improved the effector properties of T cells via decreasing expression levels of co-inhibitory molecules and decreasing frequency of regulatory T cells (figure 6). Clinically, VIPR1 receptor expression, but not VIP, provides a survival benefit (figure 7).

Conclusions VIP is a targetable mechanism of immune escape in PDAC. Inhibiting VIP receptor signaling improves effector properties of T cells and synergistically improves the anti-tumor response to checkpoint inhibitors in mouse PDAC models.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin* 2020;**70**(1):7-30.
2. Sahin IH, et al. Immunotherapy in pancreatic ductal adenocarcinoma: an emerging entity? *Ann Oncol* 2017;**28**(12):2950-2961.
3. Gonzalez-Rey E, Anderson P, Delgado M. Emerging roles of vasoactive intestinal peptide: a new approach for autoimmune therapy. *Ann Rheum Dis* 2007;**66**(Suppl 3):iii70-6.
4. Anderson P, Gonzalez-Rey E. Vasoactive intestinal peptide induces cell cycle arrest and regulatory functions in human T cells at multiple levels. *Mol Cell Biol* 2010;**30**(10):2537-51.

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MCLA-145 IS A BISPECIFIC IGG1 ANTIBODY THAT INHIBITS PD-1/PD-L1 SIGNALING WHILE SIMULTANEOUSLY ACTIVATING CD137 SIGNALING ON T CELLS

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Background MCLA-145 is a CD137 x PD-L1 bispecific antibody that releases PD-L1 mediated T-cell inhibition and activates and expands T cells through agonism of CD137. Immune checkpoint inhibitors (ICI) against PD-(L)1 have demonstrated anti-tumor efficacy in a fraction of patients across a broad range of cancers. CD137 (4-1BB, tumor necrosis factor receptor superfamily 9) is an inducible costimulatory receptor transiently expressed on T cells after TCR engagement. CD137 signaling is triggered by receptor clustering and leads to enhanced cytokine production; T cell proliferation, survival, and effector function; and immunological memory formation. Targeting of PD-L1 and CD137 with MCLA-145 may achieve synergistic activity by simultaneously blocking the inhibitory checkpoint PD-L1 and activating tumor specific T cells through co-stimulation.

Methods We performed combinatorial functional screening of bispecific antibodies generated from high affinity inhibitory Fabs binding PD-L1 combined with a large and diverse panel of agonistic CD137 Fabs.

Results MCLA-145 was selected based on its in vitro potency in multiple primary human immune cell assays. Further, it displays an ability to reverse T cell suppression mediated by M2 macrophages or Tregs. MCLA-145 binds to a unique epitope in the cysteine rich domain 2 of CD137 that overlaps with the CD137L binding region, and all potent bAbs in the screen were able to bind to this region. MCLA-145 drives activation of CD137 and the degree of CD137 agonistic activity in T

cells correlated with the expression level of PD-L1 on neighboring cells. Using proximity ligation assays and confocal microscopy we demonstrated that MCLA-145 clusters CD137 on the surface of T cells resulting in internalization. The binding location of MCLA-145 on CD137 may be optimal for the formation of 'immunological synapses' with PD-L1 expressing antigen presenting cells or tumors resulting in the potent activation of tumor specific cytotoxic T cells.

Conclusions These experiments demonstrate the dual anti-cancer activity of MCLA-145 in preclinical models: release of T-cell checkpoint inhibition through PD-L1; and activation and expansion of T cells through CD137, therefore overcoming T-cell exhaustion and increasing T-cell presence/activity (infiltration) in tumors. MCLA-145 is currently undergoing clinical development in an ongoing trial (NCT03922204).

Ethics Approval Animal experiments were performed according to guidelines for animal care of the local Animal Experiments Committee; Use of human blood cells from healthy volunteers was approved by the blood bank's Ethical Advisory Council.

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CLASS SPECIFIC HDAC INHIBITION DIFFERENTIALLY AFFECTS THE FUNCTION OF SPECIFIC T CELL SUBSETS

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Background Histone deacetylase inhibitors (HDACi) are currently being used in the clinic to treat a variety of cancer types. Despite their wide use, the mechanism by which they exert anti-tumor effects is largely unknown. Although originally posited to abrogate tumor proliferation via regulating tumor suppressor genes, responses to monotherapies of HDACi have been shown to be dependent on an adaptive immune system and to enhance responses to immunotherapy. However, whether this mechanism is driven by enhancing tumor immunogenicity or enhancing anti-tumor immune responses is unclear. Understanding this could help identify optimal combination regimens for augmenting immunotherapies. Given the role of epigenetics in regulating T cell differentiation upon antigen encounter into discrete subsets, these studies sought to determine whether HDACi differentially impact naïve from memory T cell subsets.

Methods PBMCs from healthy donors were stimulated with either anti-CD3/anti-CD28 or PMA/Ionomycin in the presence or absence of different HDAC inhibitors (OKI-005, 250 nM; Entinostat, 5 uM; and Vorinostat, 1 uM). Cells were evaluated at different time points by flow cytometric analysis to compare responses by T cell subsets for changes in cytokine production, protein acetylation and other functional responses. Supernatant was collected in separate experiments for comprehensive cytokine bead arrays.

Results Cytokine analysis of supernatants showed clear differences in response to HDACi as while most cytokines decreased, others were either unaffected or increased. We next performed ICS with surface markers to determine if these changes in cytokine production levels were subset specific. Comparisons of naïve and memory subsets found decreased IL-2 levels was primarily attributed to loss of production by naïve T cells. Furthermore, gain of TNFα was almost completely restricted to naïve cells. The preferential responses by naïve T cells was further verified during global changes in acetylated protein levels. Lastly, we found differences between

inhibitors on their effects on T cells. As these differences remained even after controlling for potency, this suggests the specificity profiles toward individual HDACs was responsible for their unique effects.

