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468 ENHANCERS AND REPRESSORS OF IMMUNOTHERAPY: TRANSLATIONAL PERSPECTIVES ON GENE-MEDIATED CYTOTOXIC IMMUNOTHERAPY IN GLIOBLASTOMA

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Background Gene-mediated cytotoxic immunotherapy (GMCI) is a local tumor immunotherapy that uses aglatimagene besadenovec (a non-replicating serotype 5 adenovirus, expressing HSV1 thymidine kinase) with the prodrug ganciclovir to induce DNA double strand breaks (DSB), leading to immunogenic tumor cell death and intratumoral immune cell invasion. Here we investigate potential repressors and enhancers of GMCI's effectiveness. GMCI is currently in clinical trials in combination with immune checkpoint blockade in glioblastoma. Thus we set out to identify potential areas to improve this approach for future application. Dexamethasone is used in symptomatic treatment of glioma patients, although it is known to cause immune suppression. However, the influence of dexamethasone on the efficacy of GMCI has not been explored. In contrast, DNA damage response inhibitors like the ATR inhibitor (ATRi) AZD6738 might not only amend the cytotoxic but also the immunogenic profile of GMCI, rendering it an attractive combination partner.
Methods We investigated the effects of ATR-inhibition and dexamethasone on GMCI in vitro using cytotoxicity, flow cytometry and T-cell-killing assays in glioblastoma cell lines. The impact of dexamethasone and ATRi in vivo was assessed in an orthotopic syngeneic murine glioblastoma model. Tumor immune infiltrates were analyzed with flow cytometry.
Results Cytotoxicity assays showed that dexamethasone has a slight impact on GMCI in vitro. In T-cell-functional assays, we observed a significantly impaired tumor cell killing. Immune cell response assays revealed a reduced immune cell proliferation after co-culture with supernatant from dexamethasone or combination treated glioblastoma cells. In vivo, while treatment with GMCI alone resulted in longer median symptom-free survival (39.5d) versus no treatment (23d), the combination of GMCI and dexamethasone resulted in the significant reduction of this effect (29d vs 39.5d ; p = 0.0184).
The combination of ATRi with GMCI proved to be synergistic in cytotoxicity assays. Flow cytometry revealed a significant increase in DSB-associated H2AX foci as well as an improved immune profile by downregulation of GMCI-induced PD-L1 expression. In vivo, the combination with ATRi led to an increase in long-term surviving animals (66.7%) compared to GMCI (50%) and proved to be highly significant compared to the untreated control (p=0.0022).
Conclusions Our data suggest that dexamethasone may decrease the efficacy of immunotherapy for glioma through impaired T cell function: this emphasizes the need in identifying alternatives to dexamethasone to prevent attenuated responses in immunotherapies. The combination of GMCI with ATRi however points to additional therapeutic benefit through enhanced cytotoxic efficacy, improved immunogenicity in vitro and increased long-term survival in vivo, making it a promising future approach for the treatment of glioblastoma.
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469 COOPERATION BETWEEN CHECKPOINT INHIBITORS TARGETING THE PD-1/PD-L1 AXIS AND ATRC-101, A NOVEL CLINICAL-STAGE CANDIDATE FOR THE TREATMENT OF SOLID TISSUE MALIGNANCIES

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Background We have previously described ATRC-101, a fully human, engineered IgG1 antibody binding a tumor-restricted ribonucleoprotein (RNP) complex as its target. ATRC-101 is currently under evaluation in the clinic as a monotherapy for solid tumors. Following target engagement, ATRC-101 functions in an Fc-mediated fashion to deliver the target to the innate immune system, which modifies the tumor microenvironment and generates an adaptive immune response involving CD8+ T cells leading to anti-tumor activity in syngeneic mouse models. Binding of ATRC-101 appears restricted to malignant tissues in both mouse models and human, across a range of cancer histologic phenotypes, including carcinomas that are known candidates for anti-PD-1 treatment. In the EMT6 mouse model, representing a T cell-excluded phenotype in which anti-PD-1 agents display limited activity, ATRC-101 monotherapy was uniformly vigorous with persistent anti-tumor memory. When co-administered at a lower dose with anti-PD-1, the combination of therapy demonstrated a robust and heightened anti-tumor response relative to either agent dosed as monotherapy at similar concentrations.
Methods To gain insight into the mechanisms that contribute to the anti-tumor effect with combination therapy, in vivo experiments in the EMT6 syngeneic mouse model were performed to determine temporal and spatial patterns of infiltrates and assessed tumors by using whole exome sequencing following administration of ATRC-101 vs. vehicle control. Within naive human tumor samples, coincident immunoreactivities of ATRC-101 and PD-L1 were also characterized.

Results In mice treated with ATRC-101, analysis by immunofluorescence revealed a significant increase in the percentage of PD-1 reactive T cells within the tumor microenvironment. Elevated transcripts for PD-L1 also were detected in tumors from mice administered ATRC-101 vs baseline levels or vehicle control. When human tumor tissues were characterized for coincident expression of these targets, a high prevalence of ATRC-101 immunoreactivity was noted in both PD-L1 reactive and non-reactive tumor cores. Across multiple indications, ATRC-101 immunoreactivity was apparent in > 50% of PD-L1+ cores.

Conclusions In situ studies suggest the target of ATRC-101 may co-locate with PD-L1, and in vivo studies indicate that ATRC-101 administration increases PD-L1 transcripts and PD-1-positive infiltrates in mouse tumor. Altogether, our data support studies to combine ATRC-101 with agents targeting PD-1 in the clinical treatment of solid tissue malignancies.

