ZL 1218 TARGETS THE MOST SUPPRESSIVE INTRATUMORAL TREG SUBPOPULATION TO AVOID PERIPHERAL TOXICITIES

Jing Zhang*, 1Linda Liu, 1Grace Wang, 1Joshua Zeng, 1Tao Geng, 1Chakrapani Tripathi, 1Lei Wang.
1Zai Lab (US) LLC, Menlo Park, CA, USA; 2Zai Lab (China) LLC, Shanghai, China

**Background** Accumulating evidence demonstrates that the removal of Treg cells is able to evoke and enhance anti-tumor immune response. However, systemic depletion of Treg cells may concurrently elicit deleterious autoimmunity. CCR8, a chemokine receptor expressed by tumor infiltrating Treg cells, is associated with poor cancer prognosis. We have recently reported that ZL-1218, a humanized therapeutic antibody targeting CCR8, exerts its anti-tumor effect as a monotherapy and in combination with PD-1 blockade treatment. In the current study, we aimed to understand the underlying biology of CCR8+ regulatory T cells and the mechanism of action of ZL-1218.

**Methods** Human and mouse dissociated tumor cells (DTCs) were immuno-profiled using multi-color flow cytometry. The CCR8 expression was quantified by both flow cytometry and immunohistochemistry (IHC)/ISH assay. The 10x Genomics single cell RNAseq (scRNAseq) was conducted, and the data was analyzed using unsupervised K-means clustering. nTreg cells were isolated from human buffy coat using EasySep™ Human CD4+CD127lowCD25+ Treg Isolation Kit.

**Results** We demonstrated that CCR8 is highly expressed on intratumoral FoxP3+ Treg cells in multiple cancers and is absent on other major intratumoral immune cell populations or any immune cell population in the peripheral blood. The percentage of CCR8+ Treg cells and CCR8 expression level indicated large donor-to-donor variations. In vitro anti-CD3/28 stimulation of nTregs suggested that Treg activation could be a trigger for CCR8 expression. Next, we assessed whether CCR8 is part of a larger Treg activation program using the scRNAseq analysis of selected DTC samples. CCR8+ tumor infiltrating Tregs indeed showed a significantly higher surface expression level of Treg activation markers such as GITR, OX-40, 4-1BB, CTLA-4 and TIGIT, which are also the markers for a highly suppressive Treg subpopulation. This implies that CCR8+ Treg cells represent a highly activated and suppressive Treg population. Importantly, we further demonstrated that ZL-1218 depleted about 50% Treg cells from selected DTC samples, which is consistent with the percentage of CCR8+ Treg cells in these samples. Additionally, in human CCR8 knock-in mice, ZL-1218 treatment depleted comparable intratumoral Tregs leading to increased CD8T/Treg ratios without affecting peripheral Tregs. The toxicology study of ZL-1218 in non-human primates following 5 weekly intravenous infusion administrations demonstrated no safety concerns up to 100 mg/kg.

**Conclusions** These findings suggest that ZL-1218 antibody may deliver optimal tumor-targeted Treg depletion in the clinic while limiting peripheral toxicities to avoid autoimmune response.

**Ethics Approval** The study was approved by IACUC committee in Zai Lab (US) LLC (Protocol approval number 2020-11_1).