Oral Presentations

Late-Breaking Abstracts I

1. AAV1-hOTOF Gene Therapy for Children with Autosomal Recessive Deafness 9

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Background: Autosomal recessive deafness 9 (DFNB9), caused by mutations of the *OTOF* gene, is characterized by congenital or prelingual, severe-to-complete bilateral hearing loss. However, no drug treatment is available for hereditary deafness. AAV1-hOTOF, adeno-associated virus (AAV) serotype 1 carrying a human *OTOF* transgene, was designed to treat patients with DFNB9. Here, we report the safety and efficacy of AAV1-hOTOF gene therapy as the treatment of children with DFNB9 via unilateral or binaural injection.

Methods: In this single-arm trial, patients (1-18 years of age) with severe-to-complete hearing loss were eligible. AAV1-hOTOF was administered into one or two cochleas through the round window. The primary outcome was dose-limiting toxicity (DLT). Adverse events (AEs), immune response, auditory function and speech perception were evaluated.

Results: From October 2022, to September 2023, 11 patients with greater than 95 dB of average auditory brainstem response (ABR) thresholds at 0.5-4 kHz were enrolled. Six patients received unilateral injections, of which patient #1 received 9×10^{11} vg dose and patients #2-6 received a 1.5×10^{12} vg dose. Subsequently, patients #7-11 received binaural injections at a dose of 1.5×10^{12} vg in each ear. Follow-up visits ranged from 4 to 26 weeks. No DLT was observed. 80 adverse events were observed, with 97.5% (78/80) being grade 1 or 2, and 2.5% (2/80) being grade 3. Ten children had hearing recovery. In patient #1, the average ABR threshold was recovered to 68 dB at 4 weeks, 53 dB at 13 weeks, and 45 dB at 26 weeks. In patients #3-6, the average ABR thresholds were recovered to 48, 38, 40, and 55 dB at 26 weeks, respectively. In patients #7-9, the average ABR thresholds of left (right) ear was reduced to 58 dB (58 dB), 85 dB (75 dB), 50(55 dB) at 26 weeks. In patients #10-11, the average ABR thresholds of left (right) ear were reduced to 78 dB (75 dB), 63 dB (63 dB) at 13 weeks. Speech perception was improved in participants who had hearing recovery. The ability of sound source localization was improved in all patients who received binaural injections.

Conclusions: The AAV1-hOTOF gene therapy is safe and efficacious as a novel treatment for patients with DFNB9.

2. Results from GALILEO-1, A First-in-Human Clinical Trial of FLT201, an AAV-Gene Therapy, in Adults with Gaucher Disease Type 1

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Introduction: FLT201 is an investigational AAV gene therapy for the treatment of Gaucher disease Type 1 (GD1). FLT201 contains a *GBA1* transgene that encodes an engineered variant of glucocerebrosidase (GCase-85) under control of a liver-specific promoter. GCase-85 has two amino acid substitutions that increase its stability by approximately 6-fold in serum and 20-fold at lysosomal pH compared to wild-type GCase. FLT201 uses a proprietary capsid (AAVS3) constructed by rational design that efficiently transduces human hepatocytes and enables high expression with low vector doses. A one-time infusion of FLT201 has the potential to lead to durable endogenous expression of a highly stable form of GCase, thereby eliminating the need for chronic treatment with enzyme replacement therapy (ERT) or substrate reduction therapy (SRT). The increased stability of GCase-85 may also increase its tissue coverage compared to ERT.

Methods: GALILEO-1 is a first-in-human, open-label, dose-finding study of a single IV infusion of FLT201. Eligible patients have GD1, are aged 18 years or older, are receiving an approved GD1 therapy and have a negative AAVS3 neutralizing antibody test. Study objectives are to assess safety and tolerability of FLT201 and to investigate the relationship between FLT201 dose and GCase-85 expression as well as effects on disease-relevant clinical parameters. A prophylactic immune management regimen is implemented for all subjects. The starting dose of FLT201 for cohort 1 is $4.5\text{x}10^{11}$ vector genomes per kilogram bodyweight, with subsequent dose escalation as needed based on observed safety and efficacy. Participants are followed for 38 weeks after treatment before entering long-term follow-up. ERT/SRT can be stopped once expressed GCase levels are within the normal range.

Results: Since June 2023, five subjects have been successfully dosed in cohort 1 and FLT201 has been well tolerated with no infusion related reactions or serious

adverse events. FLT201 rapidly generates supraphysiological levels of GCase in plasma, leading to normalisation of leukocyte GCase activity by 4 weeks with clear evidence of reduction in lyso-Gb1 concentration following withdrawal of background ERT or SRT. The first subject dosed has passed 8 months of follow-up.
Conclusion: FLT201 is the furthest advanced investigational AAV-gene therapy for Gaucher disease Type 1 currently under development. Emerging data from the participants dosed in the GALILEO-1 study so far demonstrates an acceptable safety profile of FLT201, with robust, durable expression of GCase alongside evidence of lyso-Gb1 clearance. A description of FLT201, a potentially transformative therapy for people with Gaucher disease type 1, and an overview of the GALILEO-1 study including data from all participants dosed will be presented.

3. Multi-Year Enzyme Expression in Mucopolysaccharidosis Type VI Patients After Liver-Directed Gene Therapy

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Mucopolysaccharidosis type VI (MPS VI) is an autosomal recessive disease due to deficiency of the lysosomal enzyme arylsulfatase B (ARSB) that results in multi-organ accumulation of glycosaminoglycans (GAG). Limitations of current treatments prompted the development of a clinical trial on liver-directed gene therapy (GT) for MPSVI. We report the follow-up of MPSVI patients enrolled in the high dose cohort (6×1012 gene copies/kg) of a phase 1/2 open label, dose-escalation, multicenter clinical trial investigating the safety and efficacy of a single intravenous infusion of a recombinant adeno-associated virus serotype 8 (AAV8) vector expressing the human arylsulfatase B (ARSB) gene under the control of a liver-specific promoter (AAV8.TBG.hARSB) (NCT03173521). Following enzyme replacement therapy (ERT) discontinuation, median follow-up time in patients in the high-dose cohort (n=4, mean age at administration: 8.75 ± 2.50 years, age range 5-10 years) was 48 months (range 39-53 months). Patients showed sustained serum ARSB activity (38-67% of mean normal values), modest increase in urinary GAG and no relevant changes in endurance and pulmonary function. In one out of the four patients, ERT was restarted because of elevation of GAG without reduction in serum ARSB about 2.5 years after gene therapy. No late emergent safety events were observed in any of the four patients. Liver and spleen size remained within reference ranges, albeit an increase in spleen size centile was observed in one patient. No significant changes in endurance and cardiac function were detected in all four patients. Patients at the high dose cohort showed sustained ARSB expression for at least 3 years post-gene therapy. The increase in urinary GAG was modest in all four patients and only in one patient required restarting of the ERT. Liver-directed GT performed in five-year old patients or older without loss of transgene expression. In conclusion, a single intravenous administration of AAV8.TBG.hARSB was safe and effective in the majority of patients, thus supporting liver-directed gene therapy for MPSVI.

4. Development and Translation of a Novel CRISPR Genome Editing Therapy to Induce Fetal Hemoglobin for Sickle Cell Disease

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Sickle cell disease (SCD) is a severe genetic blood disorder caused by a missense mutation in the *HBB* gene. Re-activation of silenced fetal hemoglobin (HbF) using CRISPR-Cas is a promising genome editing approach for autologous SCD therapy. We have previously shown that the disruption of the repressor-binding motifs BCL11A (-115) in the γ-globin gene promoters can effectively induce HbF that mimics naturally occurring hereditary persistent fetal hemoglobin variants. Here we present clinical scale up, optimization, pharmacology, toxicology, to initiate a genome editing clinical trial, St. Jude Autologous Genome Edited Stem Cell (SAGES1), as part of an IND submission recently cleared by the FDA. To assess the therapeutic efficacy of -115 γ-globin promoter editing approach for SCD, we electroporated plerixafor-mobilized $CD34^+$ HSPCs from one healthy donor and three individuals with SCD and transplanted them into NBSGW mice. High indel frequencies were maintained in $CD34^+$ HSPCs before (85.6% ± 0.9) and after 17 weeks of transplantation (74.2% ± 2.9) with robust levels of HbF (27.6% ± 1.7) in erythroid progeny derived from mice bone marrow (BM). By single-cell western blot, 49.3% to 58.2% of edited erythroid cells expressed γ-globin. Importantly, in vitro differentiated edited cells under hypoxia showed 3-fold reduction in sickling.We optimized clinical scale mobilization and editing of human donor CD34+ HSPCs with Cas9 and the HBG -115 gRNA with Maxcyte electroporator. We demonstrated that Cas9/HBG-115 editing rate of CD34+ HSPCs and HbF expression

correlates directly with increasing Cas9 concentration, and that at optimal concentrations, we can achieve >80% editing and ~25% HbF expression in erythroid cells *in vitro*. We conducted preGMP engineering runs using plerixafor mobilized HSPCs (n=3) from healthy African American donors edited with GMP-like Cas9 protein and sgRNA, and xenotransplanted edited cells into NBSGW mice for both *in vivo* pharmacology and *in vivo* toxicology studies. We obtained ~92% editing in the bulk HSPCs prior to transplantation and high indel rates (70-84%) maintained in BM after transplantation. We observed HbF levels of 17.1-25.9% from erythroid cells isolated from the mice BM. In *In vivo* toxicological studies, we did not detect any adverse effects in the mice. To evaluate Cas9 mediated genotoxicities associated with -115 γ-globin promoter editing, we performed CHANGE-seq (preGMP engineering runs) donors and identified potential off-target sites reproducibly present in 2 or more donors. By rhAMPseq, we confirmed there are no detectable off-targets above background at 277 candidate sites designated by CHANGE-seq and 11 in silico sites nominated by CasOFFinder. To characterize spectrum of large deletions, we used long-range PCR based PacBio sequencing and detected dominant 4.9kb deletion products generated due to simultaneous double strand beaks in *HBG1* and *HBG2* promoters. By digital droplet PCR, we observed 29.8 % ± 1.3 of this 4.9kb deletion occurring in bulk edited CD34+ HSPCs. To determine genome-wide structural re-arrangements, we applied the UDiTaS and found no reproducible translocations at day 5 and 14 in bulk edited HSPCs from preGMP engineering runs. In sum, we describe rigorous IND-enabling studies of our approach to induce potentially therapeutic levels of HbF for treatment of SCD. These represent one path to address FDA guidance to characterize gene therapy products involving genome editing.

