Effective Depletion of Tumor-infiltrating Tregs by a Novel Anti-CCR8 Antibody (LM-108): Addressing Resistance Associated with Immune Checkpoint Inhibitors

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Background

- Although immune checkpoint inhibitors (ICIs) have altered the treatment paradigm for different tumors, its therapeutic efficacy is limited due to primary or acquired resistance.[1]
- Decreasing regulatory T cells (Tregs) and increasing effector T cells (Teffs) in tumors may prevent the development of immunotherapy resistance.
- C-C motif chemokine receptor-8 (CCR8) is exclusively expressed on tumor infiltrated Tregs, especially on the sub-population Tregs with most suppressive activity,[2] making it a promising target in cancer immunotherapy.[3]
- LM-108 is a novel humanized anti-CCR8 monoclonal antibody (mAb) with engineered IgG1 that enhances antibody dependent cell mediated cytotoxicity (ADCC) and antibody dependent cellular phagocytosis (ADCP).
- In the preclinical study, LM-108 showed strong anti-tumor activity as single agent by depleting tumor infiltrated Tregs. It also synergized with PD-1 antibody in PD-1 resistance model with long lasting anti-tumor memory.
- LM-108 is currently under phase 1 clinical development for advanced solid tumor (NCT05255484 and NCT05199753).

Mechanism of Action

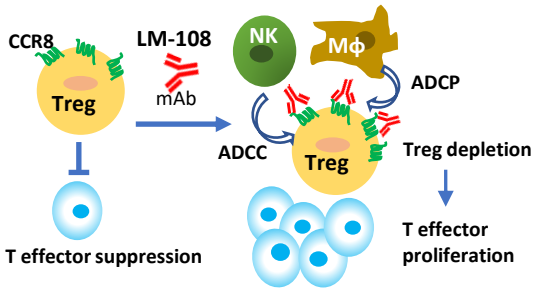

Figure 1. Binding of CCR8 mAb to Treg cells leads to ADCC and ADCP resulting in Treg depletion. This in turn lifts the suppression of Teffs leading to proliferation, and increases activity of natural killer (NK) and macrophage (Mϕ), preventing the development of immunotherapy resistance.

References

1. Jenkins RW, et al. Br J Cancer. 2018; 118(1):9-16.
2. Whiteside TL. Immunotargets Ther. 2015; 4: 159-171.
3. Campbell JR, et al. Cancer Res. 2021; 81(11): 2983-2994
4. Zheng et al., Science. 2021; 374 (6574): abe 6474

CCR8 is Specifically Expressed on Tumor Infiltrated Tregs

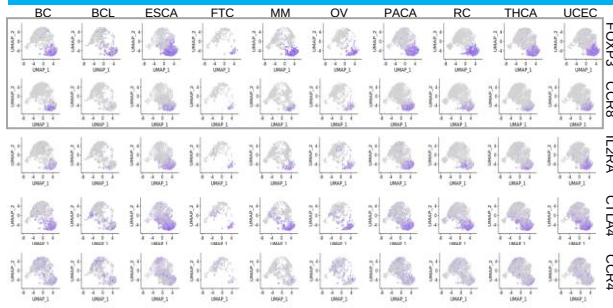

Figure 2A. CCR8 gene expression is mainly in Tregs in the tumor-infiltrating lymphocytes (TILs), and is highly correlated with FOXP3 than other markers such as CTLA-4 and CCR4. UMAPs represent single cell RNA sequencing gene expression profile localized to tumor infiltrated Tregs on 47 cancer patients by re-analyzed published data[4], as indicated. BC (breast cancer); BCL (B-cell lymphoma); ESCA (esophageal cancer); FTC (fallopian tube carcinoma); MM (multiple myeloma); OV (ovarian cancer); PACA (pancreatic cancer); RC (renal carcinoma); THCA (thyroid carcinoma); UCEC (uterine corpus endometrial carcinoma)

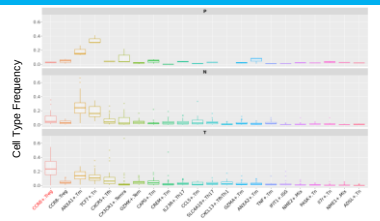

Figure 2B. CCR8+ Tregs are specifically existed in tumor but not peripheral blood. **P:** peripheral blood; **N:** Normal tissue; **T:** tumor. Box-Whisker plots represent CD4+ T cells from patients (blood n = 15, normal n = 51, tumor n = 163) by re-analysis of the published data[4].

