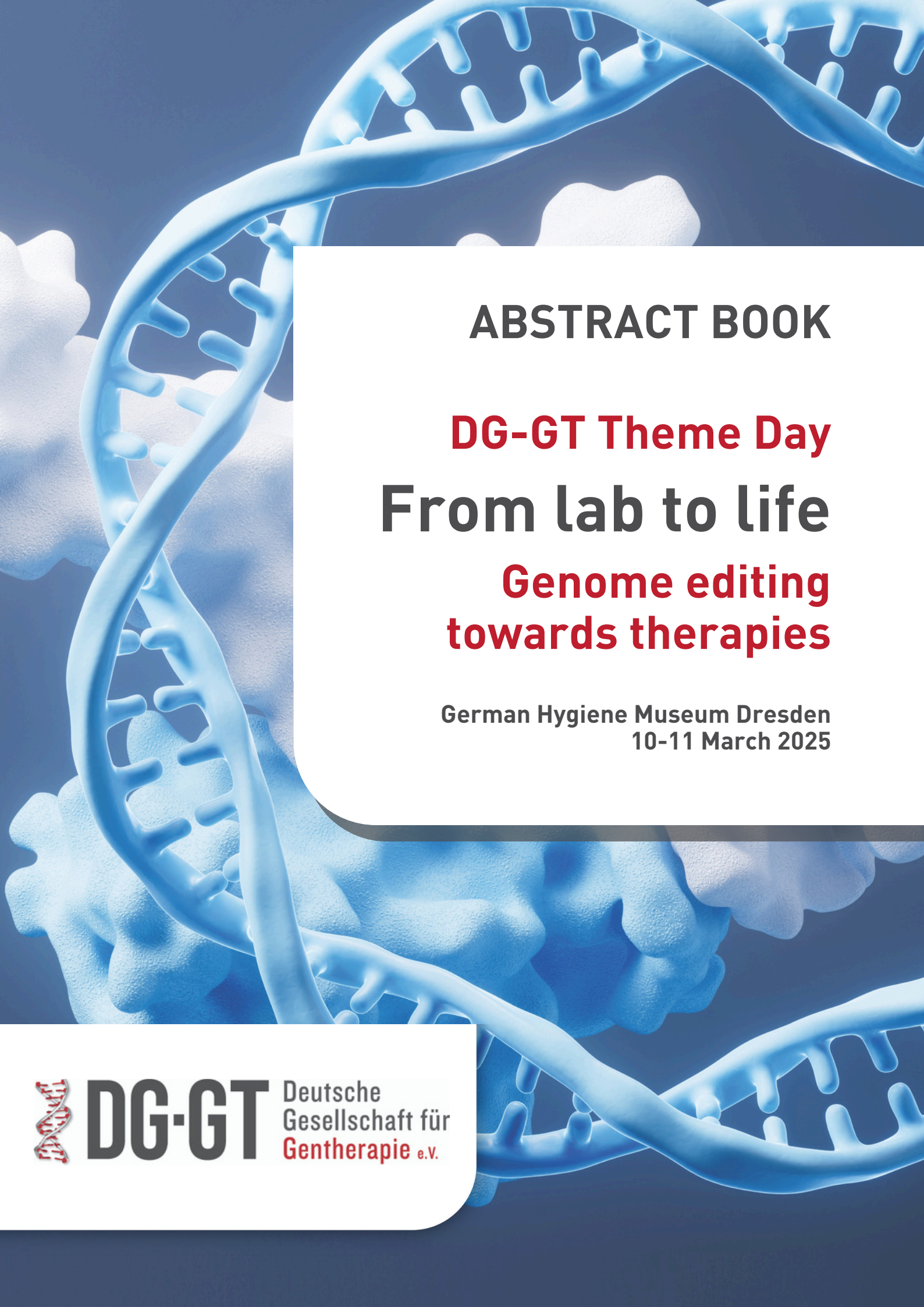

ABSTRACT BOOK

DG-GT Theme Day

From lab to life

Genome editing towards therapies

German Hygiene Museum Dresden
10-11 March 2025

DG-GT Deutsche Gesellschaft für Gentherapie e.V.

Invited Speakers

INV02

Comprehensive Interrogation of Synthetic Lethality in the Human DNA Damage Response

J Fielden [1] SM Siegner [1] JE Corn [1]
1: ETH Zürich

The DNA damage response (DDR) is a multi-faceted network of pathways that preserves genome stability. Because endogenous damage is an existential and continual threat, cells achieve robustness by engaging multiple overlapping pathways to deal with it. Addressing this complexity is a daunting challenge because gene functions in essential DNA repair processes can be masked and overlooked when pathways use completely different mechanisms to complement one another.

To tackle this problem, we undertook the most systematic genetic interaction investigation of the human DDR to date. We used dual guide CRISPR interference (CRISPRi) screening to query pairwise interactions of all core GO-annotated DDR genes spanning all DDR pathways, interrogating 679,233 unique guide RNA pairs and 149,878 gene combinations. Thus, we generated a comprehensive genetic interaction map of the human DDR, which we made available at https://spidrweb.org/. Our data yield fundamental insights into genome maintenance, provide a springboard for mechanistic investigations into new connections between DDR factors, and pinpoint synthetic vulnerabilities that could be exploited in cancer therapy.