5. A Phase 1 Clinical Trial of High Dose AAV1.SERCA2a in Patients with Heart Failure: Modulation of SERCA2a of Intra-myocytic Calcium trafficking in Heart Failure with Reduced Ejection Fraction (MUSIC-HFrEF)

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Despite advancements in medical and device therapy for Heart Failure with reduced Ejection Fraction (HFrEF), patients continue to experience high morbidity and mortality. Improved understanding of the molecular and cellular mechanisms of heart failure has identified specific targets that can be used for therapeutic purposes. A critical characteristic of failing hearts is abnormal intracellular Ca^{2+} handling, which is due to a decrease in expression and function of the cardiac sarcoplasmic reticulum calcium ATPase pump (SERCA2a). Delivery of an adeno-associated type 1 vector carrying SERCA2a improved contractile function in a dose dependent manner in many animal and human engineered tissue models of heart failure. In patients, low doses were safe but led to marginal outcomes due to poor transduction. A key to success is to titrate for a dosage high enough for efficacy without causing immunogenicity or adverse events. Our ongoing phase 1 clinical trial for the treatment of HFrEF delivers higher doses of AAV1.SERCA2a, compared to earlier trials (3E13 viral genome(vg)/patient and 4.5E13 vg/patient) through intracoronary infusion in both ischemic and non-ischemic patients with left ventricular ejection fraction of 35% or less. To date six patients have received a dose of 3E13 vg of AAV1.SERCA2a and the follow-up period has been 1 week to 18 months. There have been no gene therapy or procedure–related adverse events in the first 6-12 months post infusion. Three of the four patients have shown improvements in NYHA from Class 3 prior to treatment to Class 2 at 6 and 12 months. Clinically meaningful improvements in LVEF (+4% to +11%), 6MWT (+55 to +86m), decrease in Pro-BNP (-49 pg/ml) and decreases in troponin I (-10.6 to - 1.7 pg/mL) from basline to 12 months post-treatment. The early clinical efficacy at a dose of 3E13vg/patient of AAV1.SERCA2a will be followed by enrollment of patients at a higher dose at 4.5E13 vg/patient. These encouraging results of high dose AAV1.SERCA2a in patients with HFrEF not observed previously may offer alternative treatments to patients with severe heart failure where a large unmet need remains.

6. Harnessing Chromatin Architecture and Post-Transcriptional Regulation to Benefit the Safety of the Lentiviral Vector Platform

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Self-Inactivating Lentiviral Vectors (SIN.LVs) are widely used in gene therapy to integrate therapeutic transgenes into patient cells' genomes to cure a variety of diseases. While demonstrating notable efficacy and safety in preclinical and clinical settings, indications of genotoxicity mediated by LV integration have been reported, as dominant clones with LV targeting cancer-related genes were observed in Hematopoietic Stem/Progenitor Cell gene therapy and in Chimeric Antigen Receptor (CAR) T cell cancer immunotherapy trials. Moreover, the recent emergence of T-cell malignancies in CAR T cell therapy has prompted attention by the Food and Drug Administration. While the risk-benefit valuation of these therapies remains positive, with significant benefits for patients, these events underscore the need for mitigating potential risks associated with vector integration. To this aim, we devised a strategy to overcome activating insertional mutagenesis events by leveraging i) chromatin insulators (CI) to reduce the interactions between the vector enhancers and cellular promoters, and ii) sequences complementary to micro-RNA (miRT), to trigger degradation of oncogenic aberrant/chimeric transcripts. To test our strategy, we used an *in vivo* assay of liver-oncogenesis sensitive to LV-

mediated insertional mutagenesis, where neonatal WT mice are injected with LVs and treated with CCl_4 cancer promoting agent. Mice are euthanized after 1-year of follow-up to collect tissues for histopathology and integration sites analysis (ISA) to define the molecular culprits of oncogenesis. We generated LVs with the liver-specific Enhanced Transthyretin (ET) enhancer-promoter driving an antisense oriented GFP-expression cassette (SIN.AS), a configuration allowing the enhancer to be immediately upstream the 3' LTR, thus making this LV more genotoxic compared to standard SIN LVs. To this reference LV, we included liver-active and hematopoietic specific miRT, to degrade aberrant transcripts from both tissues, and CI. As CI operate by forming chromatin loops between convergently oriented sites, we cloned these elements in convergent or divergent orientations in the LV, to shield host genes either by confining the ET-enhancer in a LV-internal loop or by excluding the enhancer from external loops mediated by genomic CI. An active ET LTR LV was used as genotoxic control. Histopathological analysis identified 34 liver tumors (out of 30 mice) from the genotoxic ET.LTR LV group and 14 masses (out of 27 mice) from SIN.AS LV treated mice. Differently, the LVs armed with CIs and miRTs showed a significantly lower incidence of liver tumors, with only 3 and 1 tumors (out of 34 and 28 mice) respectively, indicating a superior safety profile. This observation was confirmed by ISA on DNA from liver-derived masses. In the ET.LTR group, we observed robust targeting of *Rlt1-Rian* locus, a well know player of insertional mutagenesis in this model. In liver masses marked by SIN.AS LV, fewer *Rtl1-Rian* integrations were present and we observed the emergence of clones with integration targeting *Lingo2, Cchcr1 and Diap3*, whose upregulation might be involved in proliferation and transformation. Differently, mice treated with the LVs carrying CI and miRT, did not show targeting of *Rtl1-Rian* locus and we found one marked tumor with an insertion in *Erh* gene, which has a reported role in proliferation. Overall, the reduced frequency of tumors induced by the safety-improved LVs and the absent targeting of genes commonly found in liver genotoxicity, indicate that the combination of miRT and CI allows to improve the safety of the LV platform.

7. The AMETHYST (Advanced Mesenchymal Enhanced cell THerapY for SepTic) Trial: A First-in-Human, Dose Escalation Phase 1 Safety Trial of Genetically Enhanced MSCs (GEM00220) Appeared Safe and Well Tolerated in Patients with Septic Shock

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Background: Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host immune response to infection. It is a global health priority as a leading cause of morbidity and mortality in these critically ill patients. Despite advancements in care and more than 100 clinical trials in the past 3 decades, no new product has been approved. The combination of immunomodulatory mesenchymal stem/stromal cells (MSCs) and genes linked to sepsis survival has been shown to significantly improve survival in E. coli and polymicrobial preclinical sepsis models.

Methods: A cryopreserved, allogeneic, genetically-enhanced MSC therapy product (GEM00220) was tested in adult septic shock patients in an open-label, dose-escalating trial using a 3x3x3x3 design to determine the safety and maximum feasible tolerated dose (MFTD). Participants with severe sepsis were enrolled within 48 hours if they had cardiovascular failure and at least 1 other organ failure, despite optimization of all other interventions. The primary objective was the safety and feasibility of GEM00220 in diluent for infusion to patients with septic shock, given at 4 ascending dose levels: dose level A - 15 x 10^6 cells, dose level B - 60 x 10^6 cells, dose level C - 150 x 10^6 cells, and dose level D - 300 x 10^6 cells, given as two doses of 150 x 10^6 cells, separated by 24 hours. Biomarker samples were collected periodically throughout the first 72 hours. A data safety monitoring committee (DSMC) meeting was convened between each cohort before escalating to the next subsequent dose. Safety and tolerability were also assessed by monitoring adverse events up to 28 days, with day 90- and 1-year survival assessment.

Results: Eleven participants (3 each in cohorts A-C; and 2 in cohort D, of which only 1 participant received 2 doses) were enrolled with a median age of 58 years old (range: 28 to 81); median SOFA score of 15 (range 5 to 18) with a median predicted mortality risk of **80%** (range: <10% to >90%). Mortality was **36%** at 28 days. Infusion for all doses was well tolerated. No stopping rule criteria were met for any dose cohort. There was no concern on the feasibility of receipt of the cryopreserved product, its preparation, or infusion at the bedside in an acute care setting.

Conclusions: Infusing cryopreserved GEM00220, up to 300 million cells, into patients with severe septic shock seemed safe and feasible. Further work is needed to establish the efficacy of GEM00220 in this patient population.

Late-Breaking Abstracts II

8. REKLAIM, A Phase Ib Clinical Trial Using a Novel Immune Modulation Strategy for Systemic Administration of FBX-101 (AAVrh10.GALC) After Umbilical Cord Blood Transplantation for the Treatment of Infantile Krabbe Disease

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Background: REKLAIM is a novel intravenous AAVrh10.GALC gene therapy administered during myeloablation/ immune suppression after infusion of umbilical cord blood transplantation (UCBT) for Infantile and Late Infantile Krabbe disease (IKD, LIKD). We report the results of the first 5 subjects with IKD treated with a low dose intravenous FBX-101 (1.6 x10^{13} gc/kg). IKD is a fatal neurodegenerative disorder due to galactocerebrosidase (GALC) deficiency that results in psychosine toxicity to myelinating cells in the brain and peripheral nervous system. If untreated, death occurs at a median of 2 years. Currently, the standard of care for pre-symptomatic neonates is treatment with umbilical cord blood transplantation (UCBT) that halts the brain disease, but motor function continues to decline due to progressive peripheral neuropathy. We hypothesized that FBX-101 administered during myeloablation for UCBT will override the antibody response to the vector's capsid and transgene. UCBT provides a healthy immune system from the donor that does not recognize GALC as an antigen. Recently newborn screening for Krabbe disease was recommended by the Advisory Committee for Heritable Disorders for Newborns and Children to the Recommended Uniform Screening Panel substantiating the need to treat this devastating disorder.
Methods: REKLAIM is a Phase I/II dose-escalating intravenous gene therapy to evaluate safety and efficacy of FBX-101 administered systemically more than 21 days after UCBT while the subject is myeloablated or later when immune suppressed (i.e. prevention of graft versus host disease). Each subject's immune suppression is individually evaluated by PI and a proposed regimen is approved by an independent committee of immunology experts. The protocol includes adjustments with Rituximab, Sirolimus and Prednisolone according to the subject needs.

Results: FBX-101 in 5 patients receiving the low dose was well tolerated, with no treatment-related serious adverse events and follow up ranging from 6 to 24 months. No antibodies to the transgene developed. In the two subjects treated during myeloablation, there were no antibodies to AAV, plasma and CSF GALC significantly increased, psychosine dropped below the level of detection and subjects achieved normal white matter growth, peripheral nerve conduction velocity and gross motor skills. The three subjects treated during immune suppression developed minimal total antibodies to AAV with no signs of humoral or cellular toxicity and improved gross motor skills.
In summary, FBX-101 leverages the myeloablation and immune suppression after UCBT, resulting in efficient AAV transduction and providing increased GALC enzyme that supports brain myelination and gross motor development. All 5 patients are walking independently.

