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# CD200AR-L: mechanism of action and preclinical and clinical insights for treating high-grade brain tumors

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## 1. Introduction

The characterization of negative immune regulators (‘immune checkpoints’) has spurred the development of immunotherapeutic drugs, which are chiefly monoclonal antibodies or recombinant proteins [1]. The clinical success of immune checkpoint blockade (ICB) with monoclonal antibodies has led to an estimated 3000 immuno-oncology trials, enabling durable control of previously incurable cancers such as melanoma, non-small cell lung renal cell, bladder, and head and neck cancer [2]. However, response rates to ICBs vary widely from essentially no benefit to greater than 52% in the most sensitive cancers [3]. These therapies, especially in combination, often cause serious immune-related adverse events including death, resulting in discontinuation of ICB in many cases.

The University of Minnesota has a long history of developing novel treatments for adult and pediatric patients with CNS tumors. We developed an allogenic glioblastoma cell line derived in hypoxic conditions to maintain stemness, which has been used in multiple phase 1 trials for adult and pediatric tumors and recently for treating patients with low-grade astrocytoma [4]. However, post hoc evaluations of patients in a previous clinical trial revealed high concentrations of an immune suppressive protein, CD200.

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Declaration of interest

M Olin and C Moertel are officers in OX2 Therapeutics, a spinoff company of the University of Minnesota studying the CD200 checkpoint and possible therapeutic interventions based on exploitation of the CD200 pathway. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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The CD200 protein works through a unique immunological checkpoint. In contrast to CTLA-4 and PD-1/PD-L1, the CD200 protein regulates the immune system through a paired receptor system inducing inhibitory or activation signals [5–8]. The inhibitory signals are activated through binding of the native form of the CD200 protein to the inhibitory receptor, CD200R1. In contrast, the mechanism of ligands inducing an activation signal through binding to CD200 activation receptor(s) (CD200AR) is not well defined. Nevertheless, using macrophage cell lines void of specific CD200ARs has led to the hypothesis that ligand binding works through a complex of multiple CD200ARs [9].

Rigorous evidence from prior studies reported that targeting the CD200 checkpoint enhances immunotherapy [9–14].

The most advanced of these studies was a 2008 clinical trial of relapsed refractory B-cell chronic lymphocytic leukemia and multiple myeloma (NCT00648739) that evaluated the humanized monoclonal antibody samalizumab against the CD200 protein on tumors [10]. The authors, however, reported only a 10% reduction in bulk disease, which may be due to the CD200 protein being shed from the tumor, binding samalizumab and inhibiting the ability of the antibody to target the tumor-bound CD200. Current checkpoint inhibitors passively block receptor/ligand interactions in the immune checkpoint pathways.

The CD200 protein is secreted from human glioblastoma (manuscript in preparation), inducing systemic and tumor microenvironment immunosuppression. In addition, we discovered that CD200 is upregulated in tumor-associated vascular endothelial cells in glioblastoma, forming an ‘immunologic blood–brain barrier’ [11]. We suggest that the CD200 protein is a mechanism for the tumor to evade the immune system. In recent experiments, we discovered that knocking out the CD200 protein from the tumor cells allowed immune competent mice to spontaneously reject 100% of tumors (unpublished data). This response is immune mediated, since none of the CD200KO tumors were affected in immune incompetent mice. Therefore, we hypothesize that targeting the CD200 immune checkpoint will significantly enhance immunotherapy.

## 2. CD200AR-L binding controls multiple immune checkpoint pathways

Because current checkpoint inhibitors passively block receptor/ligand interactions in immune checkpoint pathways, we took an alternative approach by designing a peptide ligand to the CD200 activation receptor (CD200AR-L). A close look at the CD200 protein resulted in the identification of multiple sequences within the protein that are inaccessible to the activation receptors in its native form. However, we discovered multiple metalloprotease cleavage sites including an ADAM15 cleavage site in the CD200 protein that, when cleaved, releases peptide fragments with sequences that are capable of binding to activation receptors.

