Results Systematic structure-activity relationship (SAR) investigation identified novel EP2 and EP4 dual antagonists. The most promising compound KT-00113 possesses high potency against both EP2 and EP4, while maintaining high selectivity over other prostanoid receptors. In vitro and in vivo ADMET studies show that KT-00113 has a favorable profile, apt for further examination in in vivo cancer models and immune cell function in tumors.

Conclusions KT-00113, a highly potent and selective EP2/4 dual antagonist has strong potential to become the best-in-class immune suppression lifting cancer immunotherapy and may be suitable for further development in a clinical setting.

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708 APPLICATION OF A NOVEL MSENS DRUG DELIVERY TECHNOLOGY FOR MRNA THERAPEUTICS

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Background Successful clinical translation of mRNA therapeutics requires an appropriate delivery strategy to overcome instability of mRNA and facilitate cellular uptake into the cells.[1] Several lipid based nanoparticle approaches that encapsulate mRNA, notably lipid nanoparticle (LNP), have been developed, but their efficiency for delivery to certain target tissues and toxicity profiles still have room for improvement. The application of a novel polymer based nanoparticle technology platform, so called Stability Enhanced Nano Shells (SENS) for mRNA (mSENS) as a mRNA delivery platform for a cancer vaccine was demonstrated.

Methods The physicochemical properties of mSENS formulation, particle size and encapsulation efficiency, were characterized using dynamic light scattering (DLS) and gel retardation assay. Using luciferase-encoding mRNA, the protein expression levels in vitro and in vivo were evaluated by luciferase assay or bioluminescence imaging (BLI), respectively. For cancer vaccine studies, antigen (tyrosinase-related protein 2 (Trp-2))-specific T cell responses were assessed by immunophenotyping mouse splenocytes using flow cytometry and by the enzyme-linked immunosorbent spot (ELISPOT) assay. The anti-tumor efficacy was studied in B16F10 lung tumor model in C57BL/6 mice. Liver and systemic toxicity of mSENS treated mice was evaluated through blood chemistry and complete blood count (CBC) tests.

Results A library of mSENS formulations complexed with luciferase-encoding mRNA, were characterized for their particle size, surface charge, encapsulation efficiency, colloidal stability, and in vitro and in vivo luciferase protein expression level. Upon systemic administration in mice, varying biodistribution profiles were observed, implicating the potential for tailored delivery to target tissues. Particularly, cancer vaccine application was further developed leveraging the formulation with preferential spleen delivery. Following vaccination with Trp-2 mRNA encapsulated with mSENS (Trp-2 mRNA-mSENS) in B16F10 tumor bearing mice, strong Trp-2 antigen-specific IFN-γ T-cell responses were observed. Generated anti-tumor immunity also marked suppression of B16F10 lung tumors were observed in Trp-2-mSENS immunized mice compared to non-immunized controls, demonstrating the potential of mSENS as a mRNA delivery platform for the application for vaccine.

Conclusions Proprietary biodegradable polymer based-mSENS platform offers an attractive delivery strategy for mRNA by tailoring to specific therapeutic applications. Depending on the application, whether it's a vaccine or protein replacement, a rationally designed mSENS formulation can efficiently distribute mRNA to specific tissues. In particular, application of a splenic mSENS formulation for a cancer vaccine has been demonstrated in murine tumor model. In summary, mRNA delivery through mSENS platform is expected to provide significant opportunities in clinical development for mRNA therapeutics.

Ethics Approval The study was approved by Samyang Biopharmaceuticals' IACUC (Institutional Animal Care and Use Committee), approval number SYAU-2027.

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709 EXOSOME SURFACE DISPLAY OF IL-12 RESULTS IN TUMOR-RETAINED PHARMACOLOGY WITH SUPERIOR POTENCY AND LIMITED SYSTEMIC EXPOSURE

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Background The promise of Interleukin-12 as a cancer treatment has yet to be fulfilled with multiple tested approaches being limited by unwanted systemic exposure and unpredictable pharmacology. To address these limitations, we generated exoIL-12™, a novel, engineered-exosome therapeutic that displays functional IL-12 on the surface of an exosome.

Methods IL-12 exosomal surface expression was achieved via fusion to the abundant exosomal surface protein PTGFRN. Potency was assessed in vitro using human PBMCs or murine splenocytes and in vivo using mouse subcutaneous tumor models. Local versus systemic pharmacology was determined with intratumoral injection in mice and subcutaneous injection in monkeys. All studies were benchmarked against recombinant IL-12 (rIL-12).

Results Exosomes engineered to express either murine or human IL-12 had equivalent potency in vitro to rIL-12 as

demonstrated by IFNγ production. Following intratumoral injection, exoIL-12 exhibited prolonged tumor retention and greater antitumor activity than rIL-12. Moreover, exoIL-12 was 100-fold more potent than rIL-12 in tumor growth inhibition. In the MC38 tumor model, complete responses were observed in 63% of mice treated with exoIL-12; in contrast, rIL-12 resulted in 0% complete responses at an equivalent IL-12 dose. This correlated with dose-dependent increases in tumor antigen-specific CD8+ T cells. Re-challenge studies of exoIL-12 in complete responder mice showed no tumor regrowth. Moreover, depletion of CD8+ T cells completely abrogated the antitumor activity of exoIL-12. Following intratumoral administration, exoIL-12 exhibited 10-fold higher intratumoral exposure than rIL-12 and prolonged IFNγ production up to 48 hr. Retained, local pharmacology of exoIL-12 was further confirmed using subcutaneous injections in non-human primates.

