HLA-A*02:01/p53 R175H pHLA complex with exquisite specificity. It effectively activated T cells and lysed tumor cells both in vitro and in vivo. This approach could in theory be used to target cancers containing mutations that are difficult to target in conventional ways.

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630 PD-L1/CD47 TUMOR DIRECTED B-BODYTM BISPECIFIC ANTIBODIES DEMONSTRATING SIGNIFICANT ANTI-TUMOR ACTIVITY WITH NO TOXICITY IN PRECLINICAL MODELS

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Background Tumor cells have been shown to utilize both innate and adaptive checkpoints to evade anti-tumor immune responses. CD47 and PD-L1 are two targets widely expressed on the cell surface of tumor cells and are predicted to coordinately suppress innate and adaptive sensing respectively to evade immune control. PD-L1 dampens T cell-mediated tumor killing (via PD-L1/PD-1 signaling) while CD47 protects tumor cells from phagocytosis (via CD47/SIRP-alpha signaling). Targeting each of the above pathways with monoclonal antibodies has shown promise with PD-L1/PD-1 inhibition showing durable responses and extended overall survival for several approved products, whereas the molecules targeting CD47 pathway are in early clinical trials. Given that a significant number of patients are either resistant or relapse on PD-L1/PD-1 therapy, combinations with anti-CD47 antibodies are being explored. However, the expression of CD47 on many normal cells such as hematopoietic cells, red blood cells (RBCs) and platelets provides a widespread antigen sink which impacts the PK and adverse event profile of these agents.

Methods Here, we describe the generation and testing of a large panel of bispecifics with combinations of different affinities to PD-L1 and CD47 using the B-Body™ bispecific screening platform. The bispecific antibodies were screened in various in vitro activity and developability assays. Selected leads from the screen were tested in multiple in vivo models with differential expression of CD47 and PD-L1.

Results The lead bispecific antibodies showed significant blockade of SIRPa/CD47 and PD-L1/PD-1 signaling in vitro and tumor growth inhibition in vivo. The studies also showed no significant binding to RBCs and induced minimal RBC phagocytosis in vitro. A summary of screened candidates and characterization of a lead candidate being developed further will be presented.

Conclusions We have identified multiple CD47/PD-L1 bispecific antibodies with favorable efficacy and safety profiles. Selection of a lead for further IND and clinical development is underway.

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631 DEVELOPMENT OF HIGHLY EFFICACIOUS AND SAFE TARGETED CANCER IMMUNOTHERAPY VIA IL12-BASED TMEKINE™ PLATFORM

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Background We developed tumor microenvironment-targeting immunocytokine or TMEkine™ utilizing strong anti-tumoral effect of interleukin 12 (IL-12). In this effort, we created a bi-specific 1+1 antibody fusion with conventional knob-in-hole technology where anti-CD20 was paired with IL-12 fc fusion arm. A couple of IL-12 muteins were used in our therapeutic molecules to reduce systemic toxicity. IL-12 has been known for a key orchestrator in immune response. The main actions of IL-12 include the induction of CD4+ Th0 cells toward Th1 type and enhancement of IFN-γ production, stimulation of cytotoxicity and growth of natural killer (NK) cells and CD8+ T cells. For these reasons, IL-12 has long been considered as a potential therapeutic molecule for treating cancers by enhancing immune activity toward tumor cells. However, systemic administration of IL-12 showed poor efficacy and severe adverse effects. With our therapeutic approach of tumor targeting and attenuated IL-12 mutein, we expect that our IL12-based TMEkine™ holds great promise for the future of cancer immunotherapy.In this study, we targeted CD-20 expressing cancers such as B-cell lymphoma with our anti-CD20/IL-12 mutein TMEkine. We evaluated the biological activity of our molecules with in vitro and in vivo efficacy and safety.

Methods The target specific binding to CD20 and IL-12 receptor was analyzed by FACS and ELISA. Biological activities as signaling transduction and T cell activation were confirmed in vitro using HEKblue IL12 cell line, primary human T cells and NK cells. The anti-tumor efficacy of TMEkine (CD20-IL-12) was assessed in A20 lymphoma syngeneic mouse model. To demonstrate long term protection to A20, the cured five mice after TMEkine administration were re-challenged with A20 and 4T1 cells.

Results First, we analyzed the specific binding of our TMEkine molecules to CD20 expressing B-cell lymphoma cell lines (such as Raji). We showed that TMEkine (CD20-IL-12) binds to Raji and Ramos, which express CD20, but not to Jurkat, which does not express CD20. We also showed that TMEkine molecules bind to IL-12 receptor in a dose-dependent manner. pSTAT4 alphaLISA assay revealed that TMEkine (CD20-IL-12) transduces STAT4 signaling. In our IL-12 mutein, key residues for heparin binding were mutated. The biological activity of our mutein molecule was attenuated due to this change in human PBMC. In addition, our TMEkine molecules significantly induced IFN-γ secretion from primary human T cells and NK cells. An A20 B-cell lymphoma syngeneic mouse model was utilized to investigate the anti-tumor activity of TMEkine (CD20-IL-12). TMEkine molecules were injected three times with Q3D intraperitoneally. Tumor growth was

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 ProTriTAC 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 ANTI-TUMOR 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 FcγR-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-κB/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-κB/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