

ENHANCING THE ANTI-TUMOR EFFICACY OF BISPECIFIC T-CELL ENGAGERS VIA SIALIDASE FUSION

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Background New strategies exploiting the potential of immune cells to recognize and destroy cancer cells in a targeted manner have ushered in a new era of cancer treatment. Bispecific T cell engagers (BiTEs) are off-the-shelf agents that recruit endogenous T cells for eradicating tumor cells. Although five bispecific engagers have received FDA approval for treating B-cell malignancies, myeloma, and uveal melanoma, the promise of these engager molecules in treating solid tumors has proven challenging. In addition to the problem of limited tumor accessibility, BiTE-based therapies must overcome the immunosuppressive tumor microenvironment, where the suppression of effector cell functions is orchestrated by tumor cells and the neighboring stromal, myeloid, and lymphoid cells. One distinctive characteristic of cancer is aberrant glycosylation, wherein tumor cells often upregulate specific glycoforms featuring terminal sialic acid as 49lycol-immune checkpoints to suppress immune activation. Furthermore, the heavily sialylated glycocalyx acts as a physical barrier, impeding the effective infiltration of T cells and NK cells into the tumor site.¹ In light of this, we propose the incorporation of sialidase into BiTE molecules, resulting in BiTE-sialidase fusion proteins. This approach aims to selectively remove sialoglycans at the interface between T cells and tumor cells, thereby enhancing the ability of T cells to reach and engage target tumor cells. Consequently, T cell-mediated tumor cell cytolysis may be augmented, offering a potential avenue for the treatment of solid tumors.

Methods To test this hypothesis, we developed BiTE-sialidase fusion proteins by introducing a sialidase to either the N- or the C-terminus of BiTE molecules.

Results We observed that targeted desialylation induced by BiTE-sialidase results in improved T cell activation and cytokine production. As a result, BiTE-sialidase fusion proteins show remarkably enhanced efficacy in inducing T cell-dependent tumor cell cytolysis in response to target antigens compared to the parent BiTE molecules alone. We demonstrated that the enhanced tumor cell cytolysis is independent of the inhibitory sialoside-Siglec signaling but results from a stronger immunological synapse (IS) formation induced by BiTEs. In several preclinical models of blood and solid tumors that BiTE-sialidase fusion proteins exhibit superior efficacy in controlling tumor proliferation and prolonging survival *in vivo* in comparison to the parent BiTE molecules.

Conclusions In conclusion, we demonstrate that targeted desialylation benefits the BiTE-based therapy, which so far has only had limited success in the treatment of solid tumors. Our findings highlight BiTE-sialidase fusion proteins as promising candidates for the development of next-generation bispecific T-cell engaging molecules for cancer immunotherapy.

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REFERENCE

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