9. Therapeutic Vaccination for the Elimination of HPV16+ High Grade Cervical Dysplasia: A Phase I Clinical Trial of the DNA Vaccine pNGVL4aCRTE6E7L2, Administered via the TriGrid™ Electroporation Device

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Objectives: While surgical procedures can effectively treat high grade dysplasia caused by high-risk human papillomavirus (hrHPV), there is currently no effective treatment targeting the etiologic agent. Here, we evaluate the safety and tolerability of a novel therapeutic vaccine targeting HPV16 E6, E7 and L2 when delivered intramuscularly followed by electroporation, and its effects on histology and viral clearance.
Methods: In an ongoing Phase I 3+3 dose escalation study (NCT04131413) we evaluate the safety, tolerability, and efficacy of the DNA vaccine pNGVL4aCRTE6E7L2, administered via the TriGrid Electroporation Device (Papivax). There are 2 separate cohorts, one for HIV negative (HIV-) women with HPV16+ CIN2/3, and one for women living with HIV (HIV+) with CIN2/3, VIN2/3 or VAIN2/3. Participants were administered vaccine at weeks 0, 4, and 8 at doses of 0.3 mg, 1.0 mg, or 3.0 mg for each cohort, respectively. At week 12, patients underwent Pap/HPV co-test, colposcopy, biopsy, and ECC to ensure absence of progression. At 6 months, all patients underwent a LEEP for definitive evaluation, and at 12 months, all patients underwent evaluation with Pap/HPV co-test (Cobas), colposcopy, biopsy, and endocervical curettage. HPV16 RNA was evaluated in histologic specimens by in situ hybridization. Descriptive statistics were used to evaluate histologic and viral responses.
Results: To date, 15 women have been enrolled in the clinical trial (5 in the HIV+ cohort and 10 in HIV- cohort). Eleven patients (2 in the HIV+ arm and 9 in the HIV- arm) have completed their 6-month evaluation and are available for analysis. All enrolled patients tolerated each vaccine

dose well. Only limited grade 1 toxicities were noted, primarily discomfort at the injection site. All self-resolved within 1 week. At the 6-month visit, 8 of 11 evaluable patients converted from HPV16+ to HPV16- and 7/10 cleared their high-grade dysplasia. One patient who cleared HPV16 was HIV+. In evaluating the timing of HPV16 clearance, only 2 of 11 evaluable patients had cleared HPV16 at the 3-month visit, implying it frequently takes several months to eliminate HPV16 infection. However, 3 additional patients had benign histology (both biopsy and ECC) at 3 months even though the swab was still HPV16+. Patients who have completed their 12-month visit (7 patients) were clear of HPV16 suggesting that the effect is sustained, and that patients do not become re-infected. Interestingly, for the woman living with HIV, who had a history of HPV16+ and other hrHPV+ for several years, HPV16 was negative at the 6-month LEEP by swab and in situ hybridization.

Conclusions: Preliminary results show a high rate of clearance of HPV16 in patients with HPV16+ CIN2/3, suggesting that this DNA vaccine delivered via electroporation may not only effectively treat precancerous lesions of the cervix but may also eliminate HPV16 infection. Furthermore, this treatment was well tolerated and may be active even in patients living with HIV, a particularly challenging group to treat for HPV-related disease.

10. Interim Safety and Efficacy of Anti-CD70 CAR-NKT (CGC729) for Patients with Refractory Metastatic Renal Cell Carcinoma

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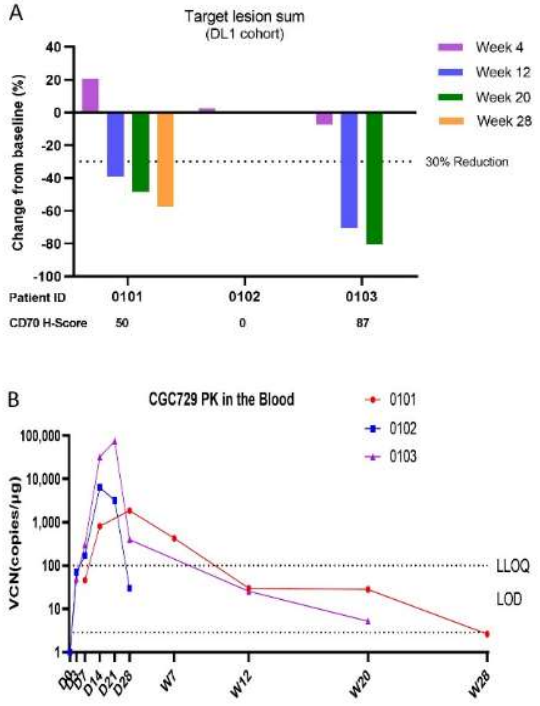
Background: Tumor heterogeneity and suppressive tumor microenvironment (TME) are major barriers to successful chimeric antigen receptor (CAR) T cell therapy in solid tumors. Natural killer T (NKT) cells, a subset of innate-like T cells, have emerged as a novel platform for the treatment of solid tumors owing to NKT's multifactorial antitumor and TME modulation properties. Here we describe interim findings from an investigator-initiated trial (IIT) of CGC729, an autologous anti-CD70 CAR-NKT product, in patients with refractory metastatic clear cell renal cell carcinoma (RCC).

Methods: This is a single-arm, open-label IIT (NCT06182735) using 3 + 3 design to evaluate three dose levels of CGC729 including $5\times10^6/m^2$ (DL1), $1.5\times10^7/m^2$ (DL2), and $4.5\times10^7/m^2$ cells (DL3). As of Feb. 2024, five patients who received at least 2 prior lines of therapy were enrolled and treated with a single infusion of CGC729 following a 3-day lymphodepletion regimen (fludarabine 25mg/m^2/day; cyclophosphamide 250mg/m^2/day). At the time of the presentation, at least six patients will have been treated with 2-9 months follow-up. Cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) were graded based on ASTCT criteria, and adverse effects were evaluated according to CTCAE 5.0.

Results: As of the Feb. 29 data cutoff, five patients (DL1=3; DL2=2) were eligible for safety evaluation. Adverse events were manageable, and no dose-limiting toxicities (DLT) were observed. The most common adverse events were lymphodepletion associated reduction of neutrophils, platelets, and white blood cells; however, no > Grade 2 cytopenia was observed by Day 28. One patient (0102) in DL1 had Grade 2 CRS resolved within 24 hours. The same patient developed Grade 2 ICANS and was resolved with corticosteroids. No CRS or ICANS was observed in the other two patients in DL1 and the two patients in DL2. The overall response rate (ORR) in DL1 at Weeks 12 and 20 was 66.7% (2/3), and the ORR among RCC CD70$^+$ patients (0101 and 0103) was 100% (2/2). Those two CD70$^+$ patients had deep tumor reduction of 57.2% at Week 28 and 80.4% at Week 20, respectively (Figure 1A). Notably, both CD70$^+$ patients had low CD70 expression (H-score < 100) in RCC indicating CGC729 may have the potential to target CD70-low patients. Patient 0102 in DL1 was RCC CD70$^-$ and experienced progress disease (PD) on Day 28. Among five patients evaluable for PK, robust CAR-NKT expansion in the peripheral blood was observed in all patients including two patients who had negative CD70 expression in RCC. CGC729 had peak expansion between Day 14 and Day 28, and it persisted up to Week 20 (Figure 1B). Moreover, CD70$^+$ T cells in the peripheral blood decreased significantly after CGC729 infusion and remained at a low level up to Week 20, indicating a durable activity of CGC729.

Conclusion: Interim analysis of this first-in-human trial of anti-CD70 CAR-NKT demonstrates a manageable safety profile and promising antitumor activity for the treatment of refractory metastatic RCC. Figure 1: CGC729 had deep anti-tumor activity (A) accompanied with robust expansion and durable persistence (B) in DL1.



11. Academic BCMA-CART Cells HBI0101, a Promising Approach for the Treatment of LC Amyloidosis

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Background: Light chain amyloidosis (AL) is a rare plasma cell disorder characterized by the deposition of misfolded immunoglobulin light chains that aggregate to form insoluble fibrils. These deposit in various organs, leading to organ dysfunction and failure. Due to the rarity of AL and the extreme frailty of this patient population, AL patients are invariably excluded from clinical studies. The treatments for AL are mostly derived from the treatments applied for multiple myeloma (MM), a related disease. While BCMA-CART drugs have been FDA approved for the treatment of MM, their application to AL patients remain off-label. HBI0101 is an academic anti-BCMA CART-based therapy developed and launched at Hadassah Medical Center for MM treatment (NCT04720313). In the phase Ib/IIa study, HBI0101 has demonstrated manageable safety profile, with therapeutic efficacy in over 100 MM patients. Although the potential toxicity of such an approach may prove challenging for AL patients, we decided to include AL patients in our study; a cohort of twelve patients, representing the largest cohort of such patients reported so far. We present here the interim results of 12 AL patients, all had clinically active, relapsing and progressive disease following a median of 5 lines of treatment (range: 3-10), 11/12 were resistant to their last line of therapy, 11/12 patients were triple refractory and 6/12 were Belantamab-mafodotin refractory. Ten of the patients had cardiac involvement. Four with MAYO-stage IIIa cardiac involvement and one with stage IIIb. Eleven patients had elevated proBNP and median value for patients with cardiac involvement was 2390pg/ml. The patients were treated within the following HBI0101 cohorts: one received 150x106 CAR+, two received 450x106 CAR+ and nine were aimed for the 800x106 CAR+ cells. Two patients were treated on a compassionate basis.
Results: Adverse events (AEs) were manageable, and included Grade 1-3 CRS observed in 10/12 patients. The median time to CRS onset was 1.5 days (range 1-3 days), with a median duration of 1.5 days (range 1-4 days). Grade 3-4 hematologic toxicities were frequent (7/12, 58%), yet short and transient. None of the patients developed immune effector cell-associated neurotoxicity syndrome (ICANS). Treatment with fresh or cryopreserved CART product had no effect on this early-onset, manageable CRS. HBI0101 therapy induced remarkable responses in these heavily pretreated AL patients, achieving a fast and efficient complete response (CR) in eight patients, a very good partial response (VGPR) in two patients, and a partial response (PR) in one patient. One patient did not respond to the treatment. A significant reduction in the difference in Involved and Uninvolved Free Light Chain (dFLC) 0-50 mg/L dFLC levels was observed in 11 patients, and flow cytometry 10-5 minimal residual disease (MRD) negativity was achieved in 6 of the patients. The time to the First hematological response was achieved after 13-28 days (median 18 days). The hematologic responses translated into clinical improvement in most patients, whereas 6 met the official criteria for organ responses. The median follow-up period was 7 months (range: 2.5-29).
Conclusion: In this largest cohort of AL patients treated with anti-BCMA-CART, we demonstrate acceptable and manageable toxicity even for advanced cardiac patients, and a remarkable hematologic efficacy, with prominent organ responses in most participants. While responses were quick and deep, survival was limited, implying for the usage of CART earlier in the course of disease. Anti-BCMA-CART may become a powerful tool to improve organ function and survival in AL.

