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NOVEL PROTEASE ACTIVATABLE LINKER WITH TUMOR TARGETING MOTIFS ENHANCES THE RETENTION OF CYTOKINE PRODRUG AND ACTIVE CYTOKINE AT DISEASE SITE AND DEMONSTRATES IMPROVED EFFICACY IN PRECLINICAL MODEL

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Background An emerging class of new protease-activatable prodrugs designed to enhance on-target activity and reduce off-target toxicity are being actively developed. Cytokines are complex immune mediators which display potent anti-tumor activity in preclinical models and have delivered clinical responses in several advanced tumor types. However, clinical development of cytokine therapies has been hampered by high systemic toxicity, a narrow therapeutic index and short circulatory half-life. To address these shortcomings, we have developed next-generation cytokine therapies, On Demand Cytokines or ODCs.

Methods ODCs are protease-activatable cytokine prodrugs in which the cytokine is linked to an inhibitory moiety via a short proprietary peptide motif. These recombinant proteins are designed to exploit the protease activity present within the tumor microenvironment (TME) and enable the local release of active cytokine to trigger an anti-tumor immune response. ODCs contain tumor stroma targeting elements to further enhance their retention and activation within the malignant tissue. We have developed an array of stromaphilic ODCs, including a panel of IL-2 prodrugs containing single or dual tumor stroma binding motifs and report their preclinical in vitro and in vivo characterization.

Results All IL-2 prodrugs were successfully manufactured and activated in vitro by Matrix Metalloprotease cleavage which triggered the release of functional cytokine. Binding of prodrugs to tumor stroma components was confirmed in vitro. The ODC-IL2 panel was tested in vivo as single agent in the subcutaneous syngeneic B16F10 melanoma model. The uncleaved drugs were retained in the tumor at 5 to 20-fold higher levels than a control cytokine prodrug lacking any tumor targeting elements. Furthermore, intratumoral levels of IL-2 and IFN γ were increased 8 to 80-fold and 10 to 40-fold respectively compared to cytokine levels measured in the control non-targeted ODC treated arm. Finally, stromaphilic ODCs displayed substantially enhanced levels in circulation over non-targeted ODC. Superior anti-tumor efficacy was observed for all stroma targeting pro-cytokines with near complete tumor growth inhibition achieved with the dual targeting site construct.

Conclusions We have demonstrated that the On Demand Cytokine platform can generate protease-activatable cytokine prodrugs with enhanced tumor retention and on-target activity, to ultimately deliver safer and more effective immunotherapies.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.713>