# B-10

## Development of Anti-SSTR CAR-T Cells for Future Treatment of NETs

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**BACKGROUND:** Neuroendocrine tumors (NETs) overexpress somatostatin receptors (SSTRs). Here, we investigate the antitumor activity of chimeric antigen receptor (CAR)-T cells directed against SSTRs.

**METHODS:** We developed a second-generation CAR-like construct containing two molecules of octreotide in the extracellular moiety and CD28 as costimulatory module. The construct was cloned in a pMSGV1-28Z retroviral vector and then transduced in CD8+ T cells from a healthy donor. BON1, CM and QGP1 NET cell lines were transduced to express luciferase (Luc) and were thus screened for membrane SSTR1-5 expression by Western blot (WB). Co-culture experiments were performed at effector/target (E/T) ratios ranging from 10:1 to 1:10 for up to 48 hrs. Tumor cell cytotoxicity was then assessed by bioluminescence imaging, while the release of IFN-γ, IL-2 and TNF-α by activated CAR-T cells was assessed by ELISA. NSG female mice (n=6/group) were subcutaneously injected with 4x106 Luc+ NET cells, and were then subjected to either intratumor or intravenous administration of anti-SSTR CAR-T cells, or untransduced (UT) T cells.

**RESULTS:** CM and BON1 cells appeared to overexpress SSTRs, whereas low amounts of these receptors were detected in QGP1 cells. Following WB confirmation of the CAR expression by transduced lymphocytes, anti-SSTR CAR-T cells were co-incubated with target cells, and induced tumor cell death in up to 40% (±8%) of CM cells when an E/T ratio of 1:1 was used. The tumoricidal effect

of CAR-T cells was time-dependent and peaked at 48 hrs. When compared with UT T cells, CAR-T cells secreted significantly higher levels ($p<0.01$) of IFN-γ (up to 1,057 pg/ml), IL-2 (up to 845 pg/ml) and TNF-α (up to 1,048 pg/ml) after co-incubation with CM cells. Experiments in mice are currently underway.

**CONCLUSION:** Anti-SSTR CAR-T cells exhibit antitumor activity against NET cell lines. The tumoricidal activity of CAR-T cells is critically influenced by the density of membrane expression of SSTRs.