substantially reduced and no cytotoxicity was observed. To further investigate the underlying mechanism, we analyzed tumor infiltrating lymphocytes (TIL) and as expected, we observed the increase in the number of CD8+ T cells in TIL, compared to control group. Interestingly, our tumor re-challenge result demonstrates that TMEkine (CD20-IL-12) protected animals from tumor recurrence implying that immunologic memory response was generated upon our TMEkine (CD20-IL-12) treatment.

Conclusions Altogether, our data suggest that TMEkine (CD20-IL-12) as an efficacious tumor targeting cytokine opening up a new avenue for the treatment of B-cell lymphoma.

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632 HPN601 IS A PROTEASE-ACTIVATED EPCAM-TARGETING T CELL ENGAGER WITH AN IMPROVED SAFETY PROFILE FOR THE TREATMENT OF SOLID TUMORS

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Background Epithelial cell adhesion molecule (EpCAM) is highly expressed in many solid tumors. However, therapeutics targeting EpCAM have had limited clinical utility or failed in clinical development likely due to the expression of EpCAM in normal tissues. For example, clinical testing of solitomab, an EpCAM-targeting T cell engager, resulted in severe dose-limiting toxicities, including elevated liver transaminases, hyperbilirubinemia, and diarrhea. Designing an EpCAM-targeting T cell engager that is only active in the tumor would expand its therapeutic window and improve its safety profile.

Methods Using a T cell engager prodrug platform named Pro-TriTAC that couples therapeutic half-life extension with functional masking, we have engineered HPN601, a protease-activated EpCAM-targeting T cell engager. HPN601 is a single polypeptide with three binding domains: anti-albumin for half-life extension, anti-CD3e for T cell engagement, and anti-EpCAM for tumor cell engagement. The anti-albumin domain contains a masking moiety and a protease-cleavable linker and keeps the molecule inert outside the tumor microenvironment. Activation by tumor-associated proteases removes the anti-albumin domain along with the masking moiety to reveal a potently active drug inside the tumor. This active drug has minimal activity outside of tumor because, without an albumin binding domain, it is rapidly cleared in circulation.

Results A humanized rodent tumor model was used to simultaneously measure anti-tumor efficacy and clinically relevant toxicity endpoints. In this model, a surrogate molecule of HPN601 was safely administered at a dose ten-fold higher than the minimal efficacious dose required for durable tumor regression. Higher doses produced toxicities including elevated ALT/AST and bilirubin, body weight loss, and evidence of tissue damage by histopathology. In contrast, a constitutively active EpCAM-targeting T cell engager could only be dosed safely up to its minimal efficacious dose. The improved safety profile of HPN601 is further supported by a toxicokinetic study in non-human primates: compared to a constitutively active EpCAM-targeting T cell engager, HPN601 had significantly attenuated cytokine production, including IFN-g, IL-2, IL-6, and IL-10.

Conclusions HPN601 is a conditionally active EpCAM-targeting T cell engager with a ten-fold improved therapeutic window compared to a constitutively active EpCAM-targeting T cell engager. An EpCAM-specific T cell engager with an improved safety profile could address unmet needs in many solid tumors and demonstrate the feasibility of using conditionally active T cell engagers to target more solid tumor antigens.

Ethics Approval The study was reviewed and approved by Harpoon’s Institutional Animal Care and Use Committee.

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633 DUAL-TARGETING OF 4-1BB AND OX40 WITH AN ADAPTIR™ BISPECIFIC ANTIBODY ENHANCES ANTITUMOR RESPONSES TO SOLID TUMOR

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Background 4-1BB (CD137) and OX40 (CD134) are critical activation-induced co-stimulatory receptors that regulate immune responses of activated T and NK cells by enhancing proliferation, cytokine production, survival, and cytolytic activity. A superagonist 4-1BB antibody has shown clinical activity but severe toxicities. APVO603, is a 4-1BB x OX40 targeting bispecific antibody with conditional agonism, activating these receptors only when both are co-engaged. The Fc portion was mutated to eliminate Fcy-R-mediated interactions. Co-stimulation through 4-1BB and OX40 has the potential to amplify the cytotoxic function and the number of activated T and NK cells in multiple solid tumor indications.1,2

Methods scFv binding domains to 4-1BB and OX40 were optimized to increase affinity, function and stability, and then incorporated into the ADAPTIR™ bispecific antibody platform to produce the APVO603 lead candidate. NF-kB/luciferase reporter cell lines expressing OX40 or 4-1BB were initially used to assess the agonistic function of APVO603’s binding domains. Primary PBMC were sub-optimally stimulated with an anti-CD3 antibody and T and NK cell proliferation was assessed using Cell TraceTM-labelled PBMC. Cytokine secretion was measured at 48 hrs using LumineX-based assays. For in vitro tumor lysis studies, PBMC were co-cultured with tumor cells expressing a tumor-associated antigen (TAA) and activated with TAA x CD3 bispecific protein. 7-AAD expression was assessed on tumor cells at 72 hrs. The in vivo therapeutic efficacy of APVO603 was evaluated using a murine MB49 bladder cancer model in human 4-1BB and OX40 double knock-in mice.

Results APVO603 stimulates 4-1BB and OX40 NF-kB/luciferase reporter activity in a dose-dependent manner, and is strictly dependent on engagement of the reciprocal receptor to elicit 4-1BB or OX40 activity. In primary PBMC assays, APVO603 induces synergistic proliferation of CD4+ , CD8+ T and NK cells when compared to OX40 or 4-1BB monospecific molecules with a wt Fc, either individually or in combination. Additionally, APVO603 enhances proinflammatory cytokine production and granzyme B expression, and augments in vitro tumor cell lysis induced by a TAAx CD3 engager. In vivo, APVO603 reduces growth of established MB49 tumors in human 4-1BB and OX40 double knock-in mice.

Conclusions APVO603 is a dual-agonistic bispecific antibody that augments the effector function of activated CD4+ and CD8+ T