sparking ongoing clinical trials, including those targeting TIGIT in combination with anti-PD-1 or anti-PD-L1. The INTASYL[™] platform is a self-delivering RNAi technology that (1) provides efficient delivery into target cells bypassing the need for specialized formulations, mechanical perturbation, or drug delivery systems; and (2) specifically and durably silence target gene expression when administered intratumorally (IT), providing in vivo tumor control. IT administration restricts pharmacokinetics to the tumor; an attractive strategy for mitigating ICB-mediated systemic irAEs. Additionally, using INTA-SYL, multiple targets can be silenced in combination. Here we demonstrate the in vivo efficacy of INTASYL specifically targeting TIGIT (PH-804), PD-1 (PH-762), PD-L1 (PH-790) alone or in combination in a CT26 model of murine colorectal carcinoma.

Methods To assess silencing activity, activated human pan-T cells were incubated in vitro with INTASYL compounds either alone or in combination and mRNA silencing was determined by qRT-PCR and protein silencing by flow cytometry. To assess in vivo tumor efficacy CT-26 cells were implanted subcutaneously into BALB/c mice. INTASYL compounds were administered IT at 1 mg/dose on Days 1, 3, 7, and 10 either as single agents (mPH-804, mPH-762, mPH-790) or in combination (mPH-804 + mPH-762 or mPH-804 + mPH-790). Controls consisted of PBS (vehicle; (IT)), and anti-TIGIT, anti-PD-1, or anti-PD-L1 antibodies (0.2 mg/dose) administered via intraperitoneal injection (IP). Tumor volumes and body weight were recorded throughout the study. Tumors were taken at the end of the study for analysis.

Results Single and combination knockdown of target molecules was validated at the mRNA level (=90%) by qRT-PCR and at the protein level (=80%) in activated human pan-T cells. In vivo, combination treatment with mPH-804 + mPH-762 or mPH-790 improved tumor control compared to individual monotherapies providing evidence of potential synergy. All treatments were well tolerated.

Conclusions n/a

Acknowledgements We demonstrate the potential of INTASYLmediated combination therapy targeting TIGIT and PD-1/PD-L1. These findings indicate that combination of TIGIT + PD-1/PD-L1 silencing improves tumor control compared to monotherapy. As INTASYL IT is efficacious and may mitigate irAEs caused by antibody ICB, INTASYL combination therapies including PH-804, PH-762 and PH-790 warrant further investigation in patients.

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199 ENHANCED IMMUNE RESPONSES IN HUMAN BREAST AND COLON CANCER FOLLOWING CHECKPOINT THERAPY IN A CD34⁺ STEM CELL HUMANIZED NCG (HUCD34NCG) MOUSE MODEL

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Background Breast and colon cancer rank second and third, respectively, in world-wide prevalence of malignancies and present a large unmet medical need. The correlation between lymphocyte infiltration into the tumor microenvironment and efficacy of anti-cancer immunotherapies has been established. Therefore, relevant and cost-saving pre-clinical models are needed for developing new treatment approaches to predominant human tumor types. HuCD34NCG mice facilitate

studying human immune responses in vivo elicited by experimental therapeutic antibodies. We characterized growth kinetics and human immune responses to checkpoint blockade in human breast and colon tumor-bearing HuCD34NCG mice. Aging, non tumor-bearing HuCD34NCG mice were also monitored for indicators of spontaneous hematopoietic cancer formation.

Methods HSC engraftment was quality controlled prior to inoculating HuCD34NCG mice with either colon adenocarcinoma (COLO 205) or triple negative breast cancer (MDA-MB-436) cells (both purchased from American Type Culture Collection, Manassas, VA). Mice were randomized into treatment groups based on tumor size, and checkpoint inhibitor antibodies were dosed twice weekly (anti-human PD-1, Bio-Xcell clone: RMP1-14 or Keytruda; anti-human CTLA-4, Bio-Xcell clone: BN13; and combination therapy). Body weights, general health status and survival were monitored. Peripheral blood (PB) and selected tissues were analyzed for the presence and composition of human immune cells by acoustic focusing flow cytometry. Non tumor-bearing aged HuCD34NCG mice (27 weeks post-engraftment) were sampled biweekly over ten weeks for lymphoma immunophenotyping.

