both adaptive and innate immune responses. As a clinical cor-
relate, a retrospective analysis of 181 consecutive IDH-wild-
type glioblastoma patients treated with PD-(L)1 blockade
revealed poorer survival among those on baseline dexametha-
sone. Upon multivariable adjustment by relevant prognostic
factors, baseline dexamethasone administration was the stron-
gest 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 5).

Conclusions We demonstrate that concurrent dexamethasone
administration, even at a low dose, limits the therapeutic ben-
efit of anti-PD-1 therapy both in mouse glioblastoma models
and in a retrospective cohort of 181 IDH-wildtype glioblas-
toma patients. Mechanistically, dexamethasone decreased intru-
tumoral T cells and systemic levels of T cells, natural killer
cells, and myeloid cells, while qualitatively impairing lympho-
cyte function. The mechanism of T cell depletion included
induction of apoptosis. These findings indicate that dexam-
ethasone 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 gener-
ating CT-2A luciferase-transduced cells, and Drs. Geoffrey
Young, Lei Qin, Xin Chen, and Jing Li for assistance in evalua-
tion 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

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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 microenvi-
ronment.\(^1\) -\(^2\) 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 can-
didate TIM-3 therapies to the clinic.

Methods Surface plasmon resonance (SPR) was used to quanti-
tify 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 main-
tained 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 recep-
tors, TIM-3 mutants, changes in receptor expression, and a
functional TIM-3 antibody, we show that preventing the inter-
action 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. Ulti-
ately, 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.

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http://dx.doi.org/10.1136/jitc-2020-SITC2020.0210

TARGETING BTN2A1 MODULATES ANTI-TUMOR
ACTIVITY OF VG9VD2 T CELLS

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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 accumula-
tion of organic phosphoantigens (pAgS) due to tumorigenesis. Recently, BTN2A1 was shown to bind to the Vg9TCR chain
allowing immune synapse between cancer and Vg9Vd2 T cells, thus initiating the anti-tumoral response. In this study, we
generated monoclonal antibodies against BTN2A1 and evaluated their ability to modulate γδT cell cytotoxicity.

Methods Anti-BTN2A1 mAbs were generated by mouse immu-
nization. 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 zoledronate or the anti-BTN3A
a
CLEC-1 IS A NOVEL MYELOID IMMUNE CHECKPOINT FOR CANCER IMMUNOTHERAPY LIMITING TUMOR CELLS PHAGOCYTOSIS AND SYNERGIZING WITH TUMOR-TARGETED ANTIBODIES

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Background Myeloid cells represent one of the most abundant immune cell types in solid tumors that impede myeloid phagocytosis by triggering ‘don’t eat me’ and ‘don’t find me’ signals. Recent literature demonstrates that C-type lectin receptors (CLRs) normally constrian immune cell-mediated tissue damage by suppressing myeloid cell activation and then promote tumor immune evasion. We previously identified the orphan (CLRs) CLEC-1 as over-expressed in situation of established immune tolerance and reported that CLEC-1 expression by dendritic cells (DCs) and macrophages is enhanced by TGFB and tempers downstream T cells responses. Furthermore, we reported that CLEC-1 is highly expressed by myeloid cells purified from human tumor micro-environment significantly more expressed by suppressive macrophages.

Methods As DCs and macrophages are professional phagocytes of dying/dead cell, we evaluated whether CLEC-1 could be a receptor of damaged cells in the phagocytosis.

Results We found that CLEC-1 fusion protein, binds specifically to late apoptotic and secondary necrotic healthy or tumor cells induced by chemotherapy, radiation (UV, X-ray) or culture stress conditions. Importantly, we observed in vivo that CLEC-1 deficient mice, but not wild-type, eradicare MC38 colorectal tumors in combination with cytotoxic and immunogenic chemotherapy (eg. Cyclophosphamide). We then generated, screened and identified different anti-human Clec-1 antagonist monoclonal antibodies (mAbs) with the capacity to block the CLEC-1/CLEC-1L interaction. We discovered that various antagonist CLEC-1 mAbs, but not non-antagonist CLEC-1 control mAbs, increase the phagocytosis of CLEC-1L-positive human tumor cells by human CLEC-1 expressing NSCLC cells (A549) when CLEC-1 is antagonized by antibodies. Furthermore, macrophages more productively engulfed Rituximab (anti-CD20 mAb)-opsonized Burkitt lymphoma cells (Raji) as well as bare NSCLC cells (A549) where CLEC-1 is antagonized by antibodies. These data indicate illustrate that CLEC-1 broadly inhibits tumor-cell phagocytosis and synergized with tumor-targeted cytotoxic monoclonal antibodies in both solid and hematological tumors.

Abstracts

USE OF A NOVEL PEPTIDE LIGAND TARGETING MULTIPLE IMMUNE CHECKPOINTS: A NOVEL APPROACH TO IMMUNOTHERAPY AGAINST CENTRAL NERVOUS SYSTEM TUMORS

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Background Cancer immunotherapy has revolutionized clinical management of malignancies by generating long-term, durable control of tumors, rendering more manageable diseases that previously had dismal prognoses. Unfortunately, these therapies often enhance autoimmunity, causing serious immune-related adverse events. In addition, little efficacy is noted in CNS tumors. Our research is focused on the CD200 immune checkpoint, which modulates the immune system through the inhibitory receptor (CD200R1) and activation receptors (CD200AR). We have demonstrated that targeting the CD200AR with a peptide ligand (CD200AR-L) activates the immune system, rendering it impervious to the inhibitory effects of CD200. In a clinical trial studying canine spontaneous high-grade glioma, CD200AR-L administered with tumor autologous tumor lysate resulted in a 20% two-year progression-free survival. No adverse effects were observed. We suggest this result is due to the ability of the CD200AR-L to modulate multiple immune checkpoints. During the characterization of the CD200AR-L, we discovered that signaling molecules are shared by CD200 and PD-1/PD-L1, suggesting these important immune checkpoints are interconnected.

Methods CD200R1KO macrophages were used to determine the connection between the CD200 and PD-1 checkpoints.