12. Oncolytic Adenovirus ORCA-010 Induces Systemic Tumor-Specific T cell Responses and Activation of the Tumor Microenvironment in Prostate Cancer: A Phase I/IIa Clinical Trial

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Background: Oncolytic immunotherapies using adenoviruses have emerged as a noteworthy treatment option in cancer therapy, illuminating substantial prospects not only for direct tumor mitigation but also for fostering an immunologically conducive tumor microenvironment. Increasingly, the role of the host immune system in achieving long-term remission is being recognized. Here, we present findings from a Phase I/IIa trial in which ORCA-010, a potent oncolytic adenovirus, is tested in early-stage prostate cancer patients as first line therapy.
Methods: In the Phase I dose escalation study, newly diagnosed patients with localized prostate cancer were treated with a single intraprostatic injection of ORCA-010, with a one-year follow-up period. The Phase IIa extension study incorporated high-risk patients, treated with two administrations of ORCA-010, followed by radical prostatectomy six-eight weeks later. The primary objectives of the study are to assess the safety of ORCA-010, and its ability to induce tumor-specific immune responses.
Results: The Phase I/IIa trial findings demonstrate that ORCA-010, administered as a single or repeated dose, exhibits a favorable safety profile without any detected dose-limiting toxicities (DLTs) or severe adverse events (SAEs). After ORCA-010 injection at the highest dose,

prostate specific antigen (PSA) levels dropped below pretreatment levels in several patients, suggesting a clinical response. Analysis of the immune response in blood samples shows a significant increase in activated (HLA-DR+) and proliferating (Ki67+) CD8+ T cells post-treatment compared to before treatment. Moreover, using blood samples from several ORCA-010 treated patients, we were able to detect tumor-specific CD8+ T cells expressing IFNγ and TNFα in response to prostate-associated antigens. Biopsies from Phase I cohorts taken one year after treatment reveal a notable rise in total intratumoral T cells, particularly CD8+ T cells. In phase IIa prostatectomy specimens, increases in CD8+ T cells and no alteration in FoxP3+ regulatory T cells were seen. Notably, ORCA-010 results in the formation of hot regions with high levels of CD8+PD-1+ T cells within the tumor region of prostatectomy samples.
Conclusions: The intraprostatic injection of ORCA-010 in treatment-naïve prostate cancer patients is not only safe (grade I/II AE's), but also boosts long term anti-tumor immune responses, demonstrated by increase in tumor-specific T cells in the blood and shifting an immunologically "cold" tumor microenvironment into "hot" regions in prostate cancer. Together, these findings highlight the potential of ORCA-010 in shaping future treatment approaches in combination with standard care modalities and/or immune checkpoint inhibitors.

13. Updated Clinical Results of Metabolically Armored CD19 CAR-T Cells for Safe and Effective Treatment of Relapsed or Refractory B-Cell Hematological Malignancies at Extremely Low Doses

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Background: Despite the undeniable success of CAR-T therapy in treating hematological malignancies, persistent challenges such as ineffectiveness and relapse after remission are observed due to T cell exhaustion and dysfunction. To address these issues, metabolically armored CAR-T cells expressing IL-10 have demonstrated significant improvements in the proliferation and persistence of CAR-T cells *in vivo*. These CAR-T cells exhibit remarkable resistance to exhaustion and elicit stem-like memory responses in diverse animal models, resulting in robust tumor eradication and enduring protection. To further evaluate both efficacy and safety, we have initiated an open-label, single-arm, investigator-initiated phase I trial (NCT05715606; NCT05747157) of IL-10 expressing CD19 CAR-T cells, denoted as Meta10-19, targeting patients with R/R B-cell malignancies.
Aims: The core aim of this phase I study is to evaluate the safety and tolerability of Meta10-19 in patients with R/R diffuse large B-cell lymphoma (DLBCL) and B-cell acute lymphoblastic leukemia (B-ALL). Concurrently, secondary objectives encompass the exploration of pharmacokinetics and the assessment of preliminary efficacy.
Methods: We enrolled and treated 18 eligible patients with R/R B-cell hematological malignancies, composing 9 with DLBCL and 9 with B-ALL recently. Patients received a single infusion of Meta10-19 across 3 dose level (DL) cohorts: DL1 at 1.0×10^5 cells/kg (n=12), DL2 at 5.0×10^4 cells/kg (n=5); and DL3 at 2.0×10^4 cells/kg (n=1). The administration followed a standard lymphodepletion regimen for 3 days. We graded cytokine release syndrome (CRS) and immune effector cell associated neurotoxicity syndrome (ICANS) according to ASTCT 2019 guidelines, immune effector cell associated hematotoxicity (ICAHT) based on EHA/EBMT consensus, and assessed adverse effects (AEs) following CTCAE 5.0 criteria.
Results: As of February 29, 2024, Meta10-19 infusion has been successfully administrated to 18 eligible patients, who subsequently underwent comprehensive safety and preliminary efficacy evaluations. The median age of the cohort was 47 years (range 17-56). Notably, the complete remission (CR) rate for all 18 patients reached 100% at 1 month (18/18) and maintained at 100% at 3 months (11/11). The longest duration of remission observed thus far is 12 months. CRS occurred in all patients, with only one patient with DLBCL assessed as grade 3 (5.6%, 1/18). ICANS at grade 1 was observed in a single B-ALL patient. Early ICAHT was noted in 15 patients (83.3%), with 7 patients (38.9%) experiencing severity ≥ grade 3. Notably, one patient encountered grade 4 neutropenia persisting for over 30 days, which was categorized as grade 4 late ICAHT. The most prevalent ≥ grade 3 AEs included neutropenia (100%, 18/18), thrombocytopenia (83.3%, 15/18) and anemia (77.8%, 14/18). It is worth highlighting that all treatment-related AEs were effectively resolved through standard of care and supportive care.
Conclusion: This first-in-human trial of IL-10 expressing CD19 CAR-T product for the treatment of R/R DLBCL and B-ALL has demonstrated encouraging preliminary efficacy and manageable safety profile at ultra-low doses. Ongoing investigations with larger patient cohorts and extended follow-up periods aim to provide further insight into the efficacy and safety parameters.

14. Allograft Overexpression of Modified PD-L1 Confers Tolerance after Heart Transplantation in a Murine Model

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INTRODUCTION: Gene therapy delivering immune suppressing molecules to a transplanted heart may reduce morbidity associated with allograft rejection and systemic immunosuppression after cardiac transplantation. Programmed death ligand 1 (PD-L1) has been implicated in cardiac allograft rejection in murine knockout studies. It remains unclear if transfer of a single gene will sufficiently prevent/delay immune rejection of the transplanted heart. We hypothesized that myocardial overexpression of PD-L1 in a fully major histocompatibility (MHC)-mismatched murine heterotopic heart transplant model could suppress tissue rejection and improve graft survival.
METHODS: Three constructs to express PD-L1 were engineered: (1) a truncated version that has the cytoplasmic tail removed but retains the transmembrane anchor (PD-L1-TM), (2) a soluble secreted PD-L1 (PD-L1-SS) and (3) a "sham" construct which PD-L1 transgene was present but not expressed, with each packaged into a proprietary Solid Biosciences AAV capsid (referred to as SLB-101). BALB/c mice were each injected with one of the viral vectors two weeks prior to transplant. Donor hearts were transplanted into the abdominal position of C57BL/6 recipients with and without concomitant co-stimulation blockade (CTLA-4 Ig 250 ug on postoperative day 0, 2 and 4). Grafts were assessed for function by abdominal palpation and echocardiography. Fulminant graft rejection, defined as complete cessation of allograft function on exam and by echocardiography, was the endpoint of the study.

RESULTS: Donor BALB/c mice injected with AAV PD-L1-TM demonstrated robust membrane-bound expression on immunohistochemistry and donor BALB/c mice injected with AAV PD-L1-SS demonstrated PD-L1 serum expression (637,670 pg/mL PD-L1-SS vs 0 pg/mL sham injected; $p<0.0001$). In the absence of other immunosuppression, transplanted control grafts were fully rejected within a median of 7 days. Graft survival from donors injected with PD-L1-TM or PD-L1-SS did not differ from controls in the absence of concomitant co-stimulation blockade (median survival 7 days, PD-L1-TM, $p=0.69$; median survival 7 days, PD-L1-SS, $p=0.13$). With the addition of concomitant co-stimulation blockade, control allografts rejected within a median of 60 days while control allografts with the sham vector rejected within a median of 53 days ($p=0.76$). Graft survival was significantly prolonged in the PD-L1-TM+CTLA-4 Ig group with 85% survival at 100 days ($p=0.02$ vs control, $p=0.01$ vs sham). Median survival among PDL1-SS grafts (90 days) was higher, though this was not statistically significant compared to control (0.99) or sham vector ($p=0.67$; Figure 1). Echocardiography demonstrated appreciable differences in graft function between control and treated PD-L1-TM expressing grafts. **CONCLUSIONS**: Overexpression of PD-L1-TM on cardiac allografts has the potential to prolong graft survival, slow the progression of immune-mediated rejection, and elicit tolerance in the setting of complete MHC mismatch when used in conjunction with co-stimulation blockade. However, PD-L1-TM alone may not be sufficient to provide a tolerogenic effect. Hence, a future therapy may consist of a combination of transgenes, including PD-L1, that target multiple immunological axes.

