor cells. We hypothesize that shifting the typical ineffective Th2 response to an inflammatory and CNS-infiltrative Th1 response will promote an anti-tumor effect that will benefit future immunotherapeutic efforts.

Supported by an NIH grant RO1 AI093369-01 and a grant to David W. Andrews from the Albert Stevens Foundation.

Poster 8

Development of an Embryonic Avian Neuronal Assay for Assessing Axon Degeneration

John Bramley[1], Christian Leatherman[1], Beni Yuzawa[1], Danny Rosenberg[1], William Buchser[1]

[1]College of William and Mary

Axon degeneration is a hallmark of many sensory impairments like glaucoma, CNS conditions like Parkinson's and Alzheimer's, and following traumatic neural injuries. Recently, SARM, a multidomain protein has been found to play a significant role in the process of Axon degeneration, and its inhibition is suspected to improve many of these conditions. While ongoing studies in drosophila and mouse are appropriate for studies of peripheral neuropathy and underlying mechanisms of this process, they are often deficient in modeling human sensory or CNS disease. Songbirds (Taeniopygia guttata) allow for the study of an animal, which like humans has exceptional vision as well as sophisticated memory. Here, we develop a novel embryonic culture system for avian neurons to first characterize wallerian degeneration. Preliminary results indicate that avian neurons are sensitive to the chemotherapy drug taxol. Subsequent examination is expected to reveal a precise time-course of delay, swelling, and axonal fragmentation.

Poster 9

An Adoptive Immunotherapy Approach for Colorectal Cancer (CRC) Patients

Marco Bregni[1], Serena del Bue[2], Paquale Ferrante[2], Silvia Carluccio[2]

[1]Ospedale di Circolo, Busto Arsizio, Italy; [2]University of Milan, Milan, Italy

Background: Adoptive T-cell transfer (ACT) refers to an immunotherapeutic approach in which anti-tumor T lymphocytes are identified, grown ex vivo and re-infused into the cancer patients. This strategy was successfully applied in metastatic melanoma and renal cell carcinoma. However, adoptive immunotherapy do not play an important role in the treatment of advanced CRC. We developed an ACT protocol for CRC using as source of tumor antigens the irradiated primary CRC cell lines, captured by dendritic cells (DC) to stimulate in vitro a specific autologus activation of patient's T cytotoxic lymphocytes (CTL) against tumor. Methods: 78 CRC patients were enrolled. Tumor tissues were obtained at surgery, together with 100 ml of heparinized peripheral blood. Tumors were mechanically dissociated to a single-cell suspension and cultured to obtain a primary cell line from each patient. DCs were generated from monocytes, cultured with recombinant human Interleukin-4 and recombinant human Granulocyte- Macrophage Colony-Stimulating Factor for 6-7 days. Anti-tumor CTLs were elicited in co/micro-cultures using DCs, autologous apoptotic tumor cells and T CD8+ lymphocytes enriched effectors, in presence of weakly irradiated T CD4+ lymphocytes, Interleukin 7 and Interleukin 12, with weekly stimulation. CTLs Interferon-γ (IFN-γ) secretion was assessed by ELISpot assay. Results: Primary tumor cell lines were obtained from 20 out of 78 patients, with a success rate of 25.6%. DCs were generated from 26 patients, and 6 had the corresponding primary tumor cell line. Co/micro-cultures were set up for 6 patients. ELISpot results showed a strong and significant IFN-γ secretion at the third, fourth and fifth stimulations for one patient and at the second for another patient, whereas for three patients a weak secretion was detected during the second and third stimulations. Conclusions: The percentage of success in the establishment of primary colon cell lines was higher than those reported in literature, although the intestinal flora adversely affected their obtainment. The generation of tumor-specific CTLs could be useful for supporting an ACT approach in CRC.

ACC/ISS, Fondazione Veronesi

Poster 10

Adoptive Transfer of IL13Ra2-specific T cells for the Treatment of Glioblastoma: Building on Clinical Achievements with Second-Generation CARs

Christine E. Brown[1], Renate Starr[1], Brenda Aguilar[1], Wen-Chung Chang[1], Lihong Weng[1], Brenda Chang[1], Annie Sarkissian[1], Yubo Zhai[1], William Brezlaff[1], Araceli Naranjo[1], Julie Ostberg[1], Massimo D'Apuzzo[2], Michael E. Barish[3], Michael C. Jensen[4], Behnam Badie[5], Stephen J. Forman[1]

Departments of [1]Hematology & Hematopoietic Cell Transplantation, [2]Pathology, [3]Neurosciences, [5]Neurosurgery; Beckman Research Institute and City of Hope National Medical Center, Duarte, CA 91010; [4]Center for Immunity and Immunotherapies, Seattle Children's Research Institute, Seattle, WA 98195.

The prognosis of patients with high-grade glioma remains grim despite improvements in standard therapies including surgery, radiation and chemotherapy. Immunotherapy approaches targeting brain tumors offer promise over conventional treatments by harnessing the potency and specificity of the immune system. We have developed a chimeric antigen receptor (CAR) T cell immunotherapy for malignant glioma targeting IL-13 receptor a2 (IL13Ra2), a cell surface receptor over-expressed by a high percentage of high-grade gliomas, but not expressed at significant levels by normal brain tissue. Bioinformatic studies demonstrated that expression of IL13Ra2 is associated with increased glioma malignancy grade, mesenchymal signature gene expression, and reduced patient survival. Previous pre-clinical efforts employing 1st-generation T cell clones comprised of membrane-tethered IL13 ligand and an intracellular CD3-zeta endodomain (termed IL13-zetakine) demonstrated potent MHC-independent IL13Ra2 anti-glioma activity against both bulk glioma populations, and glioma stem-like tumor propagating cells. Importantly, completion of two FDA-authorized Phase I clinical trials, evaluating both autologous and allogeneic IL13-zetakine T cell clones in 9 patients, demonstrates the feasibility of this approach, absence of serious side-effects, and provide evidence for transient anti-glioma tumor responses for patients with IL13Ra2pos glioblastomas. Building on this experience, we have now developed an optimized CAR T cell product incorporating enhancements in CAR design and T cell engineering, which improve T cell persistence and antitumor potency. These include a second-generation CAR containing the 41BB (CD137) costimulatory signaling domain (IL13BBzetakine-CAR), and a manufacturing strategy using an enriched central memory T cell (Tcm) population for genetic engineering. In orthotopic IL13Ra2+ glioma mouse models, single injection of optimized IL13BBzetakine-CAR Tcm resulted in robust antitumor activity and significantly improved T cell persistence compared to first-generation IL13Ra2-CAR T cells. These findings support the potential for treating patients with high-grade glioma with IL13Ra2 -specific CAR-engineered T cells.

Poster 11

Regulation of anti-tumor cytotoxic T lymphocyte trafficking by vascular disruption in vivo

Brandon J. Burbach[1], Brendan G. Coutu[1], Lynn Heltemes-Harris[1], Jason S. Mitchell1, Julie M. Curtisinger[1], Matthew F. Mescher[1], and Yoji Shimizu[1]

[1]Center for Immunology, University of Minnesota, Minneapolis, MN

Recent advances in immunotherapy remain hampered by inefficient trafficking of tumor-specific cytotoxic T lymphocytes (CTL) to the tumor and low numbers of immune cell infiltrates are associated with poor clinical responses to immunotherapy. Here we demonstrate that the initial accumulation of tumor infiltrating CTL is associated with impaired tumor vasculature and reduced entry of additional CTL into the tumor. Adoptive transfer of optimally primed OT-I or Trp2.Hi CTL specific for B16.OVA or endogenous Trp2 protein on B16, respectively, promoted early tumor regression but restricted the number of spleen-derived CTL that arrive in tumors 7 days after the initial CTL influx. Live animal imaging by ultrasound Doppler measurements indicated that blood flow was reduced beginning ~4-6 days following CTL adoptive transfer compared to untreated controls. Histological evaluation of PECAM-1 staining patterns at this time point revealed reduced microvascular lumen number and area in CTL treated tumors. Vascular disruption required CTL specific tumor antigen expression but not CTL derived gamma IFN. Recent studies have indicated that vascular normalization via anti-angiogenesis therapy promotes increased CTL access to tumor. Preliminary experiments using the VEGFR2 inhibitor axitinib indicate that pre-normalized tumor vasculature is resistant to CTL mediated vascular destruction. Together, these results suggest that reduction in late phase CTL influx may reflect prior CTL-mediated vascular impairment, and that the efficacy of adoptive T cell transfer can be improved by combining pre-treatment and coincident treatment with angiogenesis inhibitors to allow sustained CTL access to tumors.

Poster 12

Feasibility of lymphocyte harvesting and reinfusion in patients with newly diagnosed high grade gliomas

Jian Li Campian[1], Xiaobu Ye[2], Douglas E. Gladstone[2], Prakash Ambady[3], Ivan Borrello[2], Marc Golightly[4], Karen E. King[2], Matthias Holdhoff[2], Judith Karp[2], Stuart A. Grossman[2].

[1]Washington University in St Louis; [2]Johns Hopkins University; [3]Neuro-oncology, NCI; [4]Stony Brook Medical Center.

Background: Standard radiation, temozolomide, and dexamethasone cause severe treatment-related lymphopenia (TRL) (total lymphocyte counts (TLC) <500cells/mm3) 2-month after initiating therapy in patients with newly diagnosed high grade gliomas (HGGs). Published studies suggest that severe TRL is related to radiation of circulating lymphocytes and is associated with shorter survival due to tumor progression. This study was designed to evaluate the feasibility of harvesting lymphocytes before radiation and reinfusing them after radiation is completed. Methods: Ten patients with newly diagnosed HGGs and baseline TLC >1000 cells/mm3 were enrolled. Lymphocytes were harvested with a single 120-minute apheresis procedure via peripheral veins, cryopreserved, and reinfused immediately after radiation was complete. Weekly blood counts were followed for 20 weeks. A post-reinfusion TLC rise >300 cells/mm3 was desired. Results: 10 patients were harvested and 8 received lymphocyte reinfusion. Median age was 55.5 (range 40-67); median dexamethasone dose was 3 mg/day (range 0-4mg/day), and 70% was glioblastoma multiforme. Their baseline median TLC was 1980 cells/mm3 (range: 1060-2290) and a median of 8.9x107 lymphocytes/Kg (range 3.4-11.1x107) were harvested. After 6 weeks of therapy, the TLC fell 63% (median 605 cells/mm3, p<0.0001). A median of 7.0x107 lymphocytes/Kg (range: 2.7–9.5x107) were reinfused. Four weeks after reinfusion the average increase in TLC was 274 cells/mm3 (STD: ±298, p=0.045). Overall, 7 of 8 patients (88%; 90%CI: 53-99%) had an absolute increase in TLC >300 cell/mm3 in the 14 weeks following reinfusion and this persisted in 4 of 7 (57%) patients. No adverse events occurred related to harvest/reinfusion. Conclusions: Lymphocyte harvest and reinfusion is feasible in HGG patients on dexamethasone and no toxicities were noted. An increase in TLC >300 cells/mm3 was seen in 88% of reinfused patients during the 14-week observation period post reinfusion. Future studies are being designed to document the role of lymphocyte reinfusion in the increase in TLC.

Support from Johns Hopkins School of Medicine Clinical Pharmacology's T32 Grant.

Poster 13

Therapeutic treatment with Cryoablation and Meriva generates T cell responses to multiple TAAs and eliminates metastatic breast cancer almost completely

Chandra D*, Jahangir A, Lydie Meheus**, Klara Rombauts**, Francisco Cornelis***, Gravekamp C*

*Albert Einstein College of Medicine, Department of Microbiology and Immunology, Bronx, USA; **Anticancer Fund, Strombeek-Bever, Belgium; Hopital Pellegrin, CHU Bordeaux, France

Immune suppression in the tumor microenvironment (TME) is a major problem in cancer vaccination. Here we generated a multi-tumor-associated antigen (TAA) vaccine of host's own tumor and reduced IL-6-mediated immune suppression by a combination therapy of cryoablation and Meriva. Cryoablation of tumors involves killing of tumor cells by the insertion of a probe into a tumor nodule to generate tissue-ablative cold temperatures. Its mechanism of action has been attributed to the mechanical forces of crystallization, the osmotic changes due to crystallization, and the ischemic effects of microvascular injury. This leads to immunogenic tumor cell death resulting in cross presentation of multiple TAA of the cryo-killed tumor cells by antigen-presenting cells (APC) to the immune system. In turn, this will recruit TAA-specific T cells. However, these T cells are non-functional because of the strong immune suppression in the TME. Interleukin (IL)-6, frequently produced in the TME of breast and other cancers, is a cytokine that strongly inhibits T cell responses. We found that Meriva, a Curcumin derivate with high bioavailability, reduced immune suppression by IL-6, and when combined with cryoablation, resulted in the activation of T cell responses to TAA Mage-b and Survivin (highly expressed by tumors and metastases of 4T1 model), and a nearly completely eliminated the metastases and primary breast tumor in the 4T1 model. In addition, the overall survival of mice that received Cryoablation and Meriva was significantly increased compared to the saline group. This approach not only avoids the need of developing cancer vaccines with TAA but also may overcome the heterogeneity of the tumors. In summary our results demonstrate that the combination of Cryoablation and Meriva is highly effective against metastatic breast cancer, and perhaps against other IL-6-producing cancers as well.

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Poster 14

Reduced CD73 Expression by TGF-β Independent Th17 Cells Improves Tumor Control

Shilpak Chatterjee[1], Krishnamurthy Thyagarajan[1], Pravin Kesarwani1, Jin H. Song[2], Myroslawa Soloshchenko[1], Jianing Fu[3], Stefanie Bailey[3], Andrew Kraft[2], Chrystal M. Paulos[3], Xue-Zhong Yu[3], Shikhar Mehrotra[1]

Departments of [1]Surgery, [2]Biochemistry & Molecular Biology, [3]Microbiology & Immunology, Hollings Cancer Center, Medical University of South Carolina, Charleston, 29425.

T helper (Th)-17 subsets hold promise in adoptive T cell transfer therapy for cancer. However, ex vivo programming of Th17 cells in presence of TGF-β increases cell surface expression of ectonucleotidases CD39 and CD73, that in turn increases susceptibility to immunosuppression and reduces effector functions. Our data shows that ATP mediated suppression of IFN-γ production by Th17 cells can be overcome either by genetic ablation of CD73 or by generating TGF-β independent Th17 in presence of IL-1β. Th17 cells cultured in IL-1β are also highly polyfunctional, express high level of effector molecules and exhibit better short-term control of B16-F10 murine melanoma, despite reduced stem cell like properties. Adding TGF-β at low dose that does not upregulate CD73 expression, but induces stemness, drastically improves anti-tumor function of IL-1β cultured Th17 cells. It is likely that effector property of IL-1β dependent Th17 is due to their high glycolytic capacity, since generating IL-1β dependent Th17 cells in pyruvate containing media impaired glycolysis and its anti-tumor potential. Thus, our data suggests that due to induction of ectonucleotidase expression by TGF-β, ex vivo culture conditions for generating Th17 cells need to be reconsidered for exploiting their full potential in adoptive T cell therapy.

The work was supported in part by funds from Department of Surgery (MUSC) and NIH R01CA138930 and PO1 CA154778.

Poster 15

Cyclophosphamide and anti-TGFß antibody synergistically enhanced the immunogenicity and efficacy of a cancer cell vaccine in a mouse breast cancer model

Xin Chen[1,2], O.M. Zack Howard[2], John M. McPherson[3], Lalage M. Wakefield[4] and Joost J. Oppenheim[3]

From [1]Basic Science Program, Leidos Biomedical Research, Inc.; [2]Laboratory of Molecular Immunoregulation and [4]Laboratory of Cancer Biology and Genetics; Center for Cancer Research, National Cancer Institute, Frederick, Maryland, USA. [3]Genzyme Corporation, Framingham, Massachusetts, USA

Previously we reported that the combination of cyclophosphamide (CY) and 1D11, an antibody against mouse TGFß (1,2,3), effectively inhibited the development of mouse 4T1 mammary carcinoma and therefore represented a novel and promising modality of chemoimmunotherapy. This beneficial effect of combination treatment was at least partly based on the mobilization of anti-tumor immune responses. We therefore hypothesized that the combination of CY and 1D11 may have a synergistic effect in increasing efficacy of a therapeutic cancer vaccine. To test this idea, Balb/c mice bearing established 4T1 tumor were treated with one dose of CY and six doses of 1D11. The cancer cell vaccine, e.g., irradiated 4T1 cells, were injected s.c. at day 8, day 15 and day 22 after initial inoculation of 4T1 cancer cells. The results showed that a combination of CY+1D11 markedly inhibited the growth of 4T1 tumor ($p<0.05$), consistent with our previous report. To our surprise, inoculation of cancer vaccine by itself promoted the growth of 4T1 tumor, suggesting that irradiated tumor cells may induce a tolerogenic reaction, rather than a desirable stimulatory effect on tumor immunity. However, the combination of CY+1D11 was able to overcome the tolerogenic effect and consequently revealed the immunogenicity of cancer cell vaccine, because the combination of CY+1D11 plus cancer vaccine resulted in a greater inhibition of tumor growth ($p<0.01$, as compared with CY+1D11). Therefore, chemoimmunotherapy with CY and 1D11 may prove to be useful in enhancing the efficacy of therapeutic vaccine in tumor patients.

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Poster 16

Therapeutic effects of Romidepsin, a histone deacetylase inhibitor (HDACi), alone and in combination with natural killer cells against pediatric Burkitt Lymphoma

Yaya Chu[1], Ashlin Yahr[1], Janet Ayello[1], Carmella vandeven[1], Mitchell Cairo[1,2,3,4,5]

[1]Pediatrics, [2]Medicine, [3]Pathology, [4]Microbiology and Immunology and [5]Cell Biology and Anatomy, Maria Fareri Children's Hospital, New York Medical College, Valhalla, NY

Purpose: The outcome for children with Burkitt Lymphoma (BL) has improved significantly but for patients who relapse or progress, the prognosis is dismal due to chemo-radiotherapy resistance (Cairo, J Clin Oncol, 2012). Our group has successfully engineered expanded peripheral blood Natural Killer cells (exPBNK) cells with an anti-CD20 chimeric antigen receptor (CAR+ exPBNK) to target relapsed/resistant CD20+ BL (Chu & Cairo, ASH, 2013). Romidepsin, a histone deacetylase inhibitor, enhances NKG2D ligand expression on tumor cells (Chu & Cairo, EMBT, 2013). We investigated the anti-tumor effect and mechanisms of Romidepsin against BL; and the combination effect of Romidepsin with CAR+ exPBNK cells against CD20+ BL cells in NSG mice. Method: CD20+ BL cells were treated with 10ng/ml Romidepsin, provided by Celgene. Cell viability, MICA/B expression, cell cycle, and signal pathway changes were analyzed by flow cytometry. Raji-Luc or Raji-2R-Luc cells were injected into NSG mice. CAR+ exPBNK, Romidepsin or combination was given to each mouse once a week for 3 weeks. The cumulative luciferase signals and tumor size were measured with the IVIS-200 system and caliber. Results: Romidepsin induced strong cell death in rituximab rsensitive Raji ($p<0.001$), cell cycle arrest in rituximab resistant Raji-2R and Raji-4RH ($p<0.01$), and reducing p38 phosphorylation. Consistent with our in vitro observation, Rituximab significantly inhibited the growth of BL tumor cells in xenografted NSG mice ($p<0.05$). We further found MICA/B expression was significantly enhanced in Romidepsin treated BL cell lines ($p<0.001$) and the anti-CD20 CAR can further significantly enhanced the cytoxicity of exPBNK against romidepsin treated BL in vitro ($P<0.05$). Moreover, we found that CAR+ exPBNK cells combined with Romidpesin significantly increased the survival of Raji-Luc xenografted NSG mice compared to the controls ($p<0.01$). Conclusions: Romidepsin has dual-therapeutic effects in BL by inducing cell death, cell cycle arrest, and enhancing CAR+ exPBNK cytotoxicity against CD20+ BL.

