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 Vγ9Vδ2 T cell killing of cancers cells.

**Results** We generated 7 anti-BTN2A1 mAbs and tested their effect on Vγ9Vδ2 T cell degranulation against Daudi cells with or without zoledronate. Six out of 7 anti-BTN2A1 mAbs significantly inhibited basal Vγ9Vδ2 T cell degranulation against Daudi up to 17-fold, and 5 of them were able to inhibit Vγ9Vδ2 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 Vγ9Vδ2 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 Vγ9Vδ2-induced tumor cell apoptosis was also shown in 3D endometrial cancer spheroids. In co-cultures of Vγ9Vδ2 T cells with primary AML blasts, the anti-BTN2A1 7.48 inhibited Vγ9Vδ2 T cell degranulation as well as TNFα, IFNγ production and killing of AML blasts.

**Conclusions** Antagonist antibodies to BTN2A1 highlighted its critical role in Vγ9Vδ2 anti-tumor responses. BTN2A1 is involved in Vγ9Vδ2 T cell anti-tumoral activity and can constitute an interesting therapeutic target for gITI 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 131368-B-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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**212 CLEC-1 IS A NOVEL MYELOID IMMUNE CHECKPOINT FOR CANCER IMMUNOTHERAPY LIMITING TUMOR CELLS PHAGOCYTOSIS AND SYNERGIZING WITH TUMOR-TARGETED ANTIBODIES**

**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 TGFβ 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, eradicate 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 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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**213 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.