Background CD73, a pivotal ectoenzyme responsible for the conversion of adenosine monophosphate (AMP) to adenosine, exhibits widespread expression on tumor cells and promotes accumulation of adenosine within the tumor microenvironment (TME). Adenosine acts as a potent immunomodulatory factor, effectively attenuating anti-tumor immune responses in the TME by binding to adenosine receptors expressed on diverse immune cell populations. Consequently, the elevated levels of adenosine pose significant barriers to the efficacy of various anti-cancer therapies, including chemotherapy, targeted therapy, and immunotherapy. Despite the considerable potential of interleukin-2 (IL-2) as an immunocytokine for stimulating cytotoxic lymphocytes, IL-2 demonstrates limited efficacy in overcoming immunosuppression induced by high levels of adenosine.

Methods We generated GI-108, a novel bifunctional fusion protein. The anti-CD73 antibody was selected from clones screened in the single-chain variable fragment (scFv) phage library based on its CD73 blocking efficacy. The IL-2 variant which lacks binding affinity to the IL-2Rα to minimize selectivity to regulatory T cells, was then fused to the anti-CD73 antibody.

Results GI-108 showed comparable binding affinity to human CD73 (hCD73) as Oleclumab, despite targeting different binding epitopes. Compared to Oleclumab, GI-108 demonstrated superior maximum efficacy in blocking both membrane-bound and soluble CD73 without inducing any hook effects. By attenuating its affinity to IL-2Rα, GI-108 selectively increased CD8+ T cells while minimizing its impact on regulatory T cells. In non-human primates, the administration of GI-108 led to a significant increase in cytotoxic lymphocytes. Similarly, a greater activation of the IL-2-STAT5 signaling axis by GI-108 was observed in CD8+ T cells than regulatory T cells. Importantly, the phosphorylation of STAT5 in CD8+ T cells by GI-108 was greater than that by wild-type IL-2, Proleukin. This observation was attributed to the binding of GI-108 to CD73-high CD8+ T cells in cis-binding manner, facilitating the transmission of a stronger signal to IL-2Rβγ. GI-108 effectively restored the proliferation or activity of CD8+ T cells in the presence of high levels of AMP. The reinvigorated cytolytic CD8+ T cells demonstrated sufficient cytotoxicity against CD73high tumor cells (MDA-MB-231). Administration of GI-108 to a humanized mouse bearing MDA-MB-231 induced significant inhibition of tumor growth.

Conclusions GI-108 is a potent bifunctional fusion protein with the ability to selectively target to the tumor site to reinvigorate immunosuppressed immune cells in the TME. As such, this innovative IL-2-based bifunctional agent provides a promising therapeutic strategy for the treatment of cancer.

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