Results Both tumor-bearing models showed significant antihPD-1 and anti-hCTLA-4 responses, but combination therapy only enhanced growth reduction significantly in MDA-MB-436 tumors. Flow cytometric analysis identified viable human leukocytes in tumor and spleen at study termination. These tumor-infiltrating lymphocytes (TIL) and splenocytes from surviving COLO 205 and MDA-MB-436 mice consisted of a total T-cell phenotype (CD3⁺) with proliferating (Ki67⁺), CD4⁺, CD8⁺ and Treg subsets. Additionally, myeloid cells (CD11b⁺, CD11c⁺) and M1/M2 macrophages were detected within these infiltrates. Splenic and tumor-infiltrating T-cells readily secreted human cytokines (IFN- γ , IL-2, TNF- α) and granzyme B upon ex vivo activation exhibiting polyfunctional and cytotoxic capabilities in all treatment groups. Baseline murine and human cytokine levels were distinguished in plasma from aging, non tumor-bearing HuCD34NCGs. Their phenotypes also showed no conclusive indicators of abnormal blood cells developing or graft failure.

Conclusions Breast and colon tumor cell-line derived models were established in HuCD34NCG mice. Standard checkpoint inhibitor treatment promoted human T-cell infiltration into tumor microenvironments inhibiting growth. These results demonstrate that HuCD34NCG are a robust and relevant host for various human cell xenotransplants to advance preclinical immuno-oncology drug development.

Ethics Approval Animal studies were executed in compliance with local Charles River IACUC guidelines, IACUC number I-033.

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200 NBTXR3 NANOPARTICLE WITH IMMUNORADIATION IMPROVES SURVIVAL AND GENERATES LONG-TERM ANTI-TUMOR MEMORY IN AN ANTI-PD1 RESISTANT MURINE LUNG CANCER MODEL

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Background Although treatment with high-dose (HD) radiation (XRT) and NBTXR3 on primary tumors in combination with

systemic anti-PD1 was able to significantly improve abscopal effect in 344SQR murine metastatic lung cancer model, most of the mice eventually died due to the growth of secondary tumors. Therefore, we intended to use HD-XRT plus NBTXR3 injection into primary tumors and low-dose (LD) radiation on secondary tumors plus dual-agent immunotherapy (IT) of anti-PD1 and anti-CTLA-4 to acheive complete control of both the primary and secondary tumors in mice.

Methods Five groups of 8 mice each were inoculated subcutaneously with 5 \times 104 anti-PD1-resistant 344SQR cells in each hind leg, 3 days apart, to establish 'primary' (right) and 'secondary' (left) tumors. All mice in treatment groups received intraperitoneal anti-PD1 and anti-CTLA-4 on days 4, 7, 10, and 13, and continuing anti-PD1 treatment on days 20, 27, 34, 41, and 49 and 12Gy x3 (HD-XRT) to the primary tumors on days 7, 8 and 9. Primary tumors in groups 3 and 5 also received intratumoral NBTXR3 on day 6. Secondary tumors in groups 4 and 5 were also irradiated with 1Gyx2 (LD-XRT) on days 12 and 13. Experimental groups were designated as 1=Control, 2=HD+IT, 3=NBTXR3+HD+IT, 4=HD+LD+IT, and 5=NBTXR3+HD+LD+IT. The secondary tumors were analyzed by flow cytometry and Nanostring. On day 178, the survivor mice were rechallenged with 5 \times 104 344SQR cells on the right flank and the tumor growth was monitored for an additional 36 days.

