Methods
Female C57BL/6 recipient mice were inoculated with B16F10 or ovalbumin-expressing-B16F10 (B16-OVA) melanoma tumors. Once tumors were palpable, biweekly treatments of G100 were initiated. Splenic CD8+ T cells from OT-I and pmel mice, which carry a rearranged TCR transgene specific for an OVA or gp100 epitope, respectively, were magnetically isolated and transferred into the tumor-bearing mice. Tumor growth was monitored 2-3 times per week by caliper measurement until tumors either completely regressed or mice were euthanized due to tumor growth. In some experiments, tumors and tumor-draining lymph nodes were isolated from animals and analyzed by flow cytometry for infiltration of transferred CD8+ T cells.
Results
Mice that received both ACT and G100 treatments ("transfer-pull") experienced significantly enhanced tumor protection compared to mice that received ACT or biweekly intratumoral G100 alone. Treatment of B16-OVA tumor-bearing mice using the transfer-pull regimen resulted in complete tumor regression in 70% of the animals, whereas no tumor regression was observed for animals receiving either monotherapy. Consistent with the proposed mechanism of action, actively proliferating transferred T-cells were present in tumors as well as tumor-draining lymph nodes of transfer-pull treated mice. When targeting the less immunogenic gp100 melanoma antigen, median survival was significantly extended and complete regression observed in up to 28% of animals.
Conclusions
These data collectively demonstrate that intratumoral G100 can be effectively used in combination with adoptive cell therapy to enhance tumor rejection and survival, warranting further preclinical and clinical evaluation.

P608

ProTriTAC: a protease-activatable T cell engager platform that links half-life extension to functional masking and expands therapeutic window to enable targeting of broadly expressed tumor antigens

S. Jack Lin, PhD, Maria Rosalyn Dayao, Kendrick Kim, Sony Rocha, Kathryn Kwant, PhD, Timothy Yu, Thomas Evans, Stephen Yu, Michael Cremin, Wade Aaron, BS, Maria Gamez-Guerrero, Evan Callihan, Golzar Hemmati, Kevin Wright, PhD, Yinghua Xiao, Master Degree, Manasi Barath, Che-Leung Law, PhD, Bryan Lemon, PhD, Richard Austin, PhD, Holger Wesche, PhD
Harpoon Therapeutics, Inc., South San Francisco, CA, USA
Correspondence: S. Jack Lin (jlin@harpoontx.com)
Journal for ImmunoTherapy of Cancer 2018, **6(Suppl 1):**P608

Background
T cell engagers, such as blinatumomab, have demonstrated clinical activity in several hematological malignancies, but their use in solid tumors is limited by the low number of antigens that are expressed in tumors but not in normal tissues. Conditionally active T cell engagers that function preferentially in the tumor microenvironment offer a path to expanding the therapeutic window by reducing their on-target but off-tumor activity. Here, we describe a prodrug version of our T cell engager platform, termed ProTriTAC, that is activated by proteases in the tumor microenvironment and enables the safe targeting of broadly expressed solid tumor antigens.
Methods
ProTriTACs were engineered with three binding domains on a single polypeptide: anti-albumin for half-life extension, anti-CD3e for T cell engagement, and anti-tumor-associated antigen. They have an anti-albumin domain, comprising a masking moiety and a protease-cleavable linker, to keep the molecules inert outside the tumor microenvironment. Activation by tumor-associated proteases removes the anti-albumin domain along with the masking moiety to reveal the active drug. The masking moieties were identified using phage display. Binding studies to recombinant CD3e protein were determined using ELISA assays and to primary T cells using flow cytometry assays. T cell engager function was assessed using T cell-dependent cellular cytotoxicity (TDCC) assays with resting human T cells. In vivo efficacy studies were performed using a subcutaneous tumor xenograft model in immunodeficient NCG mice.
Results
Proof-of-concept experiments were carried out in vitro and in vivo. The protease-activated ProTriTAC had markedly increased binding to recombinant CD3e protein and to human primary T cells as well as increased T cell-redirected killing activity in TDCC assays when compared to the prodrug. Consistent with tumor-dependent activation of ProTriTACs in vivo, ProTriTACs have comparable anti-tumor activity to the unmasked molecule but significantly more anti-tumor activity than the masked non-cleavable molecule.
Conclusions
ProTriTACs are designed as long-lived inert prodrugs when in circulation and become short-lived active drugs for T cell-redirected tumor killing when activated in the tumor microenvironment. This half-life differential between the prodrug and the active drug is desirable as any aberrant activation of prodrug outside the tumor will be cleared rapidly, thereby further expanding the therapeutic window. This technology enables more T cell engager targets for solid tumors, and we are building a pipeline of ProTriTACs against these targets.
Ethics Approval
In vivo studies were reviewed and approved by Harpoon's Institutional Animal Care and Use Committee.