The authors would like to thank Celgene for providing Romidpesin, Dr. Dario Campana (St. Jude Children's Research Hospital) and Dr. Terrence Geiger (St. Jude Children's Research Hospital) for kindly providing K562-mb15 - 41BBL and anti-CD20 scFv, and Dr. Lauren Cooper for kindly providing ffLUCZeo-pcDNA3.1. This work was supported by grants from the Pediatric Cancer Research Foundation, Children Cancer Foundation, and New York Medical College Intramural Research Award.

Poster 17

Lentiviral shRNA-mediated Knockdown of Eosinophilic Galectin-10/Charcot-Leyden Crystals: A Novel Approach to Cancer Immunotherapy

Christine A. Clarke[1], Clarence M. Lee[2], Ibrahim Laniyan[1], Paulette Furbert-Harris[1]

[1]Howard University Cancer Center, Washington, D.C.; [2]Howard University, Department of Biology, Washington, D.C.

Despite the discovery of promising anti-cancer immunotherapeutic strategies such as cancer vaccines, cytokines, and T cell-based therapies, curative outcomes remain elusive. We have investigated the eosinophil as a potential anti-cancer effector cell, and have reported the ability of the eosinophils and their isolated granular proteins to inhibit prostate and breast cancer cell growth *in vitro*. In certain tumors, tumor-associated eosinophilia is marked by the deposition of a prominent eosinophil protein, galectin-10/ Charcot-Leyden crystals. siRNA-mediated knockdown studies have demonstrated galectin-10 expression in regulatory T cells (T_{reg})—cells that have been shown to be major players in regulating anti-cancer immune responses—and its mechanistic necessity in maintaining T_{reg} anergy and suppressive function on $CD4^+$ T lymphocytic proliferation. Despite having been documented at numerous tumor sites, the protein's prognostic significance requires further investigation. In the present study, we have elected to create a galectin-10 knockdown eosinophil sub-line by transfecting GRC.014.24 (an eosinophil cell line established in our laboratory), with shRNA lentiviral transduction particles and, thereafter, conduct further studies to examine eosinophilic galectin-10's potential to increase T- cell homing to tumors. Briefly, GRC.014.24 (2×10^4 cells/ml) were transfected with galectin-10-specific lentiviral transduction vectors. Puromycin was used to select stable transductants; PCR and immunofluoresence methods, to determine transduction efficiency. We show that a galectin-10-specific shRNA lentiviral particle effected 100% gene silencing; this eosinophil clone lacked both granular and cytoplasmic protein expression. The creation of galectin-10 knockdown eosinophils provides a useful model for investigating eosinophilic galectin-10's ability to modulate T cell access and homing to tumors, and a putative role, similar to Treg galectin-10, in regulating tumoral T lymphocytic proliferation can certainly be envisioned. The consideration of galectin-10 knockdown eosinophils as a singular approach to cancer immunotherapy, or in combination with other anti-cancer therapies such as adoptive T cell therapies, or therapeutic cancer vaccines, is intriguing.

Poster 18

ABSTRACT WITHDRAWN

Poster 19

PRECLINICAL OPTIMIZATION AND EVALUATION OF IMMUNOMODULATORY THERAPY FOR MELANOMA RESPONSIVENESS

Chi-Ping Day[1], Zoe Weaver Ohler[2], Rajaa El Meskini[2], Terry Van Dyke[2,3], and Glenn Merlino[1]

[1]Laboratory of Cancer Biology and Genetics, CCR, NCI; [2]Center for Advanced Preclinical Research, Leidos Biomedical Research, Frederick National Laboratory; [3]Mouse Cancer Genetics Program, CCR, NCI

Metastatic melanoma is highly aggressive and resistant to most therapeutic interventions. Recently, immunomodulatory checkpoint-blocking antibodies have produced durable responses in a subset of melanoma patients. However, most advanced patients still do not respond to these drugs. The mechanisms underlying the differences in patient responses are mostly unknown and predictive biomarkers of clinical efficacy are not available. We hypothesize that the density and/or quality of melanoma antigens determines immunomodulatory therapy (IMT) success, and that this can be driven by a patient's history of UV exposure. Our goal is to utilize genetically engineered mouse (GEM)-derived allograft (GDA) to design and establish immunocompetent melanoma models, required for IMD approaches, to evaluate efficacy and to explore mechanisms of IMT response. We will first test the relationship between non-self antigenicity and IMT efficacy by differences in the responses of antigen-expressing tumors in wildtype hosts compared to pre-tolerized recipient GEMs. To assess the role of UV-enhanced antigen diversity on IMD efficacy, we will determine the impact of UV dose on melanomagenesis in GEM models. Melanomas derived from high, low, or no UV treatments will be subjected to preclinical testing in our GDA model systems and genomic analysis, the results of which will be compared with all available human databases to identify predictive biomarkers. Using these newly developed models, we will evaluate therapeutic outcomes and biomarker associations using a series of inhibitors targeting the MAPK/PI3K networks in combination with IMTs. These studies will complement clinical studies correlating patient IMT therapeutic response with their UV exposure history.

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Poster 20

Characterization of Ex-Vivo Expanded Allogeneic NK Cells for Use in Combination with hu14.18-IL2 to Treat Children with Relapsed or Refractory Neuroblastoma

Kenneth DeSantes[1], Diana Drier[2], Kim McDowell[1], Myriam Bouchlaka[3], Erika Heninger[3], Derek Hei[2], Joyce Johnson[4], Dagna Sheerar[3], Christian Capitini[1], Mario Otto[1], Catherine Leith[4], Neehar Bhatia[2], Laurie Larson[2], Ross Martin[2], Timothy Sparks[2], Connor Lyons[2], John Centanni[5], Dario Campana[6], Cliona Rooney[7], and Paul Sondel[1].

[1]Department of Pediatrics, UW-American Family Children's Hospital, Madison,WI; [2]Waisman Biomanufacturing Facility, Madison,WI; [3]UW Carbone Comprehensive Cancer Center, Madison,WI; [4]Department of Pathology, UW Hospital and Clinics, Madison,WI; [5]Department of Medicine, UW Hospital and Clinics, Madison,WI; [6]National University Cancer Institute, Singapore ; [7]Baylor College of Medicine, Houston,TX

We have designed a clinical trial to assess the feasibility of administering multiple cycles of ex-vivo expanded allogeneic NK cells along with the hu14.18-IL2 immunocytokine to treat children with relapsed/refractory neuroblastoma. Apheresis products were obtained from healthy donors and 3 separate qualification runs were performed. PBMC were isolated by ficoll density gradient centrifugation. Cells were incubated in a WAVE bioreactor with a K562 cell line genetically modified to express IL15 and 41BBL. After 11-12 days, cells were concentrated by tangential flow filtration, and incubated with anti-CD3 immunomagnetic beads in the CliniMACS cell separation device. A 28 - 194 fold expansion of NK cells was achieved. The total number of NK and T cells in the final products were 3×10^9, 2.2×10^{10} and 3.6×10^6, 5.4×10^7, respectively. Viability of the final products ranged from 80 - 89%. Following expansion, NK cells demonstrated potent cytotoxicity against K562, M21 (melanoma) and CHLA20 (neuroblastoma) cells lines in a 51Cr-release assay, and when combined with hu14.18K322A in an antibody dependent cellular cytotoxicity assay. The cells retained cytotoxic activity following cryopreservation. Post- incubation, NK cells demonstrated up-regulation of NKGD2, granzyme B and natural cytotoxicity receptors NKp46 and NKp30, and down-regulation of KIR. Expression of CD16 (FcγRIII) remained unchanged. For the clinical trial, children will receive lymphodepletive chemotherapy with cyclophosphamide (60 mg/kg) and fludarabine (125 mg/m^2). Parents will serve as NK cell donors and undergo HLA and KIR typing in order to select the donor expected to exhibit the greatest degree of NK cell alloreactivity. The dose of NK cells will be escalated from 2×10^6/kg to 5×10^7/kg in a standard 3 x 3 design. Starting 24 hours after the NK cell infusions, children will receive hu14.18-IL2 at a dose of 24 mg/m^2 given as a continuous infusion over 10 days. Patients will receive 4 cycles of immunotherapy.

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Poster 21

Bispecific Anti-Env Chimeric Antigen Receptors As Potential Tools In Achieving Functional Cure Of HIV

Barna Dey[1], Li Liu1, Bhavik Patel[1], Mustafa H. Ghanem[1], Virgilio Bundoc[1], Zhili Zheng[2], Richard A. Morgan[2], Steven A. Rosenberg[2] and Edward A. Berger[1]

[1]Laboratory of Viral Diseases, National Institute of Allergy and Infectious Diseases; [2]Surgery Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD

Major research efforts are currently underway to achieve an HIV 'functional cure' whereby antiretroviral therapy can be terminated without resurgence of the virus. Cell-based therapies are gaining momentum as a testable approach in this front. Following the success of adoptive transfer of genetically modified T cells in the treatment of hematological cancers, Chimeric Antigen Receptors (CARs) have raised new hopes for the treatment of other types of cancers and chronic infections. We tailored CARs to target HIV-infected cells by selecting HIV envelope (Env)-binding moieties as the extracellular targeting domain; this was followed by sequences that encode a hinge and a transmembrane domain, both from CD28, and the intracellular CD28 and CD3-zeta signaling domains. Clinically successful CARs against cancers typically contain a single targeting moiety against a specific cancer antigen. For HIV, we introduced bi-specific antigen-binding domains. In our designs, one of the two Env-targeting moieties consisted of the extracellular domains 1 and 2 (D1D2) of CD4, which acts as the primary receptor for HIV gp120; the second component consisted of the scFv of the anti-gp120 human mAb 17b, which binds to the CD4-induced co-receptor-binding site on gp120. Alternatively, the second domain consisted of a human gp120-binding protein. We analyzed recombinant CAR variants, containing either CD4 alone (mono-specific) or CD4 linked to a second gp120-recognizing moiety (bi-specific) via a flexible linker. Our in vitro results suggest that compared to CD4 CAR, the CD4-17b CAR exerts superior potency. Difference in performance due to the length (10 aa or 35 aa) of the linker connecting CD4 to the 17b moiety hinted on the importance of serial triggering for CAR potency. Moreover, the potent bi-specific CARs are devoid of the undesired activity seen with the mono-specific CD4 CAR of rendering CD8 T-cells (also CCR5-positive) susceptible to HIV infection.

Poster 22

Effect of Directly Injecting Cytokine-containing Microbeads on an Endoscopically Initiated Model of Colon Cancer

Gerald Dryden[1], Shunmugavadiv Vanchinathan[1], Nejat Egilmez[1], Jill Suttles[1]

[1]University of Louisville, Louisville, KY

Introduction: Colorectal cancer (CRC) is a serious US health problem, with >142,800 new cases of CRC diagnosed yearly along with >50,000 cancer-related deaths. Five-year survival for metastatic CRC remain dismal. Immunological reactions against cancer have been long recognized and are increasingly harnessed as therapy, prompting our interest in using immunotherapy to alter the immunophenotype of tumor associated macrophages (TAMs) with cytokine therapy. Methods: Groups (n=6) of 8 week old C57/Bl6 mice were inoculated with tumor under endoscopic control by injecting luciferase-expressing MC-38 cells into the colonic submucosa. After allowing maturation for 3 weeks, microbeads containing either interleukin (IL)-12, IL-12+granulocyte-macrophage colony stimulating factor (GM-CSF), or vehicle were directly injected into tumors. After 7 days animals were euthanized. Tumors were resected and macerated. TAMs were harvested by magnetic beads and cultured overnight. Supernatants were analyzed for cytokine production and mRNA expression. Distant metastatic lesions were counted. Results: IL-12 or IL-12+GM-CSF microbead-treated animals gained weight while controls lost. Treated tumors decreased by an average of 21%, while controls increased. Treated tumors contained higher levels of Cd11b+ macrophages (IL-12+GM-CSF = 34%, IL-12 = 27%) than controls (23%). TAMS from cytokine treated tumors produced significantly higher levels of IFN-γ and TNF-α and lower levels of IL-10. The prominent metastases seen in control animals were not visually apparent in IL-12+GM-CSF treated animalism, which was confirmed by microscopy. Discussion: We demonstrated evidence of an anti-tumor effect by cytokine containing microbeads in murine model of CRC. The cytokines influenced tumor-associated TAM functional plasticity. The beads created a sustained *in vivo* exposure to proinflammatory cytokines, flipping the phenotypic switch of TAMSs from a role supporting tumor growth to a cancer-killing mode capable of direct tumor cytolysis. The cumulative effect of immune cell activation caused tumor regression while reducing metastatic lesions.

Poster 23

Combination therapy with an immune checkpoint inhibitor and an IL-12/ anti-DNA immunocytokine results in enhanced anti-tumor efficacy in vivo

Jonathan K. Fallon[1], Amanda J. Lyon[1], Robert Schmitz[1], Robert Tighe[2], Giorgio Karadjian[2], Wilson Guzman[2], Anna Bernhardt[2], Berend Neuteboom[2], Kenneth W. Hance[2], Yan Lan[2], Helen Sabzevari[2], Jeffrey Schlom[1], and John W. Greiner[1]

[1]Laboratory of Tumor Immunology and Biology, CCR, NCI; [2]EMD Serono Research and Development Institute, Billerica, Massachusetts USA

Antibodies that block PD-1/PD-L1 interactions appear to be safe and effective therapies against many PD-L1-expressing tumors. These antibodies may be even more effective at controlling tumor growth when combined with other immunotherapeutic agents. NHS-IL12 is an IL-12/anti-DNA fusion protein that has a longer serum half-life and induces lower, safer levels of IFN-gamma than rIL-12 in cynomolgus macaques. NHS-IL12 was found to be more effective than rIL-12 in three murine s.c. tumor models (MC38, LLC, and B16), and its anti-tumor activity is independent of tumor PD-L1 expression levels. A novel human IgG1 anti-PD-L1 antibody, MSB0010718C, was used to investigate the combined effects of NHS-IL12 treatment and PD-L1 blockade. Concurrent therapy with NHS-IL12 and MSB0010718C resulted in additive anti-tumor effects in both the MC38 and MB49 murine s.c. tumor models. Treatment of mice bearing MC38, MB49, or MCA-205 s.c. tumors with NHS-IL12 alone resulted in the upregulation of tumor PD-L1 expression as determined by immunohistochemistry. This upregulation is likely a response to serum IFN-gamma induction by NHS-IL12, and studies to investigate this possible mechanism are ongoing. Higher levels of tumor PD-L1 expression following NHS-IL12 treatment may increase MSB0010718C binding to tumor cells, thereby increasing their sensitivity to CTL-mediated killing. NHS-IL12 and MSB0010718C are currently being evaluated in separate phase I clinical trials (#NCT01417546 and #NCT01772004). The results reported here provide a rationale for combining these two therapeutic agents in a subsequent phase II trial.

Poster 24

Dendritic cells function disability promoted by cancer might begin at their hematopoietic precursors

Ana Carolina Franco Ferreira[1], Bruna Zelante Barbosa[1], Jose Alexandre Marzagao Barbuto[1]

[1]Department of Immunology, ICB, USP, Sao Paulo, Brazil

Dendritic cells (DCs) are highly efficient antigen-presenting cells that show functional disability in cancer patients. This phenomenon may be an important tumor escape mechanism, since functional modification of DCs can prevent the development of an effective immune response against tumors. Interestingly, DCs that are generated in vitro, derived from cancer patients' precursors, also have these deficiencies, indicating a systemic effect of cancer on DC precursors. The understanding of the tumors' molecular mechanisms of action on immune response through DC is essential for a more efficient use of cancer immunotherapeutic treatments. In this context, the family of nuclear factor kappa-B (NF-kB) is a fundamental target of research, since it is involved in DCs differentiation and maturation. Thus, the objective of this project is to evaluate the influence of tumors on in vitro DC differentiation, also evaluating the role of NF-kB in this process. With this purpose, the myelomonocytic cell line THP-1 was co-cultured with tumor cell lines and the effect of this setting on the THP-1 cells phenotype and NFkB signalling was evaluated. The results show that after co-culture with MDA or SKBR3 (breast cancer cell linages) THP-1 cells express more CD54, CD40 and CD14 at cellular membrane. Besides, both tumor cell lines did not alter the expression of HLA-DR and CD11c. MDA and SKBR3 particularly increased CD80 and CD116 expression, respectively. Using only the supernatant of these tumor cell lines, the effects upon THP-1 molecules surface was distinct. Together with these phenotype modifications, the co-culture promoted a rise of Rel proteins expression in THP-1 cytoplasm and changes the amount and the kinetics of NF-kB nuclear translocation after LPS stimulation. These results suggest that tumor cells, somehow, disturb NF-kB intracellular signalization in THP-1 cells and this phenomenon might affect the differentiation of these monocytic cells into DC like cells.

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Poster 25

DNA-sensor signaling in the tumor microenvironment provides anti-glioma immunity

Arundhati Ghosh[1], Takayuki Ohkuri[2], Akemi Kosaka[2], Jianzhong Zhu[1], Maki Ikeura[2], Hideho Okada[2] and Saumendra N Sarkar[1]

[1]Cancer Virology Program, and [2]Brain Tumor Program, University of Pittsburgh Cancer Institute, [1]Department of Microbiology and Molecular Genetics and [2]Departments of Immunology, and Neurological Surgery University of Pittsburgh School of Medicine, Pittsburgh, PA 15213

While type-I interferons (IFNs) are critical in developing antiviral innate and adaptive immunity, the mechanism of IFN induction in the sterile tumor microenvironment and its direct impact on tumor-infiltrating immune cells remains to be elucidated. We report that both human and de novo mouse gliomas show increased expression of type-I IFN mRNA. Since, human and mouse glioma tissues contain necrotic areas with dying or dead cells derived genomic DNA that are heavily infiltrated by macrophages, we focused on the DNA-sensor signaling mediated by the common adaptor STING (stimulator of IFN genes). In mice, CD11b+ brain-infiltrating leukocytes (BILs) were the main source of type-I IFNs. IFNs were induced partly in a STING-dependent manner in these cells. Consequently, glioma-bearing StingGt/Gt mice showed shorter survival, and lower amounts of Ifn mRNA compared with wild-type mice. Furthermore, BILs of StingGt/Gt mice showed increased CD11b+ Gr-1+ immature myeloid suppressor and CD25+ Foxp3+ regulatory T (Treg) cells, while decreased IFN-γ-producing CD8+ T cells compared to Wt mice. Concomitantly, IFN-signaling positive CD4+ and CD8+ T cells demonstrated lesser degrees of regulatory activity and increased levels of antitumor activity, respectively. Finally, intratumoral administration of a STING agonist (cyclic diguanylate monophosphate; c-di-GMP) improved the survival of glioma-bearing mice associated with enhanced IFN signaling, Cxcl10 and Ccl5 and T cell migration into the tumor. In addition to STING, we now have evidences for the involvement of upstream components of the DNA-sensor signaling, such as Oasl2 in providing anti-glioma immunity. These data demonstrate substantial contributions of DNA-sensor mediated IFN induction to antitumor immunity via enhancement of the IFN signaling in the tumor microenvironment, and imply a potential use of STING agonists for development of effective immunotherapy.

