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A novel hormone based anti-SSTR bispecific T-cell engager for the treatment of neuroendocrine tumors

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BACKGROUND

Somatostatin receptor 2 (SSTR2) is overexpressed in well-differentiated NETs. We designed a novel bispecific T-cell engager targeting SSTR2 via Somatostatin-14, the hormone that physiologically binds the SSTR2, linked with a scFV-based anti-CD3.

METHODS

The recombinant protein was expressed in Trichoplusia-ni cells, isolated and characterized by chromatography. Flow cytometry and Image Stream flow cytometry were used to determine the interaction of the molecule with CD3 and SSTR2. Target 293T cells were stably transduced to concurrently express SSTR2 and GFP or GFP only. Effector CD3+ T cells and target cells were co-incubated in the absence or presence of the engager at different concentrations. The formation of immune synapses was assessed by measuring actin rearrangement in T-cell target cell doublets and LFA-1 expression using ImageStream and IncuCyte. The engager-induced T-cell activation and cytotoxicity were evaluated by ELISA and real-time quantitative live-cell imaging. Tumor-infiltrating lymphocytes (TILs) from different tumor regions and autologous tumoroids from pancreatic NET liver metastasis were cocultured in the presence of the molecule at serial concentrations and the engager-induced activation of TILs was measured by ELISA.

RESULTS

The T-cell engager was detected by flow cytometry on approximately 85% of T-cells at a concentration of 100nM. The engager interaction with SSTR2+ and its subsequent internalization was detected by image stream between 100nM and 20nM. The induction of immunological synapses by the engager, measured as actin rearrangement and LFA-1 expression on T-cells, was significantly higher in the presence of the receptor compared to the control. IFN-γ, TNF-a and Granzyme-B secretion was significantly higher when the T-cells were co-cultured with SSTR+ 293T cells in the presence of the engager at 100nM and 20 nM as compared with conditions using SSTR- 293T cells or in absence of the molecule. Additionally, the 20nM and 100nM engager exhibited cytotoxic activity when added to SSTR+ 293T cell cultures in the presence of T-cells in a dose dependent way. The engager was also able to elicit IFN-gamma secretion by TILs when co-cultured with autologous tumoroids at concentrations of 20nM, 60nM, and 100nM. Notably, this effect was retained when TILs failing to show cytotoxic activity per se were co-cultured with autologous tumoroids.

CONCLUSIONS

To our knowledge, this is the first T-cell engager to incorporate a hormone in one binding site, exerting a dose-dependent cytotoxic activity against SSTR2-expressing cells. This molecule can elicit a TIL response against autologous tumoroids from patient with well differentiated NET, restoring the antitumor potential of bystander TILs.

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