both adaptive and innate immune responses. As a clinical correlate, a retrospective analysis of 181 consecutive IDH-wild-type glioblastoma patients treated with PD-L1 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 5).

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.

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210 REGULATION OF TIM-3 BY PHOSPHATIDYLSTERINE
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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 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.1 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-1.1 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.

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211 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 accumulation of organic phosphoantigens (pAgs) due to tumorigenesis. Recently, BTN2A1 was shown to bind to the Vg9TCHR 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 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 zoleodronate or the anti-BTN3A

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mAb 20.1. These readouts were measured by flow cytometry. Endometrial cancer spheroids were used to assess the ability of the anti-BTN2A1 antagonistic mAb to inhibit Vg9Vd2 T cell killing of cancers cells.

**Results** We generated 7 anti-BTN2A1 mAbs and tested their effect on Vg9Vd2 T cell degranulation against Daudi cells with or without zoledronate. Six out of 7 anti-BTN2A1 mAbs significantly inhibited basal Vg9Vd2 T cell degranulation against Daudi up to 17-fold, and 5 of them were able to inhibit Vg9Vd2 T cell degranulation against Daudi in presence of zoledronate. Consistently, anti-BTN2A1 mAbs abrogated zoledronate and anti-BTN3A 20.1-induced apoptosis with different efficiencies. The level of apoptosis inhibition after zoledronate and 20.1 treatment were correlated. Anti-BTN2A1 7.48 mAb was the clone with the highest inhibitory potential. Increasing concentrations of 7.48 abrogated not only Vg9Vd2 T cell degranulation (IC50= 0.033±0.0003 μg/mL) but also TNFα (IC50= 0.03±0.006 μg/mL) and IFNγ (IC50= 0.015±0.004 μg/mL) secretion against Daudi cells in presence of pAgS. The ability of anti-BTN2A1 antibodies to inhibit Vg9Vd2 induced tumor cell apoptosis was also shown in 3D endometrial cancer spheroids. In co-cultures of Vg9Vd2 T cells with primary AML blasts, the anti-BTN2A1 7.48 inhibited Vg9Vd2 T cell degranulation as well as TNFα, IFNγ production and killing of AML blasts.

**Conclusions** Antagonist antibodies to BTN2A1 highlighted its critical role in Vg9Vd2 anti-tumor responses. BTN2A1 is involved in Vg9Vd2 T cell anti-tumoral activity and can constitute an interesting therapeutic target for gfoT cell response immunomodulation in cancer or immune diseases treatment.

**Ethics Approval** The research was approved by the relevant institutional review boards (ethic committee and ANSM, HEMATO-BIO IPC 2013-015, Ref ANSM 131368B-11, Sponsor Institut Paoli Calmettes N° ID RCB 2013-A01437-38).

Consent Informed consent was obtained from all donors in accordance with the 121 Declaration of Helsinki.

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**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 constrain 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, eradicates MC38 colorectal tumors in combination with cytotoxic and immunogenic chemotherapy (eg. Cyclophosphamide). We then generated, screened and identified different anti-human Clec-1 antigen 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 TGFβ-polarized DCs or macrophages. Indeed, TGFβ-polarized DCs phagocytosed more efficiently Rituximab (anti-CD20 mAb)-opsonized Burkitt lymphoma cells (Raji) as well as bare NSCLC cells (A549) when CLEC-1 is antagonized by antibodies. Furthermore, macrophages more productively engulfed Rituximab-opsonized Raji cells as well in the context of CLEC-1 blockade (2–3 fold increase). Moreover, Cetuximab opsonized colon carcinoma cells (DLD-1; EGFR+) and Trastuzumab opsonized mammary carcinoma cells (SK-BR-3; Her2+) were likewise more phagocytosed by CLEC-1 blocked macrophages.

**Conclusions** Altogether, 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.

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**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** CD200AR-L macrophages were used to determine the connection between the CD200 and PD-1 checkpoints.