Poster 26

Oncolytic Immunotherapy with Recombinant Poliovirus

Matthias Gromeier[1], Michael Brown[1], Ross Walton[1], Smita Nair[1], Annick Desjardins[2], John Sampson[1,3], Henry Friedman[1,2], Allan Friedman[1], and Darell Bigner[1,3]

[1]Dept of Surgery, Div of Neurosurgery, [2]Dept of Medicine, [3]Dept of Pathology, Duke University Medical Center

We are investigating the oncolytic polio:rhinovirus chimera, PVSRIPO, with basic mechanistic approaches in vitro and clinical investigations in patients with recurrent GBM. PVSRIPO is the type 1 (Sabin) live attenuated poliovirus vaccine replicating under control of a foreign human rhinovirus type 2 internal ribosomal entry site (IRES). The heterologous IRES causes severe neuronal incompetence of PVSRIPO, eliminating poliovirus' inherent neuropathogenicity. Yet, PVSRIPO retains full cytotoxic potential in malignant cells. PVSRIPO exhibits natural tumor tropism due to widespread ectopic expression of the poliovirus receptor, the onco-fetal cell adhesion molecule Necl5/CD155 on tumor cells and in tumor stroma (tumor-associated macrophages/dendritic cells). Incidentally, CD155 has been identified as a key ligand for the DNAM1/CD226 activating receptor on natural killer cells. PVSRIPO infection of tumor cells results in rapid, abrupt cytotoxicity, dominated by protein synthesis initiation shut-off within ~90 min after infection. Infection forcefully exposes pathogen- (due to activation of innate, MDA5-triggered interferon responses) and danger-associated molecular patterns (due to immunogenic tumor cell killing) within the tumor itself and in tumor stroma. A currently active phase-1 clinical trial tests PVSRIPO with convection-enhanced intratumoral administration in patients with recurrent GBM. Patients previously failed aggressive standard therapy, including resection, chemotherapy (temozolomide), radiation therapy and/or bevacizumab. We have recorded intriguing complete clinical and radiographic responses in the first enrolled patients >24 months post PVSRIPO infusion. Clinical, radiographic and serologic studies are consistent with a host immunogenic response to intratumoral virus infusion that may reduce/contain tumor burden, lessen symptoms and produce durable clinical remissions in patients with recurrent GBM, a diagnosis with dismal prognosis.

Poster 27

Va24-invariant NKT Cells as a Platform for Cancer Immunotherapy with Chimeric Antigen Receptors

Andras Heczey[1], Daofeng Liu[1], Gengwen Tian[2], Amy N. Courtney[1], Jie Wei[1], Ekaterina Marinova[1], Xiuhua Gao[1], Linjie Guo, Eric Yvon[3], John Hicks[2], Hao Liu[4], Gianpietro Dotti[3], and Leonid S. Metelitsa[1]

[1]Texas Children's Cancer Center, Department of Pediatrics; [2]Department of Pathology and Immunology; [3]Center for Cell and Gene Therapy; [4]Division of Biostatistics, Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX, 77030

Advances in the design of chimeric antigen receptors (CARs) have improved the anti-tumor efficacy of redirected T cells. However, functional heterogeneity of CAR T cells and their restriction by polymorphic HLA molecules limit their therapeutic potential and are associated with toxicity, especially in allogeneic settings. We proposed that CAR expression in Va24-invariant NKT cells (NKTs) can build upon the natural antitumor properties of these cells while their restriction by monomorphic CD1d limits toxicity. Primary human NKTs were engineered to express a CAR against the GD2 ganglioside, which is highly expressed by neuroblastoma. We compared CAR constructs that encoded the CD3ζ chain alone, with CD28, 4-1BB, or CD28 and 4-1BB co-stimulatory endodomains. CAR expression rendered NKTs highly cytotoxic against neuroblastoma cells without affecting their TCR-induced proliferation and CD1d-dependent killing of M2-polarized macrophages. We observed a striking Th1-like polarization of NKTs by 4-1BB-containing CARs in vitro and in mice. Expression of both CD28 and 4-1BB endodomains in the CAR maximized persistence of NKTs in NSG mice with neuroblastoma xenografts. These NKTs, expressing CAR with two co-stimulatory endodomains localized to the tumor site more effectively than T cells with the same CAR ($p < 0.001$). Three weekly injections of 107 CAR NKTs prolonged survival of humanized (hu)NSG mice with metastatic neuroblastoma (75.2 ± 6 days) compared to a single injection (52.1 ± 4.3 days, $p = 0.007$) or untreated control (35.4 ± 1.2 days, $p < 0.0001$). A single injection of CAR T cells was associated with tumor regression early after treatment but resulted in lethal graft-versus-host disease (GvHD) within 5 weeks that prevented evaluation of the anti-tumor activity. In contrast, CAR NKTs did not induce GvHD, even after repeated injections. These results establish the potential of NKTs to serve as a safe and effective platform for CAR-directed cancer immunotherapy.

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Poster 28

Disrupting central tolerance augments the anti-tumor effects of peripheral immune checkpoint blockade

Lee Hong[1], Meng-Lei Zhu[1], Imran Khan[2], Maria Mouchess[2], Ajay Gulati[1,3], Yafei Hou[4], Lawrence Fong[4], Mark S. Anderson[2], Maureen A. Su[1,5]

[1]Department of Pediatrics and Microbiology/Immunology, School of Medicine, University of North Carolina at Chapel Hill; [2]Diabetes Center, University of California, San Francisco; [3]Center for Gastrointestinal Biology and Disease, University of North Carolina at Chapel Hill; [4]Division of Hematology/Oncology, Department of Medicine, University of California, San Francisco; [5]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill

Ipilimumab (anti-CTLA-4 IgG1) blocks peripheral (extrathymic) T cell tolerance induction and is an approved treatment for advanced melanoma. However, only a minority of patients experience therapeutic benefit, and therapies to enhance CTLA-4 blockade are urgently needed. Proposed strategies to date, such as anti-PD-1 antibody, rely on antagonizing additional peripheral tolerance mechanisms. However, blocking central (thymic) tolerance as a strategy to enhance anti-CTLA-4 treatment has not been explored. Autoimmune Regulator (Aire) mediates central tolerance mechanisms that restrict the generation of melanoma-reactive T cells. We show here that Aire deficiency and CTLA-4 blockade have additive effects in activating pro-inflammatory T cells, slowing melanoma growth, and improving survival in melanoma-challenged hosts. Mechanistically, this is due to increased CTLA-4 expression among tumor-infiltrating effector T cells in Aire deficient mice compared to wildtype mice. Because the therapeutic index of CTLA-4 blockade is limited by immune-mediated adverse events (irAEs), we tested whether central tolerance blockade would exacerbate T cell-mediated colitis, the most frequent irAE among melanoma patients treated with ipilimumab. Importantly, Aire deficiency did not exacerbate immune-mediated colitis in recipient mice treated with anti-CTLA-4 antibody. Finally, therapeutic administration of anti-RANKL antibody, which depletes Aire-expressing cells in the thymus of adult mice, similarly enhanced the effects of CTLA-4 blockade in slowing melanoma growth and improving survival. These findings provide a framework for combining central and peripheral tolerance blockade to increase the efficacy of cancer immunotherapy.

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Poster 29

Targeted TLR9-activation/STAT3-blocking abrogates immunosuppressive functions of myeloid-derived suppressor cells from late-stage prostate cancer patients

Dewan Md Sakib Hossain, Priyanka Duttagupta, Dayson Moreira, Qifang Zhang, Stephen Forman, Sumanta K. Pal, Marcin Kortylewski

Beckman Institute, CITI, City of Hope, CA , Dept of Urology, City of Hope, CA

Clinical advances emphasized that elimination of tumor-induced immune-suppression is pre-requisite for successful therapy of advanced human cancers. The myeloid-derived suppressor cells (MDSCs) are one of the major populations limiting T cell-mediated antitumor immune responses in late-stage solid tumors, such as castration-resistant prostate cancers (CRPCs). Targeting MDSCs proved challenging due to their heterogeneity and phenotypic similarities with other myeloid cell lineages. Here, we identify a population of granulocytic MDSCs, which accumulate in patients' blood during progression of prostate cancer from localized to metastatic disease. The CRPC-associated MDSCs potently inhibit proliferation, IFNγ and Granzyme-B production in autologous CD8+ T cells. Importantly, the MDSCs in blood and in lymph nodes of CRPC patients show high levels of activated STAT3 transcription factor, which is a crucial immune checkpoint regulator. We previously generated an original method to silence genes specifically in TLR9+ myeloid cells using CpG-siRNA conjugates. Now, we demonstrate that the CpG-STAT3siRNA is also effectively internalized by human MDSCs and induces STAT3 silencing without the need for transfection reagents. Our further studies demonstrated that two-pronged TLR9-activation/STAT3-blocking by CpG-STAT3siRNA inhibits immunosuppressive effects of CRPC-MDSCs on CD8+ T cells, while both TLR9 triggering or STAT3 inhibition alone have only minimal effects. The reversal of MDSC immunosuppression by CpG-STAT3siRNA results from reduced expression and enzymatic activity of Arginase I, a downstream STAT3 target gene. Overall, our study demonstrates the feasibility of overcoming immunosuppression in CRPC patients using CpG-STAT3siRNA strategy to alleviate effects of MDSCs. We anticipate that our strategy to reverse MDSC-mediated immune-suppression without depleting myeloid cell populations will generate safer and more effective immunotherapy for late-stage, metastatic prostate cancers and potentially other solid tumors.

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Poster 30

Listeria-based vaccination overcomes immune suppression and immune senescence in tumor-bearing mice at young and old age

Arthee Jahangir[1], Dinesh Chandra[1], Wilber Quispe-Tintaya[1], Manisha Singh[1], and Claudia Gravekamp[1]

[1]Department of Microbiology and Immunology, Albert Einstein College of Medicine, Bronx, New York

Immunotherapy may be the best and most benign option for curing metastatic cancer. However, a real breakthrough has been hampered by immunosenescence and immune suppression. In this study we evaluated various immunizations protocols with a Listeria-based vaccine in young and old mice with metastatic breast cancer (4T1) to overcome immunosenescence and immune suppression. We analyzed responses of T cells, natural killer (NK) cells and macrophages generated by Listeria vaccination. These immune responses are meaningful because we have shown in earlier studies that Listeria infects tumor cells, thereby altering the tumor cells into a sensitive target for Listeria-activated T cells and NK cells. Moreover, we also found that Listeria induces immunogenic tumor cell death, most likely through the generation of reactive oxygen species (ROS), resulting in the recruitment and stimulation of CD8+ T cells to tumor-associated antigens (TAA) of the host's tumors and metastases. While semi-therapeutic immunizations with Listeria once a week induced strong innate and adaptive immune responses specifically to Listeria at young and old age, CD8+ T cell responses to TAA Mage-b, expressed by the 4T1 tumors, were observed at young age only. However, when the same protocol was administered in an exclusively therapeutic setting once a week, innate and adaptive immune responses to Listeria and Mage-b were strongly inhibited in both young and old mice, and metastatic growth was only reduced by 20-30%, demonstrating the strong immune suppression in the tumor microenvironment (TME) at both ages. Finally, Listeria was administered only therapeutically at a high frequency every other day. This resulted not only in strong innate and adaptive immune responses to Listeria, but also to CD8+ T cell responses to Mage-b at young and old age, and most importantly, the number of metastases was reduced by 90% at young and old age.

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Poster 31

miR-155 augments CD8+ T cell anti-tumor activity in lymphoreplete hosts by enhancing responsiveness to homeostatic γc cytokines

Yun Ji[1], Claudia Wrzesinski[2], Zhiya Yu[2], Nga V Hawk[1], William G Telford[1], Jinhui Hu[1], Sanjivan Gautam[1], Madhusudhanan Sukumar[2], Douglas C Palmer[2], Zulmarie Franco[2], Rahul Roychoudhuri[2], David Clever[2], Christopher A Klebanoff[2,3], Charles D. Surh[4], Nicholas P Restifo[2], Luca Gattinoni[1]

[1] Experimental Transplantation and Immunology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA. [2] Surgery Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA. [3] Clinical Investigator Development Program, National Cancer Institute, NIH, Bethesda, Maryland, USA. [4] Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037, USA

Lymphodepleting regimens are routinely employed prior to adoptive immunotherapy to remove endogenous cellular sinks for homeostatic cytokines thus augmenting the engraftment and anti-tumor efficacy of transferred T cells. These treatment modalities, however, are often associated with severe toxicities. We found that tumor-specific CD8+ T cells constitutively expressing miR-155 displayed sustained proliferation and cytokine production resulting in enhanced anti-tumor responses in the absence of lymphodepletion. The benefit of miR-155-transduced T cells was minimized in lymphodepleted hosts or lymphoreplete animals receiving high doses of exogenous homeostatic γc cytokines, suggesting that miR-155 enhances T cell activity only under conditions of limited cytokine availability. Consistently, the increased functionality of miR-155-overexpressing T cells was virtually abrogated in mice deficient of the homeostatic cytokines interleukin-7 and -15. We found that miR-155 promoted the activity of Akt and Stat5, two pivotal pathways downstream of homeostatic cytokine signaling, by suppressing the Akt inhibitor Ship1 and several negative regulators of Stat signaling including Socs1 and Ptpn2. Enforced expression of a constitutively active variant of Stat5a recapitulated the proliferative advantages conferred by miR-155 while constitutive Akt activation promoted sustained cytokine-release. Thus, miR-155 enhances the anti-tumor activity of CD8+ T cells by acting as a Stat5 and Akt rheostat to facilitate signaling when homeostatic cytokines are limiting. These findings indicate that miR-155 might be employed to increase the effectiveness of adoptive immunotherapies in a cell intrinsic manner without the need of life-threatening, lymphodepleting maneuvers.

Poster 32

Enhancement of anti-tumor immunity induced by OX40 ligand-expressing oncolytic adenovirus Delta-24-RGDOX

Hong Jiang, Karen Clise-Dwyer, Xuejun Fan, Joy Gumin, Farah Mukheef, Laura Bover, Carlo Toniatti, Kathryn Ruisaard, Luis Vence, WK Alfred Yung, Candelaria Gomez-Manzano, Juan Fueyo

Brain Tumor Center, 2Department of Stem Cell Transplantation, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA

We recently reported that Delta-24-RGD, a cancer-selective oncolytic adenovirus undergoing Phase I/II clinical trial in patients with recurrent glioblastomas, induces anti-glioma immunity in an immuno-competent mouse model. To further enhance the tumor-specific immunity induced by the virus, we constructed a new oncolytic adenovirus Delta-24-RGDOX which includes an OX40 ligand (OX40L) expression cassette in the genome of Delta-24-RGD. We hypothesized that the expression of OX40L on the virus-infected tumor cells stimulate and increase the population of tumor-specific T effector cells. Thus, functional study of the splenocytes and brain lymphocytes isolated from GL261 glioma-bearing mice showed that Delta-24-RGDOX significantly increased the specific reaction of the immune cells against GL261 cells with or without viral infection compared to Delta-24-RGD. Delta-24-RGDOX-stimulated immune reactions were also stronger than the ones induced by agonist anti-OX40 antibody (OX86) or the combination of Delta-24-RGD and OX86. Moreover, Delta-24-RGDOX caused more tumor-associated OVA-specific CD8+ T cells (recognized by H2 Kb OVA tetramer) filtration to the tumor site and stronger reaction against OVA epitopes from the CD8+ or CD4+ cells isolated from the spenocytes of GL261-OVA glioma-bearing mice treated with the viruses. Consequently, Delta-24-RGDOX significantly increased the survival of glioma-bearing mice compared to Delta-24-RGD ($p < 0.0001$) while the survival of OX86 and the combination treatment groups fell in between. Further evidence supporting the therapeutic effect by the virus-induced immunity is that the above treatment groups showed no significant difference from the control group in immune deficient athymic nude mice. Collectively, we demonstrate here a new generation of oncolytic adenovirus that can target immune checkpoint proteins, resulting in stronger anti-tumor immune response and therapeutic efficacy.

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Poster 33

Dendritic cell therapy prevents cancer relapse in surgically treated patients of pancreatic cancer - 10 years study on 24 patients

Jamal A. Khan[1], Sharmin Yaqin[2]

[1]Dendritic cell lab, [2]Protein Lab, Institute of Cellular Therapies, Noida, India Pin 201301

Pancreatic cancers are diagnosed at late stages in India. The usual presentation is low back ache, abdominal discomfort, weight loss and/or jaundice. In such patients, surgery remains a distant possibility. In operable patients, relapse rates remain high. Less then 5% patients survive for more then 2-3 years and 5 years survival even further. Chemotherapy deteriorates quality of life and has very little to offer in terms of extension of life span in such patients. We have treated 24 patients with pancreatic cancer earlier treated by surgery with autologous dendritic cell therapy given as 1 million DC matured with proprietary tumor associated antigen manufactured by ICT and given as 6 doses IV at 15 days interval. Post surgical profile of these patients indicated complete remission in 11 patients, 10 patients had node positive status and 3 patients had distant metastasis to liver and stomach. 7 patients are surviving for more then 5 years, 6 survived 4 years and 3 survived for 2 years and 2 between 1-2 years, 6 developed relapse and died within 1 year. 3 patients of node positive cancer are included in first group of more then 5 year survivors. It indicates the efficacy of dendritic cell therapy in this group of patients also. Dendritic cell therapy can be considered in all patients of pancreatic cancer as it is safe and effective. It can be manufactured in centers where cell culture facilities are available and sterility techniques are followed and certified by trained medical microbiologist. Patients remain free from toxic effects of chemotherapy.

We wish to thank Shahzad, Rajkumar, Dharmender and Rahul for their contribution in managing lab services and providing sterile instruments and final product to patients in the study.

Poster 34

HPV based photodynamic therapy: A new approach for anti-cancer therapy

Rhonda C. Kines[1,2], Nicolas Çuburu[2], Hisataka Kobayashi[3], John MacDougall[1], Elisabet de los Pinos[1], Peter Choyke[3], John T. Schiller[2]

[1] Aura Biosciences, Cambridge, MA USA; [2] Laboratory of Cellular Oncology, NCI/NIH, Bethesda, MD; [3] Molecular Imaging Program, NCI/NIH, Bethesda, MD

A new method of targeted photodynamic therapy (PDT) is proposed for potentiating anti-tumor immunity. Human papillomavirus (HPV) virus-like particles (VLPs) bind a broad range of tumor types both, *in vitro* and *in vivo*, but do not bind healthy, intact tissues. We have linked a photosensitizer dye, IR700, to the HPV VLPs and applied the dye-coupled particles (IR700-VLP) to tumor cells. When exposed to 690nm light, the bound conjugates induced immediate necrotic-like cell death in ovarian, lung, melanoma and cervical cancer cell lines *in vitro*, and in melanoma and lung tumors *in vivo*. We are currently expanding these studies to examine long-term tumor growth/survival and the generation of anti-tumor immunity in C57Bl/6 mice using the TC-1 tumor model, which expresses the HPV oncogenes, E6 and E7. This model allows us to track the T-cell responses generated against the HPV oncogenes, in both primary and metastatic tumor settings. Treatment with IR700-VLP and light exposure induced an influx of CD8+ and CD4+ T-cells into the treated tumors. *Ex vivo* cultures of whole tumor cell suspensions showed IFN-γ production in PMA/ionomycin-stimulated CD8+ and CD4+ T cells and to a lower extent without stimulation, suggesting that these T-cells may have been primed against the tumor antigens. Pilot studies indicate a potential correlation between Ag-specific T-cells and tumor regression. Our data suggest that local anti-tumor immunity can be induced through the rapid tumor cell death caused by IR700, in conjunction with the ability of HPV VLP to induce an innate immune response.

