DEVELOPMENT AND ENGINEERING OF HUMAN SIALIDASE FOR DEGRADATION OF IMMUNOSUPPRESSIVE SIALOGLYCANS TO TREAT CANCER

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Background Sialoglycans, a type of glycans with a terminal sialic acid, have emerged as a critical glyco-immune checkpoint that impairs antitumor response by inhibiting innate and adaptive immunity. Upregulation of sialoglycans on tumors has been observed for decades and correlates with poor clinical outcomes across many tumor types. We previously showed that targeted desialylation of tumors using a bifunctional sialidase x antibody molecule, consisting of sialidase and a tumor-associated antigen (TAA)-targeting antibody, has led to robust single-agent efficacy in mouse tumor models. In addition to tumor cells, most immune cells present substantially more abundant sialoglycans than non-hematological healthy cells, which may also contribute to immunosuppression. Therefore, we studied the impact of immune cell desialylation and evaluated the therapeutic potential of a newly developed sialidase-Fc fusion (Bi-Sialidase), which lacks a TAA-targeting moiety and consists of engineered human neuraminidase 2 (Neu2) and human IgG1 Fc region, in preclinical mouse tumor models.

Methods The first generation Neu2 variant was further optimized to improve titers and stability to constructed Bi-Sialidase. Bi-Sialidase's desialylation potency and impact on immune responses were studied in vitro using various human immune functional assays, including T-cell activation, allogeneic mixed lymphocyte reaction, antibody-dependent cellular cytotoxicity, macrophages polarization/activation, neutrophil activation, and peripheral blood mononuclear cell (PBMC) cytokine release assays. We evaluated its antitumor efficacy in mouse tumor models. Bi-Sialidase's safety profile was characterized by conducting rat and non-human primate (NHP) toxicology studies.

Results The optimized Bi-Sialidase achieved a titer of 2.5 g/L from a 15-day fed-batch Chinese hamster ovary cell culture; in contrast, the wild-type and first-generation Neu2 had no production or a low titer (<0.1 g/L) under similar conditions, respectively. We demonstrated that Bi-Sialidase led to dose-dependent desialylation of immune cells and potentiated T-cell immunity, without impacting NK, macrophage, or neutrophil activation by desialylating immune cells. Activated and exhausted T cells upregulated surface sialoglycans and Bi-Sialidase-mediated desialylation reinvigorated exhausted-like T cells as measured by IFN γ production. Bi-Sialidase treatment also enhanced DC priming and activation of naïve T cells by desialylating both T cells and DCs. Furthermore, Bi-Sialidase showed single-agent antitumor activity in multiple mouse tumor models, including MC38, CT26, A20, and B16F10. Importantly, Bi-Sialidase did not cause cytokine release in human PBMC assays and was tolerated to up to 100 mg/kg in rats and NHPs, demonstrating a wide safety margin.

Conclusions Bi-Sialidase with an optimized Neu2 offers a novel immunomodulatory approach to enhancing T-cell immunity by desialylating immunosuppressive sialoglycans for cancer treatment.

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