P609

First in man study of TK positive oncolytic vaccinia virus delivered by adipose stromal vascular fraction cells

Boris Minev, MD[1], Elliot Lander, MD[3], John Feller, MD[4], Mark Berman, MD[5], Stuart May, MD[4], Bernadette Greenwood[4], Ivelina Minev, MSc[6], Duong Nguyen, PhD[6], Antonio Santidrian[6], Dobrin Draganov, PhD[6], Mehmet Kilinc, PhD[6], Santosh Kesari, MD, PhD[7], Edward McClay, MD[8], Gabriel Carabulea, MD[9], Aladar Szalay, PhD[6]
[1]Moores University of California San Dieg, San Diego, CA, USA; [2]Moores UCSD Cancer Center, San Diego, CA, USA; [3]California Stem Cell Treatment Center, Rancho Mirage, CA, USA; [4]Desert Medical Imaging, Indian Wells, CA, USA; [5]Cell Surgical Network, Beverly Hills, CA, USA [6]Calidi Biotherapeutics, San Diego, CA, USA; [7]John Wayne Cancer Institute, Santa Monica, CA, USA; [8]cCare, Encinitas, CA, USA; [9]Ocean View Hematology and Oncology Group, San Clemente, CA, USA
Correspondence: Boris Minev (bminev@ucsd.edu)
Journal for ImmunoTherapy of Cancer 2018, **6(Suppl 1):**P609

Background
Recent oncolytic virus clinical studies have shown safety and implied anti-tumor activity. However, a major obstacle to this approach has been the rapid oncolytic virus elimination by patient's immune system. We hypothesized that oncolytic viruses would be protected and delivered efficiently to tumor sites by autologous adipose stromal vascular fraction (SVF) cells. Effective virus protection by adipose derived cells has been confirmed in preclinical studies. Here, we report the results of a first-in-man trial to determine the safety and feasibility of this approach in patients with advanced solid tumors and AML.
Methods
In this single-arm, open-label safety study, 24 patients with advanced solid tumors and 2 patients with AML were treated with a single administration of the oncolytic virus ACAM2000 (vaccinia) delivered by SVF cells. Patients received ACAM2000/SVF by intravenous application, or by a combination of intravenous and intratumoral or intraperitoneal injections. The dose for ACAM2000 was between 1.4 x 106 pfu to 1.8 x 107 pfu incubated with same number of SVF cells. The primary endpoint was safety/tolerability by incidence of dose-limiting toxicity. Secondary endpoints included evaluations of overall survival and induction of anti-tumor and anti-vaccinia immune responses. Blood samples were collected at multiple time points for quantifying vaccinia virus DNA in peripheral blood by qPCR. In addition, levels of 30 plasma cytokines and the effects on activated T cells, Tregs, memory T cells, NK cells, and MDSC were analyzed.
Results
No serious toxicities (> grade 2) were reported. Eight of the 26 subjects reported an AE: self-limiting skin lesions, lasting 7 to 18 days – an expected reaction to ACAM2000. No infusion-related AEs were reported. No AEs leading to study discontinuation were reported.

Viral DNA was detected in all patients immediately following treatment. Interestingly, in 8 patients viral DNA disappeared 1 day and reappeared 1 week post treatment, suggesting active viral replication, possibly at tumor sites. This viral DNA reappearance correlated with longer survival of these patients. No major increase in cytokine levels was observed in any of the patients. No correlation between cytokine levels and pox lesions was noted. Flow cytometry showed gradual changes suggesting improved immune cell activation status. Tumor size reduction was documented in several patients.

Conclusions

Treatment with ACAM2000/SVF in patients with advanced solid tumors and AML is safe and well tolerated, with clear antitumor effects in several patients. These promising initial clinical results merit further investigation of therapeutic utility.

Acknowledgements

Boris Minev, MD and Elliot Lander, MD contributed equally to this work.

