A novel anti-SSTR bispecific T-cell engager (BiTE^R)-like molecule for the treatment of neuroendocrine tumors

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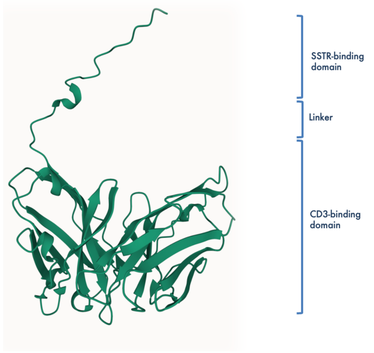
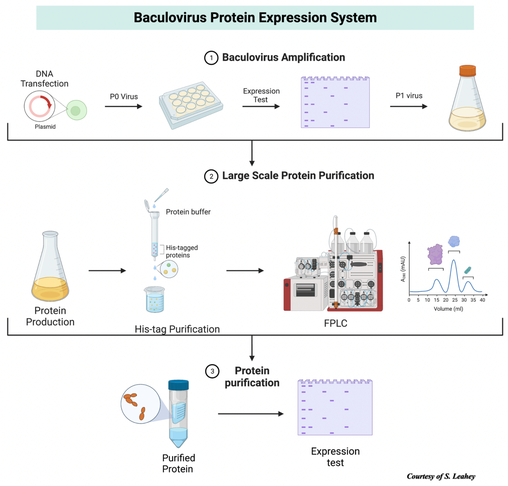
INTRODUCTION



Bispecific T-cells engagers (BiTE^R) are an emerging class of immunotherapeutic molecules that promote the formation of a cytolytic immunological synapsis between T-cells and tumor cells. Well- differentiated neuroendocrine tumors (NETs) overexpress somatostatin receptors (SSTRs). We designed a novel T-cell engager targeting SSTR with a functional domain composed of 2 molecules of Somatostatin-14, the hormone that phisiologically binds the SSTR, and inhibit NET cells proliferation.

METHODS



The optimized sequence of the protein was subcloned into a vector designed for protein expression in insect cells using Baculovirus. Trichoplusia-ni (High Five) cells were used to express the recombinant protein, which was isolated from the supernatant using nickel affinity chromatography.

Flow cytometry and confocal microscopy were used to determine the binding potential of the molecule towards CD3 and SSTR2. CD3+ T cells isolated from the peripheral blood of healthy donors were co-incubated with 293T cells stably transduced to concurrently express SSTR2 and green fluorescent protein (GFP) in the absence or presence of the molecule. The SSTR2- parental 293T cell line was used as negative control, while anti-CD3/CD28 beads were added as a positive control. The molecule-induced T cell activation was evaluated measuring the secretion of IFN-gamma and Granzyme B by ELISA and OX40, 41BB, CD25 and CD69 by flow cytometry.

RESULTS

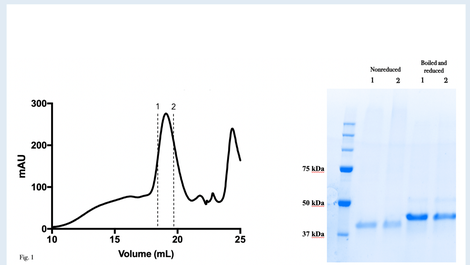


Fig. 1 Recombinant protein expression.
The recombinant protein was characterized for its purity by SDS-PAGE .
The molecular weight of the recombinant protein was around 37 kDa, as expected.

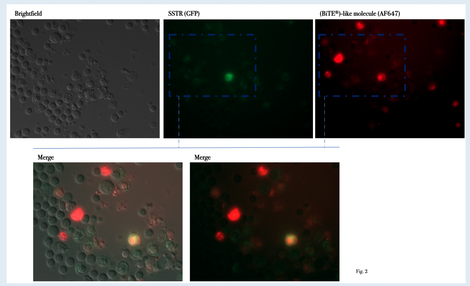


Fig. 2 Interaction of the hotmone-based T cell engager molecule with T cells and SSTR+ target cells by confocal microscopy.
The anti-SSTR hotmone-based T cell engager was stained with AF647 and the 293T cells were transfected with a vector encoding for a GFP-SSTR2 fusion protein. T cells are not stained.
The SSR2 (green) and the molecule (red) are co-expressed on SSTR2+GFP+ 293T cells.

