both adaptive and innate immune responses. As a clinical correlate, a retrospective analysis of 181 consecutive IDH-wild-type glioblastoma patients treated with PD-(L)1 blockade revealed poorer survival among those on baseline dexamethasone. Upon multivariable adjustment by relevant prognostic factors, baseline dexamethasone administration was the strongest predictor of poor survival, regardless of dose (referent no dexamethasone; <2 mg HR 2.16, 95%CI: 1.30–3.68, p=0.003; ≥2 mg HR 1.97, 95%CI: 1.23–3.16, p=0.005; table 1 and figure S). Conclusions We demonstrate that concurrent dexamethasone administration, even at a low dose, limits the therapeutic benefit of anti-PD-1 therapy both in mouse glioblastoma models and in a retrospective cohort of 181 IDH-wildtype glioblastoma patients. Mechanistically, dexamethasone decreased intratumoral T cells and systemic levels of T cells, natural killer cells, and myeloid cells, while qualitatively impairing lymphocyte function. The mechanism of T cell depletion included induction of apoptosis. These findings indicate that dexamethasone hinders both adaptive and innate immune responses, intratumorally and systemically, and that its administration should be carefully assessed among glioblastoma patients undergoing second-generation immunotherapy clinical trials. Our findings also have ramifications for brain metastasis patients where immune checkpoint inhibitors are part of standard-of-care management. Acknowledgements We thank Min Wu for assistance in generating CT-2A luciferase-transduced cells, and Drs. Geoffrey Young, Lei Qin, Xin Chen, and Jing Li for assistance in evaluation of patients’ radiographic imaging. Ethics Approval Approved under DFCI Institutional Review Board protocol 10-417. http://dx.doi.org/10.1136/jitc-2020-SITC2020.0209

REGULATION OF TIM-3 BY PHOSPHATIDYLSERINE

Courtney Smith*, Alice Li, Nithya Krishnamurthy, Mark Lemmon. Yale University, West Haven, CT, USA

Background Immune checkpoint blockade has proven effective in targeting exhausted T-cells to reactivate the immune system against cancer. However, the majority of patients fail to respond to currently available therapies, which primarily target PD-1. Thus, a key challenge for checkpoint blockade therapy is to identify and understand new therapeutic targets. Another immune checkpoint receptor is TIM-3, which – like PD-1 – is expressed on exhausted T-cells in the tumor microenvironment.1,2 TIM-3 belongs to a family of phosphatidylserine (PS) receptors, including TIM-1 and TIM-4, which have well-documented roles in the engulfment of apoptotic cells by phagocytes.3 However, the role of PS in regulating TIM-3 function is less clear. We therefore investigated how TIM-3 modulates T-cell signaling and how PS influences TIM-3 activity, with the ultimate goal of improving the translation of candidate TIM-3 therapies to the clinic. Methods Surface plasmon resonance (SPR) was used to quantify the interaction between human TIM-3 and PS. A Jurkat T-cell model was used to investigate the role of TIM-3 in T-cell receptor (TCR) signaling and to determine the role of PS in regulating TIM-3 function. Results TIM-3 bound PS-containing membranes with low micromolar affinity in vitro. In the Jurkat cell model system, high – but not low – surface levels of TIM-3 promoted T-cell signaling, suggesting a threshold of receptor expression needed to modulate T-cell signaling, similar to what has recently been reported for PD-1.4 However, chimeric receptors that maintained the TIM-3 cytoplasmic tail but were unable to bind PS failed to enhance T-cell signaling like the full-length TIM-3 receptor. Cells expressing mutant TIM-3, which displayed reduced PS binding as quantified by SPR, also displayed reduced T-cell signaling compared to cells expressing wild-type TIM-3. Importantly, treatment of TIM-3-expressing cells with a functional TIM-3 antibody that blocks PS binding also reduced T-cell signaling compared with untreated TIM-3-expressing cells. Conclusions Our results support a role for PS as a ligand capable of modulating TIM-3 activity. Using chimeric receptors, TIM-3 mutants, changes in receptor expression, and a functional TIM-3 antibody, we show that preventing the interaction between TIM-3 and PS blocks TIM-3 activity. These data suggest that blocking the PS-TIM-3 interaction is a key mechanism for functional antibodies targeting TIM-3. Ultimately, this work supports the development and use of clinical antibodies that block the interaction of TIM-3 with PS and provides new mechanistic insight into how TIM-3 modulates TCR signaling. Acknowledgements This work was supported by the PhRMA Foundation Pre-Doctoral Fellowship in Pharmacology/Toxicology.

REFERENCES

TARGETING BTN2A1 MODULATES ANTI-TUMOR ACTIVITY OF VG9VD2 T CELLS

1Carla Cano*, 1Aude de Gastass, 1Christine Pasero, 1Marie Fullana, 1Emile Granarolo, 1HOET Rene, 1Caroline Imbert, 1Laurent Gorvel, 2Antoine Briantais, 2Anne-Charlotte Le Floch, 2Daniel Olive, 2ImCheck Therapeutics, Marseille, France; 2CRCM, Marseille, France

Background Vg9Vd2 T constitute the predominant subset among gd T cells in peripheral blood. Their infiltration into malignant tissues is associated with a favorable prognosis. Their anti-tumor activity is triggered by intracellular accumulation of organic phosphoantigens (pAgs) due to tumorigenesis. Recently, BTN2A1 was shown to bind to the Vg9TCHR chain allowing immune synapse between cancer and Vg9Vd2T cells, thus initiating the anti-tumoral response. In this study, we generated monoclonal antibodies against BTN2A1 and evaluated their ability to modulate γδ T cell cytotoxicity. Methods Anti-BTN2A1 mAbs were generated by mouse immunization. Their effect on Vg9Vd2 T cell degranulation, secretion of IFNγ/TNFα, and target cell killing as depicted by caspase 3/7 cleavage, were tested in co-cultures with Daudi, HL-60 cell lines and primary acute myelocytic leukemia (AML) blasts with or without zoldebradon or the anti-BTN3A