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

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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).