Ethics Approval

The study was approved by International Cell Surgical Society's Ethics Board, approval number ICSS-2017-004

P610

Transcriptome analysis of CT26 tumors treated with HSV-1 oncolytic virus expressing multiple immune factors

Yanal Murad, PhD[1], Jun Ding, PhD[1], Erica Lee, PhD[1], Dmitry Chouljenko, PhD[1], Luke Bu, MSc[1], Guoyu Liu, MSc, MD[1], Zahid Delwar, PhD[2], Will Liu, PhD[1], Ronghua Zhao, MD[1], William Jia, PhD[2]

[1]Virogin Biotech. Inc., Vancouver, BC, Canada; [2]University of British Columbia, Vancouver, Canada

Correspondence: William Jia (w.jia@ubc.ca)

Journal for ImmunoTherapy of Cancer 2018, **6(Suppl 1):**P610

Background

Oncolytic HSV-1 (oHSV-1) treatment induces a potent immune response against tumor antigens, which can be augmented once combined with other immune stimulatory factors. Previously, we have generated an oncolytic HSV-1 virus (VG161) which carries 2 immunomodulator cytokines, IL12 and IL15/IL15RA1, along with a PD-L1 mimic peptide capable of blocking PD-1/PD-L1 interaction. These factors work synergistically to trigger and maintain an efficient anti-tumor immune response in the tumor microenvironment. In this work, we demonstrate the superior activity of the VG161 virus, compared to the back-bone virus (with no payloads) using a mouse colon cancer tumor model. We also perform a transcriptome analysis to determine the differential gene expression in the treated tumors and compare the two treatments.

Methods

We tested the efficacy of VG161 treatment in CT26 mouse model and examined the differential expression of transcriptome in these tumors collected 5 days post treatment with VG161 or the corresponding backbone virus. After extracting the RNA from the tumor samples, we performed RNA sequencing and analyzed differential expression of genes and compared them between the 2 treatments. qRT-PCR was used to validate targets identified by RNA sequencing.

Results

In the CT26 model, tumors regressed to undetectable limits upon intratumoral injection with VG161. When the treated mice were challenged with the same tumor, the tumor cells did not grow. Tumor treated with VG161 has demonstrated a higher number of tumor-infiltrating CD8 T cells, which activity against the tumor cells was also demonstrated by ELISpot assay. Differential expression of 24342 genes was performed and 18 differentially expressed genes with q-Value <0.05 were identified. These genes included chemokines and interferon response elements associated with inflammatory response, along with acute phase proteins. Some of the overexpressed genes, especially those related IFN response elements genes were also validated by qRT-PCR.

Conclusions

We have demonstrated by transcriptome analysis that VG161, a novel oncolytic virus which can induce a strong anti-tumor immunity and oncolytic activity, can induce multiple genes which result in efficacy against tumors. The efficacy of VG161 can be partially attributed to the immune response generated by the modified virus, which is likely induced by the change in the tumor microenvironment triggered by the VG161 payload. Further work is needed to dissect the function of each of the differentially expressed genes to understand the role they play in the regression of cancer upon VG161 treatment.

P611

Withdrawn

P612

Overcoming oncolytic poliovirus-mediated adaptive immune resistance by combining with anti-PD1/-PDL1 therapy in cancer

Smita Nair, PhD, Eda Holl, PhD, RAC, Michael Brown, PhD, Victoria Frazier, David Boczkowski, BS, MSc, Vidyalakshmi Chandramohan, PhD, Darell Bigner, MD, PhD, Shelley Hwang, Matthias Gromeier, MD

Duke University School of Medicine, Durham, NC, USA

Correspondence: Smita Nair (smita.nair@duke.edu)

Journal for ImmunoTherapy of Cancer 2018, **6(Suppl 1):**P612

Background

Oncolytic poliovirus (OncPV) PVSRIPO is a recombinant, non-pathogenic polio:rhinovirus chimera that targets cancer cells via CD155. PVSRIPO also targets antigen-presenting cells (APCs), including dendritic cells (DCs) and macrophages. PVSRIPO infection of APCs induces sustained type I interferon and APC activation (1). In a phase-1 clinical trial of intratumor PVSRIPO in 61 patients with recurrent glioblastoma (GBM), the survival rate at 24- months and 36-months was 21% (2). This study examines the following hypotheses: 1] Intratumor OncPV administration causes oncolysis and inflammation, which stimulates innate and adaptive immunity; 2] Immune cell activation in tumor triggers adaptive immune resistance via the PD1/PDL1 axis; 3] Blocking PD1/PDL1 in conjunction with OncPV will eliminate adaptive resistance and potentiate durable antitumor immunity.

