Abstract 465 Figure 3 Agonistic CD40 treatment improves RT+CTLA-
4 therapy. Individual tumor growth curves for untreated, RT+CTLA-4, or
RT+CTLA-4+CD40 treated mice. Color annotate group. *, **, ***, and
**** indicate p-values < 0.05, 0.01, 0.001, and 0.0001, respectively,
calculated using a linear mixed-effects model. (A) and (B) represent two
individual experiments. (Abbreviations) RT, radiation therapy; CTLA4,
radiation therapy; CTLA4, Ab; CD40, anti-CD40 Ab; mm³, cubic millimeter; d, days

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Abstract 465 Figure 2 RT+CTLA-4i increased tumor infiltration by
Gzm+b+Prf1+Lag3+Pd1+ Cd8+, Ifng+Cd40lig+Cd4+, and Ifng+Tnf+Cd8+
T cells in 4T1 tumors. (A) Design of experiment enabling single cell
analysis of T cells infiltrating 4T1 tumors. (B) Based on gene expression
levels, the T cells were divided into 17 clusters (indicated by colors)
and visualized in 2D using UMAP dimensionality reduction algorithm.
(C) Gene expression levels of selected high-level T cell markers. (D) Table
with main phenotype, key genes representative for each cluster, and the
distribution of T cells from each condition falling into the different
clusters. (E) Proportion of Cd4+ and Cd8+ T cells for the different
treatment groups. (F) The expression of cluster-specific gene signatures in
bulk 4T1 tumors for clusters 0, 2, and 4. (G) Survival curves and (H)
multivariate analysis of the association between survival and enrichment
of the gene signatures of clusters 0, 2, and 4. (I) The positioning of the all
AH1-dextramer+ Cd8+ T cell clones within the UMAP plot. Color
annotate clusters. (Abbreviations) tx, treatment; RT, radiation therapy;
Untr., untreated; CTLA4, CTLA-4 Ab; CD40, anti-CD40 Ab; mm³, cubic millimeter; d, days

survival in patients. Unexpectedly, inhibition of checkpoint
receptors expressed by a large CD8 T cells cluster did not fur-
ther improve responses to RT+CTLA4i, whereas agonistic
CD40 therapy did, suggesting new therapeutic strategies.

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Results A 78 year old women with significant cardiac disease and a St Jude tissue aortic valve, had undergone prior surgeries and radiation therapy for her recurring SCC of the face followed then by major resection, parotidectomy, flap reconstruction, and supraomohioid neck dissection; only two weeks after the latter surgery, she presented with over 20 new in-radiation field metastases (see photo below). A 90 year old woman with emphysma on home oxygen and living in a facility presented with diffuse local recurrence 4 months after orbital exenteration, parotidectomy, neck dissection, and flap. Both patients' tumors were characterized: PDL1 (clone E1L3N) 2% and 10%, respectively; scant peritumoral or intratumoral lymphocytes; tumor mutation burden high (33 and 30 mutations per megabase, respectively); epidermal growth factor receptor (EGFR) high 3+ by IHC, but with no gene mutations detected in EGFR, kras or nras; microsatellite stable. In the 78 yo woman, after two cycles of pembrolizumb, the ~ 5 mm pink nodules grew further to up to 3 cm with facial erythema, edema, scaling the eye closed. Only by criteria this was not considered pseudoprogression. Panitumumab was integrated between cycles 2 and 3, resulting in a dramatic abrupt response: the masses became centrally necrotic, flaking, pouring off her face with prompt resolution in edema and complete response (CR) within 2 months - now lasting over 18 months - a period during which pembrolizumab and panitumumab were continued for 27 and 26 cycles respectively. Her major toxicity was diffuse erythema involving ~ 30% of her torso; this resolved early on with triamcinolone 0.1% cream. She also developed scabs in her uninvolved scalp - some where other squamous and basal carcinomas had previously been resected and these all healed slowly (see photo), suggesting we were preventing similar future cancers from emerging in these areas. Similarly, the 90 yo woman achieved only a mixed response to nivolumab over 3 months with shrinking level V neck node but continued stubborn diffuse disease over her face and into the exenteration field. When panitumumab was added, however, there was clear improvement (See photo). With each of eight cycles, prolific crusting/scabbing would occur, shed, and reoccur, some in areas of the face without visible tumor, Mild acniform rash and mild hypomagnesemia were readily managed. Her performance status and appetite improved and she gained back 14 pounds. After only 6 months, with pathologically confirmed CR, treatment had to be held because she was restricted to her assisted living facility in the midst of the COVID-19 pandemic. Now after a year, the remaining scabs are largely gone (see photo).

Conclusions The excellent tolerance of multiple cycles of outpatient combination treatment in these two consecutive patients with the same diagnosis, coupled with the observed durable anti-tumor clinical activity lasting now over a year - all support further exploration of panitumumab in combination with anti-PD-1 antibody treatment. A randomized trial would be needed to establish whether outcomes are truly better with the combination. Deciding on hyperprogression versus pseudoprogression while getting anti-PD-1 antibody treatment remains a challenge. Laboratory studies would evaluate how such specific signal transduction inhibition by panitumumab might interfere with immune suppressive mechanisms in metastases, rendering them more sensitive to an induced anti-tumor cellular immune response by an anti-PD-1 antibody. Finally such combination treatment should help reduce the need for increasingly cosmetically and functionally altering surgeries.

Ethics Approval 'Per our Hartford Health Care IRB, case series of three or less patients does not constitute research.' Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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Abstract 467 Figure 1 Panitumumab + pembrolizumab for metastatic cutaneous SCC #1
Dramatic durable response in 22 metastases on face and also scabbing then healing on scalp where there was no evidence of tumor but history of prior resected squamous cell and basal cell carcinomas, suggesting effective prevention of such lesions
Abstracts


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 agalitamine besadenovec (a non-replicating serotype 5 adenovirus, expressing HSV-1 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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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.

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