A TRISPESIFIC ROR1 X CD3 T CELL ENGAGER MEDIATES IN VITRO TUMOR CELL KILLING AND IN VIVO TUMOR ERADICATION

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Background Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is expressed on a variety of difficult to treat solid and hematological malignancies. Several therapeutic concepts targeting ROR1 are currently in clinical studies, including antibody-drug conjugates (ADCs), chimeric antigen receptor engineered T cells, as well as a bispecific T cell engager. In contrast to ADCs, T cell engagers have the capacity to induce tumor cell depletion irrespective of tumor cell mitotic activity. For the therapy of ROR1 expressing tumors, we engineered a T cell engager with prolonged half-life to support convenient administration schemes.

Methods NM32-2668, a ROR1-targeting T cell engager with prolonged serum half-life was engineered by joining three humanized rabbit antibody variable region (Fv) fragments specific for ROR1, CD3 ζ , and serum albumin, into our tri-specific scMATCHTM3 format. Each Fv fragment was stabilized using the ζ -capTM technology. NM32-2668 was tested in assays for specific tumor lysis, induction of T cell proliferation, and cytokine release. These studies were performed using human T cells co-cultured with tumor cell lines and human tumor samples expressing various levels of ROR1. In vivo xenograft mouse studies were conducted using a human mantle cell lymphoma model in NCG mice engrafted with human PBMCs.

Results Here we report the design and the promising preclinical activity of the scMATCHTM3 ROR1/CD3/hSA T cell engager NM32-2668 in vitro and in vivo. Importantly, we demonstrate potent and specific cytotoxic activity in the sub-nanomolar range on tumor cell lines expressing different levels of ROR1. NM32-2668 also mediates ROR1 dependent T cell activation and cytokine release. We observe robust tumor cell killing activity of NM32-2668 over an extended time period and at multiple ratios of effectors to targets in a real time imaging-based cytotoxicity assay. This molecule also mediates T cell proliferation in response to target cell binding. NM32-2668 mediates in vitro lysis of CLL patient tumor cells, T cell activation, and cytokine release, with minimal IL-6 involvement. In an in vivo mantle cell lymphoma model (Jeko-1) engrafted with human PBMCs, we observe tumor regression and eradication.

Conclusions Collectively, these data demonstrate robust anti-tumor efficacy by NM32-2668, a scMATCHTM3 ROR1/CD3/hSA. Our results demonstrate that NM32-2668 promotes ROR1 dependent T cell activation and proliferation, as well as T cell-mediated tumor cell lysis. The activity of NM32-2668 has the potential to provide significant benefit to patients with ROR1+ malignancies on a convenient dosing schedule. We intend to rapidly progress NM32-2668 to clinical development.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.844>