Poster 35

Recombinant Human IL-12 Administration to Healthy Human Subjects Shows Differential Effects on CD56bright and CD56dim NK cells

Chris E. Lawrence, Simmy Thomas, Vernon Mar, Hue Kha, Lena A. Basile

Neumedicines Inc., Pasadena, CA 91107

HemaMax™, recombinant human interleukin-12 (rHuIL-12), has successfully completed clinical safety studies in healthy human subjects, prior to being evaluated in combination with radiotherapy, chemotherapy or immunotherapy for the treatment of cancer patients. A single subcutaneous (sc) dose of HemaMax (12micro.g) was administered to 17 healthy human subjects. Immunophenotyping of blood cell populations was conducted by FACS, on peripheral blood samples collected prior to administration, and for up to 14 days post administration. Interleukin-12 (IL-12) has potent immunoregulatory and hematopoietic properties, and exerts significant biological effects on natural killer (NK) cells. Mature CD56dimCD16bright cytotoxic NK cells are resident in the blood, and cytokine producing immature CD56brightCD16+/- NK cells are predominantly located in secondary lymphoid tissues. HemaMax caused a transient decrease in peripheral blood CD56dimCD16bright NK cells, with a nadir (60% reduction from baseline) reached on Day 2 following administration. CD56dimCD16bright NK cell levels returned almost to baseline levels on Day 5. Placebo was without effect. Conversely HemaMax caused an elevation in peripheral blood CD56brightCD16+/- NK cells, particularly between Days 2 and 3 after administration, which was sustained until a peak was reached on Day 5 (265% above baseline). Levels returned to baseline by Day 11. HemaMax did not impact the less functional CD56-CD16bright NK cell subset. The rapid fall in circulating mature CD56dimCD16bright NK cells suggests their immediate exit from peripheral blood into the tissue compartments. This could be mediated by the increase in NK cell CD62L (L-selectin) mRNA expression observed in vitro with HemaMax stimulated primary human NK cells. HemaMax administration to healthy human subjects demonstrated differential effects on the two key NK cell populations in peripheral blood, and as such, excellent potential as an immunotherapeutic and hematopoietic agent for the treatment of cancer patients, by impacting the maturation, activation, immunoregulation, and cytolytic properties of NK cells.

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Poster 36

The IL-15 Superagonist ALT-803 Enhances Antitumor Activity Mediated by Rituximab

Bai Liu, Lin Kong, Kaiping Han, Xiaoyue Chen, Emily K. Jeng, Peter R. Rhode, Hing C. Wong

Altor BioScience Corporation, Miramar, FL

ALT-803 is a complex comprised of a superagonist IL-15 variant and dimeric IL-15Rα-IgG-Fc fusion protein that effectively trans-presents IL-15 and exhibits prolonged in vivo pharmacokinetics compared to IL-15. We hypothesized that ALT-803 may augment rituximab (anti-CD20 mAb)-directed immune cell ADCC against B cell lymphomas and facilitate B lymphoma clearance. Short-term in vitro activation with ALT-803 increased the expression of the cytotoxic effector proteins, granzyme B and perforin, in human NK cells and CD8+ T lymphocytes. ALT-803 also potentiated rituximab-directed human PBMC ADCC against the CD20+ human Daudi B lymphoma cell line in vitro. In immunocompetent C57BL/6 mice, ALT-803 treatment induced significantly greater NK cell and CD8+ T lymphocyte activation and proliferation than was seen with IL-15 treatment. In addition, splenocytes from ALT-803-treated mice displayed stronger rituximab-directed ADCC activity against Daudi cells than splenocytes from control mice. In order to investigate antitumor efficacy of ALT-803+rituximab treatment, Daudi cells were engrafted into NK cell-competent SCID mice. Tumor-bearing mice were then treated (day 15,18) with vehicle, rituximab, ALT-803, or ALT-803+rituximab, and Daudi cell burden in the bone marrow (BM) was assessed at day 22. Mice treated with ALT-803+rituximab had significantly reduced Daudi cell burden in BM, compared to rituximab, ALT-803, or vehicle treatment groups. The enhanced antitumor activity of the combination therapy was ALT-803 dose-dependent. Furthermore, tumor-bearing mice treated with ALT-803+rituximab had prolonged survival compared to ALT-803 or rituximab monotherapy groups. ALT-803 was well tolerated at all of the administered dose levels in combination with rituximab. Thus, ALT-803 represents an effective IL-15 superagonist that augments immune cell cytotoxic potential and ADCC against malignant B cells in vitro, and significantly increases rituximab-triggered clearance of B cell lymphoma by immune cells in vivo. Based on these findings, a phase 1/2 clinical trial of ALT-803 plus rituximab is planned for patients with CD20+ B cell malignancies.

Poster 37

Oncolytic Poxvirus Armed with Chemokine CXCL11 Modulates the Tumor Immune Microenvironment and Enhances Therapeutic Efficacy

Zuqiang Liu, Roshni Ravindranathan, Jun Li, Pawel Kalinski, Z. Sheng Guo, and David L. Bartlett

The University of Pittsburgh Cancer Institute, and Department of Surgery, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA

In this study, we have investigated how a chemokine CXCL11-armed oncolytic vaccinia virus (vvDD-CXCL11) enhanced oncolytic virotherapy in tumor models of colon cancer and mesothelioma. Compared with control virus treatment, CXCL11-armed virus facilitated trafficking of antitumor Tc1/Th1 cells into the tumor tissue. CXCL11 expression from the tumor-selective oncolytic virus positively correlated with more granzyme B and less TGF-β, CCL22 and COX2 in the tumor microenvironment (TME), favorable indicators for antitumor immunity. Treatment with vvDD-CXCL11 induced sustained systemic tumor-specific interferon (IFN)-γ-producing CD8+ T cells, and at effector phase, more CD8+ T cells in mesentery lymph node and spleen, suggesting a sustained systemic antitumor immunity. The positive changes of immunological markers in the TME and sustained systemic antitumor immunity led to enhanced therapeutic efficacy and long term survival benefit, which was mediated by CD8+ T cells and IFN-γ, but not CD4+ T cells. In summary, our data suggested that locally delivering chemokine cxcl11 to tumor site by oncolytic virus could enhance immune cell trafficking to tumor site and make a favorable immunologic changes in tumor microenvironment, and lastly elicit systemic antitumor immunity, which indicated that oncolytic virotherapy might also be effective for distant or metastatic tumor lesion treatment via the efficient induction of antitumor immunity.

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Poster 38

A compact gene regulatory cassette supports key aspects of T-cell receptor (TCR)-α gene locus control region (LCR) activity

Jordana Lovett, Armin Lahiji, Gayathri Devi Raghupathy and Benjamin D. Ortiz

Department of Biological Sciences, City University of New York, Hunter College and Graduate Center, New York, NY, USA

A barrier to the use of stem cell transplants to deliver therapeutic gene products to T cells is the current dearth of vectors capable of driving predictable, spatiotemporally controlled gene expression during stem cell differentiation. Locus Control Regions (LCR) are cis-acting gene regulatory elements with the ability to transfer the gene expression pattern of their locus-of-origin to a linked transgene, and generate high-level transcription from virtually any integration site in the genome. We study the LCR derived from the mouse TCRα gene locus. The properties of the TCRα LCR may enable direction of robust therapeutic gene expression to the T cell progeny of engineered stem cells with predictable developmental timing during T cell development. The TCRα LCR naturally extends over 13-kb of DNA in the genome, a size too large to incorporate into gene delivery vectors. We have extensively characterized the regulatory sub-regions of the TCRα LCR. This information has enabled the design of a 4.0-kb TCRα LCR cassette that is 30% of its original DNA span in the genome. We tested the function of this mini-TCRα LCR in T cells derived in vitro from mouse embryonic stem cells, and in transfected T cell lines. We find that the cell type specificity, developmental timing and barrier insulation capacity of the mini-TCRα LCR is comparable to that of the full length LCR in these assays. It provides integration site-independence to a linked reporter transgene and maintains a euchromatin state at the transgene locus. Thus, the cassette provides long-term and spatiotemporally specific transgene expression in T cells. These data indicate that we have successfully identified the key LCR sub-regions required for its activity, and have translated this information to the creation of a gene regulatory cassette that may be accommodated within the space constraints of therapeutic gene transfer vectors.

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Poster 39

Rapid Cell Expansion (RACE) Technology for the Production of Anti-CD19 CAR Transduced Peripheral Blood Lymphocytes

Lily T. Lu[1], Omar Pugach[2], Robert Somerville[1], Mack Bell[1], Alex Iwamoto[1], Mary A. Black[1], Steve A. Rosenberg[1], James N. Kochenderfer[3], Marc Better[2] and Steve A. Feldman[1]

[1] Surgery Branch, CCR, NCI, [2] Kite Pharmaceuticals, Santa Monica, CA; [3] Experimental Transplantation and Immunology Branch, CCR, NCI

Cancer immunotherapy clinical trials targeting a variety of B cell malignancies using CD19-specific chimeric antigen receptor (CAR) engineered T cells have shown promising results. In an ongoing NCI clinical trial, we observed an overall response (OR) rate of 75% (21/28 patients) as well as a 72% OR rate in the subset of patients with diffuse large B cell lymphoma (DLBCL) (8/11). CD19 CAR-T cells for this trial we grown in an open system cell production platform with serum-containing medium. In preparation for an upcoming multicenter clinical trial, an effort was undertaken to develop a rapid serum-free closed cell manufacturing process. In this process, PBMC from a patient's apheresis product are isolated using a Sepax 2. PBMC are then stimulated in vitro for two days in bags with soluble anti-CD3 antibody and IL-2 followed by a single anti-CD19 CAR γ-retroviral vector transduction. The cells are then expanded in bags for an additional 4 days at which time the number of CD19 CAR-positive cells is determined by FACS prior to cryopreservation at the appropriate cell dose. In conclusion, we have developed a 6 day cell manufacturing process in serum-free medium that generates anti-CD19 CAR-positive T cells that are comparable to the open system currently used in the Surgery Branch, NCI CD19 clinical trials.

This work was supported by Kite Pharma, Inc. through a collaborative research and development agreement with Surgery Branch, NCI, NIH.

Poster 40

Enhancement of T cell immunity to osteosarcoma through modulation of PD-1/PD-L1 interactions

Danielle M. Lussier[1,2], Lauren O'Neill[2], Lizbeth M. Nieves[2,3], Megan S. McAfee[1,2], Susan A. Holechek[2], Andrea W. Collins[4], Paul Dickman[4], Pooja Hingorani[4], and Joseph N. Blattman[2]

[1]Molecular and Cellular Biology Graduate Program, [2]Center for Infectious Diseases and Vaccinology, [3]Molecular Biosciences and Biotechnology, School of Life Sciences, Arizona State University, Tempe, AZ, [4]Center for Cancer and Blood Disorders, Phoenix Children's Hospital, Phoenix, AZ

Osteosarcoma remains one of the most common bone cancers in children and adolescents. While conventional therapy can be effective at reducing or stabilizing primary osteosarcoma tumors, metastatic disease is typically refractory to treatment. Cytotoxic T-lymphocytes (CTL) likely play an important role in the control of developing metastatic osteosarcoma as polymorphisms in the inhibitory tumor necrosis factor receptor (TNFR) family member, cytotoxic T lymphocyte antigen 4 (CTLA4) that result in increased protein expression levels are associated with higher risk of developing osteosarcoma. We have investigated the role of PD-1, another inhibitory TNFR family member, in limiting the efficacy of CTL to mediate metastatic osteosarcoma immune control. We show that human metastatic, but not primary, osteosarcoma tumors are expressing PD-1 and that tumor infiltrating CTL express PD-1, suggesting this pathway limits CTL control of metastatic disease in patients. PD-L1 is also expressed on K7M2 tumor cells that establish metastatic disease in mice, and PD-1 is expressed on tumor infiltrating CTL during disease progression. Blockade of PD-1/PD-L1 interactions in the K7M2 mouse model of metastatic osteosarcoma dramatically improves survival outcomes by enhancing the function of osteosarcoma-specific CTL. These results suggest that blockade of PD-1/PD-L1 interactions in patients with metastatic osteosarcoma should be pursued as a means of improving prognosis in children.

Poster 41

Tumor-associated macrophages promotes extracellular matrix degradation

Daniel H Madsen, Roberto Weiger, and Thomas H Bugge

OPCB, NIDCR, NIH, Bethesda, MD

Cancer growth and invasion is closely associated with degradation of the surrounding extracellular matrix. Nevertheless, many aspects related to this turnover process in vivo are still incompletely understood – both in terms of the degradation mechanism and the involved cell types. Within a tumor, several different types of stromal cells exist including various types of inflammatory cells. Different subtypes of tumor-associated macrophages have been identified and these have often been classified as either M1- or M2-polarized macrophages. M1 macrophages have been suggested to have anti-tumorigenic functions whereas M2 macrophages have been suggested to promote tumor growth. In this study we have utilized a newly developed assay to directly visualize extracellular matrix turnover in vivo. We demonstrate that in the dermis of mice, the extracellular matrix protein collagen undergoes efficient cellular endocytosis and is routed to the lysosomes for complete degradation. Using transgenic reporter mice and whole-mount immunostaining, we show that the process is mediated by M2-like macrophages and to a smaller extent by fibroblasts. This process is dependent on the endocytic receptors uPARAP (Endo180, Mrc2) and the mannose receptor (MR, Mrc1). During carcinoma progression we observe a dramatic increase in cellular collagen endocytosis suggesting that this degradation pathway is an essential part of cancer associated matrix turnover. Collagen uptake is in this case mediated by tumor-associated macrophages, and not by the tumor cells themselves. These cells are recruited during cancer growth and display characteristics of M2-polarized macrophages. The study identifies a hitherto unknown key role of M2-like macrophages in extracellular matrix degradation and we are currently exploring whether this function of tumor-associated macrophages contributes to cancer progression.

Poster 42

Microecology of Tumors Associated with Avian Leukosis / Sarcoma Virus Group in Commercial Layer

Fazal Mahmood and Ahrar Khan

Diagnostic Laboratory, Department of Pathology, Faculty of Veterinary Science, University of Agriculture, Faisalabad Pakistan

Avian leukosis is naturally occurring retrovirus infection that can cause a variety of neoplastic disease conditions. Oncogenic viruses are variably neglected threats described in mammals and in avian species in Pakistan. The etiology and pathogenesis of these tumors is not clear in most cases. However, in chickens, a low incidence of lymphomas and histigenic sarcoma is consistently observed in subgroup J avian leukosis virus (ALV J) infections. In present study morphological characterization, Microecology and immune responders were investigated among the commercial layer. Affected birds revealed a significant enlargement of bursa with granular appearance at necropsy. Histologically marked neoplastic transformation of B cells in bursa and their metastasis in the form of diffuse and nodular to hepatic parenchyma leaving few islands of normal hepatocytes, multifocal to coalescing proliferation within the splenic white pulp, kidney and ovaries. Histologic morphology of these tumor cells was very similar to that of lymphocytes with less abundant cytoplasm, prominent nucleus and frequent mitotic figures. In some birds multiple well circumscribed diffusely spread nodules over the mesentery were observed those were also extended and embedded among ovarian follicles clutch. Nodular mass was comprise of ductal pattern with punctuation of mylocytic cells that have abundant cytoplasm contain prominent eosinophilic granules representing the mylocytomatosis. Tumors were heavily infiltrated with late immune infiltrates such as monocytes, CD3 positive cells, plasma cells, fibroblasts, and large number of tumor associated monsterous macrophages. It is concluded that pathogensis of ALV-J- induced cancers and the development of these lesions is poorly understood however, this study provides an initial evidence of circulating ALV J virus and its association with spread of neoplasms in local commercial egg type poultry thus as a potential threat of its dissemination to not only to the meat type chicken but also to the human being.

I highly acknowledge Professors Hadely and Hafiz for extending their expert opinion,

Poster 43

Biodegradable Nanoparticles for In Situ Immunization Against Lymphoma

Amani Makkouk[1], Vijaya Joshi[2], Aliasger Salem[2], and George Weiner[1,3]

[1] Interdisciplinary Graduate Program in Immunology, University of Iowa, Iowa City, IA [2] College of Pharmacy, University of Iowa, Iowa City, IA [3] Internal Medicine, University of Iowa, Iowa City, IA

In 2013, it was estimated that there would be over 69,000 new cases of non-Hodgkin's lymphoma and that over 19,000 people would die of this cancer. Innovative immunotherapeutic approaches that harness the patient's immune system are thus being tested at a fierce pace. In situ immunization aims at generating anti-lymphoma immune responses through manipulating the tumor microenvironment. Nanoparticles (NPs) made of PLGA (a biodegradable polymer) and injected intratumorally can allow controlled release of loaded agents. We designed a 3-step approach to in situ immunization: (1) Enhancing immunogenic tumor cell death by loading NPs with the chemotherapeutic drug Doxorubicin (Dox). Dox enhances the expression of 'eat-me' signals by dying tumor cells, facilitating their phagocytosis by dendritic cells (DCs); (2) Enhancing antigen presentation and T cell activation by administering systemic anti-OX40 (α-OX40). α-OX40 is an immunostimulatory antibody that enhances the function and survival of T cells; (3) Sustaining T cell responses by blocking immune checkpoints using systemic anti-CTLA-4 (α-CTLA-4). CTLA-4 is an inhibitory receptor expressed by activated T cells and regulatory T cells that regulates T cell activation. α-CTLA-4 blocks its action and can lead to long-lasting anti-tumor immunity. Using the A20 B-lymphoma cell line and bone marrow-derived DCs, we show in vitro that Dox NPs are less cytotoxic to DCs than to A20 tumor cells and can significantly enhance phagocytosis of A20 by DCs. Using mice inoculated with two A20 tumors, our 3-step therapy (Dox NPs injected into one tumor combined with systemic α-OX40 and α-CTLA-4) induced potent CD4 and CD8-dependent systemic immune responses that eradicated distant tumors and significantly improved survival. All three members of the designed approach were crucial for maximum efficacy. Our findings merit further investigation into the use of our designed 3-step therapy for lymphoma patients. Ongoing studies are aimed at dissecting immunological mechanisms involved.

This research was supported by funding from the Iowa/Mayo Lymphoma SPORE (P50 CA97274).

