## OTR4. CONSTRUCTION AND VALIDATION OF AN ACTIVATING AND INHIBITORY CHIMERIC ANTIGEN RECEPTOR (CAR) SYSTEM.

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**INTRODUCTION** The use of adoptive transfer of T cells for the treatment of cancer is hampered by low avidity and persistence of infused cells and the difficulty in isolating and expanding antitumor lymphocytes. The use of chimeric antigen receptors (CARs) avoids some of these problems. CARs consist of an antigen recognition unit (Fab fragment as scFv), a transmembrane region and an intracellular activation domain. CARs redirect the specificity of lymphocytes, recognizing the target antigen with high affinity and independently of MHC. However, on target/off tumor responses due to target recognition in normal cells limits the widespread application of this therapy. The CD19 antigen represents a good target for elimination of B-cell precursor acute lymphoblastic leukemia (BCP-ALL) and lymphocytes expressing anti-CD19 CARs (19BBz) are being used in the clinical setting. However, the expression of CD19 as a pan-B marker can lead to undesired side effects such as depletion of mature B cells.

**OBJECTIVE** As mature B cells express both CD19 and CD20 antigens, we propose the creation of an inhibitory anti-CD20 CAR, that when used in combination with 19BBz would be able to discriminate between leukemic blasts and normal B cells.

**METHODOLOGY** Three inhibitory CARs were constructed containing signaling domains of CTLA-4, PD-1 or BTLA. As a reporter system to test our hypothesis, Jurkat T cells expressing the plasmid pGL4.30 - expressing luciferase controlled by a NFAT responsive promoter - were generated. K562 cell line was used as target cells and modified to express CD19 (K5-19), CD20 (K5-20) or CD19 and CD20 (K5-19/20). The *Sleeping Beauty* transposon was used to modify primary human T lymphocytes.

**RESULTS** In coculture experiments, Jurkat cells expressing 19BBz showed induction of luciferase activity when cultured with K5-19 or K5-19 / 20. However, Jurkat expressing both activation and inhibitory CAR showed inhibition of luciferase

activity when incubated with K5-19 / 20 while maintained high activity when cultured with K5-19 cells. Furthermore, all three inhibitory CARs were able to inhibit the expression of the activation marker CD69 induced by 19BBz in Jurkat cells. In primary human T cells, the 20PD1 CAR did not inhibit proliferation or cytotoxicity induced by 19BBz. The 20PD1 CAR was also unable to inhibit the first generation 19z CAR, which lacks the 4-1BB coestimulatory endodomain, showing that PD1-mediated inhibition in our system is inefficient.

**CONCLUSION** Additional experiments are needed to evaluate the activity of 20PD1 in primary human T cells. Experiments with additional inhibitory CARs (20BTLA, 20LAG3 and 20PD1LAG3) are ongoing and can serve as an alternative to CAR 20PD1 in the construction of a conditional response system.

**KEYWORDS** immunotherapy, chimeric antigen receptor, T lymphocytes.