Poster Presentations

Wednesday Late-Breaking Abstracts

LBA-15. WITHDRAWN

LBA-16. Suppressor-tRNA Delayed Disease Progression in an Alport Syndrome Mouse Model

Lingjie Kong[1], Jianguo Zhu[1], Xiaoyu Zhang[1], Jianfeng Dong[1], Kongyun Dong[1], Rong Huang[1], Mengyao Xu[2], Jialing Yang Liang[2], Nan Liu[2], Bo Cao[3], Dan Wang[2], Dong Wei[1]
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Introduction: Mutations in over 4000 genes are known to be involved in human diseases, and about 11% of these mutations are nonsense mutations (aka, Premature Termination Codons, or PTCs) leading to protein truncations. These diseases can be treated by Suppressor-tRNAs, which are novel tRNAs engineered to readthrough PTCs to rescue full-length protein production and functions. The use of suppressor-tRNA to induce PTC readthrough delivered through adeno-associated virus (AAV) in a diseased mouse model was recently achieved in a mouse model.
Suppressor-tRNAs: We have developed multiple series of proprietary Suppressor-tRNAs targeting UAG or UGA PTCs. Selected tRNAs were engineered at anti-codons and other positions to efficiently and specifically achieve PTC readthrough, with minimal impact on tRNA integrity, endogenous tRNA pool, amino acid charge and native termination codons. When delivered through lentivirus, suppressor-tRNA candidates demonstrated significantly PTC readthrough in multiple genes and cell lines, and near completion PTC readthrough in iPSCs derived from patients with PTC in disease-causing genes. When delivered through AAV with intracerebroventricular injection in the MPS1 mouse model, suppressor-tRNA candidates were able to significantly rescue IDUA enzyme activity in the brain of the diseased mouse model.
Delayed disease progression: Alport Syndrome is the second most common cause of inherited kidney failure,characterized by hematuria and progressive kidney failure, with prevalence of at least 1 in 5,000 in the population. 85% of Alport Syndrome is X-linked and caused by pathogenic variants in the COL4A5 gene, of which nonsense mutation or protein truncations represent most severe clinical manifestation. The large size of the coding region of COL4A5 gene provides a major hurdle for AAV based gene therapy. Suppressor-tRNA provides a promising opportunity to overcome this challenge. In

multiple cell models with PTCs in COL4A5 gene, our suppressor-tRNA candidates delivered in lentivirus rescued mRNA levels and protein level of COL4A5 efficiently. In a transgenic mouse model with a PTC in COL4A5 gene, suppressor-tRNA candidates delivered in AAV significantly delayed disease progression, indicated by timing and level of proteinuria, in a dose-dependent manner.
Summary: Our data support the hypothesis that suppressor-tRNAs delivered via AAV could be developed as novel therapeutics for diseases caused by PTCs, regardless of diseases, genes and type of PTC mutations.

LBA-17. WITHDRAWN

LBA-18. Nonsense Mutation Rescue with Anticodo-Engineered tRNA for the Treatment of Severe Hemophilia A

Suchul Jang, Alan Leggett, Stefania Lenna, Deena Silton, Ruan Zhang, Sara Evke, Sagi Ravid, Lingyue Yan, Conlin O'Neil, Christopher D. Katanski, Phillip T. McGilvray, Monika Tasak, Gautam Goel, Leslie Williams, Jose M. Lora
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Background: Nonsense mutations (premature termination codons) in the critical coagulation protein, Factor VIII (FVIII) result in severe hemophilia A. Over the last few decades, significant progress has been made for the management of this genetic disease, mainly driven by replacement therapy with recombinant FVIII or AAV-mediated gene therapy, and bypass therapy with monoclonal antibodies. However, these approaches fail to restore wild type, physiological FVIII. To address this significant unmet medical need, we developed anticodon engineered tRNAs (ACE-tRNA) to enable readthrough of nonsense mutations in the endogenous mRNA transcripts by inserting a cognate amino acid, therefore restoring full length protein expression.
Methods: A DNA construct that encodes ACE-tRNAs (targeting TGA premature stop codons) is encapsulated into lipid nanoparticles (LNP) and delivered to cells in vitro and to mice in vivo. Biodistribution of LNP/DNA was tested. Suppression of a nonsense mutation was evaluated with multiple reporter- and FVIII-based systems.
Results: LNP delivered ACE-tRNAs and restored full-length protein expression in cell-based assays in a dose-dependent manner. Coagulation assays showed that rescued FVIII was biologically active. In mice, LNP encapsulated ACE-tRNAs were efficiently delivered to liver endothelial cells (the site of natural production of FVIII) after intravenous administration, and functional PTC rescue was demonstrated.
Conclusion: These data support the development of ACE-tRNA as a therapeutic agent for severe hemophilia A.

LBA-19. Brain Organoids Incorporating Microglia-Endothelia-Pericytes Reveal APOE Impact on SARS-CoV-2 Viral Loads and Vascular Damage

Jinchong Xu, Haohui Fang, Xiangyu Liao, Ruoyan Pu, Alina Yang, Noveda Chen, Will Zhang, Naomi Crane, Juliana Condoleo
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The long-term effects of SARS-COV-2 (SCV2) infection are not understood. SCV2 infection has demonstrated increased severity in those with apolipoprotein-E (APOE) variants, a genetic risk factor for Alzheimer's Disease (AD). Following SCV2 infection, there have been reported cognitive and neurological implications in individuals. However, the potential effects of SCV2 on advancing AD pathology requires further understanding. To investigate the synergistic effects of genetic predisposition to AD and SCV-2 infection, human induced pluripotent stem cell (hiPSC) assembloids derived from APOE-ε alleles, including APOE-ε4/4, APOE-ε3/3 and APOE-ε2/2 cell lines were developed. The assembloids generated were novel in the types of brain cells and connections formed consisting of excitatory and inhibitory cortical neurons, astrocytes, oligodendrocytes, microglia, vascular endothelial cells and pericytes. The hiPSCs assembloids developed provide an important development in investigating the implications of infection on the brain and will be an insightful avenue of research regarding the SCV2 infection on neuronal function. In our study, we used these models to understand the mechanism of SCV2 infection on the brain. First, hiPSCs were infected with SCV2 after 3 months of culture. Then, samples were analyzed post infection using biochemical and molecular techniques to measure AD markers including amyloid-beta (aβ) and tau in insoluble and soluble fractions, and markers of SCV2 infection including SCV2 nucleocapsid and spike protein. In our results, we found that elevated viral loads, angiogenesis, and vascular injury were present in APOE2 and APOE4 relative to APOE3 variant. Post-infection of SCV2, there was a significant increase in aβ and tau proteins in the insoluble and soluble fractionations of the APOE variants, specifically in the APOE-ε4/4 insoluble fractionations. Overall, our data shows an increase in AD biomarkers in an isoform dependent manner following SCV2 infection. Understanding the biochemical mechanisms that may be implicated by SCV2 infection in the brain, specifically for those predisposed to AD via APOE variants, can provide insight into AD pathology of the brain and avenues of treatment options for those with SCV2.

LBA-20. Discovering Rationally-Engineered AAV5-derived Capsid Variants for Superior CNS Tropism and Peripheral Tissue De-targeting in Non-Human Primate via Systemic Administration

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Adeno-associated viruses (AAVs) have emerged as versatile gene-delivery vectors, offering significant promise for therapeutic applications in both research and clinical settings owing to their well-recognized safety profile and capability for sustained expression of genetic payloads *in vivo*. However, the natural AAV serotypes face formidable challenges in achieving efficient transduction within the central nervous system (CNS), primarily due to the restrictive blood-brain barrier. Efforts to enhance CNS transduction via systemic administration of AAVs have garnered considerable interest, yet challenges persist, including limited CNS bioavailability and sequestration of viral particles in peripheral organs. Addressing these challenges is crucial for unlocking the full therapeutic potential of AAV-based gene therapy in CNS disorders. Utilizing NeuShen Therapeutic's AAV capsid discovery platform, we developed the first structure-guided rationally-designed capsid library comprising 176 novel capsid variants alongside 3 naturally occurring and 8 CNS benchmark capsids sourced from leading industry and academia entities. Each individual capsid variant was individually manufactured and vector titered, following which they were intravenously administered to two female Mauritius cynomolgus monkeys over a period of 4 weeks at a dosage of 2.7E13 vg/Kg. To enable tracking, each capsid variant was labeled with two unique barcodes. Subsequently, specific barcoded DNA and RNA corresponding to various capsid variants were retrieved from primary peripheral tissues and different CNS tissues, including cortex, cerebellum, spinal cord, brainstem, hippocampus, and deeper brain structures such as putamen, caudate, globus pallidus, and thalamus. Next generation sequencing analysis of barcode enrichment data of benchmark capsids underscored the assay reliability. The findings demonstrated an average enrishment of 25-fold over AAV9, with the highest observed enrichment reaching 216-fold over AAV9 across diverse CNS regions. Furthermore, this study identified 20 novel capsid variants (11- AAV5 variants and 9- AAV9 variants) demonstrating >10-fold improved CNS tropism compared to AAV9. Remarkably, 3 AAV5 variants exhibited over 1,000-fold enrichment across key brain regions relative to AAV9, outperforming the most effective CNS benchmark capsids by more than fivefold. Moreover, these variants exhibited reduced expression in the liver, heart, muscle, and dorsal root ganglions (DRGs). In summary, our study has unveiled a promising array of AAV5 variants demonstrating superior CNS tropism, surpassing current industry standards, while simultaneously mitigating off-targeting effects in peripheral tissues and maintaining optimal vector manufacturability. Given the low prevalence of pre-existing immunity and potential manufacturing advantages of AAV5, this discovery holds tremendous potential for improving treatment strategies for CNS disorders. The top 10 variants demonstrating substantial CNS enrichment have been selected for further evaluation in a final *in vivo* selection process.

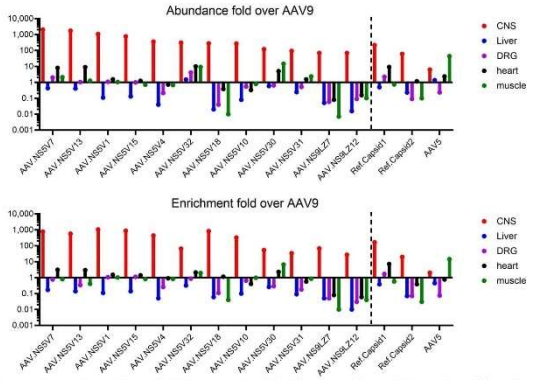

Figure 1: Comparative analysis of NGS data presenting fold difference in (A) abundance reads and (B) enrichment score (abundance reads normalized by virus input) across different variants relative to AAV9 in diverse tissues. CNS represents the average across cortex, cerebellum, spinal cord, hippocampus, putamen, caudate, globus pallidus, thalamus, and pons/medulla.

LBA-21. WITHDRAWN

LBA-22. MRI Guided Focused Ultrasound For Central Nervous System Therapies: When Clinical Data Inspires Non-clinical Research Models

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Background and Purpose: The use of non-rodents such as non-human primates (NHPs), canines and minipigs is often required to investigate the efficacy, biodistribution and toxicology of neurological drugs. Drug transport to the brain remains challenging owing to the blood-brain barrier (BBB). Multiple strategies have been established to overcome this challenge in drug development.
Methods: Intrathecal, intracerebroventricular, and convection-enhanced delivery are commonly used to treat a wide range of neurological indications. An increasing number of drugs are delivered using direct parenchymal delivery in a procedure named convection enhanced delivery (CED). We have most recently implemented MRI-guided focused ultrasound (MRIgFUS) which transiently permeabilizes the BBB in a minimally invasive manner to grant access of parenchymal brain tissue to systemically injected drugs.
Results: Parenchymal convection enhanced delivery (CED) was associated with a low rate of complication (3%) mostly associated with minor ischemic events. Intrathecal delivery in Gottingen minipigs showed one of the highest rate of success with no procedure related complications in a population of n=110 control minipigs. Non-human primate intrathecal delivery was also successful and complications were most often associated with higher dose volumes. CED in dogs showed a lower level of procedure related clinical signs than other non-rodent species. As a non-invasive approach, MRIgFUS offers a transformational methodology for CNS delivery of novel therapeutics. Applications and clinical availability of each modality are discussed to provide comprehensive guidelines for selecting the most efficient approach for gene therapy in preclinical toxicology models. Conclusions: Selecting the proper route of administration and methodology and the most appropriate

species results in a reduced risk related to CNS delivery and improves the potential for translation of preclinical studies to the clinical scenarios. MRIgFUS is emerging as a transformational methodology for the treatment of neurological indications.