Results All mice in all the groups except NBTXR3+HD+LD +IT died due to the growth of either the primary tumor or the secondary tumor by day 36. Both the primary and the secondary tumors in 4 mice of NBTXR3+HD+LD+IT group were completely eliminated. No tumor growth was observed in these mice after rechallenged with 344SQR cells. Flow cytometry data demonstrated that only the mice in the groups with NBTXR3 had significantly more CD8+ T cell infiltration in the secondary tumor collected on day 16 than the control. Both flow cytometry and Nanostring data showed that only the mice in NBTXR3+HD+LD+IT had a significantly higher CD8+ Tcell/Treg cell ratio than the control.

Conclusions The combination of NBTXR3 plus high and low dose radiation with immunotherapy effectively controlled the growth of both primary and secondary tumors, significantly extended the survival, generating long-term antitumor memory. This combination therapy induced immune-mediated control of the secondary tumor at both genetic and cellular levels.

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201 CARBOPLATIN, PACLITAXEL AND PEMBROLIZUMAB FOR THE FIRST LINE TREATMENT OF RECURRENT AND/OR METASTATIC HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Background A recent phase III study (Keynote-048) demonstrated survival benefit of platinum, 5-FU and pembrolizumab in 1st line treatment of recurrent and/or metastatic head and neck squamous cell carcinoma (RM-HNSCC).¹ However, administration of 5-FU has been a challenge for logistics and toxicities. As platinum, paclitaxel and pembrolizumab has been shown to be an effective and safe treatment for non-small cell lung cancer,² we hypothesized that carboplatin, paclitaxel and pembrolizumab would be safe and effective for 1st line treatment of RM-HNSCC.

Methods We performed a retrospective study of RM-HNSCC patients who received carboplatin, paclitaxel and pembrolizumab for first line systemic therapy, treated between December 2015 and January 2020 at the University of California, San Francisco. Patients who received at least 1 cycle of treatment with pre-treatment and post-treatment images were included in the analyses. Response to the treatment was assessed using RECIST criteria version 1.1. We also estimated overall survival and progression free survival using Kaplan-Meier method.

Results Nine patients who received carboplatin, paclitaxel and pembrolizumab as first line systemic therapy for RM-HNSCC were identified. Two patients had HPV positive oropharyngeal SCC, the other patients unknown primary SCC in head and neck (2), oral cavity SCC (2), laryngeal SCC (1), hypopharyngeal SCC (1) and SCC of orbit (1). There were 1 complete response (CR, 11%), 6 partial responses (PR, 55%), 1 stable disease (SD, 11%) and 1 progressive disease (PD, 11%). Overall response rate (ORR) was 78%, and median progression free survival and median overall survival have not reached with median follow-ups of 6 months and 8 months, respectively. Two patients discontinued chemotherapy after 1 cycle for grade 4 acute kidney injury and grade 4 anaphylaxis, yet achieved CR and PR, respectively. **Conclusions** The retrospective analysis suggests that first line carboplatin, paclitaxel and pembrolizumab for RM-HNSCC is an active regimen and can be considered in place of platinum.

an active regimen and can be considered in place of platinum, 5-FU and pembrolizumab, which merits further investigation. Ethics Approval The study was approved by UCSF's Institutional Review Board, approval number 19-29365.

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202 IN VITRO AND IN VIVO COMBINATION THERAPY OF LOW MOLECULAR WEIGHT HEPARINS, CHEMOTHERAPY AND IMMUNOTHERAPY, INDUCE ANTITUMOR ACTIVITY IN PANCREATIC CANCER

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Background PDAC is recognized as a highly thrombogenic tumor; thus, low-molecular-weight heparin (LMWH) is routinely used for PDAC patients. Based on the combinatorial therapy approach to treating highly malignant and refractory cancers such as PDAC, we hypothesized that LMWHs could augment the effectiveness of immune checkpoint inhibitors and induce an efficient antitumoral activity.¹