Conclusions This work demonstrates that tumor-restricted pharmacology of exoIL-12 results in superior in vivo efficacy and immune memory without systemic IL-12 exposure and related toxicity. exoIL-12 is a novel cancer therapeutic candidate that has the potential to overcome key limitations of rIL-12 and thereby create a therapeutic window for this potent cytokine.

Ethics Approval All animals were maintained and treated at the animal care facility of Codiak Biosciences in accordance with the regulations and guidelines of the Institutional Animal Care and Use Committee (CB2017-001).

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710 DEVELOPMENT OF IL-33 AS A NOVEL IMMUNOTHERAPY OF CANCER

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Background Immune-checkpoint-blockade (ICB) therapy has produced unprecedented survival benefits for cancer patients but such therapy has been limited by low response rates in most cancer. One major obstacle for ICB therapy is the reduced immunogenicity of tumor tissues due to genetically driven down-regulation of epithelial tissue cytokines. IL-33 is a member of the IL-1 gene family and its level is downregulated in many advanced carcinomas such as lung cancer, breast cancer and pancreatic cancer. It has recently been shown that IL-33 plays an important role in mediating cancer immune therapy. In addition, transgenic expression of the active form of IL-33 in tumor cells or administration of the recombinant IL-33 exerts strong antitumor effects. Mechanistically, IL-33 enhances the function of Th1 and CD8+ T cells in vitro and types 1 antitumor immune responses in vivo.

Methods In the current study, we have optimized the pharmacodynamics of IL-33 by engineering a fusion protein, called anti-HSA-IL-33, using IL-33 and an anti-human albumin antibody. We have used preclinical mouse tumor models to determine the efficacy and toxicity of this new molecule.

Results We have shown that anti-HSA-IL-33 has excellent antitumor activities alone and enhances the antitumor function of PD-1 mAbs. Despite causing increased inflammation, anti-HSA-IL-33 is well tolerated with limited toxicity in mice.

Conclusions These studies support further development of IL-33 as a novel cancer immunotherapy.

Ethics Approval The animal experiments have been approved by IACUC of University of Pittsburgh.

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711 HBM1022, A NOVEL ANTI-CCR8 ANTIBODY DEPLETES TUMOR-INFILTRATING REGULATORY T CELLS VIA ENHANCED ADCC ACTIVITY, MEDIATES POTENT ANTI-TUMOR ACTIVITY WITH KEYTRUDA

Shuang Lu, Shaoping Hu, Xin Gan, Yongqiang Wang, Chuchu Zhao, Yi Ding, Jinqiu He, Qing Du, Xiaocheng Lv, Beibei Qin, Yun He, Lei Niu, Yuntao Wu, Fei Chen, Yuandong Wang, Yunxing Yang, Yang Cao, Musheng Bao, Jason Noon, Wenhao Yu, Juan Liu, Joe Zhao, Yiping Rong, Shuang Lu*. *Harbour Biomed, Shanghai, China*

Background CCR8-expressing CD4 and Foxp3 positive Treg (CCR8+ Treg) has been demonstrated to be a major driver for immunosuppression in solid tumors1 <1> Superscript. Clinical studies have shown that CCR8 is selectively up-regulated by tumor resident Tregs in several tumor types including clear cell renal cell carcinoma (ccRCC)2 <2> Superscript and breast cancer3 <3> Superscript. In these tumor types, CCR8 exhibit strong expression on tumor resident Tregs while it is rarely observed on Tregs in peripheral blood mononuclear cells (PBMCs). High expression of the CCR8 in tumor-infiltrating lymphocytes-Treg cells (TIL-Tregs) was associated with poor prognosis in breast cancer patients. These results suggest CCR8 as a promising therapeutic target; and anti-CCR8 mAbs could selectively inhibit a subpopulation of tumor resident Tregs in the tumor microenvironment (TME), to augment antitumor immunity.

Methods In vitro assay:HBM1022 binding on human, cynomolgus CCR8 and TIL-Tregs are evaluated via flow cytometry. Blocking and ADCC functional assay are all based on CCR8 overexpressing cell lines.In vivo efficacy study:HBM1022 anti-CCR8 antibody was administered after implantation (≈ 100 mm^3 <3>Superscript tumor volume) alone and in combination with anti−PD-1.

Results Anti-CCR8 antibody HBM1022 specifically binds to cell lines that over-express human or cynomolgus CCR8, as well as TIL-Tregs in multiple cancer types with the high affinity. HBM1022 potently blocks CCL1 binding to both human and cynomolgus CCR8. HBM1022 inhibits CCL1-induced migration and related GPCR signaling pathways. Furthermore, with enhanced antibody-dependent cell-mediated cytotoxicity (eADCC) activity, HBM1022 exhibits potent in vitro killing activity on CCR8-expressing cells. HBM1022 shows tumor growth inhibition as monotherapy in preclinical mouse syngeneic and humanized models. Moreover, HBM1022 shows enhanced antitumor activity with the combination of Keytruda® in preclinical efficacy models.

Conclusions Our finding reveals HBM1022 as an innovative immunotherapy targeting intra-tumoral suppressive Treg cells to change suppressive tumor to hot tumor. HBM1022 presents its great potential as exciting mono or combo anti-tumor therapies.

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