LBA-23. Helper-Dependent Adenoviral Vector-Mediated Muscle Expression of a Murine LDLR/Transferrin Fusion Protein Safely Ameliorates Lipid Profile in LDLR-Deficient Mice

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Familial hypercholesterolemia (FH), a monogenic genetic disorder, leads to an inability to process low-density lipoprotein (LDL), which leads to high LDL levels and severe atherosclerosis. Therefore, without treatment, these individuals are at a higher risk for developing life-threatening conditions such as severe heart disease, leading to early mortality. To date, several treatments are available but have cons such as side effects, intolerance issues, and more importantly, rely on at least partial presence of endogenous functioning LDLRs to work effectively. In order to treat patients with no residual LDLR activity, we have developed helper-dependent adenoviral (HD-Ad) vectors for the expression of a murine LDLR/Tf chimeric protein under the control of the mCK promoter for intramuscular delivery (HD-Ad-mCK-mLDLR/mTf); this route of administration should reduce inflammatory and immunologic responses therefore increasing safety and duration of the therapy. In vitro functional analysis indicated that the fusion protein can effectively correct the phenotype of LDLR-deficient cells and can increase LDL uptake. The effects of treatment with HD-Ad-mCK-mLDLR/mTf were evaluated in LDLR-deficient mice through intravenous or intramuscular administration and the evaluation of triglycerides (TG), total cholesterol (TC), LDL-C, and HDL-C at different time points reveals a significant and long-lasting amelioration of lipid profile. Furthermore, evaluation of IL-6, IL-12, TNF-α, platelets, and D-dimer markers, commonly associated with activation of the immune response suggests a safety of HD-Ad-mCK-mLDLR/mTf administration either intravenously or intramuscularly. To monitor the hepatotoxicity we evaluate serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels 72 hours post-infection and ALT and AST levels in both mice groups fell within the accepted normal range. The results obtained until now, support the possibility of treating FH through a gene therapy approach and show the potential for additional studies that evaluate the safety of this gene therapy treatment as a long-term correction solution, which prevents activating the immune system by avoiding toxic build-up.

LBA-24. AAV-based Gene Therapy as Potential Treatment for CTNNB1 Syndrome

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CTNNB1 syndrome is a rare neurodevelopmental disorder (1:50,000) caused by loss-of-function mutations in the CTNNB1 gene that lead to a wide range of phenotypes among patients. CTNNB1 gene encodes for beta-catenin, which plays a critical role in neuronal development, synapse formation, and maturation of the brain. Consequently, these mutations lead to cognitive impairments such as intellectual disability, learning difficulties, and developmental delay as well as progressive spasticity and motor skills detoriation.

Although there is currently no treatment for CTNNB1 syndrome, the genetic root cause of disease could be addressed by using adeno-associated viral (AAV) vectors to deliver a functional copy of the gene into the cells involved in disease phenotype. This would lead to the recovery of normal protein functions and the potential amelioration or even correction of the disease phenotype. As no gene augmentation therapy had been tested before for this disorder before, we designed six different AAV-CTNNB1 constructs. Each construct included a correct coding sequence of CTNNB1 gene along with a variety of untranslated regulatory elements to enhance the expression of the gene in the target cells and inhibit expression in off-target tissues.

These constructs were individually packaged into AAV vectors that were used to transduce patient-derived neuro-progenitor cells and cortical brain organoids. We found that only one of our constructs was able to restore beta-catenin expression and function in both preclinical models (Figure 1A, B). This AAV-CTNNB1 construct was further analysed in a disease mouse model ($Ctnnb1^{+/-}$). Three increasing doses of an AAV9-Construct4 vector were inoculated to the animals intracerebroventricularly (ICV), and behavioural tests were conducted to assess phenotype correction. We observed correction of anxiety and locomotor functions at 20 weeks of age (Figure 1C, D), two phenotypes that recapitulate symptoms manifested by CTNNB1 syndrome patients.

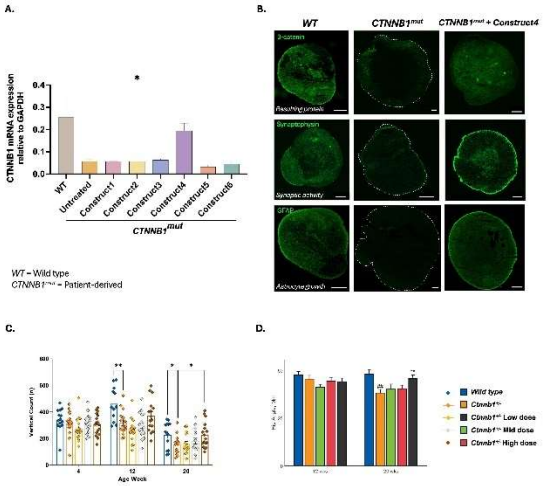

Figure 1. A) *Effect of AAV-Construct1-6 on CTNNB1 mRNA expression in patient-derived (CTNNB1^{mut}) cortical brain organoids. * p<0.05, Student's t test.* ***B)*** *Immunofluorescence staining for beta-catenin, synaptophysin, and Glial Fibrillary Acidic Protein (GFAP), (green) in WT, and CTNNB1^{mut} organoids treated or not with AAV-Construct4. Scale= 200 um.* ***C)*** *Effect of AAV-Constuct4 administration on total rearing frequency during open field test as a measure of anxiety in Ctnnb1$^{+/-}$ mice. * p<0.05, ** p<0.01, Student's t test.* ***D)*** *Hip angle in kinematic gait analysis as measurement of locomotor function. ## p<0.01, Student's t test between WT and Ctnnb1$^{+/-}$ mice, ** p<0.01, Student's t test between untreated Ctnnb1$^{+/-}$ mice and Ctnnb1$^{+/-}$ mice treated with the high dose of AAV-Construct4.*

These preclinical data demonstrate the efficacy of ICV AAV9-CTNNB1 in the restoration of beta-catenin expression and function, resulting in the correction of anxious behaviour and locomotor function in a disease mouse model, and support an investigational gene therapy clinical trial to treat CTNNB1 syndrome.

LBA-25. Generation of Allogeneic CAR-NKT Cells for Cancer Immunotherapy with Genetically Engineered CD34+ HSPCs and Xenogeneic Feeder-free Culture

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Autologous chimeric antigen receptor (CAR) T cell therapy faces challenges in manufacturing, cost, and patient selection that could be avoided by using 'off-the-shelf' products, such as allogeneic CAR natural killer T (AlloCAR-NKT) cells. Previously we reported a culture system for differentiating human CD34+ hematopoietic stem and progenitor cells (HSPCs) into AlloCAR-NKT cells, but the use of 3D xenogeneic feeder-dependent culture limits its clinical potential. Here we describe a xenogeneic feeder-free culture to generate IL-15-enhanced AlloCAR-NKT cells targeting various cancers including multiple myeloma with high yield and purity. In vivo, the cells exhibit potent antitumor efficacy, expansion, and persistence. They selectively deplete immunosuppressive cells in the tumor microenviroment and antagonize tumor immune evasion via triple targeting of CAR, TCR and NK receptors. They exhibit a stable hypoimmunogenic phenotype that we trace to epigenetic and signaling regulation. These properties of AlloCAR-NKT cells, together with their low risk of graft vs host disease and cytokine release syndrome, support their potential for clinical translation.

Thursday Late-Breaking Abstracts

LBA-26. Base Editing of Human Hematopoietic Stem and Progenitor Cells with the Pin-point™ Platform

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Hematopoietic stem and progenitor cells (HSPCs), with their ability to self-renew and restore all blood cell populations, are a foundational cell type for the development of engineered therapies for monogenic disorders and cancer immunotherapy for which the substitution of endogenous hematopoiesis with genetically corrected or healthy donor-derived cells can halt the pathogenic process or support the therapeutic outcome. Gene editing is a promising tool to engineer HSPCs, opening the possibility to more precisely correct disease-causing mutations and introduce other functional changes specific to the therapy. Since engineered HSPCs are meant to persist throughout a patient's lifetime and are particularly sensitive to DNA damage, it is important to utilize a gene editing technology which helps minimize genotoxicity. Nuclease-induced double strand breaks (DSBs) at on- and off-target sites trigger the activation of a p53-dependent DNA damage response, and their processing may lead to chromosomal aberrations. Conversely, base editors that are not reliant on DSBs represent an efficient mitigation strategy to these challenges due to their reduced genotoxic potential. We have developed the Pin-point^{TM} platform[1], which enables the modular assembly of the base editing machinery, including a DNA binding Cas and a DNA modifying deaminase, at the target locus via the interaction between an aptamer binding protein fused to the deaminase and an RNA aptamer located in the scaffold of a sequence-targeting guide RNA (gRNA). In addition to allowing modifications of the DNA without relying on the introduction of DSBs, the modularity and aptamer-dependent nature of the platform allows for high flexibility in the customization of each component to address specific editing needs. Complex genetic modifications can be now

possible in a single intervention as demonstrated in primary human T cells[2] and iPSCs, where we achieved efficient base editing at multiple sites and simultaneous targeted transgene knock-in without compromising genome integrity. The advanced safety profile of this technology makes it well suited to the development of cell and gene therapies in sensitive cell types such as HSPCs. Here we show that by optimising reagent design and delivery conditions of a Pin-point base editor composed of Rat APOBEC1 and SpCas9 nickase mRNAs in combination with a control synthetic aptamer-containing gRNA, we achieve up to 80% C to T conversion at the target base in CD34+ HSPCs. Using the optimised conditions, we then targeted two separate loci known to reactivate γ-globin expression: 1) the erythroid enhancer of the repressor BCL11A, and 2) the BCL11A binding site in the HBG promoter. We achieved a high level of base editing at both loci that corresponded with an increase in γ-globin mRNA and protein expression as a relevant therapeutic outcome. Edited HSPCs retained viability, immunophenotype, and differentiation potential toward the erythroid lineage *in vitro*. While concerns have been expressed about base editors leading to imprecise outcomes at target sites ascribed to DNA DSBs, with our technology and optimised conditions, we observe precise editing outcomes with high levels of editing purity at the target site and very low incidence of indels, an indirect measure of DSBs occurrence. The ability to base edit HSPCs efficiently and safely, while retaining high cell viability and differentiation capability, demonstrates the strength of the Pin-point™ platform as a tool for the generation of advanced cell therapies using sensitive multipotent cell types. 1. Collantes JC, Tan VM, Xu H et. al. (2021). CRISPR J, 4:58-68 (PMID 33616445) 2. Porreca I, Blassberg R, Harbottle J et. al. (2023). Preprint at bioRxiv, 10.1101/2023.06.20.545315

