BEYOND CCR8 KEY EPITOPES TARGETING DYNAMIC CCR8 CONFORMATIONAL STATES AND A DIVERSITY OF MONOCLONAL ANTIBODIES TO MODULATE THE TUMOR MICROENVIRONMENT FOR THE TREATMENT OF CANCERS

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**Background** Chemokine (C-C motif) receptor 8 (CCR8) belongs to the G protein-coupled receptor (GPCR) family. Based on the transcriptional landscape of tumor infiltrating T regulatory cells, it has been found that CCR8 positive tumor infiltrating T regulatory cells (TITR) were highly immune suppressive and defined as a specific signature molecule. Generation of functional antibodies against integral membrane proteins such as the G-protein coupled receptor CCR8 is technically challenging for several reasons, including the coverage of epitope diversity related to transcriptional and post-transcriptional regulation CCR8 transmembrane protein and its dynamic conformational according to activation cellular state or immunosuppressive cell subsets.

**Methods** Domain Therapeutics has generated wide monoclonal antibodies (mAbs) libraries targeting a diversity of separate and non-overlapping CCR8 epitopes. Using different sources of native CCR8 positive cell subsets, distinct mAbs cellular reactivity profile has been also determined. Using different approaches (BRET, ELISA, FCM, …), mAb activities were characterized in binding and several functional assays, including the inhibition of different G-proteins and B-arrestin 2 recruitment. A deep molecular and pharmacological characterization of each mAb was also performed by taking account the kinetic exposure and the nature of targeted CCR8 ligand. Some experiments were also conducted mimicking the tumor microenvironment conditions.

**Results** A panel of antibodies targeting human CCR8 was successfully generated. A wide diversity related to targeted epitopes was obtained, combined with high affinity binding properties. The requirement to target, or not, selective post-translational modifications (PTM) was also documented to trigger the highest inhibition level of CCR8 signaling. In parallel, treatment of tumor-bearing mice with cell-depleting anti-CCR8 antibody indeed eradicated established tumors with induction of potent tumor-specific effector/memory T cells. Thus, specific depletion of clonally expanding tumor Tregs is clinically instrumental for evoking effective tumor immunity without autoimmune adverse effects.

**Conclusions** Due to the high and relatively specific expression of CCR8 on tumor infiltrating Tregs, CCR8 represents an attractive immunotherapeutic target. Up to date, targeting selective CCR8 epitopes mimicking dynamic conformational CCR8 stage according to CCR8 positive cell subset or activation cellular state will be a key issue and differentiate therapeutic approach for the TME modulation in the treatment of cancer. Moreover, the use of depleting anti-CCR8 mAb with enhanced cytotoxic activity (i.e., ADCC, CDC, ADCP) can also reduce the number of CCR8 immune suppressive cells in a tumor.