Methods

OncPV-mediated immune activation was analyzed in: 1] immunocompetent murine models of orthotopic E0771 breast cancer and subcutaneous B16 melanoma; 2] human tumor cell lines and primary human tumor tissue from patients with breast cancer, melanoma and GBM; 3] human DCs and macrophages. To investigate combination PVSRIPO and PD1/PDL1 blockade, C57BL6-CD155 transgenic mice were orthotopically implanted with E0771- CD155 tumor cells. Mouse PVSRIPO (mRIPO) was injected intratumorally once in 50-100 mm3 tumors. Checkpoint antibodies were injected intraperitoneally 1-day post-mRIPO, 4-6 times every 3 days. Tumor growth was monitored.

Results

Intratumor mRIPO induced recruitment of immune cells (Figure 1), a classical acute inflammatory response and systemic antitumor cytotoxic T cell responses (1). Tumor infiltrating CD8/CD4 T cells demonstrated an effector phenotype and expressed PD1 (Figure 2). Infection of primary human tumors with PVSRIPO induced Stat1-p, IFIT1 and PDL1 expression and production of pro-inflammatory cytokines (Figure 2). Infection of human tumor cell lines with PVSRIPO induced PDL1 expression (Figure 2). PVSRIPO-infected primary human DCs and macrophages demonstrated sustained activation and PDL1 expression.Based on these data we investigated combination PVSRIPO with PD1/PDL1 blockade in murine breast cancer model. mRIPO, anti-PD1/-PDL1, and mRIPO+anti-PD1/-PDL1 significantly inhibited tumor growth compared

Background
Immunotherapy has shown impressive clinical responses, but many patients do not respond to single modality immunotherapy due to a number of non-redundant resistance mechanisms. Our lab and others have proposed that tumor cells compromise T cell function by generating a metabolically inhospitable microenvironment, suggesting that immune or tumor metabolism can be differentially modified to improve T cell responses and thus immunotherapy.
Methods
We identified the adipokine leptin as a means to remodel the metabolic state of tumor infiltrating T cells. To assess the effects of leptin in the tumor microenvironment, we generated an aggressive PTEN/BRAF melanoma line overexpressing leptin, as well as an oncolytic strain of Vaccinia virus engineered to induce tumor-specific secretion of leptin.
Results
Treatment of T cells with leptin in vitro resulted in dramatic metabolic reprogramming. In vivo, intratumoral administration of leptin resulted in enhanced T cell metabolic and effector function. We then engineered melanoma cell lines to locally secrete leptin. While there was no proliferation difference between wild-type and leptin- expressing tumor cells in vitro, these cells are controlled in vivo in a CD8+ T cell specific manner. Leptin overexpressing tumors have increased T cell infiltration compared to control tumors, and these TIL are metabolically and functionally superior. In order to translate out findings to a therapeutic setting we utilized an oncolytic virus model. Oncolytic viruses are an attractive therapeutic modality promoting tumor specific killing as well as inducing an anti-tumor immune response. While wild-type oncolytic Vaccinia resulted in some tumor regression, leptin-engineered Vaccinia had superior therapeutic efficacy inducing complete regressions in 30% of mice. TIL from these tumors have improved T cell infiltration and function. We profiled immune infiltrates by single cell RNAseq and TCR sequencing. Data revealed the influx of new T cells by vaccinia which was characterized by a polyclonal repertoire. On the other hand, T cells from tumors treated with leptin-expressing virus showed a reduced polyclonal phenotype indicative of specific clonal expansion. This clonal expansion is associated with a more memory like state, and indeed leptin-engineered VV induced a greater percentage of CD127hi memory precursors than the oncolytic VV alone.
Conclusions
Taken together, these data suggest metabolic modulators like leptin can be therapeutically exploited to bolster intratumoral T cell function using the oncolytic virus platform. Our goal is to further design novel therapeutic strategies using oncolytic viruses.