LBA-27. WITHDRAWN

LBA-28. WITHDRAWN

LBA-29. Anti-Cancer Efficacy and Safety of hTERT Splicing Ribozyme-based RNA Editing Therapy for Glioblastoma Multiforme

Seungryul Han[1], Sarah Yoon[1], In Hye Jeong[1], Hye Rim Park[1], Chae Won Song[1,2], Kum Hee Noh[1], Ji Hyun Kim[1], Bomi Kim[1], Sungwoo Hong[1], Seong-wook Lee[1,2]
[1]Rznomics Inc., Seongnam-si, Korea, Republic of,[2]Dankook University, Yongin-si, Korea, Republic of

Group I intron-based trans-splicing ribozyme enables RNA editing of the entire downstream part of targeted RNA site by catalytically sensing and reprogramming target RNA into the gene of interest. Previously, we proposed human telomerase reverse transcriptase (hTERT) targeted trans-splicing ribozyme harboring therapeutic suicide gene for hepatocellular carcinoma (HCC) therapy and received IND approval from both Korea and US FDA for first-in-human phase 1/2a clinical trials of the trans-splicing ribozyme. Glioblastoma multiforme (GBM) is one of the most aggressive cancers with a 5-year survival rate of only 7.2%. However, effective treatment options are currently lacking. In most GBM, hTERT promoter mutations are observed, and the resulting increased hTERT expression plays an essential role in GBM growth. In this study, we expanded and observed the preclinical anti-cancer effects, biodistribution, and toxicity of the RNA editing approach based on the genetically modified replication-incompetent adenoviral vector encoding the hTERT-targeted trans-splicing ribozyme, called RZ-001, for GBM. RZ-001 treatment combined with ganciclovir (GCV) induced cytotoxicity in human GBM cells in a dose-dependent manner. Moreover, intratumoral single dose treatment of RZ-001 followed by the administration of GCV significantly reduced tumor volume and increased overall survival in orthotopic GBM mouse xenograft model, compared with control group or treatment group with adenoviral vector encoding the suicide gene. Importantly, RZ-001 administration followed by GCV treatment efficiently improved median survival rate in PDX model established with tumor tissues from recurrent and temozolomide (TMZ)-resistant GBM patient, compared with groups treated with control or TMZ. Biodistribution and safety study were performed in ICR mice following intrastriatal single-dose treatment of RZ-001. RZ-001 level was shown to decrease in the brain (injection site) from week 2. At week 24, RZ-001 was completely cleared out in all organs of all animals. No lesions indication toxicity of RZ-001 were observed in the safety study including histopathological examinations. Taken together, RNA editing strategy mediated by hTERT-targeted trans-splicing ribozyme could provide a clinically relevant, safe, and effective approach for GBM therapy. Based on the results, RZ-001 received IND approval for phase 1/2a clinical trials from the Korean Ministry of Food and Drug Safety and the US FDA for recurrent GBM patients. Recently, RZ-001 received Fast Track designation from US FDA for GBM patients. This study and recent recognitions by the US FDA may raise the potential of splicing ribozyme-based RNA editing approaches as a safe and effective therapeutic option for patients with highly unmet medical needs.

LBA-30. Repurposed DNA Repair Inhibitors to Enhance Targeted Integration Drastically Increases Chromosomal Instability

Nathan White, Yi-Ting Hu, Alex Chalk, Adrian Thrasher, Giandomenico Turchiano
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The efficacy of therapeutic gene editing strategies, which aim to integrate a donor template using designer nucleases and exogenous sequences, can be compromised by undesired repair outcomes such as indels via the non-homologous end-joining (NHEJ) pathways. To mitigate sub-optimal integration efficiency, compounds that inhibit the end-joining repair pathways have been increasingly repurposed to direct cells to preferentially repair via the homology-directed repair (HDR) pathway. However, the effect of systemic repair inhibition and how this impacts the repair products generated from off-target double-strand breaks has not been thoroughly investigated. To explore

this, we cultured CRISPR-Cas9 edited haematopoietic stem and progenitor cells (HSPCs) with an end-joining repair inhibitor cocktail (ART558 and AZD7648) and performed CAST-Seq and digital PCR (dPCR) to quantify aberrations. To our surprise, dPCR revealed up to 60% of total alleles harbouring double-strand breaks at known off-target loci before inhibitors were removed. Following this, we observed a 15-fold increase in both the total number of identified translocations and mapped events with the on-target locus in repair inhibitor-treated cells using CAST-seq. By quantifying the absolute frequency of three previously identified translocations using dPCR, we found these translocation combinations alone accounted for 1.6% of the total alleles, up from 0.24% in the absence of repair inhibitors. Integrating a donor sequence into a specific locus is a promising corrective gene therapy strategy. However, our latest findings reiterate that careful strategy design should be at the forefront of designer nuclease-based gene therapies. Seeking to adopt repair-inhibiting compounds may improve integration efficacy but at the cost of genome destabilisation and increasing the risk of generating genotoxic by-products. We propose that repair-inhibiting compounds may be better suited as sensitivity-enhancing agents to improve the resolution of existing off-target and aberration-detecting techniques.

LBA-31. A Targeted Non-Viral CRISPR-Based In Vivo Gene Editing Strategy for the Treatment of Sickle Cell Disease

Christophe LECHAUVE, Sheryl WANG, Patricia B. Berthelette, Andrew DRABEK, Kiki IGNATIADIS, Hong WANG, Cathleen Cornell, Grace ACKWII, Douglas Drager, William KUHLMAN, Jasmine BLOOM, Eyoung SHIN, Aaron GRISET, Zhaohua HUANG, Martin Goulet, Christian Mueller, Christopher Borges, Tongyao Liu
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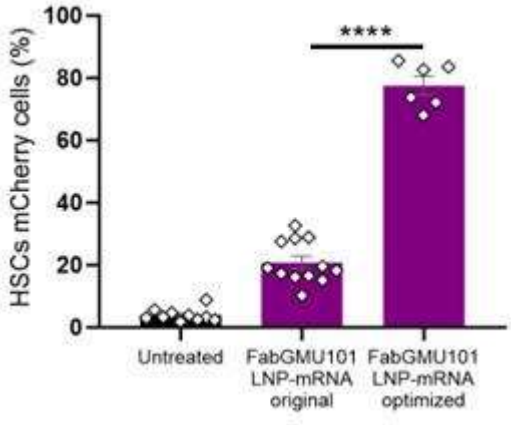
Hematopoietic stem cells (HSCs) reside in the bone marrow (BM), where they divide throughout life to produce all cells of the blood and immune system through their self-renewing capacity. HSC modification using CRISPR/Cas9 technology has demonstrated tremendous promise to cure sickle cell disease (SCD) by preventing red blood cell sickling and vaso-occlusive events through genetic modification of regulatory elements of *BCL11A,* a repressor of fetal γ-globin (fetal hemoglobin; HbF). We have developed a non-viral gene therapy strategy that leverages CRISPR nuclease mRNA and an associated guide RNA (gRNA) to be encapsulated by an LNP as its cargo and be efficiently delivered in vivo into human HSC in the bone marrow environment. We analyzed on- and off-tissue transfection using HSC-targeted lipid nanoparticles (LNPs) encapsulating nucleoside-modified messenger RNA (mRNA) by a single dose injection.

Here, we describe HSC-targeted lipid nanoparticles (LNP) conjugated to a previously selected antigen binding fragment (ASGCT 2023) encapsulating mRNA (FabGMU101/LNP-mRNA) to efficiently transfect HSCs in vivo. LNPs were engineered, using different ionizable lipids and formulated with variable compositions of an ionizable lipid, helper lipid, cholesterol, and PEG-lipid to efficiently transfect HSCs in vivo. Using human $CD34^+$ hematopoietic stem cells cultured in vitro and hu-CD34 engrafted NSG™ mice, we observed increased transfection efficiency, from 65% to >90% in vitro, and from 20% to ~80% in vivo (Figure 1). Moreover, using our optimized formulation, we measured a 2-fold increase of mCherry mean fluorescent intensity of HSCs in vivo.

The improved FabGMU101/LNP-mRNA formulation which enabled improved binding and transfection of HSCs in combination with CRISPR nuclease-mRNA, will form the foundation of a transformative platform to eliminate some limitations and challenges of ex vivo therapy and to successfully engineer human HSCs in vivo for the treatment of SCD that will be highly effective, economically viable, and safe.

Figure 1 legend: **In vivo HSC targeted-LNP**. hu-CD34 engrafted NSG™ mice were infused with LNP encapsulated mCherry mRNA, i.v. LNP were conjugated with the selected FabGMU101 antibody specific for long-term hematopoietic stem cells (LT-HSC). Original and optimized LNP formulation are presented to illustrate the improvement of LT-HSC transfection efficiency in vivo measured by flow cytometry analysis of BM 24 hours following transfection.



LBA-32. Long-Circulating Lipid Nanoparticles (LCLNP) Effectively Deliver Nucleic Acids to Hematopoietic Stem and Progenitor Cells in the Bone Marrow

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NanoVation Therapeutics, Vancouver, BC, Canada

Hematopoietic stem and progenitor cells (HSPCs) are the precursors of most immune cells. Specifically, haematopoietic stem cells (HSC) possess the ability to self-renew and to give rise to downstream differentiated cell populations. These features make HSPCs of particular interest to treat and potentially cure rare genetic diseases. HSPCs mainly reside within the bone marrow and are considerably inaccessible by current LNP formulations following intravenous administration. Reported methods of achieving HSPC delivery rely on approaches that are not clinically relevant, such as incorporation of targeting ligands or charged lipids. Therefore, designing formulations

capable of delivering to extrahepatic tissues remains an important hurdle to effectively manipulate HSPC populations *in vivo*. Here, we have developed the long-circulating LNP (lcLNP), which is engineered to have an extended circulation time and deliver nucleic acids to extrahepatic tissues, including the bone marrow. These lcLNP formulations consist of an ionizable lipid, helper lipid, cholesterol, and PEG-DMG, and typically contain elevated levels of phospholipids. Using a rational design approach, we have demonstrated our lcLNP formulations are capable of functional delivery of nucleic acids to extrahepatic tissues, such as the bone marrow, and demonstrate robust expression in HSPCs. When compared to a benchmark LNP with the composition 50/10/38.5/1.5 (ionizable lipid/DSPC/cholesterol/PEG-DMG in mol%), lcLNPs displayed significantly increased reporter expression in several HSPC populations, specifically reaching 30-40% of long-term HSCs post intravenous administration in mice. These studies show that a combination of an appropriately designed formulation, including selection of lipids and compositions, results in significant improvements of delivery to HSPCs in the bone marrow compared to clinically approved LNPs. Finally, this work demonstrates a new generation of LNPs capable of enhanced HSPC delivery, suggesting an important development in the field of gene therapy.

