strategy to mitigate DRG toxicity in addition to using a local ROA. Additional AAV, ROA, and procedure experiments and analyses are ongoing to evaluate the risk-benefit profile of local pancreatic gene therapy for metabolic disease.

313 Preclinical Safety Assessment of an Investigational Gene Replacement Therapy for the Treatment of *RPE65*-Mediated Inherited Retinal Dystrophies

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Background: Inherited retinal dystrophies (IRD) are a group of progressive blinding genetic diseases caused by mutations in any one of the over 250 causative genes. An estimated 1000-2000 people in the United States have the IRD disease caused by the mutations in *RPE65* gene, which encodes all-*trans* retinyl ester isomerase, an enzyme critical to the visual cycle. We developed an investigational gene therapy product, HG004, containing AAV-mediated human retinal pigment epithelium 65kDA protein (AAV-*hRPE65*) gene. Here we reported the results of its preclinical safety assessment study after injection in non-human primates (NHPs). **Methods:** HG004 was assessed in a 3-month good laboratory practice (GLP) toxicology study in NHPs after bilateral subretinal injection at three dose levels. Clinical observation, ophthalmoscopic examination (including optical coherence tomography, intraocular pressure, fundus photography, fundus fluorescein angiography, electroretinogram, and etc.), detection of vector shedding and immune response assessment were conducted during the study period. Histopathology and vector biodistribution in tissues were evaluated at 4-week and 13-week termination as well. **Results:** In terms of pharmacokinetic properties, HG004 was mainly distributed in the retina tissues, especially in the retina and choroid (above 10^6 copies/μg) (Fig.1a). The copy numbers of DNA and mRNA in other tissues were much lower than those seen in the retina tissues. No HG004 distribution was detected in the reproductive system on D92 (Fig.1a), and HG004 basically no longer entered the environment with the excretion 1-week post-injection (Fig.1b). Additionally, there were no drug-related systemic toxicity of HG004 observed after 4-week and 13-week post-injection. The adverse reactions (such as retinal injury at the site of needle entry, vitreous inflammatory cell exudation, and locally relevant changes in the area covered by the drug) mainly occurred in the retinas of both vehicle and tested group of monkeys, suggesting that these events were mainly caused by the injection procedure. Finally, the results of immunotoxicity assessment showed that there were no drug-related abnormalities found in whole blood samples of monkeys after injection, and no AAV or RPE65 antigen-specific T cells immune response was detected. **Conclusions:** Overall, HG004 was generally well tolerated, and there were no systemic toxicity reported. Therefore, HG004 is relatively safe for subretinal injection as a potential treatment of inherited retinal dystrophies due to *RPE65* dysfunction. The results of this China-manufactured gene therapy study for *RPE65*-associated inherited retinal dystrophies support the recent investigational new drug (IND) clearance from FDA in January 2023.

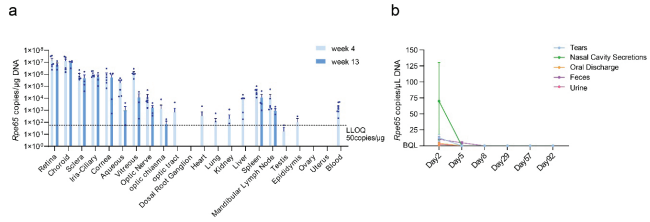

Figure 1. Biodistribution and vector shedding of HG004 in NHPs

314 *In Vitro* Toolbox to Investigate AAV-Induced Immune-Associated Hepatotoxicity

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Adeno-associated virus (AAV) vectors have emerged as promising in vivo gene delivery tools for gene therapy. They are mostly non-integrative, are able to transduce various tissues and have a low immunogenic profile. Despite these advantages, there are still reports of severe immune-associated hepatotoxicity after AAV administration in clinical trials. The mechanism behind this liver immune response is still not clearly understood. Here, we aimed to investigate the effect of AAVs on liver cells and to better understand which cell populations and by which mechanisms they may elicit a hepatic immune response. We used human liver in vitro models: HepaRG (hepatocyte cell line), hTERT-HSC (hepatic stellate cell line) and THP-1 (macrophage cell line, used as a Kupffer cell surrogate) that were exposed to different AAV-GFP serotypes (AAV2,3,8 & 9) and titers (MOI 10^3 - 10^6). Transduction efficiency was investigated by PCR, fluorescence microscopy and image quantification. Cellular responses were studied using cell viability and gene expression assays, immunofluorescent staining, and multiplex cytokine panel screens. Titer- and serotype-dependent decreases in cell viability were observed in HepaRG and hTERT-HSC but not in THP-1. Hepatic cells could be transduced using AAV, as the vector genome (GFP gene) could be detected in all three cell lines transduced with all four tested serotypes. However, protein expression of GFP was cell and serotype dependent. At a MOI of 10^6, AAV2 was able to transduce all three tested cell lines, whereas AAV3, 8 and 9 were able to transduce only HepaRG and THP-1. Transduction also elicited varied cellular responses: AAV2 increased αSMA gene and protein expression and decreased TGF-β gene expression in hTERT-HSC suggesting a direct activation of HSC. THP-1 appeared also activated by AAV2 and AAV8 as indicated by increased gene expression of CD80, CD206, HMOX and CXCL10 (IP-10). Immunostimulatory potential of AAV2 and AAV8 on hepatic cells was also suggested by the increased secretion of various pro-inflammatory cytokines and chemokines in all three cell lines, with IL-6 levels strongly increased in HepaRG and THP-1. Based on these studies in liver cell surrogates, an immune response to AAV-mediated liver gene therapy may be potentially driven by the activation of hepatic stellate cells and Kupffer cells, and increased secretion of

pro-inflammatory cytokines and chemokines by parenchymal and non-parenchymal cells. Further studies will focus on more complex 3D human in vitro models to gain insight into AAV-induced immune-associated hepatotoxicity and eventually help improving the safety of AAV vectors in gene therapy.