Poster 44

Enhanced efficacy of IL15-based ALT-803 superagonist complex in combination with immune checkpoint inhibitors in a metastatic murine colon carcinoma model

Warren D. Marcus, Wenxin Xu, Peter Rhode, and Hing C. Wong

Altor BioScience Corporation, Miramar, FL

Recent advances in achieving highly durable clinical responses via inhibition of immune checkpoint molecules have revolutionized the outlook for cancer immunotherapy. However, such responses are only observed in a minority of patients, suggesting that strategies to further augment antitumor immune activity may provide additional clinical benefit. ALT-803 is an IL-15 superagonist:IL-15Rα-Fc complex capable of stimulating T cell and NK cell responses without inducing T-reg activity. Mechanism-of-action studies showed that ALT-803 is capable of inducing both innate effector-like and adaptive T cell antitumor activity, suggesting that the immunostimulatory effects of ALT-803 could compliment the activity of checkpoint inhibitors to block tumor immune evasion. To assess this, we utilized a metastatic murine CT26 colon carcinoma model that was previous used to show increased efficacy of IL-15+anti-PD-L1+anti-CTLA-4 mAb treatment. Notably, ALT-803+anti-PD-L1+anti-CTLA-4 mAb treatment provided a significantly greater survival benefit to mice with metastatic CT26 tumors than treatment with the IL-15+anti-PD-L1+anti-CTLA-4 mAb combination. ALT-803 monotherapy also resulted in prolonged survival of tumor-bearing mice compared to treatment with PBS or IL-15, consistent with the enhanced capacity of ALT-803 to stimulate NK and T cell antitumor responses. Our experiments also demonstrated that CTLA-4 inhibition was the major contributing mechanism responsible for the increased efficacy in the combination therapy. This was an expected result since flow cytometric analysis of the CT26 cell line showed a higher expression of CD80/86 than PD-L1. In this model, combination therapies of ALT-803 and anti-checkpoint Abs were well tolerated. Characterization of immune cell activity responsible for improved antitumor efficacy is underway. Overall, these results confirm that enhanced antitumor responses can be achieved by combining immune checkpoint inhibitors with ALT-803, warranting evaluation of these strategies in the clinical setting.

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Poster 45

Turning a Tumor into a Vaccine Factory: In Situ Vaccination for Low-Grade Lymphoma

Thomas Marron[1], Nina Bhardwaj[1], Elizabeth Crowley[2], Tibor Keler[2], Thomas Davis[2], Joshua Brody[1]

[1]Division of Medical Oncology, Mount Sinai School of Medicine, New York, NY; [2]Celldex Therapeutics, Needham, MA

BACKGROUND: Lymphomas are the 5th most common cancer and most are incurable. Previously, we completed three trials of 'in situ vaccination' - combining low-dose radiotherapy (XRT) with intratumoral administration of TLR9 agonist. We demonstrated induction of antitumor CD8 T cell responses and clinical remissions of patients' non-irradiated sites of disease, lasting beyond 4 years. One limitation was the paucity of intratumoral dendritic cells (DC). DC are uniquely able to endocytose dying tumor cells for cross-presentation to anti-tumor CD8 T cells. METHODS: Flt3L - the predominant DC differentiation factor- induces tumor leukocyte infiltration and tumor regression pre-clinically and mobilizes human BDCA-1 and BDCA-3 DC; these subsets respond to multiple TLR agonists and cross-present antigens more effectively than TLR9-responsive (plasmacytoid) DC. We initiated a phase I/II study of a new iteration of in situ vaccine, adding Flt3L-priming and replacing the TLR9 agonist with the TLR3 agonist poly-ICLC. The vaccine consists of administering: -intratumoral Flt3L to increase DC within the tumor - low-dose XRT to induce immunogenic tumor cell death and release tumor-associated antigens, - intratumoral poly-ICLC to activate tumor antigen-loaded DC. RESULTS: Six patients have enrolled, two patients have completed therapy. Treated patients had 2-200-fold increases in BDCA1 and BDCA3 intratumoral DC after Flt3L and marked DC activation after XRT/poly-ICLC. Both treated patients have had partial remissions of untreated sites, persisting or improving >6 months post-vaccine. These include regressions of bulky lymph nodes, as well as peripheral blood and bone marrow disease. A patient with blood tumor burden experienced >10-fold decrease in malignant B cells with concurrent increase in non-tumor B cells, suggesting cell specificity in the tumor-killing mechanism. CONCLUSIONS: Flt3L-primed in situ vaccine appears safe and immunologically and clinically active, warranting further study.

Poster 46

Dextran Sodium Sulfate -Induced Colitis in Dendritic Cell Deletion Mice

Junko Masuda[1,2], Atsuhi Kitani[1], Ivan Fuss[1], Masaharu Seno[2], and Warren Strober[1]

[1]Mucosal Immunity Section, LHD, NIAID, NIH, Bethesda, MD, USA; [2]Department of Biotechnology, Graduate School of Natural Science and Technology, Okayama University, Kita-ku, Okayama, Japan

Dendritic cells (DC) in lamina propria are critical for converting to CD4+Foxp3+ regulatory T (Treg) cells from CD4+Foxp3- in the presence of retinoic acid and TGF-beta. However, role of DC and Treg in immunological tolerance with commensal bacteria that express Toll-like receptor and NOD-like receptor ligands is still controversial. To address this question, we bred constitutively CD11c+ DC-depleted mice on C57BL/6 background. Although CD4+ T cells isolated from splenocytes showed normal in vitro differentiation into Th1 and Th17 cells, these DC-depleted mice were more protective than control against dextran sodium sulfate (DSS) -induced colitis. These results suggest that other cell populations regulate gastrointestinal system in both static and chronic inflammatory state in DC-depleted mice.

Poster 47

Loss of SOCS3 in Myeloid Cells Delays Tumor Growth and Prolongs Survival in a Syngeneic Model of Glioblastoma

Braden C. McFarland, Margaret P. Marks, Amber L. Rowse, Magda Gerigk, Phyllis M. Walker, Hongwei Qin, and Etty N. Benveniste

Department of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, AL, USA

Glioblastoma (GBM), a particularly devastating type of brain tumor, remains a challenging and difficult disease to treat. Despite aggressive therapies, patients diagnosed with GBM have a median survival of 12-16 months. On a cellular level, GBM tumors are extremely heterogeneous, consisting of resident tumor cells, tumor initiating cells, infiltrating immune cells, endothelial cells, and other tumor associated stromal cells, which makes developing targeted therapies a challenge. In GBM, microglia (MG) and macrophages (MP) are the largest population of tumor-infiltrating cells. GBM tumor cells recruit MG/MP via the secretion of chemo-attractants; however, upon arrival at the tumor, the MG/MP adopt an anti-inflammatory phenotype, and in turn accidentally aide in tumor promotion through the secretion of various cytokines. We have previously determined that macrophages with deletion of Suppressor of Cytokine Signaling 3 (SOCS3), the negative regulator of Signal Transducer and Activator of Transcription 3 (STAT3), have an enhanced M1 (pro-inflammatory) response. Herein, we sought to determine the role of SOCS3 in MG/MP functionality in GBM. In this report, we utilized a conditional model in which SOCS3 deletion is restricted to the myeloid cell population. We found that deletion of SOCS3 in the myeloid cell population delays intracranial tumor growth and increases survival of mice bearing orthotopic glioma tumors. Intracranial tumors from mice with SOCS3 deficient myeloid cells have an increase in the number of infiltrating immune cells, including monocytes, neutrophils and macrophages. Lastly, SOCS3 deficient bone marrow derived macrophages display enhanced and prolonged expression of pro-inflammatory M1 cytokines (TNF-alpha, IL-6) when exposed to tumor cell conditioned medium in vitro. Therefore, loss of SOCS3 in MG/MP in the tumor promotes an inflammatory, immune response early and thus delays tumor growth. These preliminary findings highlight an important role of the STAT3/SOCS3 signaling axis in MG/MP in GBM tumors.

Poster 48

Bortezomib enhances anti-tumor T cell immunity by remodeling Notch system

Thounaojam C. Menaka[1], Duafalia F. Dudimah[1], Samuel T. Pellom Jr.[1,3], Roman V. Uzhachenko[1], David P. Carbone[4], Mikhail M. Dikov[4], Anil Shanker [1,2,*]

[1]Department of Biochemistry and Cancer biology, School of Medicine, Meharry Medical College, Nashville, TN, USA. [2]Host-Tumor Interactions program, Vanderbilt- Ingram Comprehensive Cancer Centre, Vanderbilt University, Nashville, TN, USA. [3]School of Graduate Studies and Research, Meharry Medical College, Nashville, TN, USA. [4]Department of Medicine, James Cancer Centre, The Ohio State University, Columbus, OH, USA

The immunosuppressive tumor microenvironment perturbs numerous immune regulatory networks and usurps host antitumor immunity. We found that tumor interferes with host hematopoietic Notch system in lung cancer patients. The resultant decrease in immune Notch signaling could be a major causative link in the adequate induction of antitumor immunity. Interestingly, we observed that administration of the FDA-approved proteasome inhibitor drug bortezomib - which also sensitizes tumors to death signals - to tumor bearing mice can restore Notch signaling in lymphoid cells without increasing tumor cell proliferation or clonogenicity. Moreover, bortezomib administration altered Notch receptor and ligand expression pattern and increased the expression of Notch target genes Hes1, Hey1 and deltex1 in thymus, lymph node and spleen. Bortezomib administration in tumor bearing mice sustained T cell activation and increased IFN-γ production by T cells while the proportion of regulatory T cells was decreased. Data indicate that the activation of Hes1 and Hey1 is mediated via inhibition of NFκB pathway while deltex1 activation is mediated by PI3K pathway. In another set of experiments, we observed that administration of bortezomib along with adoptive CD8+ T cell transfer to tumor-bearing mice resulted in the reduction of tumor nodules, increased apoptosis and improved overall survival of mice. Our results clearly indicate that combining bortezomib with adoptive T cell therapy can sustain T cell activation and function and, thus, enhance tumor immune surveillance. The potential of bortezomib to modulate anti-tumor Notch signaling and to enhance T cell activity presents exciting opportunities. Therapeutic restoration of immune Notch signaling by bortezomib could help break tumor resistance, enhance immune surveillance and sustain robust anti-tumor responses.

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Poster 49

Treatment with anti-PD-1 antibody may lead to different bystander immune response

Mikayel Mkrtichyan[1], Zuzana Berrong[1], Grigori Okoev[1], Adelaida Matevosyan[1], Samir N Khleif[1]

[1]Georgia Regents University Cancer Center, Augusta, Georgia

Tumors induce multiple suppressive mechanisms that inhibit cancer immunotherapies. One of the main strategies that tumors utilize to escape immune surveillance is via the interaction of the Programmed Death receptor-1 (PD-1), expressed on T-cells, with its ligands, PD-L1 and PD-L2, expressed on a wide array of cells including many cancers. PD-1/PD-L1 interaction leads to inhibition of T-cell proliferation, decreased cytokine production, anergy and/or apoptosis. Accordingly, we and other have shown that use of different PD-1-engagement blocking strategies can lead to tumor eradication. We also demonstrated that PD-1 binding agents can function in ways that may be different. We reported previously that B7-DC-Ig (PD-1-targeting fusion protein) works through decreasing the number of tumor-infiltrating Treg cells and by decreasing the percentage of exhausted CD8 T-cells, suggesting the existence of additional, "non-canonical" mechanisms of action mediated through PD-1 signaling. Here we show that another PD-1-targeting molecule, anti-PD-1 monoclonal antibody, CT-011(pidilizumab; CureTech Ltd, Israel), differently affects CD4 T-cell subsets. Our data show that, conventional (Tconv) and regulatory (Treg) T cells respond to CT-011 treatment differently. We demonstrate that in vitro treatment with CT-011 has no direct effect on Tregs activation/proliferation, but, in contrast to other anti-PD-1 antibodies, significantly stimulates CD4 Tconv cells, although both subsets express similar levels of PD-1 at activated stage. We believe that this effect might be due to shown differences in downstream regulatory signaling pathways between these two subsets according to our recent publication. In addition, our in vivo experiments show that CT-011 treatment in combination with vaccine enhances antigen-specific immune responses only when T-helper epitope is incorporated into treatment (CD4-dependent vaccine model), confirming selective stimulatory effect of CT-011 on CD4 T cells. We believe that discovery of this new mechanism is crucial for further development of strategies to target PD-1 axis and designing appropriate mechanism-based combinational cancer immunotherapies.

Poster 50

HAGE-derived peptide sequences for development of cancer vaccine for patients with breast cancer

Divya Nagarajan, Stephanie Mc Ardle, Graham Pockley, Robert Rees

John van Geest Cancer Research Centre, Nottingham Trent University NG11 8NS

Background: The delivery of specific immunotherapies for malignant tumours requires the identification of relevant tumour antigens and sequences from these which can be used to stimulate protective T cell-mediated immunity. HAGE (DDX43) is a cancer testis antigen belonging to the DEAD box family of helicases. We have found it to be over-expressed in many solid cancers (Mathieu et al., 2010) and immunogenic (Mathieu et al., 2007). Moreover, we have recently shown that HAGE is strongly associated with aggressive breast cancer and indicate a poor prognosis, as women with breast cancer considered to be at low risk, but with a high expression of HAGE, were twice as likely to die, or suffer a relapse, than those with 'HAGE negative' tumours. Our study also revealed that HAGE can predict a patient's response to chemotherapy, as the incidence of relapse following chemotherapy in patients with 'HAGE positive' breast cancer was lower than that in patients whose cancer did not express HAGE (Abdel-fatah et al., 2014). The presence of HAGE also appears to limit the effectiveness of hormone therapy, as high risk patients with HAGE negative breast cancer lived longer with such therapy. The aim of this study is to identify strongly immunogenic HAGE-derived sequences which can be used for the development of a therapeutic vaccine for HAGE positive cancer.

Experimental Design: The HAGE-derived sequences were identified and assessed after: (i) using a computer-based epitope predictive tool; (ii) determining the affinity of the predicted peptide with HLA-A2.1 molecules using a peptide-binding assay; (iii) confirming the natural endogenous processing of identified peptides using HHDII/DR1 transgenic mice; (iv) confirming the efficacy of peptide-induced cytotoxic T cells to target cells expressing HAGE and HLA-A2.1 molecules.

Results: Two HAGE-derived sequences (a 24 amino acids long and a 30 amino acids long) encompassing several immunogenic epitopes and exhibiting a broad HLA binding spectrum have been identified and their immunogenicity assessed.

Conclusions: The unique and elevated expression of HAGE in several cancers makes it a promising molecule on which to base the development of new T cell-based immunotherapeutic strategies. The novel HAGE-derived sequences identified here might therefore facilitate the development of long peptide-based, broadly applicable cancer vaccines.

Poster 51

Th17 cell engraftment is improved by host lymphodepletion but does not depend on IL7

Daniel Neitzke[1], Chrystal Paulos[2], David Cole[1], Mark Rubinstein[1]

[1]Department of Surgery, Medical University of South Carolina; [2]Department of Microbiology & Immunology, Medical University of South Carolina

Adoptive T cell therapy (ACT) has shown promise in clinical trials for multiple malignancies. Preclinical data indicate that using the polarized T cell subsets Tc1 or Th17 instead of unpolarized T cells can improve tumor control. However, little is known about which cytokines these subsets require to engraft and kill tumor cells. By characterizing the cytokine requirements of the Tc1 and Th17 subsets we hope to provide important information for the design of a new generation of ACT clinical trials utilizing these cells, as well as a more rational and targeted approach to cytokine administration in the clinic. We have recently found that polarizing CD8 T cells to the Tc1 lineage with IL12 increases their dependence on IL7 in lymphodepleted hosts. To evaluate the cytokine requirements of Th17 cells we polarized CD4 cells with TGFb and IL6 and tracked their persistence after transfer into B6 mice. We first determined the effect of lymphodepletion by comparing persistence in mice receiving 6 Gy total body irradiation with persistence in control mice. Engraftment of Th17 cells in the lymphodepleted mice was much more efficient. Donor cells retained the ability to produce IL17 although the IL17 positive fraction decreased relative to the pre-transfer level. To test whether Th17 engraftment in a lymphodepleted host depends on IL7, we tracked donor persistence in irradiated mice which were depleted of IL7 via monoclonal antibody. We found that Th17 engraftment in lymphodepleted hosts did not depend on IL7, in contrast to our observations with Tc1. We are investigating the roles of other cytokines including IL15 as we seek to elucidate which properties of the lymphodepleted host potentiate Th17 engraftment. Answering these questions will pave the way for more effective clinical trials utilizing Th17 cells in combination with cytokine supplementation.

Poster 52

Multifunctional CD26hi Th17 cells eradicate large human tumors

Michelle H. Nelson[1], Stefanie R Bailey[1], Logan Huff[1], Sreenath Kundimi[1], and Chrystal M Paulos[1]

[1] Department of Surgery, Medical University of South Carolina, Charleston

Human CD4+ T cells differentiate into multiple effector subsets, but their distinct roles in anti-tumor immunity remain elusive. CD4+ T cell subsets enriched from bulk human CD4+ T cells [Th1 (CXCR3+), Th2 (CCR4+), and Th17 (CCR6+ or CD26hi)] were stimulated with anti-CD3 beads bearing agonists to either CD28 or ICOS and were then engineered with a chimeric antigen receptor that recognizes human mesothelioma. In vitro, ICOS costimulation proved superior to CD28 for augmenting the function of human Th1, Th2, CCR6+ Th17 and CD26hi Th17 cells, as indicated by elevated production of IFN-γ, IL-4 and IL-17A, respectively. Moreover, CD26hi Th17 cells possessed strikingly enhanced poly-functionality compared to Th1, Th2 or CCR6+ Th17 cells, as demonstrated by their heightened capacity to co-secrete IL-17A, IFN-γ, IL-22, IL-2, and TNF-α simultaneously. Compared to other enriched T cell subsets, a greater percentage of CD26hi Th17 cells exhibit an effector memory phenotype. In vivo, CD26hi Th17 cells more efficiently reconstituted immunodeficient hosts and persisted long-term. Furthermore, CD26hi Th17 cells possessed a superior ability to kill large human tumors (>150mm2) when infused into mice compared to Th1, Th2 or CCR6+ Th17 cells. These results suggest that the generation of multifunctional, long-lived human Th17 populations could be instrumental to the design of novel, effective T cell-based cancer therapies.