P617

A cell-based platform to protect and enhance oncolytic virus therapies

Antonio Santidrian, Dobrin Draganov, PhD, Duong Nguyen, PhD, Okyay Kilinc, Ivelina Minev, MSc, Boris Minev, MD, Aladar Szalay, PhD
Calidi Biotherapeutics
Correspondence: Antonio Santidrian (asantidrian@calidibio.com)
Journal for ImmunoTherapy of Cancer 2018, **6(Suppl 1):**P617

Background
Different types of viruses, including vaccinia virus (VACV), can selectively replicate in cancer cells and trigger antitumor immunity. Oncolytic virotherapies, as a monotherapy or in combination with other immunotherapeutics, have shown safety and exciting proof-of-concept results in pre-clinical studies as well as in different clinical trials. The therapeutic potential of oncolytic viruses, however, can be severely restricted by multiple innate and adaptive immune barriers that can be overcome using cell-based delivery approaches. Mesenchymal stem cells are particularly attractive carriers of oncolytic viruses due to their unique immunosuppressive properties allowing protection of the virus from complement/antibodies-mediated neutralization and to overcome antiviral cellular immunity in both autologous and allogeneic settings
Methods
As carriers of oncolytic VACV, we used cells a) freshly isolated from adipose tissue stromal vascular fraction (SVF), and b) SVF-derived cultured Adipose-Derived Mesenchymal Stromal/stem Cells (AD-MSC). We analyzed the ability of those carrier cells to take up, protect, amplify the virus as well as to overcome innate and adaptive immune barriers by flow cytometry, microscopy and virus plaque assays of ex vivo co-cultures of cells infected with VACV in the presence of human serum or peripheral blood mononuclear cells from healthy donors. Comparative analyses were performed to establish statistically significant correlations.
Results
We have demonstrated that autologous SVF cells did protect VACV against serum-inactivation. Cell sorting demonstrated that supra adventitial-adipose stromal cells (SA-ASC; CD235a-/CD45-/CD34+/CD146-/CD31-), and pericytes (CD235a-/CD45-/CD34-/CD146+/CD31-) were the two cell populations of SVF cells that were efficient facilitating the delivering of VACV to the tumor cells, validating their clinical use as a tool to potentiate oncolytic virus therapies in autologous settings. We further analyzed the potential of using cultured AD-MSC (derived from CD34+ SA-ASC) as a delivery vehicle in allogeneic settings. AD-MSCs demonstrated ability to protect against serum-inactivation as well as to amplify the virus in the presence of human PBMCs in both autologous and allogeneic settings. This activity can be linked to their intrinsic immunosuppressive properties and the evasion of allogeneic rejection. Moreover, these cells demonstrated ability to provide transient immunosuppression by inhibiting antiviral responses originating from both innate (NK)- and adaptive (T)-immune cells, thus augmenting viral oncolysis and the generation of anti-tumor immunity.
Conclusions
Overall, our findings indicate the feasibility to significantly potentiate oncolytic virotherapy by using either a simple autologous or a more scalable off-the-shelf allogeneic cell-based delivery technology allowing rational design of virus-based therapies that are not dramatically eliminated by immune barriers.
Ethics Approval
The study was approved by International Cell Surgical Society Ethics Board; IRB# ICSS-2016-024

P618

Selective delivery of exosome-mediated STING agonist to antigen presenting cells results in significantly improved potency and reduced toxicity

Su Chul Jang, PhD[1], Raymond Moniz[1], Christine Sia[1], Joyoti Dey, PhD, MPH[2], Rane Harrison[1], NIKKI ROSS, PhD[1], Ke Xu[1], Kevin Dooley[1], Nuruddeen Lewis[1], Christine McCoy[1], Agata Villiger-Oberbek[1], Scott Estes[1], Jorge Sanchez-Salazar[1], Kyriakos Economides[1], Sriram Sathyanarayanan[1]
[1]Codiak Biosciences, Cambridge, MA, USA; [2]Presage Biosciences, Seattle, WA, USA; [3]Codiak, Cambridge, MA, USA
Correspondence: Sriram Sathyanarayanan (sriram.sathy@codiakbio.com)
Journal for ImmunoTherapy of Cancer 2018, **6(Suppl 1):**P618

Background
Emerging research has established the role of exosomes as an efficient natural messenger system to deliver macromolecules between cells. We have leveraged this capacity to develop a novel, engineered exosome therapeutic, to selectively deliver agonists of the Stimulator of Interferon Gene (STING) pathway to tumor resident antigen presenting cells (APC).
Methods
ExoSTING is composed of exosomes, which are molecularly engineered to over-express an exosomal membrane glycoprotein, and which are loaded ex vivo with a STING agonist (SA).
Results
In vitro assays with human PBMCs showed ExoSTING enhanced the potency of dendritic cell and monocyte activation and IFN_beta