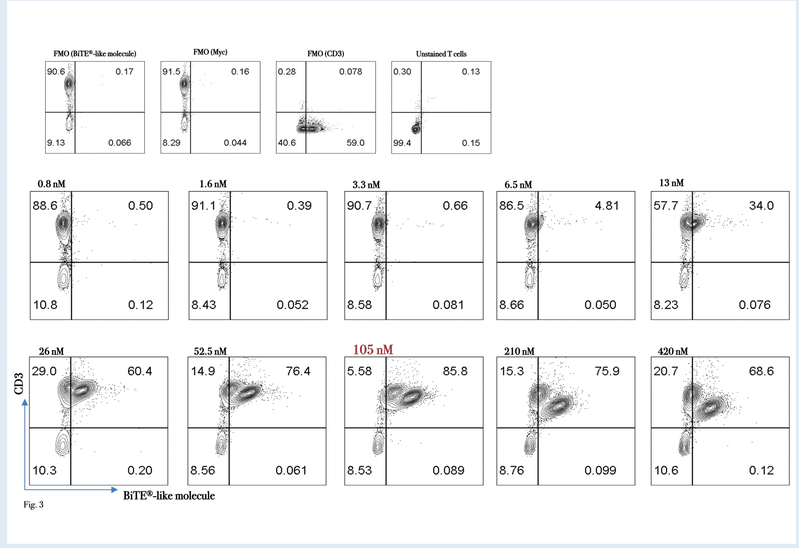


Fig. 3 Interaction of the molecule with the CD3
At 100 nM, the hormone-based T cell engager bound the CD3 receptor of approximately 85% of T cells.

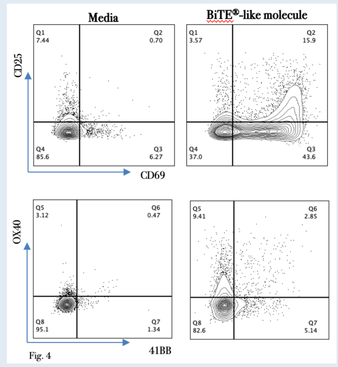


Fig. 4 Activation markers
After incubation with the hormone-based T cell engager, the t cells upregulate the expression of CD25 and CD69.

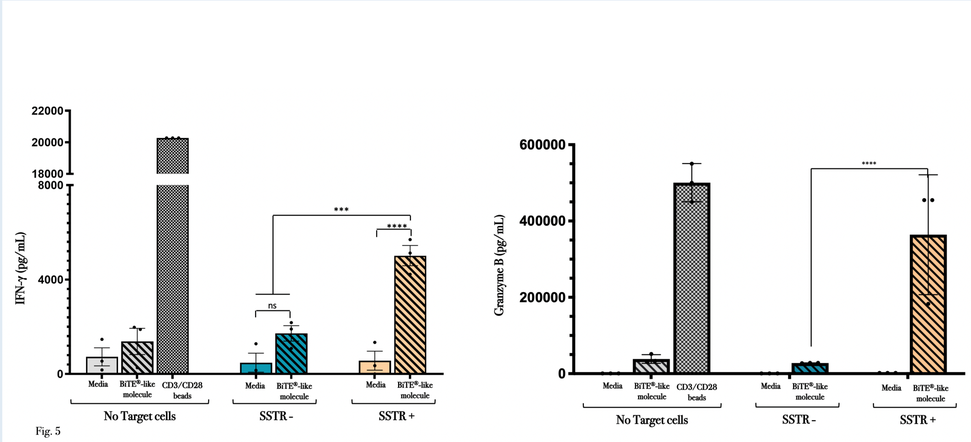


Fig. 5 Hormone-based T cell engager molecule mediates SSTR-specific T cell activation.
TFN-gamma and Granzyme B secretion was significantly higher when the T cells were co-cultured with SSTR+ 293T cells in the presence of the hotmone-based T cell engager as compared with parallel preparations with SSTR- 293T cells or without the molecule, suggesting that the T cell activation is specific.

CONCLUSION

To our knowledge, this is the first BiTE to incorporate a hormone in one binding site, which efficiently engages SSTR2 and T cells enabling the formation of immune synapsis.