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Poster 53

Proximal TCR signaling and CD8+ immunity to infectious disease and cancer is regulated by Cish

Douglas C. Palmer[1], Geoffrey C. Guittard[1], Zulmarie Franco[1], Shashank J. Patel[1], Heather D. Hickman[2], Yun Ji[1], Nicholas Van Panhuys[2], Dorina Frasheri[1], Lindsay M. Garvin[1], Christopher A. Klebanoff[1], Nicolas Acquavella[1], Luca Gattinoni[1], Madhusudhanan Sukumar[1], Joseph G. Crompton[1], Robert L. Eil[1], David Clever[1], Rahul Roychoudhuri[1], Rajat Varma[2], Ena Wang[3], Francesco M. Marincola[3], Lakshmi Balagopalan[1], Lawrence E. Samelson[1] and Nicholas P. Restifo[1]

[1]National Cancer Institute, Bethesda, MD, USA, [2]National Institute of Allergy and Infectious Disease, Bethesda, MD, USA, and [3]Sidra Medical and Research Center, Doha, Qatar

CD8+ T cells are potent killers of pathogens and tumors, specifically recognizing their targets through the T cell receptor (TCR). Upon encountering peptide-bound major histocompatibility complexes on target cells, engaged TCRs activate phospholipase Cγ1 (PLCγ1), actuating NFAT and NFκB activity, initiating a T cell activation program. While much has been revealed about the activation of TCR signaling, the negative regulation of this critical pathway remains unclear. We report that Cish plays an inhibitory role in immunity to infection and cancer and that it physically interacts with PLCγ1, targeting it for degradation following TCR engagement. Cish downregulation of PLCγ1 resulted in decreased Ca2+ flux and inhibition of CD8+ T cell polyfunctionality. Furthermore, the adoptive transfer of Cish-deficient CD8+ T cells resulted in the elimination of solid cancer. Our data establishes a central role for Cish as a negative regulator of TCR-mediated signaling and CD8+ T cell immunity and identifies a new targetable interaction for immune-based therapy of infectious disease and cancer

Poster 54

Isolation of T cell receptors specifically reactive with mutated tumor associated antigens

Maria Parkhurst[1], Paul Robbins[1], and Steven Rosenberg[1]

[1]Surgery Branch, NCI

The adoptive transfer of tumor infiltrating lymphocytes (TIL) can mediate the regression of metastatic melanoma. In addition, the adoptive transfer of lymphocytes expressing T cell receptors (TCRs) specifically reactive with antigens expressed on melanoma cells can mediate tumor regression. Many T cells from TIL recognize mutated antigens expressed only on the autologous patient's tumors. Therefore, we attempted to isolate TCRs reactive with unique mutated antigens to treat patients with autologous T cells genetically modified to express those TCRs. Exome and RNA sequencing were used to identify mutated antigens highly expressed in tumors. We used 2 methods to isolate TCRs reactive with mutated antigens as follows: 1. TIL were stimulated overnight with autologous dendritic cells (DCs) electroporated with in vitro transcribed RNAs encoding mutations. CD3+CD8+CD137+ T cells were FACS sorted and expanded in vitro. 2. Peptides encompassing mutations with high predicted binding affinities to HLA-A2 were used to stimulate peripheral blood lymphocytes (PBL) from autologous patients. PBL were stimulated in vitro with peptide-pulsed autologous DCs and restimulated with peptide-pulsed PBMCs. CD137+ T cells after stimulation with HLA-A2+ target cells expressing the mutation were sorted and expanded in vitro. Recognition of target cells by resulting T cell populations was evaluated on the basis of IFNγ secretion and CD137 expression. For populations containing mutation reactive T cells, TCRα and β chain sequences were identified using 5' RACE, and retroviruses encoding those TCRs were used to transduce human PBL. Using these techniques, we identified, enriched, and expanded T cell populations that recognized mutated tumor associated antigens. We identified dominant TCR α and β chains in these enriched populations and demonstrated these TCRs mediated recognition of the expected mutated tumor associated antigens. We are currently developing clinical reagents to treat patients with TCRs that recognize unique mutations on autologous tumor cells.

Poster 55

Effects of bortezomib on lymphocyte stimulatory cytokines and anti-tumor function

Samuel Pellom[1,2], Duafalia Dudimah[1], and Anil Shanker[1]

[1]Laboratory of Lymphocyte Function, Department of Biochemistry and Cancer Biology, School of Medicine; [2]Department of Microbiology & Immunology, Meharry Medical College, Nashville, TN

The tumor microenvironment can suppress the immune system, support tumor growth, and promote therapeutic resistance. With the goal of reducing tumor burden and enhancing immune mechanisms against solid tumors, we are currently investigating 4T1, an inflammatory murine mammary tumor model that represents stage 4 human breast cancer, along with a mildly inflammatory kidney cancer model, Renca. We administered bortezomib, a proteasome inhibitor drug that sensitizes tumor cells to apoptotic signals in mice bearing 4T1 or Renca tumors. We characterized the impact of bortezomib treatment on lymphoid and myeloid cells and their cytokine and chemokine production in the tumor microenvironment. Our data show that in the spleen of 4T1 tumor-bearing mice, bortezomib treatment increases immuno-stimulatory interleukins IL-12 and IL-15. However, in the spleen of Renca tumor-bearing mice, we found that bortezomib treatment did not significantly affect these immunostimulatory interleukins. Based on the increase of these cytokines in 4T1 tumor-bearing mice, we assessed whether bortezomib can modulate the outcome of cytokine signaling by evaluating the expression of total and phosphorylated proteins of downstream components of signaling pathways. We observed that bortezomib modulated the phos-phorylation of Jak1, Tyk2, STAT3, and STAT5 proteins in 4T1 tumor-bearing mice. We also assessed the effects of bortezomib on natural killer (NK) cell activating receptor NKG2D and IL-15 receptor signaling. We found increased expression of NKG2D ligand, Rae1$_{\gamma}$, and IL-15 receptor in the lymphoid organs of bortezomib administered 4T1-bearing mice. These data predict the potential of administering bortezomib to modulate the tumor microenvironment by inducing differential expression of cytokines and chemokines, and enhance their signaling by intersecting with their downstream signaling pathways, and promoting the function of immune effector cells in solid tumors.

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Poster 56

Immunostimulatory cancer therapy using dendritic cell-tumor cell hybrids

Mariana Pereira Pinho[1]; Patricia Cruz Bergami-Santos[1]; Jose Alexandre Marzagao Barbuto[1]

[1]Immunology Department, Biomedical Sciences Institute, University of Sao Paulo, Sao Paulo, Brazil

Dendritic cell-tumor cell hybrids (heterokaryons) are under investigation as a means for tumor immunotherapy since they should express MHC class I molecules from both cell types and, thus, present tumor antigens within an immunogenic context. The aim of this work was, therefore, to evaluate the immunostimulatory potential of the heterokaryons in order to understand and boost the efficiency of the vaccines based on these cells. To generate the hybrids, mature dendritic cells (mDCs) differentiated from peripheral blood of healthy donors were fused with cells from the breast cancer cell line MDA-MB-231 by applying an electric pulse. Flow cytometry analysis showed that the hybrids expressed both the tumor antigen Her2 and the mDC marker CD11c (percentage of double-positive cells; Mix: 1.9 ± 0.3; Fusion: 16.4 ± 2.8; $p<0.0001$; n=14) and high levels of the molecules CD40, CD83, CD80 and CD86. The hybrids also had higher expression of MHC class I molecules (median fluorescence intensity; Mix: 17.8 ± 3.4; Fusion: 33.2 ± 7.2; $p<0.05$; n=4) which was proved to be due to the co-expression of molecules from both cell types. The lymphostimulatory ability of the hybrid cells were evaluated after co-culture with autologous T lymphocytes. INF-γ was detected in the supernatant of the co-culture of T cells with the mix and the hybrids but not with the cells individually. T cell proliferation was detected in all groups. After re-stimulation with the hybrids, memory T cells were observed. Hybrids also induced a lower percentage of FoxP3+ regulatory T cells (13.1%), when compared to the untreated (18.0%) or mix-stimulated T cells (20.8%). Cytotoxic T cells capable of killing tumor cells were also observed after priming with the heterokaryons. So, these data indicate that dendritic cell-tumor cell hybrids are phenotypically competent to present antigens, express the costimulatory molecules and are able to prime T cells.

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Poster 57

Tumor microenvironment induces suppressive CD163highPD-L1highIL-10high M2-like macrophages and alters monocyte precursor's differentiation in primary breast and ovarian cancer patients

Rodrigo Nalio Ramos[1-3], Celine Rodriguez[3,4], Sophie Leon[4], Isabelle Treilleux[4], Carola Ries[5], Emilie Lavergne[4], Sylvie Chabaud[4], Christophe Caux[2-4], Jose Alexandre M. Barbuto[1], Nathalie Bendriss-Vermare[*3,4] and Christine Menetrier-Caux[*,3,4]

[1]Depart. of Immunology, Institute of Biomedical Sciences - University of Sao Paulo, Brazil; [2]University Claude Bernard Lyon-1, France; [3]INSERM U1052, Centre de Recherche en Cancerologie de Lyon, France; [4]Centre Leon Berard, France; [5]PRED, ROCHE Diagnostics, Germany, [*]equal contribution

The accumulation of Tumor-Associated Macrophages (TAM) is correlated to poor patient's survival in cancer. However, the understanding of their differentiation, phenotype and function remain unclear in humans. In breast (BC) and ovarian cancer (OC), TAM are the most frequent subpopulation within CD45+MHC-II+ cells and could be found in variable frequency of CD64+CD163low (7.3±2%) and CD64+CD163high (4.5±1.3%). Interestingly, we observed an upregulation of PD-L1 and IL-10 production by LPS-activated CD64+CD163high TAMs. Additionally, tumor-conditioned media from primary BC and OC tumours' skewed in vitro differentiation of healthy blood monocytes towards a M2-like phenotype (CD163highPD-L1highIL-10high) those were able to efficiently suppress T cell expansion. Importantly, BC patients' blood monocytes failed to fully differentiate into M1 in presence of GM-CSF/IFNγ and maintained an altered M1/M2 mixed phenotype. An IHC study on a retrospective BC cohort with 10-year follow-up reveals a strong correlation between higher intra-tumor CD163+ TAM and poor patients' survival. Altogether our data suggest that tumor microenvironment favor the differentiation of suppressive M2-like macrophages and acts systemically on blood monocytes, putting forward the importance of clinical approaches targeting CD163high TAMs differentiation in neoplasia.

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Poster 58

Biologic Response of Donor Lymphocyte Infusions in the Absence of Macro-chimerism

John L. Reagan[1], Loren D. Fast[1], Martha Nevola[1], Andrew Schumacher[2], Kayla Rosati[3], Matthew Quesenberry[1], Eric S. Winer[1], Peter J. Quesenberry[1]

[1]The Warren Alpert Medical School of Brown University, Providence, Rhode Island; [2]Lifespan Office of Clinical Research, Providence, Rhode Island; [3]Brown University Oncology Group, Providence, Rhode Island

Background/Objective: Allogeneic stem cell transplantation (allo-SCT) is an effective treatment modality for leukemia and lymphoma that exerts much of its therapeutic benefit via a graft versus tumor effect. Its applicability is limited by toxicity from preconditioning chemoradiation and graft versus host disease. We present data in which haploidentical allogeneic cells are infused without prior chemotherapy or radiation. The goal is to generate a robust allogeneic response that breaks host tumor tolerance without the toxicity profile seen with allo-SCT. **Methods**: Eligible patients have refractory acute leukemias and aggressive systemic lymphomas without curative options. HLA haploidentical donors are identified then undergo leukapheresis without stem cell mobilization. 1-2x108 CD3+ cells/kg are infused unprocessed immediately following collection. Peripheral blood samples collected 1, 24, 48, 72 and 168 hours after infusion are examined for effector cell populations, stimulatory/inhibitory signals, and cytokine release profiles. Results Three patients have been infused haploidentical cells. Two developed hyperpyrexia following treatment that persisted for 48 hours. One of three patients demonstrated a decrease in peripheral blood and bone marrow blast counts following therapy. No Grade 3 or 4 toxicities or durable chimerism was seen. Host T cells demonstrated cytolytic effector molecule expression but little cytolytic activity possibly secondary to rapid up-regulation of PD-1 on host T cells and PD-1 ligands on leukemic cells. **Discussion:** Haploidentical cellular infusions are well tolerated and demonstrate biological activity in relapsed leukemia. Lab correlative studies suggest potential future mechanisms to augment response. Our method provides a true bench to bedside approach utilizing adoptive immunotherapy created via a host allogeneic rejection response.

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Poster 59

Mast cell modulate autologous immature monocyte-derived DCs

Cecilia Pessoa Rodrigues, Jose Alexandre M. Barbuto

Institute of Biomedical Sciences, Department of Immunology, University of Sao Paulo, Sao Paulo, Brazil

Tumor microenvironment is a factor that affects significantly the immune response against tumors. It is composed of transformed and immune cells, vascular components and mesenchymal cells. These different cells may have beneficial, harmful or dual roles in the antitumor reaction, depending on various factors that range from cellular interactions to cytokines present therein. One example of this functional ambivalence is found in the dendritic cell. These cells, though able to initiate effective immune response, when activated by danger signals, fail to do so in the tumor microenvironment. In this environment, DCs are found in an immature state, thus not only failing to induce an anti-tumor response, but even contributing to the tumor evasion of the immune system. Recent studies demonstrate that cancer cells recruit mast cells in early stages of tumor development. This study, thus, aims to contribute to the understanding of the mast cells role. To that end, our first step was to induce mast cells differentiation in vitro, from peripheral blood CD34 cells. After 52 days, as confirmed by flow cytometry, we were able to obtain an enriched cell population with mast cell phenotype. These cells were, then, co-cultured with autologous immature monocyte-derived DCs. After 2 days, we evaluated the surface molecules expression on DCs and noticed that in the presence of mast cells, DCs had decreased CD80, PDL1, CCR7 expression. Also, we observed that mast cells culture supernatant induced IL-10 production by iDCs. Finally, we noticed that after co-culture, iDC induced high levels of FOXP3 expression and down regulated allogeneic T-lymphocytes proliferation. Taken together these data suggest that mast cells within the tumor microenvironment could have a role in tumor evasion from the immune system through their effects upon DCs that, under their influence seem to have a bias towards the induction of regulatory T cells.

Center for Research Facilities (CEFAP) of University of Sao Paulo, Sao Paulo, Brazil.

Poster 60

The Role of Transcription Factor NF-κB in the Process Dendritic Cells Differentiation

Roberta Neves Gago Rodrigues[1], Ana Carolina Franco Ferreira[1], Jose Alexandre Marzagao Barbuto[1]

[1]Tumor Immunology Laboratory, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil

Dendritic cells are a unique type of immune cell, given their integral role in the induction and regulation of immune response, making them most interesting targets for cancer immunotherapy. This crucial role requires DCs to rapidly respond to alterations in their microenvironment, activating signaling pathways that will lead to the synthesis of new molecules. One of these signaling pathways is the NF-κB pathway. Nuclear Factor κB is a family of transcription factors which regulate a great number of genes involved in the immune response. In dendritic cells, the contribution of NF-κB in the process of DC maturation is fairly well described. However, its role in DC differentiation is not entirely known, hence this study to better understand the part that this transcription factor takes in the process of in vitro DC differentiation from monocytes. For that purpose, we differentiated DCs in the presence of the NF-κB inhibitor sodium salicylate in different concentrations (up to 15mM) and we analyzed the resulting membrane phenotype of these DCs in comparison to the control group concerning the following cell markers: CD1a, CD116, CD11c, CD80, CD86, CD40, CD83, CCR7 and CD123. The results indicated that the inhibitor sodium salicylate at 15mM significantly alters the membrane phenotype of human dendritic cells differentiated in vitro from monocytes, modifying the expression of the CD123, CD11c, CD116, CD1a, CD80, CD86, CD83 and CD40 markers, suggesting an extremely complex role of the NF-κB transcription factor in the process of in vitro differentiation of dendritic cells from monocytes.

Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil. Bioscience Institute, University of Sao Paulo, Sao Paulo, Brazil. The Sao Paulo Research Foundatio - FAPESP

Poster 61

Recovery of immune competence after tumor removal assessed by the evaluation of monocyte-derived dendritic cells phenotype and function

Maria Alejandra Clavijo Salomón[1], Rodrigo Nalio Ramos[1], Celia Regina Pizzo Pinto[1], Patricia Cruz Bergami-Santos[1] and Jose Alexandre Marzagao Barbuto[1].

[1]Laboratory of Tumor Immunology, Department of Immunology, Institute of Biomedical Sciences, University of Sao Paulo, Sao Paulo, Brazil

The immune system plays a major role protecting the body from cancer in early steps of tumoral development. After an oncologic surgery, it may also play an important role in preventing recurrence or metastasis. Dendritic cells (DCs) are responsible for recognition of homeostatic disturbances and prime T cells that will maintain immune responses throughout life. When a tumor is established, DCs and T cells are victims of escape mechanisms that prevent them from functioning properly and promote tumor growth. Although these mechanisms are well documented, DCs and T cells status after oncologic surgeries remain less studied. We report the phenotypic and functional changes of monocyte-derived dendritic cells (Mo-DCs) of a 34-year-old male before and after a partial nephrectomy due to chromophobe renal cell carcinoma. CD14+ monocytes from the patient's peripheral blood, one week before and three months after partial nephrectomy, were differentiated into Mo-DCs by culture and then harvested and co-cultured with allogeneic T cells. The phenotype of Mo-DCs and their capacities to stimulate lymphocyte proliferation and Foxp3+ T cell induction were determined by flow cytometry. Supernatants from all cultures were collected for cytokine analysis. After surgery, Mo-DCs decreased the expression of CD205, HLA-DR, CD80, CD86 and PD-L1. Also, Mo-DC's capacity to stimulate T cells proliferation increased along with improvement on CD4/CD8 ratio, expression of IL- 2Rα and production of IFN-γ. Finally, Mo-DC's capacity to induce de novo Foxp3+ T cells decreased after surgery. One-year postoperative follow-up shows no tumor recurrence. Our results showed that, in our patient, the presence of a tumor impaired Mo-DCs to exhibit a mature phenotype, stimulate T cell anergy and induce Foxp3+ T cells. However, tumor resection completely revert this scenario. These findings highlight the close interaction between tumors and the immune system and may provide new parameters for the clinical management of patients.

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Poster 62

Clinical Assessment of Autologous Human Cytomegalovirus-specific T-cell Therapy as Consolidative Treatment for Recurrent Glioblastoma Multiforme

Andrea Schuessler[1], Corey Smith[1], Leone Beagley[1], Glen M. Boyle[2], Sweera Rehan[1], Katherine Matthews[1], Linda Jones[1], Tania Crough[1], Vijayendra Dasari[1], Kerenaftali Klein[3], Amy Smalley[4], Hamish Alexander[1,4], David G. Walker[4] and Rajiv Khanna[1]

[1]QIMR Centre for Immunotherapy and Vaccine Development and Tumour Immunology Laboratory; QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia. [2]Cancer Drug Mechanisms Group; QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia. [3]Statistics Unit, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia. [4]Newro Foundation, The Wesley Hospital, Brisbane, Queensland, Australia

Glioblastoma multiforme (GBM) is one of the most aggressive human brain malignancies. Even with optimal treatment, median survival is less than six months for patients with recurrent GBM. Immune-based therapies have the potential to improve patient outcome by supplementing standard treatment. Expression of human cytomegalovirus (CMV) antigens in GBM tissues provides the unique opportunity to target viral antigens for GBM therapy. Here we report findings of a formal clinical assessment of safety and potential clinical efficacy of autologous CMV-specific T cell therapy as a consolidative treatment for recurrent GBM. From a total of 19 recurrent GBM patients, CMV-specific T cells were successfully expanded from 13 patients (68.4%) and 11 of whom received up to four T cell infusions. Combination therapy based on T cell infusion and chemotherapy was well tolerated and we detected only minor adverse events. The overall survival of these patients since first recurrence ranged from 133 to 2428 days with a median overall survival of 403 days. Most importantly, four out of 10 patients that completed the treatment remained progression free during the study period. Furthermore, molecular profiling of CMV-specific T cell therapy from these patients revealed distinct gene expression signatures which correlated with their clinical response. Our study suggests that that a combination therapy with autologous CMV specific T cells and chemotherapy is a safe novel treatment option and may offer clinical benefit for recurrent GBM patients.

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Poster 63

Investigating Nuclear Autophagy and Localization in Human Cancer Cell Lines

Nazila Shafagati, Daniel Rosenberg, William Buchser

William and Mary, Williamsburg, Virginia

Autophagy can be utilized by stressed cells to survive challenging conditions. Autophagy is one of the two cellular processes for degradation of intracellular material, but has classically been thought to occur exclusively in the cytoplasm. A newly described subset of autophagic processes known as nuclear autophagy or nucleophagy degrades nuclear material. Nucleophagy has been shown to play a role in cancer and other diseases such as Charcot-Marie-Tooth, some muscular dystrophies, and Bloom syndrome. Here, we have established an assay to measure nucleophagy by automated microscopy and cell-based analysis for nuclear 'holes' and LC3 puncta. Compounds from the DTP NCI diversity set are being used to uncover novel nucleophagy inhibitors. We are also investigating the role of nuclear import and export in the process of nucleophagy.