LM-108 Has High Binding Affinity to Human CCR8 Expressing Cells

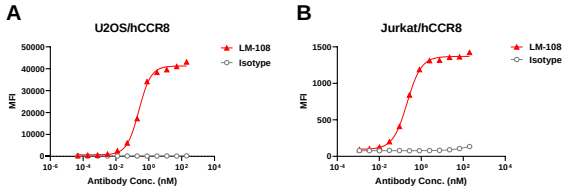

Figure 3. LM-108 mAb binds specifically to human CCR8 (hCCR8) in both **(A)** CCR8 high-expressing U2OS [EC_{50} = 0.25 nM] cells and **(B)** CCR8 low-expressing Jurkat [EC_{50} = 0.21 nM] cells in a dose dependent manner. The hCCR8 overexpressing cells were incubated with titrated LM-108 and isotype control, followed by staining with fluorescence conjugated secondary antibody and determined by flow cytometry analysis.

ADCC Effect of LM-108 Towards Human CCR8 Over-expressing Cells

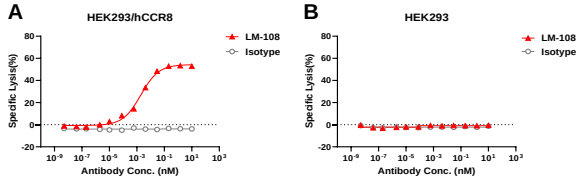

Figure 4. ADCC dose response curves towards CCR8 expressing HEK293 cells. LM-108 specifically depleted hCCR8 expressing HEK293 cells **(A)** [EC_{50} = 0.002 nM] but not in parental HEK293 cells **(B),** co-cultured with primary hPBMCs. Cell lysis was determined by the released LDH level in the supernatant after co-culture of effector cells (hPBMCs) : target cells = 50:1 and titrated LM-108 for 6 hours.

Specific and Dose Dependent Phagocytosis of LM-108 Towards Human CCR8 Over-expressing Cells

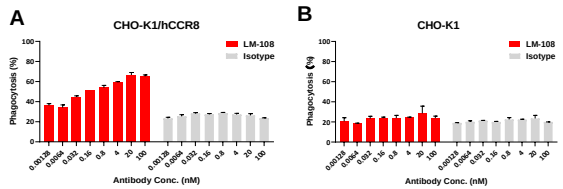

Figure 5. Antibody-dependent cellular phagocytosis (ADCP) of LM-108. LM-108 specifically depleted hCCR8 expressing CHO-K1 cells **(A)** compared to parental CHO-K1 cells **(B)**, by mixed with monocyte-derived macrophages (MDMs). The MDMs, CFSE-labeled target cells (CHO-K1/hCCR8, CHO-K1), and titrated LM-108 or isotype control were incubated for 2 hours. Cells were stained with APC-conjugated anti-human CD14 antibody to identify MDMs and then subjected to flow cytometry. Phagocytosis index was determined as the percentage of CD14-APC+/CFSE+ double positive cells in total CD14+ positive cells. Each data bar represents means of replicates ± SDs of phagocytosis,%.