LBA-33. Peripheral Nerve Remyelination and Restoration of Neuro-Muscular Function by Allogeneic Schwann Cell-like Therapy: Preclinical Models of Charcot-Marie-Tooth Type 1 Neuropathy and Phase 1 Trial in CMT1 Patients.

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The peripheral nervous system retains a significant capacity for repair and regeneration, yet, through genetic inheritance, trauma, infection or chronic disease, many patients are unable to mount an appropriate regenerative response and may be subject to lifelong pain and disability. Cellatoz Therapeutics has developed a novel tonsil MSC-derived neuronal regeneration-promoting cell therapy (CLZ-2002), which is fully differentiated to display multiple Schwann cell-like characteristics, including the remyelination and regeneration of damaged nerves. CLZ-2002 is an allogeneic therapy manufactured under GMP as an 'off-the-shelf' treatment and, as such, highlights the potential for therapeutic intervention in a range of peripheral nerve indications. Our primary indication is Charcot-Marie-Tooth disease Type 1, a rare peripheral neuropathy where impaired transmission of nerve signals gives rise to sensory deficits and muscle wasting in the extremities. CMT Type 1A represents the largest sub-group of patients and is caused by duplication of the peripheral myelin protein 22 (PMP22) gene. Overexpression of PMP22 in CMT1A Schwann cells leads to defective myelination of peripheral nerves and impaired nerve conductance. We utilized the C22 mouse model of CMT1A which harbours duplication of the human PMP22 gene and displays a severe neuropathy phenotype. At 12 weeks following bilateral injection into C22 mouse gastrocnemius muscle, CLZ-2002 promoted sciatic nerve remyelination, restored nerve conductance, gastrocnemius muscle morphology and markedly improved motor functions. It is of note that, as a cell-based therapy, CLZ-2002 was able to normalise PMP22 overexpression in C22 mouse sciatic nerves in a dose-dependent manner. Repeat CLZ-2002 dosing gave rise to further gains in efficacy. Improvements in C22 mouse mobility persisted for up to 9 months following a single CLZ-2002 administration. Allogeneic CLZ-2002 therefore represents an innovative scientific and logistical approach to intervene in the progression of disability associated with peripheral neuropathy. Based on this positive preclinical data, CLZ-2002 was progressed to a Phase 1, open-label, prospective, dose-finding study for the evaluation of safety and tolerability of intramuscular injections of CLZ-2002 in patients with Charcot-Marie-Tooth Type 1 disease.

LBA-34. The Preclinical and First-in-Human Study of iPSC-Derived Human Forebrain Neural Progenitor Cell Injection-hNPC01 in Treating Chronic Ischemic Stroke

Jing Fan[1], Anxin Wang[2], Fang Ren[1,2], Yinggang Yan[1,2], Shuning Zhang[1,2], Jasmine Wang[3]
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The hNPC01 injection, is an injection containing Human Forebrain Neural Progenitor Cells (hFNPCs) derived from induced Pluripotent Stem Cell (iPSC) and manufactured under GMP condition. This allogenic cell therapy product contains more than 95% hFNPCs and its progeny cells, and was able to differentiate further into functional cortical neurons and glia cells both *in vitro* and *in vivo*. In previous mechanism and preclinical studies, hNPC01 showed potential in promoting functional recovery of ischemic stroke rat and cynomolgus monkey models and forming neural circuitry *in vivo*. The preclinical safety study programs including tumorigenicity study in NOG mice up to 52 weeks indicated a good safety profile of hNPC01. A phase I open-lable dose-escalation clinical study of hNPC01 injection treating chronic ischemic stroke by intracerebral injection is approved by NMPA and currently undergoing. The current observation of first dose cohorts showed good safety and tolerance of hNPC01, as well as sign of motor function improvement in volunteer subjects with ischemic stroke onset 17 to 40 months prior to the enrollment. It is expected that the 12 month safety observation and exploration of evaluation tools will provide more valuable information for the future development of iPSC cell therapy for stroke or traumatic brain injury caused brain injury patients with chronic symptoms.

LBA-35. FT522: A CAR NK Cell with the Unique Ability to Target Multiple Pathogenic Cell Types and Circumvent Lympho-conditioning in Systemic Autoimmunity

Alec Witty[1], Amanda Sims[1], Alan Williams[1], Daniel Morales-Mantilla[1], Yijia Pan[1], Amber Chang[1], Christine Y. Chen[1], Karina Palomares[1], Lauren Fong[1], Nicholas Brookhouser[1], Berhan Mandefro[1], Sajid Mahmood[1], Frank Cichocki[2], Jodie P. Goodridge[1], Jeffrey S. Miller[2], Anil Bagri[1], Bob Valamehr[1]
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CD19-targeted chimeric antigen receptor (CD19 CAR) T cells represent a potential paradigm shift for the treatment of B-cell mediated autoimmune diseases (AID). In a recently published series, patients with refractory AID treated with conditioning chemotherapy and autologous CD19 CAR T cells achieved drug-free remission with evidence of immunological reset. These results have led to the investigation of a number of CD19 CAR-targeted cell therapies in AID. However, drivers of pathology can include additional immunological mediators including plasma and T cells. Also, the use of conditioning chemotherapy, which has been necessary for effective treatment with cell therapy, is associated with significant toxicities including poor immune reconstitution, increased susceptibility to infections, and secondary malignancies. Therefore, a next-generation cell therapy would aim to maintain the same B-cell depletion activity, target additional diseased cell types, reduce or eliminate the need for standard patient conditioning, and enable combination with existing standard of care therapies. To address these challenges, we developed FT522, a multiplexed-engineered, off-the-shelf CAR natural killer (NK) cell, which is derived from a clonal, CD38 knockout (KO) human induced pluripotent stem cell (iPSC) line that expresses a CAR targeting CD19; an alloimmune defense receptor (ADR) targeting 4-1BB; a high-affinity, non-cleavable CD16 (hnCD16) to maximize antibody-dependent cellular cytotoxicity (ADCC); and an interleukin (IL)-15/IL-15 receptor fusion protein (IL-15RF) for enhanced function. FT522 exhibited CD19-specific cytotoxicity toward CD19+ B cells through multiple rounds of target cell re-stimulation in a manner similar to the primary CD19 CAR T cells (Fig 1A). Additionally, in combination with rituximab or daratumumab, FT522 elicited potent ADCC against CD20+ and CD38+ B cell populations, respectively, while primary CD19 CAR T cells failed to eliminate these same populations in the absence of CD19 expression (Fig 1B). Similar observations were seen in vivo, as FT522 effectively depleted CD19+ B cells in a mouse model, and was further enhanced when delivered in combination with rituximab or daratumumab (Fig 1C). Notably, CD38+ populations, including T cells, which can drive pathology in advanced AIDs, were depleted in combination with daratumumab. Furthermore, in a mixed lymphocyte reaction assay using unmatched PBMCs from SLE-diagnosed donors, FT522 maintained persistence while eliminating CD19+ B cells and alloreactive T cells (Fig 1D,E) as compared to CD19 CAR NK cells without ADR arming, indicating that the functionality of FT522 can be uniquely maintained in the presence of an unmatched host immune system. Our data suggest that FT522 has the unique ability to elicit durable elimination of CD19+ B cells, target multiple pathogenic cell types in combination with therapeutic antibodies, and eliminate the need for conditioning chemotherapy with uncompromised effector function. Collectively, FT522 represents a promising off-the-shelf cell therapy strategy for treatment of numerous autoimmune diseases while eliminating toxicities associated with patient conditioning.

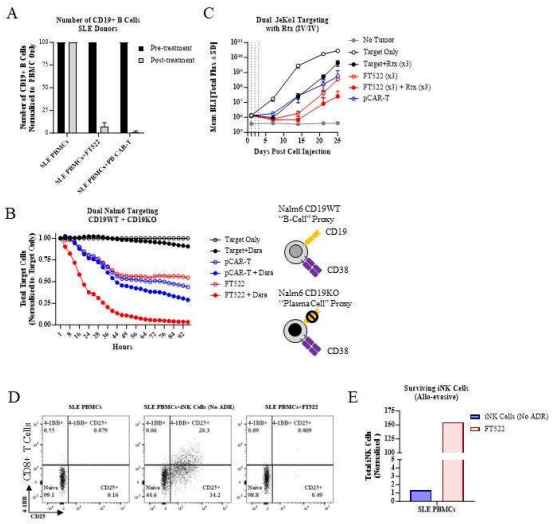

Figure. FT522 is uniquely able to target multiple cell types in the presence of unmatched PBMCs from AID donors.

A) FT522 depletes CD19+ B cells from unmatched, SLE-diagnosed donor PBMCs in a manner comparable to primary CD19 CAR T cells (pCAR-T) when co-cultured in vitro. B) FT522 demonstrates robust targeting of both CD19WT (B cell proxy) and CD19KO (plasma cell proxy) NALM6 cells in combination with daratumumab (Dara) through CAR and ADCC-mediated cell killing in vitro. In comparison, pCAR-T cells demonstrate CD19WT NALM6 cell killing, but fail to target CD19KO Nalm6 cells in combination with Dara. C) FT522 demonstrates dual targeting of JeKo1 cells, a CD19+CD20+ B cell proxy, in combination with rituximab (Rtx) with greater cytotoxicity than pCAR-T alone in an in vivo NSG mouse model. D) In a mixed lymphocyte reaction model, FT522 controls and suppresses alloreactive 41BB+ CD8+ T cells from an unmatched, SLE-diagnosed donor PBMCs through ADR, whereas CD19 CAR iNK cells without ADR fail to control 41BB+ T cells. E) In a mixed lymphocyte reaction model using unmatched, SLE-diagnosed donor PBMCs, FT522 demonstrates persistence whereas CD19 CAR iNK cells without ADR are depleted.

LBA-36. WITHDRAWN