1375 A HORMONE-BASED BISPECIFIC T CELL ENGAGER (BITE)-LIKE MOLECULE FOR THE TREATMENT OF NEUROENDOCRINE TUMORS

[1]Eleonora Pelle*, [2]Mauro Cives, [1]Elliot Medina, [1]Charlotte Mason, [1]Sebastian Snedal, [1]Xiomar Bustos Perez, [1]Leticia Tordesillas, [1]Miguel Gomez Fontela, [1]Renata Rossetti, [3]Gabriele Maiorano, [1]Vincent Luca, [1]Patrick Hwu, [1]Daniel Abate-Daga, [1]Jonathan Strosberg. *[1]Moffitt Cancer Center, Tampa, FL, USA; [2]University of Bari Aldo Moro, Bari, Italy; [3]National Research Council, Lecce, Italy*

Background We designed a novel bispecific T-cell engager (BiTE) targeting the somatostatin receptor (SSTR), which is overexpressed by well-differentiated neuroendocrine tumors (NETs). In our BiTE, the single chain variable fragment (scFV) based anti-SSTR domain is replaced by 2 molecules of somatostatin-14 (SST14), the hormone that physiologically activates the SSTR.

Methods The sequence of the SST14-scFV anti-CD3 BiTE was optimized and cloned into a pAcGP67avector. The recombinant protein was expressed by Trichoplusia-ni (High Five) using Baculovirus and isolated from the supernatant using nickel affinity chromatography. The binding potential of the BiTE towards CD3 and SSTR was determined by flow cytometry and confocal microscopy. The SSTR-specific T cell activation and the BiTE-induced cytotoxicity were measured after coincubation of $CD3^+$ T cells isolated from the peripheral blood of healthy donors with 293T cells stably transduced to concurrently express SSTR and GFP, in presence of the BiTE. The same conditions in absence of BiTE or with the $SSTR^-$ parental 293T were used as negative control, while anti-CD3/CD28 beads were added as a positive control. The BiTE-induced cytotoxicity was assessed by real-time quantitative live-cell imaging using the Incucyte system. The BiTE-induced T cell activation was evaluated measuring the secretion of IFN-γ, and granzyme-B by ELISA.

Results At a concentration of 100 nM, the BiTE bound the CD3 receptor of approximately 85% of T cells. By confocal microscopy, the BiTE was found to coat $SSTR^+$293T cells. When added to $SSTR^+$293T cell cultures, the BiTE by itself exerted antiproliferative activity ($p < 0.0001$), possibly as result of an agonist activity on the SSTR. Such a cytotoxic effect was significantly more pronounced when T cells were also present in the cultures ($p<0.0001$). IFN-γ and granzyme-B secretion was significantly higher when the T cells were co-cultured with $SSTR^+$293T cells in the presence of the BiTE as compared with parallel preparations with $SSTR^-$293T cells or without the BiTE, suggesting that the BiTE-induced T cell activation is specific.

Conclusions To our knowledge, this is the first BiTE to incorporate a hormone in one binding site. Our preclinical data indicate that the BiTE specifically engages the SSTR, the T cell receptor and induces a high level of cytotoxicity in the presence of T cells.

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