

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-BTN2A1 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 ( $IC_{50} = 0.033 \pm 0.0003 \mu\text{g/mL}$ ) but also  $TNF\alpha$  ( $IC_{50} = 0.03 \pm 0.006 \mu\text{g/mL}$ ) and  $IFN\gamma$  ( $IC_{50} = 0.015 \pm 0.004 \mu\text{g/mL}$ ) 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  $TNF\alpha$ ,  $IFN\gamma$  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 gdT 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 TGF $\beta$  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 TGF $\beta$ -polarized DCs or macrophages. Indeed, TGF $\beta$ -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

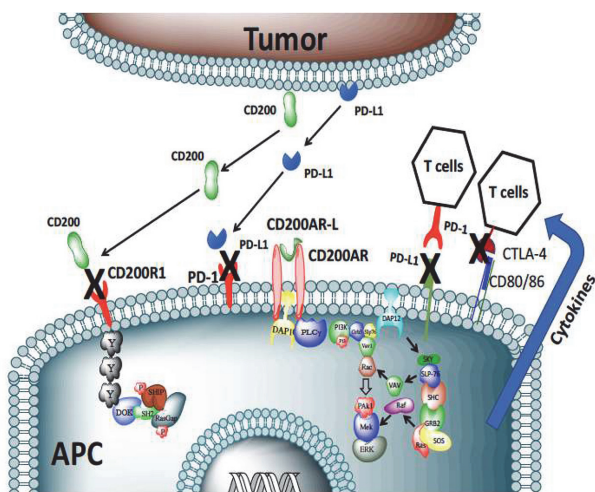
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**Background** Cancer immunotherapy has revolutionized clinical management of malignancies by generating long-term, durable control of tumors, rendering more manageable diseases that previously had dismal prognoses. Unfortunately, these therapies often 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** CD200R1KO macrophages were used to determine the connection between the CD200 and PD-1 checkpoints.

Next, we analyzed signaling molecules activated in CD11b cells pulsed with PD-L1 ± CD200AR-L, followed up with in vitro and in vivo effects of CD200AR-L on the expression of PD-1/PD-L1 and CTLA-4. Finally, we analyzed the ability of the CD200AR-L to surmount the suppressive effects of PD-L1.

**Results** Our studies demonstrate that the inhibitory CD200R1 and PD-1 mediate immune checkpoint signaling activities through SHIP1 protein. Moreover, CD200AR-L overpowers the suppressive effects of CD200 and PD-L1, which are both shed by tumors, by downregulating the inhibitory CD200R1 and PD-1 on both antigen-presenting cells (APC) and T-cells (figure 1). In addition, CD200AR-L downregulates PD-1 on APCs and inhibits the upregulation of PD-L1 and CTLA4.



**Abstract 213 Figure 1** Mechanism of the CD200 Checkpoint Ligand

**Conclusions** These studies led to the discovery that this novel peptide modulates the CD200, PD-1/PD-L1 and CTLA-4 pathways, providing the basis for the translatable development of a novel CD200 peptide inhibitor for clinical use against multiple tumors, including gliomas. These studies led to the FDA approval for the first in human peptide checkpoint inhibitor to initiate a phase I single center, open-label, dose-escalation clinical trial in adult patients with recurrent glioblastoma, to be followed by a clinical trial for children with recurrent malignant brain tumors.

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**THE EFFECT OF ANTI-PD-1 THERAPY ON MEDIAN OVERALL SURVIVAL AND PROGRESSION FREE SURVIVAL IN GLIOBLASTOMA MULTIFORME PATIENTS WITH CERTAIN TUMOR MARKERS**

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**Background** Almost 1 in 6 malignant brain cancers are Glioblastoma Multiforme, relative to most other brain cancers it is the most aggressive and prevalent by the numbers.<sup>1</sup> Even with the best treatment options median Overall Survival(OS) remains morbid at 14.6 months and Progression Free Survival (PFS) remains 6.9 months.<sup>2</sup> Telomerase Reverse Transcriptase promoter mutations,<sup>3</sup> Isocitrate Dehydrogenase(IDH)

mutations,<sup>4</sup> and Tumor Mutation Burden(TMB)<sup>5</sup> are three prominent tumor markers that are known to be associated with better PFS and OS; markers like these in the presence of new therapies maybe prove crucial to the development of novel therapies. Immunotherapy has been dubbed a ‘game changer’ in certain hematological and solid malignancies. Specifically, PD1 is a glycoprotein that is a strong negative regulator of the immune system, by blocking this glycoprotein Anti-PD-1 agents harness a strong response by the immune system to fight a malignancy<sup>6</sup>. In conjunction with these new found tumor markers, Anti-PD-1 agents maybe the solution that could dramatically improve OS and PFS in these patients.

**Methods** The goal of this study was to retrospectively analyze patients’ charts who had received Anti-PD-1 therapy and had TERT promoter mutations, IDH mutations, different TMBs, and other markers and to compare their OS and PFS outcomes with conventional therapies and their response to immunotherapy.

**Results** Upon analyzing the data the presence of a TERT promoter 124C>T mutation, IDH wildtype, and lower TMB gave much better OS and PFS after treatment in patients on Anti-PD1 therapy.

**Conclusions** Although this was a small study, these results certainly can be used to examine larger subsets of patients with these markers receiving immunotherapy because they had definitively better outcomes as compared to status quo treatment options.

**Ethics Approval** The study was approved by Washington University Ethics Board, approval number 201111001.

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**AO-176, A HIGHLY DIFFERENTIATED CLINICAL STAGE ANTI-CD47 ANTIBODY, PREFERENTIALLY BINDS TUMOR VERSUS NORMAL CELL CD47 WHEN COMPLEXED TO  $\beta$ 1 INTEGRIN**

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**Background** Overexpression of CD47 by tumor cells exploits an immune checkpoint preventing tumor recognition and destruction by innate immune cells. Binding of tumor CD47 to SIRP $\alpha$  on macrophages and dendritic cells triggers a ‘don’t eat me’ signal that inhibits phagocytosis and allows escape from innate immune surveillance. Blockade of the CD47/SIRP $\alpha$  axis, however, enables immune recognition and phagocytic clearance of tumor cells. We have developed a clinical stage CD47 targeting antibody AO-176 that is highly differentiated among agents in this class. AO-176 not only blocks the