315 Investigating the Impact of Empty AAV Capsids on Safety and Efficacy Following Intracisterna Magna Administration in New Zealand White Rabbits

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Recent reports of treatment-emergent serious adverse events from recombinant AAV-mediated gene therapy have highlighted the need to better understand the effect of quality attributes of the AAV material on safety and efficacy outcomes. A particularly important topic which has been receiving a lot of attention is the impact of empty AAV capsids. Empty AAV capsids are process-related impurities generated during AAV production which lack the transgene DNA that is present in "full" AAV particles. There are significant gaps in understanding how the presence of empty capsids impacts safety and efficacy of AAV preparations. Additionally, there are no definitive regulatory guidelines on acceptable levels of empty capsids. Route of administration (ROA), dose, target, and indication can all potentially influence the impact of empty capsids, further increasing the complexity of this issue. To support an internal therapeutic program and using a rabbit model, we investigated the potential impact empty capsids may have on AAV performance *in vivo*. New Zealand white rabbits were dosed with 1.0E+13 genome copies of an AAV vector by intracisterna magna (ICM) injection. This AAV vector expresses a small RNA under the control of ubiquitous promoter and knocks down expression of human superoxide dismutase 1 (SOD1). Study materials were prepared by transient transfection in HEK293 cells followed by affinity chromatography purification. Cesium chloride (CsCl) gradient ultracentrifugation was then applied to isolate and enrich full and empty capsid populations. Target percentage full:empty ratios were achieved by combining these enriched populations and confirmed through analytical characterization. Rabbit study groups (n = 3 per group) were administered vehicle control, enriched full (1.0E+13 viral particles), 75% full and 25% empty (1.33E+13 viral particles), 50% full and 50% empty (2.0E+13 viral particles), and enriched empty capsids (1.0E+13 viral particles). The three groups which were administered full capsids all received the same genome copy dose. Findings were compared across groups to assess both transduction and safety endpoints. In-life observations, clinical chemistry, and hematology analyses showed no significant differences across study groups. Additionally, blood and cerebrospinal fluid (CSF) analysis of neurofilament (NF), a biomarker for neurotoxicity, showed elevations in study groups which received any percentage of full capsids, but no difference across these groups. Conversely, the vehicle control group and the group which received enriched empty capsids saw no elevations in NF. Biodistribution, transgene expression, and histopathology analyses are on-going. These results demonstrate that ICM co-injection of up to 1.0E+13 empty AAV capsid particles to rabbits does not significantly impact common safety endpoints. We will continue to evaluate the potential impact of empty capsids on transduction and microscopic pathology.

316 Intraparenchymal Dosing in Beagle Dogs Using Convection Enhanced Delivery Guided by Real Time MRI

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In the clinic, convection-enhanced delivery (CED) is routinely used in patients for targeted intraparenchymal brain dosing, spanning a multitude of neurological indications. As non-clinical toxicology studies require drug delivery methods to closely mirror those used in the clinic, expanding the number of non-rodent preclinical models for CED proves valuable. To achieve CED, standard procedures incorporate real-time MRI to target and verify local delivery to desired brain regions by employing a step-infusion paradigm coupled with a contrast agent such as gadolinium. We have established this procedure in non-human primates (NHPs) and have successfully delivered novel neurological therapeutics to multiple brain regions tailored to each respective indication. Expanding on this progress established in NHPs, we have developed a Beagle dog model as an additional non-rodent non-clinical alternative. Up to six different parenchymal sites per animal have been dosed at rates of 120 to 300 μL per hour with dose volumes ranging between 30 to 50 μL per site. The radial error in relation to the planned target is the submillimetric range. By incorporating real-time MRI, it is possible to accurately target relevant brain regions while also monitoring dose distribution over time. As observed in NHPs, Beagle dogs recover well from the surgeries and presented with minimal procedure-related clinical signs including mild tremors, cutaneous changes, and decreased activity which were expected after surgery and resolved within approximately 4-days after the procedure. From an ethical standpoint, Beagle dogs represent an advantageous alternative to non-human primates. This work demonstrates an additional non-clinical model for CED using real-time MRI, with a workflow that utilizes clinically relevant methods and devices, ultimately addressing the need for alternative non-rodent non-clinical models for assessing toxicology of neurological therapeutics.

317 Target Cell and Tissue Specificity of a Novel CD8-Targeted Fusosome for Direct *In Vivo* Delivery of CD19 or a CD20 CAR to CD8+ T Cells

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To overcome conventional *ex vivo* CAR T limitations, Sana is developing a novel gene therapy platform that can deliver CAR transgenes directly to T cells via systemic administration of a fusosome (a novel integrating viral vector whose target specificity can be altered by engineering viral envelope proteins). Although anti-CD19 CAR T cells have been clinically validated to be effective with an acceptable