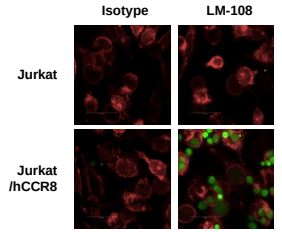

Figure 6. Representative images of LM-108 induced ADCP *in vitro*. LM-108 induced phagocytosis by MDMs in hCCR8 expressing Jurkat cells (bottom right). MDMs were labeled with an anti-human CD14-APC antibody (red) and target tumor cells were labeled with CFSE (green), images were acquired in a fully automated manner using 40× wide angle lens equipped microscope (Operetta, PerkinElmer).

In Vivo Anti-tumor Efficacy of Surrogate Antibody LM-108m in PD-1 Sensitive and Resistant Models

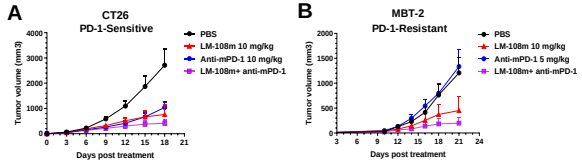

Figure 7. Tumor growth curve of CT26 tumor-bearing Balb/c mice **(A)** and MBT-2 tumor-bearing C3H/He mice **(B)** post administration of LM-108 murine surrogate antibody (LM-108m) and anti-mPD-1 mAbs. LM-108m showed effective anti-tumor activity as a single agent. In combination, LM-108m and anti-mPD-1 resulted in additive effects in the PD-1 sensitive model of CT26, and synergistic effects in the PD-1 resistant model of MBT-2.

LM-108 Shows Potent Anti-tumor Activity by Depleting Tumor Associate Tregs in MC38 Model with hCCR8 Knock-in Mice

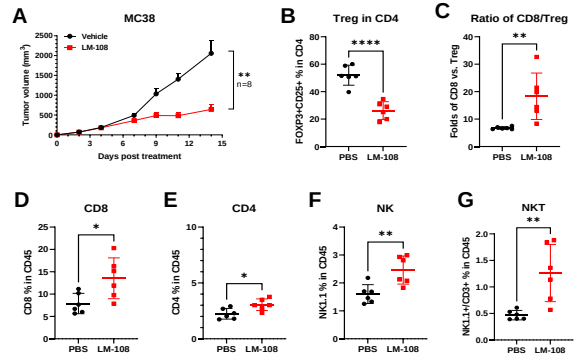

Figure 8. LM-108 at 10 mg/kg significantly inhibited tumor growth (TGI = 68.77%) in MC38 syngeneic model with hCCR8 KI mice as compared to vehicle **(A)**. TILs were analyzed after treatment with LM-108, Treg cells were significantly reduced, whereas CD8+ T cells, CD4+ T cells, cytotoxic natural killer (NK) cells and natural killer T (NKT) cells were significant elevated in TILs **(B-G)**. *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$.

LM-108 Synergizes with PD-1 Antibody Resulting in Strong Anti-tumor Activity in the PD-1 Resistant Model with hCCR8 Knock-in Mice

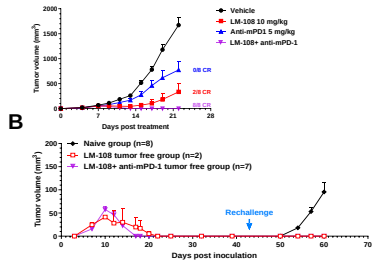

Figure 9. Anti-tumor activity of LM-108 in hCCR8 KI mice. **(A)** LM-108 as a single agent potently inhibited tumor growth (TGI = 79.97%, 2/8 CR), and combined treatment with anti-mPD-1 antibody provided a synergistic anti-tumor effect (TGI = 100%, 8/8 CR) *in vivo*. **(B)** Tumor free mice were re-challenged by tumor cells engraftment (blue arrow). These mice were resistant to rechallenge indicating that LM-108 therapy evoked long-lasting anti-tumor memory.

Summary

In vitro and *in vivo* findings indicate that LM-108 is a novel Fc-optimized CCR8 antibody that selectively depletes tumor infiltrating Tregs, improves anti-tumor immune response as monotherapy or combination therapy, and is hence a promising therapeutic approach to overcome resistance to ICIs.