Poster 64

Immunosuppressive Exosomes Present in Human Ovarian Tumor Micro-environments Rapidly and Reversibly Arrest CD4+ and CD8+ T Cell Activation

Gautam N. Shenoy[1], Raymond J. Kelleher Jr.[1], Sathy Balu-Iyer[2], Jenni Loyall[1], Anthony Sacca[1], Vandana Balu-Iyer[2], Anas M. Fathallah[2], Charles S. Berenson[3], Paul K. Wallace[4], Joseph Tario[4], Kunle Odunsi[5] and Richard B. Bankert[1]

[1]Departments of Microbiology and Immunology and [2]Pharmaceutical Sciences at the School of Medicine, University at Buffalo; Buffalo, NY; [3]Infectious Disease Division, Department of Veterans Affairs Western New York Healthcare System, State University of New York at Buffalo School of Medicine, Buffalo, New York; [4]Departments of Flow Cytometry and [5]Gynecologic Oncology Roswell Park Cancer Institute; Buffalo, NY

The identification of immunosuppressive factors within human tumor microenvironments and the ability to block these factors would be expected to enhance patients' anti-tumor immune responses by re-activating hypo-responsive tumor-associated T cells. We previously established that an unidentified factor or factors present in ovarian tumor ascites fluids reversibly inhibited the activation of T cells. We have now identified that one of the inhibitory factors is a phospholipid, phosphatidylserine (PS), and report here that the inhibitory activity is mediated by small, 50-120nm, extracellular vesicles (exosomes) that are present in ovarian tumor ascites fluids and in solid tumor tissues. Tumor ascites fluids act directly upon CD4+ and CD8+ T cells to induce a rapid and reversible arrest in their activation. The exosomes present in the ascites fluids are surrounded by a lipid bilayer in which PS is expressed in its outer leaflet. Phenotyping by flow cytometry reveals that these tumor-associated exosomes are heterogeneous and express surface markers associated with both tumor and tumor-associated non-malignant cells, indicating that they are derived from multiple cellular sources. We report that the tumor-associated exosome-induced T cell signaling arrest is dependent upon PS, as a blockade of PS with anti-PS antibodies significantly reverses the inhibitory activity of the exosomes. The inhibitory activity is overcome by the addition of diacylglycerol kinase inhibitors, suggesting a possible mechanism for the T cell inhibition and also potential molecules to be targeted in developing approaches to reverse it. We conclude that exosomes present within ovarian tumor microenvironments represent a novel T cell checkpoint, and are a potentially viable target to block and thereby enhance anti-tumor immune responses in ovarian cancer patients.

Poster 65

Recurrent mutations in ATM and common gamma chain signal transducers in peripheral T cell lymphoma

Haley M. Simpson[1], Rashid Z. Khan[1], Chang Song[1], Deva Sharma[1], Kavitha Sadashivaiah[1], Aki Furusawa[1], Luke J. Tallon[2], Xinyue Liu[2], Qing C. Chen[3], Ferenc Livak[4], Aaron P. Rapoport[1], Amy Kimball[1], and Arnob Banerjee[1]

[1]Program in Oncology, Greenebaum Cancer Center, Center for Stem Cell Research and Regenerative Medicine, Department of Medicine, University of Maryland School of Medicine, Baltimore, MD; [2]Genome Resource Center, Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD; [3]Department of Pathology, University of Maryland School of Medicine, Baltimore, MD; [4]Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD

Peripheral T cell lymphoma (PTCL) is a heterogeneous malignancy with poor response to current therapeutic strategies and incompletely characterized genetics. We conducted whole-exome sequencing of matched PTCL and non-malignant samples from 12 patients, spanning 8 subtypes, to identify somatic mutations in PTCL. Analysis of the mutations identified using computational algorithms: CHASM, PolyPhen2, PROVEAN, and MutationAssessor to predict the impact of identified mutations on protein function and PTCL tumorigenesis revealed 17 somatic mutations that were selected by all four algorithms. We identified recurrent somatic mutations in LAMA1, KCNH8, ATP9B, CELSR1, JAKMIP1, NTRK3, PCSK5, and SIRPA. We also identified somatic mutations in ATM in 4 out of the 12 samples and mutations in the common gamma chain signaling pathway (JAK3, IL2RG, STAT5B) in 3 out of the 4 samples with ATM mutations. Our findings contribute insights into the genetics of PTCL and suggest a relationship between gamma chain signaling and ATM in T cell malignancy.

Poster 66

Targeting Myeloid Cells in the Tumor Microenvironment Enhances Vaccine Efficacy in Epithelial Ovarian Cancer

Kelly L Singel BS[1], Anm Nazmul H Khan MBBS PhD[2], Nonna Kolomeyevskaya MD[3], Melissa J Grimm PhD[2], Kirsten B Moysich PhD[4], Scott I Abrams PhD[1], Kunle Odunsi MD PhD1,[3] and Brahm H Segal MD[1,2,5]

[1]Immunology, [2]Medicine, [3]Gynecologic Oncology, and [4]Cancer Prevention and Control, RPCI, Buffalo New York; [5]Medicine, University at Buffalo School of Medicine, Buffalo NY

Epithelial ovarian cancer (EOC) is typically diagnosed at advanced stages, and is associated with a high relapse rate following primary cytoreductive surgery and adjuvant chemotherapy. Patients in remission with minimal disease burdens are ideal candidates for anti-tumor immune augmentation strategies aimed at cure or prolonging disease-free periods. However, immunosuppressive pathways in the tumor microenvironment are obstacles to durable antitumor immunity. Using an orthotopic syngeneic mouse model of epithelial ovarian cancer, we found that immunosuppressive macrophages and myeloid-derived suppressor cells (MDSCs) accumulate in the local tumor environment, correlating with disease burden. In addition, resident peritoneal macrophages from non-tumor-bearing mice were highly immunosuppressive, abrogating stimulated T cell proliferation in a cell contact-dependent fashion. Immunization with stimulatory microparticles comprising TLR9 and NOD-2 ligands (MIS416) significantly prolonged survival in tumor-bearing mice. The strategy of MIS416 immunization followed by anti-CD11b myeloid cell depletion further delayed tumor progression, thereby establishing the proof of principle that myeloid depletion can enhance vaccine efficacy. In patients with advanced EOC, ascites analysis showed substantial heterogeneity in the relative proportions of myeloid subsets and their immunosuppressive properties. Together, these findings point to immunosuppressive myeloid cells in the EOC micro-environment as targets to enhance vaccination. Further studies of myeloid cell accumulation and functional phenotypes in the EOC microenvironment may identify patients who are likely to benefit from vaccination combined with approaches that deplete tumor-associated myeloid cells.

Poster 67

CD47 in the tumor microenvironment limits synergy between anti-tumor T cell immunity and radiation therapy

David R. Soto-Pantoja[1], Masaki Terabe[2], Arunima Ghosh[1] Lisa A. Ridnour[3], William G. DeGraff[3], David A. Wink[3], Jay A. Berzofsky[2], and David D.Roberts[1].

[1]Laboratory of Pathology, [2]Vaccine Branch, and [3]Radiation Biology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892

Ionizing radiation is utilized in the treatment of a majority of cancers, but achieving a curative response is limited by its detrimental side effects. We previously demonstrated that CD47 deficiency or blockade in syngeneic mouse models confers radioprotection to normal tissues while enhancing tumor radio-sensitivity. We now report that CD47 blockade directly enhances immunosurveillance by CD8+ cytotoxic T cells. Blockade of CD47 in combination with irradiation did not alter 15-12RM fibrosarcoma growth in T-cell deficient mice. However adoptive transfer of tumor-specific CD8+ T-cells restored the cooperative interaction between CD47 blockade and irradiation to significantly decrease tumor growth. Blockade of CD47 on either target or effector cells enhanced antigen-dependent CD8+ CTL-mediated killing of tumor cells in vitro. Furthermore radiation treatment of syngeneic B16 melanomas growing in a CD47-/- host resulted in a >50% reduction in volume relative to irradiated tumors in WT mice, indicating that CD47 deficiency in the tumor microenvironment is sufficient to enhance tumor radiosensitivity. Increased numbers of CD8+ cytotoxic T-cells infiltrated tumors growing in a CD47-/- microenvironment. Lack of or blockade of CD47 in combination with irradiation significantly increased granzyme B mRNA and protein levels. These results indicate that T cell immunity is a direct target of CD47 blockade that enhances the efficacy of radiation therapy and may increase the percentage of curative responses.

Poster 68

Very Low Avidity Endogenous Memory CD8+ T Cells Control Tumor Growth

Katherine Stagliano[1,2], Ziqiang Zhu[1], Arthur A. Hurwitz[1]

[1]CIP, CCR, NCI; [2]George Washington University

Endogenous Memory (EM) T cells are proposed to arise from naïve T cells in the absence of activation by foreign antigen. These EM-T cells have been documented in unprimed, germ-free, wild-type mice as well as in unmanipulated RAG-/- TCR transgenic mice. EM-T cells are a very rare population of cells which are proposed to contribute to primary immune responses, but have not been studied in cancer models. In this study, we describe mice bearing a very low avidity TCR transgene (TCRvlo) specific for the melanoma antigen tyrosinase-related protein 2 (TRP-2180-188). Remarkably, a majority (>50%) of TCRvlo CD8+ T cells displayed a phenotype of CD44hi, CXCR3hi, Gr1hi, GITRhi, CD69lo, CD25lo, CD49dlo which is consistent with the expression pattern for EM-T cells. This phenotype was not observed in the thymus, but was detected in peripheral lymphoid tissues, as well as in the bone marrow of TCRvlo mice. In our preliminary tumor studies, TCRvlo transgenic mice displayed delayed subcutaneous B16 melanoma growth compared to wildtype animals. When TCRvlo CD8+ T cells were used in an adoptive transfer model, B16 tumor growth was also delayed compared to untreated animals. Ongoing studies are focused on characterizing the functional response of the TCRvlo CD8+ T cells to antigen and cytokines. Future studies will examine the ability of TCRvlo CD8+ T cells to resist or delay tolerance induced by the tumor microenvironment. Overall, these preliminary studies suggest that endogenous memory T cells may provide a novel tool for use as cancer immunotherapeutics.

Poster 69

Foxp1 regulation of T cell effector functions in the tumor microenvironment

Tom Li Stephen, Melanie R Rutkowski, Michael J Allegrezza, Jose R. Conejo-Garcia

Tumor Microenvironment and Metastasis Program, The Wistar Institute, 3601 Spruce St., Philadelphia, PA 19104, USA

T cells are emerging as the principal mediators of anti-tumor immunity. However, T cell anti-tumor immunity is sub-optimal and is overridden by the immunosuppressive tumor microenvironment. In this study, we report that the transcription factor Foxp1 upregulation on anti-tumor effector CD8 lymphocytes prevents their proliferative and effector functions in an anergy and exhaustion-independent mechanism. Accordingly, Foxp1-deficient T cells are superior in their IFNγ and Granzyme B responses in the tumor. Furthermore, immunotherapy with Foxp1-deficient T cells rejected incurable tumors and resulted in protection against tumor re-challenge. Mechanistically, we identify that Foxp1 expression enhances T cell susceptibility to microenvironmental TGF-β. Immunoprecipitation analysis shows that Foxp1 is an integral component of Smad2/3 nuclear complex, the key signaling mediator of TGF-β. Therefore, Smad2/3-mediated c-Myc repression requires Foxp1 expression in T-cells. Furthermore, Foxp1 directly mediates TGF-β-induced c-Jun transcriptional repression, which abrogates T-cell activity. Collectively, our study identifies a new targetable transcriptional regulatory mechanism that impairs T cell anti-tumor immunity.

Poster 70

Co-Administration of IL-12 Conditioned CD8+ T cells and IL-2 Antibody Complexes Leads to Complete Tumor Regression in Lymphoreplete Hosts

Wern Su[1], Caitlin Moore[1], Samantha Suriano[1], Neizel Songalia[1], Bryce Johnson[1], Daniel Neitzke[1], Chrystal Paulos[2], David Cole[1], and Mark Rubinstein[1]

[1]Department of Surgery, Medical University of South Carolina, Charleston, South Carolina; [2]Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, South Carolina

To improve the outcome of adoptive T cell therapy (ACT) in patients with metastatic disease, ACT is commonly accompanied by IL-2 administration. However, engagement of the high affinity IL-2 receptor subunit CD25, not only promotes the survival and proliferation of tumor-reactive T cells but regulatory T cells (Treg) as well. As IL-15, another member of the gamma-chain cytokine family can signal through the intermediate affinity IL-2 receptor (CD122/CD132) without CD25, we hypothesized that IL-15 will serve as a better adjuvant therapy to ACT than IL-2. To test this hypothesis, we activated pmel-1 TCR transgenic CD8+ T cells in the absence (Tc0) or presence (Tc1) of IL-12 and adoptively transferred these cells into mice bearing established B16 melanomas. In order to circumvent dose-related toxicity issues, we administered IL-2 and IL-15 in the form of complexes, as they possess a greater half-life in vivo than their unadulterated forms. In mice that received Tc0 cells, both IL-2 and IL-15 complexes failed to significantly delay tumor growth. However, in mice that received Tc1 cells, IL-2 but not IL-15 complexes induced complete and durable responses. Post-infusion, donor Tc1 cells in IL-2 complex-treated mice persisted for greater than 100 days without losing their antigen-specific responsiveness. This finding is of great clinical significance as it was achieved without lymphodepletion, a pre-conditioning therapy commonly used to increase the success of ACT in clinical trials. To uncover the underlying basis for the synergy between Tc1 cells and IL-2 complexes, we are focusing our attention on CD25 as it is expressed at extraordinarily high levels on Tc1 but not Tc0 cells. Altogether, we present evidence for a combinatorial therapy that delivers long-term curative responses in mice with aggressive melanoma.

Poster 71

Immunostimulating Gene Therapy Using an Oncolytic Adenovirus Armed with CD40 Ligand Potently Kill Tumor Cells, Activates Dendritic Cells and Induces T Cell Responses

Svensson Emma[1], Moreno Rafael[2], Milenova Ioanna[1], Christiansson Lisa[1], Alemany Ramon[2], Loskog Angelica[1,3]

[1]Uppsala University, Dept of Immunology, Genetics and Pathology, Uppsala, Sweden. [2]IDIBELL-Institut Catala d'Oncologia, L'Hospitalet de Llobregat, Barcelona, Spain. [3]Lokon Pharma AB, Uppsala, Sweden

CD40 ligand (CD40L) is a potent immune stimulator and tilts a suppressive tumor milieu toward Th1 immunity. CD40L can also reduce the level of myeloid suppressor cells and M2 macrophages in favor of M1 macrophages and activated dendritic cells. We have previously demonstrated in various experimental models, in dog melanoma patients and in clinical trials on bladder cancer and melanoma that a replication deficient adenovirus serotype 5 vector carrying the CD40L gene induces robust immune responses and has effect on the tumor. However, to increase efficacy and applicability, an oncolytic version was developed. Further, viral infection was enhanced by changing the Ad5 fiber (Ad5M). A CMV promoter was inserted to autonomously drive a modified CD40L transgene cassette to allow for transgene expression also by the tumor stroma cells. The Ad5M-CD40L virus infected both tumor cell lines and human dendritic cells (DCs) differentiated from CD14+ monocytes with high efficacy and both the cell lines and the DCs expressed the transgene even if the virus did not replicate in the DCs. The transgene cassette did not interrupt oncolysis since the Ad5M-CD40L virus demonstrated an equal oncolytic capacity as an Ad5M-Mock virus without the transgene. The transduced immature DCs rapidly increased their expression of CD83, CD86 and IL12 upon CD40L gene transfer. These matured DCs could efficiently stimulate the expansion of antigen-specific T cells using a CMV-pp65 in vitro system. Taken together, Ad5M-CD40L can kill tumor cells via oncolysis but also stimulate adaptive T cell responses via activation of dendritic cells. Hence, Ad5M-CD40L is a novel oncolytic virus with immunostimulatory capacity.

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Poster 72

The global genomic organizer SATB1 regulates dendritic cell differentiation and immunostimulatory functions in cancer

Amelia J. Tesone, Melanie R. Rutkowski, Tom L. Stephen, Eva Brencicova, Michael J. Allegrezza, Alfredo Perales-Puchalt, Nikolaos Svoronos, Jenny Nguyen, and Jose R. Conejo-Garcia

Tumor Microenvironment and Metastasis Program, The Wistar Institute, Philadelphia, PA. USA.

Special AT-rich sequence- binding protein 1 (SATB1) is a master genomic organizer that controls genome-wide transcriptional programs. The regulation of Satb1 activity has a critical role in driving T-cell development and differentiation. However, the role of Satb1 in myeloid lineages is far from being understood. In the present study, we investigated the expression pattern of Satb1 during Dendritic Cell (DC) differentiation and tumor progression. We found that constitutive Satb1 deficiency in hematopoietic progenitors results in a significant defect in the generation of myeloid subsets in competitive reconstitution experiments. Under steady-state conditions, Satb1 expression follows a dynamic pattern during DC differentiation, reaching its peak during the transition from pre-DCs to MHC-II+ DCs, and progressively disappearing thereafter. Importantly, Satb1 is required for sustained up-regulation of MHC-II, but ensuing down-regulation is necessary for effective antigen presentation and immunostimulatory function. However, Satb1 remains significantly up-regulated in the nuclei of tumor-associated DCs. We found that Satb1 is up-regulated in response to inflammatory S100A9, overexpressed in cancer. Correspondingly, silencing SATB1 in vivo selectively in tumor-associated DCs reverses their immunosuppressive phenotype, boosting anti-tumor immunity and enhancing survival. Our results identify sustained Satb1 overexpression as a major driver of defective antigen presentation in cancer-bearing hosts, which could have remarkable implications for development of improved immunotherapies.

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Poster 73

Preventing tumor escape by targeting a post-proteasomal trimming independent epitope

Ana Textor[1], Karin Schmidt[2], Peter-Michael Kloetzel[2], Bianca Weissbrich[3], Cynthia Perez[1], Jehad Charo[1], Kathleen Anders[1], John Sidney[4], Alessandro Sette[4], Ton N. Schumacher[5], Christin Keller[2], Dirk Busch[3], Ulrike Seifert[2,6], Thomas Blankenstein[1,7]

[1]Max-Delbrück-Center for Molecular Medicine, Berlin, Germany ; [2]Institute for Biochemistry, Charité, Campus Mitte, Berlin, Germany; [3]Institute for Medical Microbiology, Immunology and Hygiene, Technical University, Munich, Germany; [4]La Jolla Institute for Allergy and Immunology, La Jolla, USA; [5]The Division of Immunology, The Netherlands Cancer Institute, Amsterdam, The Netherlands; [6]Institute for Molecular and Clinical Immunology, Otto-von-Guericke-Universität, Magdeburg, Germany; [7]Institute of Immunology, Charité, Campus Buch, Berlin, Germany

Adoptive T cell therapy (ATT) can achieve regression of large tumors in mice and humans, however tumors frequently recur. High target peptide-MHC I (pMHC) affinity and T cell receptor (TCR)-pMHC affinity are thought critical to prevent relapse. Here we show that targeting two epitopes of the same antigen in the same cancer cells by monospecific T cells, which have similar pMHC and pMHC-TCR affinity, results in eradication of large established tumors when targeting the sub-dominant but not the dominant epitope. Only the 'escape' but not the 'rejection epitope' required post-proteasomal trimming, which was regulated by IFNγ, allowing IFNγ-unresponsive cancer variants to evade. The data describe a novel immune escape mechanism and better define suitable target epitopes for ATT.