We reported that intradermal injection of CD200AR-L activates local immune cells and boosts chemokine and cytokine production, which results in recruitment of other immune cells to the injection site [12]. In subsequent *in vitro* studies, we determined that CD200AR-L binding to a complex of CD200ARs upregulates the DAP10 and DAP12 signaling pathways [14]. Following the characterization of the CD200AR signaling pathway, we

formed a scientific premise that we are able to control the CD200R1, CTLA-4, PD-1/PD-L1 pathways with the use of our CD200AR-L. This premise is based on previous studies, which show that the inhibitory CD200 protein is upregulated with PD-L1[7] and that the CD200 checkpoint negatively regulates the immune system similarly to CTLA-4 and PD1/PD-L1/2 through the PI3K-Akt pathway and Ras-MEK-ERK signaling [5–8]. Our studies show that knocking out the inhibitory CD200R1 pathway inhibits the ability of PD- L1 to suppress the immune system and the administration of the CD200AR-L *in vivo* downregulates the inhibitory CD200R1 and PD-1/PD-L1 molecules (unpublished data). We reported that using autologous brain tumor lysate in combination with a canine CD200AR-L significantly extended survival of companion dogs with high-grade gliomas in a canine clinical trial [13]. In a subsequent study monitoring the dogs' immune responses, we observed a downregulation of Tregs ($p = 0.001$), MDSCs ($P = 0.004$), and Bregs ($P = 0.013$) in addition to down regulation of PD-1 expression on both CD4 ($P = 0.001$) and CD8 ($P = 0.019$) T-cells (Figures 1(a,b)). In our ongoing Phase I dose escalation trial (NCT04642937) of CD200AR-L combined with an allogeneic tumor lysate, GBM6- AD, we learned that we could elicit an immune response against CNS cancers (preliminary results presented in an abstract at the 2021 Society of Neuro-Oncology). In this trial, we observed a downregulation of the inhibitory CD200R1, PD- 1, PD-L1, and CTLA4 in the patient population having favorable responses (Manuscript in preparation). These data, in conjunction with published data, suggest that multiple signaling molecules are shared by the CD200 and PD-1/PD- L1 pathways [5–8], hence the CD200 checkpoint is the next logical target for immunotherapy.

## 3. CD200AR-L extends survival

We believe that the ability to target multiple immune checkpoints with the CD200AR-L is how we enhanced survival in a high-grade glioma model [9,11–14]. In a murine glioma trial conducted by Dr Maria Castro, her gene therapy model exhibited a superior response when combined with CD200AR-L compared to the concurrent use of anti-PD-1 or anti-CTLA4 checkpoint blockade (Figure 2(a)).

Given that CD200AR-L is a peptide targeting the immune system, we tested the ability of the CD200AR-L to extend survival in a breast carcinoma model. Balb/c mice were inoculated with 5,000 luciferin-positive 4T1 tumors in the lower fat pad. On day 7, mice were treated with the murine CD200AR-L + 4T1 tumor lysates (TL). We observed an increase in survival for mice receiving the CD200AR-L in combination with TL compared to mice receiving TL alone (Figures 2(b–e)) extending survival (Figure 2(f)). Eight weeks following tumor regression, mice were rechallenged with 10,000 luciferin-positive 4T1 tumor cells in the contralateral fat pad. In contrast to the naïve control group, none of the mice previously receiving the CD200AR-L + TL exhibited tumor growth (Figures 2(g,h)).

## 4. Expert opinion

It has been 11 years since the use of immune checkpoint blockade (ICB) was approved for clinical use. These monoclonal antibodies have demonstrated the importance of protecting the immune system from exploitation by tumor cells. ICB as a first- or second-line therapy has been shown to provide a survival advantage in the clinical management of multiple

cancers. However, like an aircraft, the immune system contains redundant systems often requiring a combination of immune checkpoint inhibitors to be effective. In addition, current ICB is designed to passively find its target to block the suppressive molecule that inhibits an antitumor response.

Many immune checkpoints have been established, including, but not limited to, PD-1, PD-L1, CD80, CD86, VTCN1, HHLA2, TNFRSF14, PVR, CD200, LGALS9, ICOSLG, TNFSF9, TNFSF4, CD70, TNFSF18, and CD48[15]. To date, the most effective combination of checkpoint inhibitors is anti-PD-1 combined with anti-CTLA-4. However, with new efforts to combine ICB antibodies to augment efficacy, immune-related adverse events and greater autoimmune-associated toxicities have also become more prevalent [2,3]. To overcome the issue of toxicity with multiple ICBs, we took the approach of downregulating multiple immune checkpoints with a single peptide ligand. We believe that this unique mechanism is how we achieved our superior survival results in our preclinical murine and canine trials.

