

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.

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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

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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.

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

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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

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

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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.

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Withdrawn

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

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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