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Trial Registration NCT04244552

Ethics Approval The study was approved by WIRB (Western Institutional Review Board) on Jun 11, 2013. The WIRB study number is 20130121.

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470 TARGETING PAN-TUMOR ASSOCIATED ANTIGEN B7H3 VIA COMBINATION OF TRI-SPECIFIC KILLER ENGAGER AND OFF-THE-SHELF NK CELL THERAPY ENHANCES SPECIFICITY AND FUNCTION AGAINST A BROAD RANGE OF SOLID TUMORS

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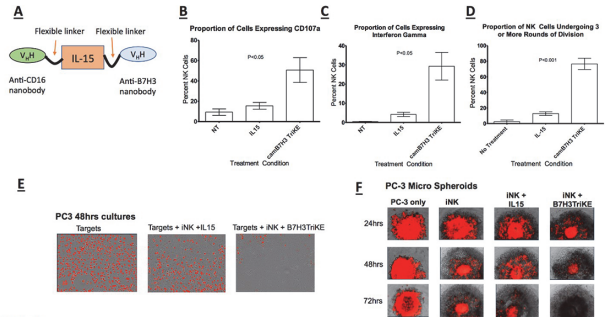
Background B7H3 is a tumor associated antigen (TAA), found on numerous malignancies including prostate, lung, and breast cancers. High levels of B7H3 expression are correlated with late stage disease and poor prognosis. Furthermore, B7H3 is minimally expressed on normal tissue, making it an ideal TAA for broad cancer treatment strategy. We developed a tri-specific killer engager (TriKETM) consisting of a nanobody anti-CD16, IL-15, and nanobody anti-B7H3 joined by flexible linkers (camB7H3 TriKE) (figure 1A). The combination of B7H3 TriKE with an off-the-shelf NK cell therapy presents an appealing therapeutic strategy for the treatment of solid tumors with decreased risk of toxicity in allogeneic settings compared to T-cell derived products.

Methods An anti-B7H3 nanobody was developed via biopanning and cloned into a TriKE vector. TriKE was produced in Expi293 cells and affinity purified using poly-His tag. NK cells were co-incubated with cell lines exhibiting a range of B7H3 expression and with 3nM of camelid B7H3 TriKE or control. We have previously derived NK cells expressing high affinity non-cleavable hnCD16, CD38 KO, and IL-15/IL-15R fusion from clonal master engineered iPSC lines. Engineered iNK cells were tested in conjunction with the TriKE. A repeated measures ANOVA was used for statistical comparisons as noted in figure legends

Results Engineered iNK cells co-incubated with camB7H3 TriKE and C4-2 prostate cells significantly increased degranulation (CD107a) and cytokine production (IFN-gamma) compared to controls (figure 1B/C, $P<0.05$, $n=3$). camB7H3 TriKE directly bound C4-2 cells with an estimated EC50 of approximately 3nM. camB7H3 TriKE increased percentages of engineered iNK cells dividing robustly (3 or more times) compared to corresponding IL-15 doses at 3 nM (figure 1D, $P<0.001$, $n=3$). Furthermore, camB7H3 TriKE enhanced cytotoxic activity of engineered iNK cells against a variety of tumor cells in 2D and spheroid format independent of cytokine support (figure 1E-F). Engineered iNK cells incorporating an anti-B7H3 chimeric antigen receptor (CAR) is also being developed and will be discussed.

Conclusions camB7H3 TriKE dramatically increases function and activation on endogenous NK cells as well as engineered iNK cell, which can be adoptively transferred to patients with a broad range of cancers, including prostate cancer. TriKE



Abstract 470 Figure 1 A) Schematic of TriKE molecule demonstrating spatial relationship of anti-CD16 nanobody, IL-15, and anti-B7H3 nanobody with flexible linker regions. B) Percent of PB NK cells CD107a as a marker of NK degranulation. Unselected PBMCs were stimulated with 3nM camB7H3 TriKE, 3nM IL-15, or no treatment with B7H3-expressing C4-2 prostate cancer cell lines Cells were stimulated for 5 hours and evaluated for degranulation (surface CD107a) by flow cytometry, gating on the NK (CD56+CD3-) population (displayed). Graphs display mean ± SEM. $P<0.05$ using repeated measures ANOVA. C) Percent of PB NK cells expressing intracellular interferon-gamma. Unselected PBMCs were stimulated with 3nM camB7H3 TriKE, 3nM IL-15, or no treatment with B7H3-expressing C4-2 prostate cancer cell lines Cell were stimulated for 5 hours and evaluated for degranulation (surface CD107a) by flow cytometry, gating on the NK (CD56+CD3-) population (displayed). Graphs display mean ± SEM. $P<0.05$ using repeated measures ANOVA. D) Percent of PB NK cells dividing robustly (3 or more times) over a 7 day stimulation with 3nM camB7H3 TriKE, 3nM IL-15, or no treatment. PBMCs were incubated with Cell-Trace Violet reagent prior to stimulation. Graphs display mean ± SEM. $P<0.001$ using repeated measures ANOVA. E) Representative 2D IncuCyte images of PC3 prostate cancer cell lines transduced with NucLight Red. Cells were co-incubated with iNK alone, iNK with 3nM IL-15 or iNK with 3 nM antiB7H3 TriKE. Images represent remaining NucLight Red transduced PC3 cells after 72 hours of co-incubation as noted above. F) Representative microspheriod IncuCyte images of PC3 prostate cancer lines transduced with NucLight Red. Cells were co-incubated with iNK cells alone, iNK with 3 nM IL-15 or iNK with 3 nM antiB7H3 TriKE. Images represent remaining NucLight Red transduced PC3 cells after 72 hours of co-incubation as noted above