In our phase I clinical trial described above, the CD200AR-L has potential to demonstrate efficacy in one of the hardest tumors to fight. In addition, our preclinical murine studies to date suggest that the CD200AR-L may also benefit patients with breast carcinoma [12], lung cancer, and melanoma (unpublished data). Exploitation of the CD200 checkpoint pathway has the potential to be a powerful force in the future development of cancer immunotherapy. We have shown that the activation of CD200AR by a synthetic peptide ligand downregulates multiple immune checkpoints (unpublished data). This approach has the potential to be less expensive and less toxic than the current immune checkpoint antibody blockade approach.

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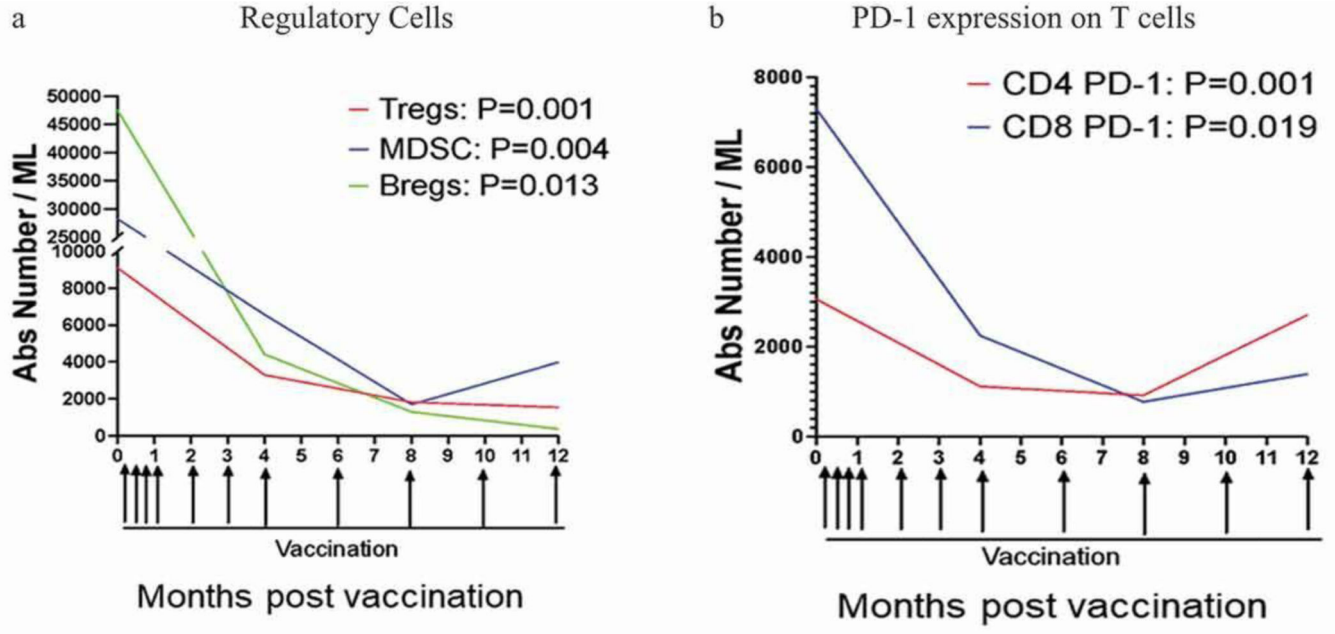
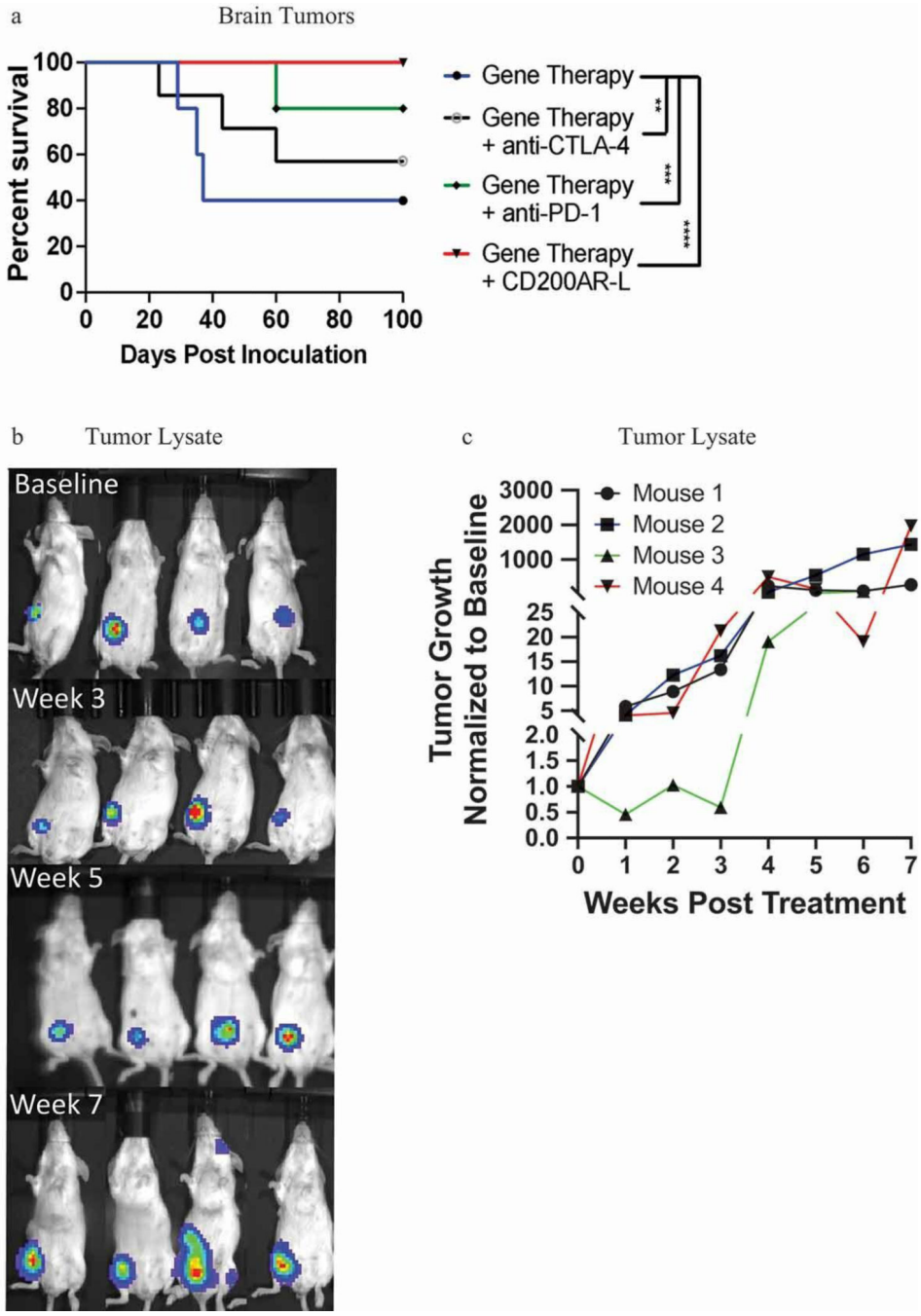



