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.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0631

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**Abstracts**

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.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0632

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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-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
A380


and NK cells in a dose-dependent manner, and reduces growth of established tumors in vivo. This preclinical data, demonstrates conditional dual stimulation of 4-1BB and OX40 and supports further development of APVO603, a promising immuno-oncology therapeutic with potential for benefit in solid tumors.

Ethics Approval Treatment of study animals was in accordance with conditions specified in the Guide for the Care and Use of Laboratory Animals, and the study protocol (ACUP 20) was approved by the Institutional Animal Care and Use Committee (IACUC).

REFERENCES


http://dx.doi.org/10.1136/jitc-2020-SITC2020.0633

634 PRODUCTION AND TESTING OF A NOVEL BSPECIFIC NANOBODY CONSTRUCT TARGETING NK CELLS AND EGFR EXPRESSING MALIGNANCIES

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Background The ability to kill tumor cells with an acceptable toxicity profile, makes Natural Killer (NK) cells promising assets for cancer therapy. However, strategies to enhance the preferential accumulation and activation of NK cells in the tumor microenvironment would likely increase the efficacy of NK cell-based therapies.

Methods In this study, we show a novel bispecific nanobody-based construct (biVHH) targeting both CD16A (low-affinity Fc receptor: FcRγIIIA) on NK cells and EGFR on tumors of epithelial origins.

Results Higher levels of NK cell activity and subsequent tumor cell lysis were found in vitro in the presence of the biVHH and were dependent on the expression of both CD16A and EGFR while they were independent of the KRAS mutational status of the tumor. Increased NK cell activity was found in NK cells derived from colorectal cancer (CRC) patients when co-cultured with the biVHH and EGFR expressing tumor cells. Finally, higher levels of cytotoxicity were found against patient-derived metastatic CRC cells in the presence of the biVHH and autologous peripheral blood mononuclear cells or allogeneic NK cells.

Conclusions Based on our results, the bispecific CD16A and EGFR targeting VHH construct could be a useful tool in combination with various NK cell-based therapies.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0634

635 A NOVEL SITE-DIRECTED CHEMICAL CONJUGATION TECHNOLOGY CONFERS ANTITUMOR ACTIVITY VIA NATIVE FC RECEPTOR TO PLASMA IMMUNOGLOBULIN BY ATTACHING TUMOR BINDERS


Background We describe KPMW101, which was created by chemical conjugation of a CD38-specific binder to clinical grade intravenous immunoglobulin (IvIg) pooled from healthy donors. Kleo’s MATE™ technology enables efficient site-directed chemical conjugation to ‘off-the-shelf’ IvIg and allows the development of antitumor agents with rapidly introduced target specificity. Our platform allows for chemical engineering of existing IvIg in a cost-efficient manner. This technology relies on synthetic compounds that consists of antibody binder with react-and-release mechanism.

Methods Design of synthetic chemical reagents included antibody binding group capable of covalent bond formation with specific lysine, CD38 binding moiety proven to work in our clinical candidate KP1237, and tunable non-cleavable linker. Conjugation efficiency to polyclonal IvIg was evaluated using LC-MS analysis of IdeZ-digests. The binding of CD38, CD16a, and FcRn were determined by ELISA and BLP for in vitro ADCC assays, PBMCs provided NK effector function. Daudi (CD38+) B lymphoblast cells were treated with KPMW101 or IvIg, PBMCs were introduced and incubated for 18h, and target cellular death was measured. For an in vivo IP macrophage lavage model of ADCP, SCID mice were implanted IP with CFSE-labeled Daudi cells. Mice were injected with IvIg or KPMW101 (0.21, 0.625, 1.875 mg/kg) SQ, and tumor cell counts were measured by flow cytometry. The pharmacokinetic profile of in vivo KPMW101 was determined from blood and analyzed utilizing a human Ig isotyping array.

Results Synthetic chemical reagents with multiple linker types have been conjugated to IvIg and evaluated in biochemical assays. KPMW101 showed the highest conjugation efficiency. Binding affinity of KPMW101 to CD38 was 27nM. ELISA results show KPMW101 binds to CD16a and FcRn, indicating that conjugation does not interfere with FcR binding. In vitro ADCC results demonstrate that KPMW101 elicited CD38+ target cell killing with an EC50 of 0.91–2.09nM. In vivo studies showed that KPMW101 resulted in a 49.9–63.5% reduction of tumor cells. Pharmacokinetic profile showed stability of KPMW101 throughout the 144-hour study, whereby IgG1, IgG2, IgG3, and IgG4 isotypes were detectable.

Conclusions KPMW101 is created by chemical conjugation of CD38-specific binder to IvIg using our proprietary MATE™ technology, maintaining native binding to FcRs via the Fc domain. This ensures the stability of the molecule and retains immune-mediated mechanisms of action. KPMW101 induces IvIg to adopt Fc effector mechanisms like ADCC and ADCP. Our in vitro data and in vivo studies confirm KPMW101 ability to kill tumor cells, making IvIg into an active antitumor therapeutic agent.

http://dx.doi.org/10.1136/jitc-2020-SITC2020.0635

Immunotherapy toxicities

637 IMMUNE-RELATED ADVERSE EVENTS (IRAEs) MAY INDICATE A FAVORABLE PROGNOSIS IN METASTATIC RENAL CELL CARCINOMA (M RCC) PATIENTS TREATED WITH IMMUNE CHECKPOINT INHIBITORS (ICI)

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Background Immune checkpoint inhibitors (ICI) have become an increasingly utilized treatment in metastatic renal