Conclusions These studies demonstrate clear differences in the effect of HDACi on cytokine production by distinct T cell subsets. Ongoing studies are aimed at elucidating the specific HDACs responsible for regulating T cell effector functions and tumor immunogenicity when targeted. Ultimately, understanding this could help identify inhibitors with the desired specificity profile for combining with immunotherapy.

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LOCAL RADIOTHERAPY SYNERGIZES WITH TUMOR-SPECIFIC TCR REDIRECTED T CELLS IN THE REJECTION OF PROSTATE CANCER

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Background Adoptive T cell therapy (ACT) has become a promising option for cancer patients. While tumor-infiltrating lymphocytes were initially exploited as a source of tumor reactive lymphocytes, T cells genetically redirected to the tumor by TCR/CAR gene transfer are now in clinical validation. In the case of solid tumors, unfavorable immunosuppressive microenvironments remain recognized barriers to therapeutic efficacy. We have recently reported that the therapeutic activity of ACT against poorly immunogenic and indolent prostate cancer is improved by the concurrent targeting of the tumor stroma by mean of T cells redirected to an ubiquitously expressed minor histocompatibility antigen or a tumor vessel targeted TNF derivative. We have now taken the concept further and hypothesized that local radiotherapy (RT), might also synergize with ACT by promoting lymphocyte endothelial transmigration and tumor recognition, and ultimately favor abscopal effects.

Methods We investigated the combination of local RT and ACT in TRAMP (Transgenic Adenocarcinoma of the Mouse Prostate) mice and in mice bearing subcutaneous B16/B16-OVA (MO4) or TRAMP-C2/TRAMP-C2-OVA tumors. Local RT was delivered by X-RAD SmART (the Small Animal Radiation Therapy) microirradiator in single dose or hypo-fractionated regimens. ACT consisted of T cells engineered with tumor-specific TCRs. Immunogenic consequences were analyzed by Real-Time PCR, and flow cytometry (FACS) analyses. Prostate tumor debulking was evaluated by histological analyses.

Results We found that local hypofractionated RT and ACT, while individually inefficacious in controlling tumor growth, concurred to the debulking of advanced prostate adenocarcinoma when used in combination in treating TRAMP mice. Mechanistically, exposing isolated tumor cells, or the TRAMP mouse prostate to hypo-fractionated RT regimens induced stronger type-I interferon (IFN-I) responses, when compared to single high dose. Acutely, hypofractionated RT promoted better immune tumor infiltration, among which TCR redirected effector cells.

Conclusions Data support feasibility and efficacy of combining hypo-fractionated local RT with ACT in the form of TCR

engineered T cells to promote prostate cancer recognition and eradication. Tumor debulking was observed in the absence of treatment-related toxicity. Systemic recirculation of TCR redirected T cells was observed. We are now investigating therapeutic effects at distal (metastatic) sites.

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Ethics Approval The studies involving animals were approved by The Institutional Ethical Committee (IACUC#999).

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CD4 T CELLS ARE ESSENTIAL FOR AN ANTI-TUMOR EFFECT IN A B78 MURINE MELANOMA TUMOR MODEL

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Background Mice bearing B78 melanoma tumors can be cured using an in situ vaccine (ISV) regimen that includes radiation (RT) together with immunocytokine (tumor-targeting mAb conjugated to IL-2). B78 melanoma cells, derived from B16 cells, express minimal to no MHC-I but express MHC-II upon IFN- γ /TNF- α stimulation. Although B78 cells are primarily MHC-I deficient, an increased CD8 T cell infiltration into the tumor microenvironment (TME) has been shown following ISV.¹ To further investigate the potential role of specific immune cell lineages in the B78 anti-tumor response to ISV, immune subset depletion studies and flow cytometric analyses were performed.

Methods C57BL/6 mice bearing B78 tumors were depleted of immune cell subsets with mAbs (anti-CD4, anti-CD8, anti-NK1.1, or Rat IgG control) for 3 weeks during the course of treatment. Treatment groups included no treatment, RT (12 Gy), or ISV (RT D0 and immunocytokine D5-D9). 6 mice/group (repeated three times) were followed for survival/tumor growth, and flow cytometry studies included 4 mice/group, sacrificed on D8 and D13 following the start of ISV.

Results Mice depleted of CD4 T cells during the course of ISV showed a significant reduction of anti-tumor effect as compared to mice treated with ISV/Rat IgG (p

Conclusions These studies suggest that CD4 T cells are essential for an anti-tumor response in the B78 melanoma model. In vivo depletion data show that CD4 T cells, but not CD8 or NK cells, are required for a decrease in tumor growth via ISV. Flow cytometric analyses suggest an interplay between CD4 and CD8 T cells as indicated by a decrease in CD8/IFN- γ expression following ISV in the absence of CD4 T cells. The role that MHC-I and MHC-II expression plays in this CD4/CD8 T cell anti-tumor response is under investigation. In future studies, B78 melanoma may serve as a critical syngeneic model for development of more effective immunotherapy treatment regimens.

Ethics Approval All animal experiments were performed in accordance with protocols approved by Animal Care and Use Committees of the University of Wisconsin-Madison.

REFERENCE

1. Morris Z, Guy E, Francis D, et al. In situ tumor vaccination by combining local radiation and tumor-specific antibody or immunocytokine treatments. *Cancer Res* 2016;**76**(13):3929-3941.

<http://dx.doi.org/10.1136/jitc-2020-SITC2020.0823>