Poster 74

Loss of sphingosine kinase 1 enhances melanoma-specific CD8+ T cell responses

Krishnamurthy Thyagarajan[1], Amir Al-Khami[1], Suriyan Ponnusamy[2], Beichu Guo[3], Besim Ogretmen[2], Shikhar Mehrotra[1]

[1]Department of Surgery, MUSC, Charleston. [2]Department of Biochemistry and Molecular Biology, MUSC, Charleston. [3]Department of Microbiology and Immunology, MUSC, Charleston

Sphingosine kinase 1(Sphk1) is a key enzyme in sphingolipid metabolism that is involved in the production of sphingosine-1-phosphate (S1P). S1P controls many cellular processes such as cell proliferation, migration and cytokine production. We sought to address the role of Sphk1 in regulating T cell responses to a tumor specific 'self' antigen gp100, in a mouse model of melanoma by generating TCR transgenic mice pMel on Sphk1 deficient background. We found that Sphk1-/-pMel mice showed reduced tumor progression on subcutaneous administration of B16-melanoma. Tumor progression was slower when T cells from Sphk1-/-pMel mice were adoptively transferred to treat Rag-/- mice with established subcutaneous tumors. There was an increased infiltration of Sphk1-/-pMel T-cells at the tumor site as compared to pMel cells. This suggests that loss of Sphk1 from T-cells enhanced their ability to target the tumor site. We also found TGF-beta mediated suppression of IFN-gamma secretion was poorer in Sphk1-/- T-cells suggesting better functionality of effector T cells infiltrating the tumor site. A comparative evaluation of T cells from WT and Sphk1-/-pMel showed similar cell surface markers, activation status and proliferation and Tc1 cytokine response. However, we found that Sphk1-/- T-cells showed greater IL-17 secretion. It is possible that the enhanced anti-tumor T cell immunity noticed in Sphk1-/- mice is dependent on the innate Tc17 phenotype and lesser susceptibility to suppression.

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Poster 75

Investigation of Anti-Cancer Entirely Carbohydrate Constructs Tn-PS A1 and TF-PS B

Kevin R. Trabbic, Mengchao Shi, Jean Paul Bourgault and Peter Andreana

The University of Toledo, Department of Chemistry and Biochemistry

Antibodies have emerged as promising cancer immunotherapies by binding specifically to tumor cells. The generation of both the Tn-PS A1 and TF-PS B constructs represents entirely carbohydrate constructs that can assist in tumor binding and killing.[1] The polyclonal anti-serum generated from these constructs, demonstrated binding to the glycoside portion of both the Tn and TF antigens, in ELISA studies. Since bacterial polysaccharides generate a robust IgM response, IgM's have demonstrated binding to glycosides better than their IgG counterparts through higher avidity.[2] Using sera that contains both IgM and IgG, specific for aberrant glycosylations represents a unique therapeutic approach to target TACAs in tumor models. Since, TACAs are present on almost all cancers, having an immunotherapy that generates a polyclonal response that can recognize specific glycosides may prove to be an efficient strategy against cancer. Importantly, IgM antibodies have been showed to be efficient at mediating complement directed killing of tumor cells, this approach potentially offers a therapeutic benefit; a current pursuit of ours.[3]

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Poster 76

Overcoming resistance to antibody targeted therapy in cancer

Katarzyna Urbanska, Rachel Lynn, Jenessa B. Smith and Daniel J. Powell Jr.

Ovarian Cancer Research Center, Department of Pathology and Laboratory Medicine, Abramson Cancer Center, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA

Targeted therapies utilizing monoclonal antibodies (mAbs) are now the major class of successful therapeutics for treating a variety of malignancies. Mechanistically, mAbs can have direct antitumoral activity but often their effectiveness relies upon antibody-dependent cellular cytotoxicity (ADCC). For instance, Trastuzumab is a therapeutic mAbs directed against HER2 that is well-tolerated and useful in the treatment of HER2-positive breast cancer, where efficacy is mediated in part by ADCC. In contrast, Trastuzumab is relatively ineffective in the treatment of HER2-positive epithelial ovarian cancer. Poor response to mAb therapy in EOC and other types of cancers may be explained in part by diminished numbers and cytotoxic potential of NK-cells in cancer patients. Therefore, we hypothesize that the development of potent effector cells with the capacity to bind tumor-bound mAb and mediate strong antibody-directed cellular cytotoxicity would markedly improve the efficacy of mAb-targeted therapy. In order to expand applications for T-cell-based immunotherapy and to enhance ADCC, we developed novel effector T-cells engineered to express Fc-binding-immune-receptors (FcIR) containing human Fc receptor of low FcγRIIIA (CD16), intermediate FcγRIIA (CD32) or high affinity FcγRI (CD64) molecules fused to intracellular TCR and co-stimulatory signaling domains in order to enable cytotoxic T-cells to mediate mAb-directed cytotoxicity. Following lentiviral transduction, all forms of FcγRs were expressed on the surface of primary human T-cells. Trastuzumab-armed FcIR T-cells specifically recognized HER2+ cancer cells, as did unarmed FcIRs but only when the cancer cells were pre-bound with Trastuzumab. In addition, CD64-FcIRI T-cells exhibited stronger specific lytic activity than NK-cells, even at low antibody concentrations. Further, co-administration of CD64FcIR-28z T-cells with immunotherapeutic mAb, Trastuzumab completely eliminated HER2+ tumor in vivo. In summary, our results show that ADCC can be enhanced by T-cells engineered to express an FcIR and that this novel approach may overcome issues of resistance to mAb-targeted therapies.

Poster 77

Generation of cancer antigen specific T cells from human induced pluripotent stem cells

Raul Vizcardo[1], Linda Tran[1], Nicholas Klemen[1], Nicholas Restifo[1]

[1]Surgery Branch, CCR, NCI

Human induced pluripotent stem (iPS) cells can be derived from several types of somatic cells by reprogramming with Yamanaka factors (Oct4, Sox2, Klf4, and Myc). iPS cells have reported similarities to embryonic stem cells in terms of their gene expression signature and multipotency, and as such, have tremendous therapeutic potential in regenerative medicine. We have previously shown that human iPSCs can be derived from mature cytotoxic T cells specific for the melanoma epitope MART-1. Subsequent co-culture with OP9 stromal cells induced human iPS differentiation into hematopoietic progenitor cells characterized by expression of CD34+CD43+. Moreover, co-culture with OP9/DLL1 stromal cells generated mature and functional CD8SP T cells specific for the original MART-1 epitope. In the present study, we plan to develop iPS cells from tumor-infiltrating lymphocytes (TIL) isolated from patients with metastatic cancer. Because iPS cells from lymphocytes maintain antigen-specificity, TIL-derived iPS cells may be used as a progenitor source of tumor-specific T cells used in adoptive cellular immuno-therapy (ACT) for advanced cancer. Our current aim is to develop a xeno-free *in vitro* differentiation method capable of yielding therapeutic numbers of progenitor T cells and evaluate their *de novo* potential to form tumor-specific naïve T cells after adoptive transfer. Because naïve T cells have enhanced capacity for self-renewal and multipotency, this approach of reprogramming TIL to a naïve-like state may significantly improve the therapeutic efficacy of ACT for cancer.

Poster 78

A Chimeric Antigen Receptor (CARs) based upon a Killer Immunoglobulin-like Receptor (KIR) Triggers Robust Cytotoxic Activity in Solid Tumors

Enxiu Wang[1], Liang-Chuan Wang[2], Edmund Moon[2], Ching-Yi Tsai[1], Vijay Bhoj[1], Steven Albelda[2], Michael C. Milone[1]

[1]Department of Pathology and Laboratory Medicine, [2]Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104

Chimeric antigen receptors (CARs) based upon a single chimeric molecule bearing an antigen binding domain linked in cis to the cytoplasmic domains of CD3ζ and costimulatory receptors CD28 or 4-1BB provide a potent method for engineering T cell cytotoxicity towards tumors. We describe a simple chimeric receptor based upon a killer immunoglobulin-like receptors (KIRs) normally expressed by natural killer (NK) cells. Constructed by fusing a single chain variable fragment (scFv) to the transmembrane and cytoplasmic domain of a KIR, we show that a KIR-based CAR targeting mesothelin (SS1-KIR) triggers antigen-specific cytotoxic activity and cytokine production that is comparable to CD3ζ-based CARs. Using a xenograft model of mesothelioma, we further demonstrate that a KIR-based CAR targeting mesothelin exhibits more potent anti-tumor activity compared with T cells bearing mesothelin-specific CD3ζ-based CARs with costimulation despite superior in vivo persistence of the latter CAR-modified T cells. The ability of a KIR-based CAR to induce regression of a tumor in which second generation CD3ζ-based CARs show limited activity supports the future clinical evaluation of a KIR-based CAR in mesothelioma and other tumors.

Poster 79

Targeting Soluble NKG2D Ligand Prevents Cancer Progression and Eliminates Metastasis in a 'humanized' Spontaneous Pre-clinical Cancer Model

Jennifer Wu[1], Shengjun Lu[1], Fahmin Basher[1], Xiao Gang[1]

[1]Medical University of South Carolina and Hollings Cancer Center, Charleston, SC 29425

Expression of the MHC I-chain related molecules A and B (MICA/B) on epithelial cell surface in response to transformation or DNA damage can signal the immune system of the abnormality and thus initiate active immune surveillance by Natural Killer (NK) cells and cytotoxicity T cells. We and others have shown that malignant tumor cells can shed MICA/B to down regulate NKG2D expression and negatively impact NK and CD8 T cell function in cancer patients. Since no MIC homolog was identified in rodents, we thus generated a humanized 'MIC' transgenic animal model in which human native MIC was expressed concurrently with oncogenic transformation in an organ-specific manner. With this model, we not only recapitulate the biology in patients that cancer progression is associated with elevated serum sMIC, but also uncovered a novel cancer immune evasion mechanism that high levels of serum sMIC perturb NK cell homeostatic maintenance. These findings suggest that we have generated a bona fida pre-clinical model for cancer immunotherapy which enables us to take account of the impact of NKG2D ligands. These findings also suggest that sMIC is a therapeutic biomarker and target for advanced cancers. With a sMIC-specific antibody, we further demonstrated that targeting sMIC can effectively inhibit the progression of primary tumor and eradicate established metastatic tumors through restoring NK peripheral homeostasis and potentiate CD8 and CD4 T cell anti-tumor immunity. Furthermore, we found that the enhanced CD4 and CD8 anti-tumor response is NK-dependent. Together, our study suggests that: 1) the impact of sMIC should be accounted in current cancer immunotherapy trials and practice; 2) NK cells play an important role in sustaining adaptive immunity against cancer.

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Poster 80

Fas expression in memory CD8+ T cell subsets augments cellular differentiation and effector function

Tori N. Yamamoto[1,2], Anthony J. Leonardi[2], Hui Liu[3], Ena Wang[3,4], Luca Gattinoni[5], Richard M. Siegel[6], Nicholas P. Restifo[1], and Christopher A. Klebanoff [1,7]

[1]Center for Cancer Research, NCI/NIH; [2]Immunology Graduate Group, University of Pennsylvania, Philadelphia, PA; [3]Infectious Disease and Immunogenetics Section, Department of Transfusion Medicine, Clinical Center, NIH; [4]Sidra Medical and Research Centre, Doha, Qatar; [5]Experimental Transplantation and Immunology Branch, NCI/NIH; [6]National Institute of Arthritis and Musculoskeletal and Skin Diseases, NIH; [7]Clinical Investigator Development Program, NCI, NIH.

Memory CD8+ T cells (TMem) have the capacity to provide lifelong protection against intracellular pathogens and cancer. Despite phenotypic and functional heterogeneity among TMem, the expression of Fas, a tumor necrosis family receptor (TNFR) superfamily member conventionally known as a death receptor, is held in common among all TMem subsets across multiple species. As Fas has also been shown to mediate other effects besides death signaling, we therefore set out to elucidate the role of Fas signaling in defined TMem subsets, including T stem cell memory (TSCM), T central memory (TCM), and T effector memory (TEM). We found that augmenting Fas signaling in stimulated TSCM using an oligomerized form of its ligand FasL (lz-FasL) resulted in augmented cellular differentiation and a corresponding loss in IL-2 secretion capacity. Conversely, deprivation of Fas signaling in TCM using a blocking, non-agonistic antibody to FasL (aFasL) retarded cellular differentiation. Gene expression analysis demonstrated that TMem expanded with aFasL expressed greater levels of memory-associated transcription factors relative to IgG-treated controls. Moreover, preventing Fas-signaling significantly altered the metabolic state of activated TMem, most notably by limiting the acquisition of glycolytic metabolism. When used in vivo, TMem expanded with aFasL showed greater on-target immunity compared to IgG controls. These studies demonstrate that Fas signaling promotes not only cell death but also TMem differentiation towards a more effector-like state, a finding which has implications for the design and execution of clinical trials T cell-based immunotherapies in patients with cancer and infectious disease.

Poster 81

SOCS3 Deficiency in Myeloid Cells Promotes Prostate Cancer Development

Hao Yu, Yudong Liu, Braden C. McFarland, Etty N. Benveniste and Hongwei Qin

Departments of Cell, Developmental and Integrative Biology, University of Alabama at Birmingham, Birmingham, Alabama

Suppressor Of Cytokine Signaling (SOCS) proteins are negative regulators of the JAK/STAT pathway, and function as tumor suppressors and tumor promoters, depending on cellular context. We report for the first time that genetic deletion of SOCS3 specifically in myeloid cells significantly enhances prostate tumor growth, which correlates with elevated levels of myeloid-derived suppressor cells (MDSC) in the tumor microenvironment, and diminished CD8+ T-cell infiltration in tumors. Furthermore, SOCS3-deficient bone-marrow-derived cells exhibit heightened STAT3 activation and preferentially differentiate into the MDSC phenotype. Importantly, we identify granulocyte-colony stimulating factor (G-CSF) as a critical factor secreted by the tumor microenvironment that promotes development of Gr-1+CD11b+ MDSC via a STAT3-dependent pathway. Abrogation of tumor-derived G-CSF reduces the proliferation and accumulation of MDSC and inhibits prostate tumor growth. These findings highlight the critical function of SOCS3 as a negative regulator of MDSC development and function, which may contribute to the tumor suppressor attributes of SOCS3.

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Poster 82

Tryptophan catabolites in glioma: relevance to diagnostics and immunotherapeutics'

Lijie Zhai[1], Martin Kelly Nicholas[2], Maciej Lesniak[3], Robert McCusker[4], Derek Wainwright[1]

[1]Department of Neurological Surgery, Feinberg School of Medicine, Northwestern University, Chicago; [2]Department of Neurology, University of Illinois, Chicago; [3]Section of Neurosurgery, University of Chicago, Chicago; [4]Department of Animal Science, University of Illinois, Urbana.

Glioblastoma is an incurable primary brain tumor with no effective strategy for significantly increasing survival, even though aggressive treatments that include surgical resection, radiotherapy and chemo-therapy are used as a standard-of-care. To create new potentially effective therapies, we have turned our attention to the immune system as a high-value medium for causing a patient to spontaneously-reject his/her own tumor. However, for immunotherapy to be maximally-effective, the potent immuno-suppression caused by the brain tumor must be addressed. In this investigation, we have collected and analyzed the peripheral blood from brain tumor patients, as well as a high-fidelity transgenic mouse model that faithfully mimics malignant glioma in patients. We were surprised to find that the genetic deletion of an immunosuppressive gene, indoleamine 2,3 dioxygenase 1 (IDO1), resulted in decreased survival in the transgenic glioma model. Moreover this deletion was associated with a normal recruitment of immunosuppressive regulatory T cells (Treg); a pathogenic immune cell subset that we and others have previously shown to be associated with malignant glioma progression. We also identified the gene, IDO2, as a likely candidate that compensates for the loss of IDO1. Thus, a future immunotherapeutic strategy will be to co-inhibit IDO1 and IDO2, simultaneously, since the individual blockade of IDO1 had a limited effect on survival. These data significantly expand our knowledge of primary and secondary (i.e., compensatory) immunosuppression in malignant brain tumors and provides a strong foundation for further pursuit of effective immunotherapies against brain cancer.

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Poster 83

Functional analysis of monoclonal antibodies recognizing various epitopes of glypican-3 on liver cancer cells

Yi-Fan Zhang[1] and Mitchell Ho[1]

[1]Laboratory of Molecular Biology, CCR, NCI

Glypican-3 (GPC3) is a cell surface proteoglycan that is overexpressed in hepatocellular carcinoma and is a potential target for antibody therapy. Our lab has generated a group of monoclonal antibodies that recognizes different epitopes in GPC3. One (HN3) targets a conformational epitope while several others (e.g., YP7) bind the C terminus. By comparing their Fv-pseudomonas exotoxin fusion proteins (immunotoxin), we found that the anti-C terminus immunotoxins had stronger tumor cell binding but weaker cytotoxicity than the HN3 immunotoxin. In addition, although YP7 immunotoxin can bind GPC3 both inside and outside of the detergent resistant membrane (DRM), the HN3 antibody can only bind GPC3 that is outside of the DRM, suggesting that the endocytosis of HN3 may be mediated by clatharin rather than caveolin. In cell surface co-immunoprecipitation, the anti-C terminus immunotoxins can pull down unglycosylated, lightly glycosylated and heavily glycosylated GPC3, whereas the HN3 immunotoxin can only pull down lightly glycosylated surface GPC3, which are located outside of DRM. This evidence showed that the HN3 immunotoxin targets a unique portion of cell surface GPC3, which may have a higher internalization rate than other GPC3 molecules. On the other hand, the anti C-terminus immunotoxins target a larger population of cell surface GPC3, suggesting that their antibodies are more suitable for therapies based on naked antibodies than HN3. We humanized the group of anti C-terminus antibodies, and showed that these antibodies can induce more ADCC and CDC than HN3 in target cells. Among these humanized antibodies, humanized YP7 (hYP7) had the highest affinity and is being tested in a hepatocellular carcinoma xenograft model in nude mice.

Poster 84

Self-Assembled DNA Immuno-Nanoflowers for Immunostimulation in Cancer Therapy

Guizhi Zhu[1,2], Liqin Zhang[2], Lei Mei[3], and Weihong Tan[2,3]

[1]NIBIB, NIH, Bethesda, MD. [2]Department of Chemistry, Health Cancer Center, University of Florida Gainesville, FL. [3]Molecular Science and Biomedicine Laboratory, Hunan University, Changsha China

Immunotherapy exploits the host immune systems for disease intervention. Synthetic unmethylated cytosine-guanine (CpG) oligodeoxynucleotides are immunostimulatory motifs and have shown tremendous promise as vaccines or adjuvants for treatment of cancers and infectious diseases. However, it is highly desirable to efficiently deliver these nucleic acid therapeutics into target immune cells while protecting them from nuclease degradation. Taking advantage of DNA nanoflowers we have developed before, here we report novel immuno-nanoflowers (NFs), which are self-assembled from long DNA encoded with tandem CpG through rolling cycle replication, for efficient CpG delivery and protection from nuclease degradation. In a model macrophage, these CpG-NFs proved potent immunostimulation by stimulating macrophage proliferation and releasing immunostimulary cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-10 (IL-10). Preliminary study demonstrated the capability of CpG-NFs to induce cancer cell apoptosis and necrosis.