**Figure 1. CD200AR-L extends survival.**

Following surgical resection of the tumors, serial vaccinations of autologous tumor lysate and canine-specific CD200AR-L were administered to each dog. Disease status was followed using magnetic resonance imaging (MRI). Peripheral Blood Mononuclear Cells were isolated at time of treatments and analyzed for (a) Tregs, Bregs, and MDSCs and (b) $CD4^{+}$PD-1 and $CD8^{+}$PD-1 T-cells.



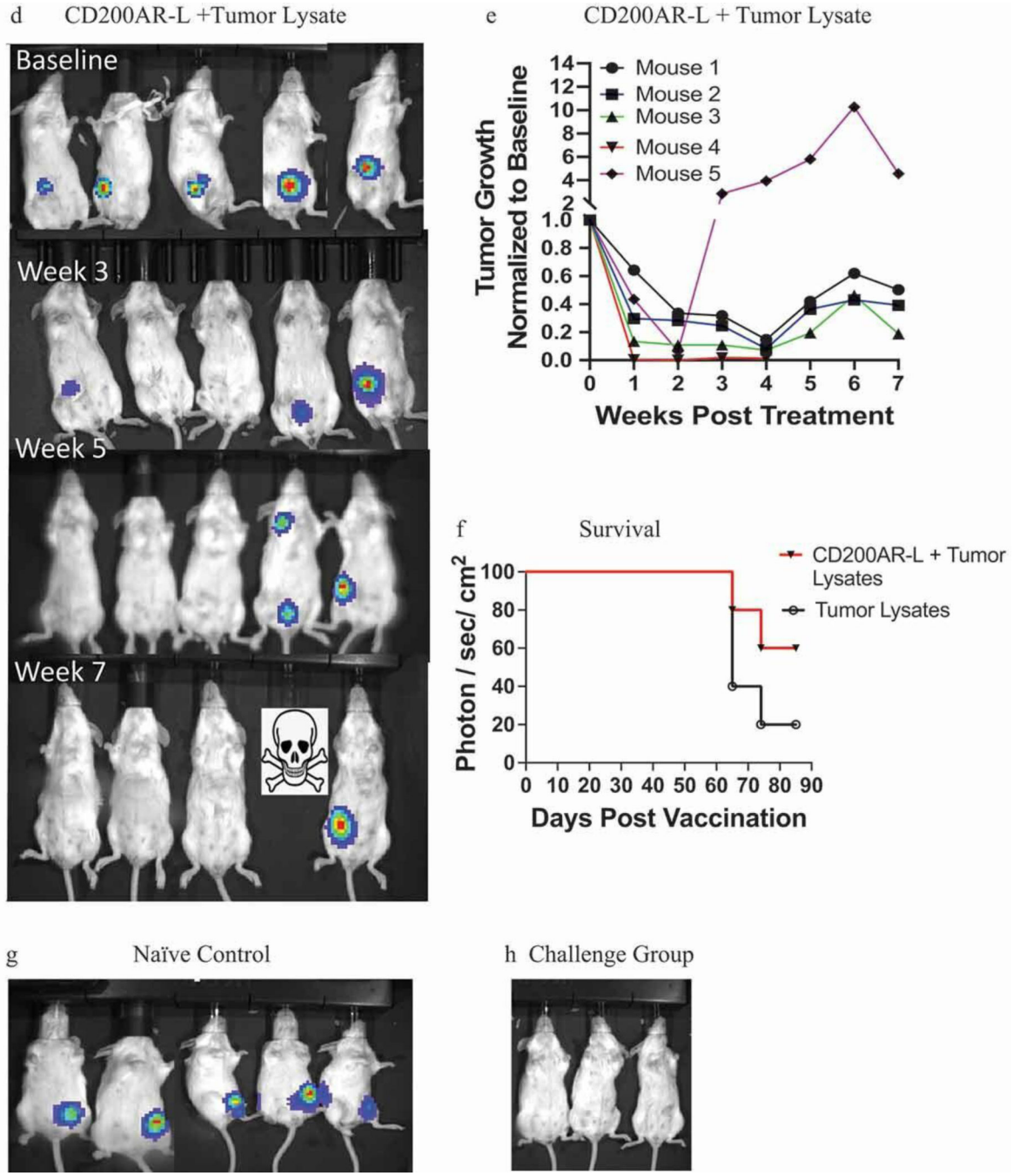



**Figure 2. CD200AR-L enhances survival in murine and canine CNS glioma studies.**

(a) Twelve days post GL261 inoculation, mice were treated with $5 \times 10^8$ plaque- forming units (pfu) of adenoviral (Ad)-Flt3L and $1.0 \times 10^8$ pfu of Ad-TK followed by the administration of ganciclovir. Mice were treated with CD200AR-L (2.5 mg/kg) (s.c.) twice weekly for 4 weeks. PD-L1-neutralizing or CTLA-4-blocking antibodies were administered twice, 6d and 11d post gene therapy; animals were monitored for survival. Experiments were performed separately with appropriate controls. $^{**}p < 0.01$, $^{***}p < 0.005$, by log-rank (Mantel-Cox) test, MS, median survival. Breast carcinoma mice were vaccinated weekly

with CD200AR-L + autologous tumor lysates. Mice were imaged weekly and monitored for (b-e) tumor growth and (f) Survival. (g) Mice were rechallenged in the contralateral fat pad with and monitored for 14 days for tumor growth.