1071 CHARACTERIZATION OF A TUMOR-TARGETING AND ACTIVATABLE T-MASK PLATFORM TO ENHANCE TUMOR ACCUMULATION AND TOLERABILITY OF POTENT IMMUNE MODULATORS

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Background The novel T-MASK (Targeted Metallo/protease Activated SuperKine) platform involves fusion of a dual tumor-targeting/masking domain to potent immune modulator (s) via a metallo-protease (MMP) sensitive linker (PSL) to achieve the following objectives: (1) reduce/fine-tune the potency of the immune modulator by steric hindrance to increase systemic tolerability and (2) promote retention in the tumor microenvironment (TME) to maximize MMP cleavage and restore full potency at the intended target site. As proof of concept, we selected as the tumor-targeting/masking domain an IL-13 superkine (MDNA213) with high selectivity and affinity for the IL-13 decoy receptor IL-13Ra2, a tumor associated antigen expressed in many aggressive solid tumors. MDNA213 is fused via a PSL to MDNA11 and MDNA223, both containing a not-alpha, beta-enhanced IL-2 fused with albumin or anti-PD1 antibody respectively. We present preliminary results on characterization of both T-MASK constructs demonstrating conditional fine-tuning of IL-2R agonism.

Methods T-MASK optimization included assessment of PSL linker and orientation of the tumor-targeting/masking domain. *In vitro* IL-2 and PD-1/PDL-1 reporter assays were performed to evaluate IL-2R stimulation and anti-PD1 blockade respectively. *In vitro* MMP assay was used to validate cleavage of T-MASK constructs and restoration of full potency.

Results T-MASK constructs showed approximately 10–40 fold reduction in potency compared to respective non-masked versions in IL-2R induced p-STAT5 reporter cell assay. Extent of fine-tuning can be adjusted by length and composition of PSL and orientation of the domains, providing versatility to the T-MASK platform and potential to engage the complete repertoire of peripherally circulating immune cells. In the case of MDNA223 (anti-PD1-IL-2^{Superkine}), fusion of MDNA213 to generate the T-MASK construct MDNA113 resulted in reduced IL-2R agonism but had no effect on potency of PD1/PDL-1 blockade as expected. Unmasking of MDNA113 by MMP-mediated cleavage fully restored its IL-2R signaling activity to the same level as the non-masked MDNA223 construct. Similar data were obtained with the masked version of MDNA11, demonstrating robustness of the T-MASK platform. *In vivo* studies are ongoing to evaluate the effect of T-MASK constructs on peripheral immune cell expansion (systemic response), tumor retention (maximize activation) and tumor growth inhibition (targeted response).

Conclusions MDNA113 is a novel T-MASK construct designed to increase tolerability while leveraging the synergy between PD1/PDL-1 blockade and IL-2R agonism for immunotherapy. Ongoing studies investigate alternative tumor-targeting/masking domains and immune modulators, including other cytokines and potent therapeutic agents, to potentially broaden the utility of the T-MASK platform.

Ethics Approval Animal studies are conducted in concordance with institutional guideline and approval.

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