I will summarize our CRISPRi screening work and highlight its many applications. I will also present data defining the molecular mechanism for one of the strongest, novel synthetic lethal interactions we identified between the DNA translocases, SMARCAL1 and FANCM.

INV10

Programmable recombinases to treat genetic diseases

F Lansing [1]
1: Seamless Therapeutics, Dresden

Programmable recombinase technology represents a transformative advancement in the field of gene editing, offering unprecedented precision and versatility for genomic modifications. Unlike traditional approaches, which often rely on CRISPR-based systems, other nuclease-dependent methodologies and cellular DNA repair, programmable recombinases achieve targeted DNA edits without inducing double-strand breaks or relying on cellular DNA repair mechanisms. This unique capability might lower the risk of off-target effects and enhance the fidelity of genetic modifications.

Programmable recombinases allow for precise insertion, deletion, and replacement of large DNA sequences at predetermined genomic loci, thereby expanding their applicability across a broad

spectrum of therapeutic areas. From correcting pathogenic mutations in monogenic diseases to engineering complex genomic rearrangements, our platform addresses key challenges in developing durable and effective gene therapies.

We will share some of our initial data on programmable Large Serine Recombinases (LSRs) for targeted integration of DNA to potentially allow for treatment of multiple diseases, and our approach to use large deletions to treat blood disorders. Our platform research is focused on optimising recombinase engineering to further enhance their specificity and programmability, with the goal of translating these innovations into clinical therapeutics.

INV13

The ethics of human genome editing

K Alex [1 2]
1: AG Winkler, Section Translational Medical Ethics, Department of Medical Oncology, National Center for Tumor Diseases (NCT), NCT Heidelberg, a partnership between DKFZ and Heidelberg University Hospital, Heidelberg University, Medical Faculty Heidelberg, Germany 2: Philosophy Seminar, Heidelberg University, Germany

A central issue in recent debates about genome editing (GE) is the question whether germline GE (GGE) should be translated from lab to life, too (cf. position papers by the US' & UK's International Commission on the Clinical Use of Human GGE, and by the German Ethics Council). The ethics of human GE are primarily concerned with this question and have been since before the advent of CRISPR/Cas. In this talk, selected aspects of the more recent debate are presented. First, the numerous ethical flaws of the "He Jiankui Affair" (as Henry Greely calls it), which can be taken as reasons against proceeding from lab to life with GGE, are summarized. Second, insights into current ethical debates are offered by outlining an argument for the position that GGE is very unlikely to affect the edited embryo (or the person it becomes) in either a beneficial or harmful manner since it is quite probable that this person never came into existence without GGE. This argument has been independently formulated by several bioethicists (e.g., Robert Sparrow, Thomas Douglas, Christoph Rehmann-Sutter and me). Third, implications of this finding for determining whether we should proceed from lab to life with GGE are discussed. Since the ethical debate has been primarily concerned with interventions at the germline level ever since its inception, the focus of the presentation is chosen accordingly, but ethical challenges relating both to somatic and germline interventions (e.g., risks, equity of access, and cost coverage) will be considered in a final outlook.

Oral Presentations

OR01

Establishment of a stable episomal replicon system for HBV to screen novel RNA-based antiviral gene therapies

P Mallick [1] S Ambike [1] J Kornherr [1] MZ Masood [1] J Grünewald [1] U Protzer [1]
1: Technische Universität München

Chronic Hepatitis B virus (HBV) infection is a significant global health issue, affecting approximately 254 million people and resulting in 1.1 million deaths annually. While nucleos(t)ide analogs control virus replication, they do not cure HBV and require lifelong treatment. Therefore, there is an urgent need to develop a curative therapy. To directly target HBV that persists episomally, it is essential to have an *in vitro* system where an HBV episome is present and replicated in every hepatocyte as in natural infection. In this study, we took advantage of plasmid pEpi in which a matrix attachment region sequence allows episomal persistence and replication in a copy number of 5-10 molecules per cell comparable to episomal HBV-DNA in infected hepatocytes. We integrated a replication-competent 1.3-fold HBV genome and created a stable cell line, Huh7-pEpiH1.3, and showed that they support the full life cycle of HBV and produce infectious virus from the episome. To target HBV therapeutically, we designed guide RNAs (gRNAs) compatible with cytosine base editors (CBEs) and adenosine base editors (ABEs) that enable precise DNA base conversions, allowing for the generation of missense and nonsense mutations without causing double-stranded breaks in the HBV genome. The *in-silico* design of gRNAs ensures compatibility with the predominant HBV genotypes circulating globally. Our novel Huh7-pEpiH1.3 screening system will allow the identification of the most effective gRNAs. We aim to validate our findings in hepatoma cell lines, primary human hepatocytes infected with HBV and in *in-vivo* models of HBV infection.

OR02

A direct comparison of the Sleeping Beauty Transposon system and the CRISPR/Cas9 machinery for genetic engineering

S Erkens [1 2] J Dietz [1 2] E Ehrke-Schulz [1 2] A Ehrhardt [1 2] W Zhang [1 2]
1: Witten/Herdecke University 2: Centre for Biomedical Education and Research (ZBAF)

Background: Genome editing technologies, including the Sleeping Beauty (SB) transposon system and CRISPR/Cas9 machinery, have caused a paradigm shift in both research and therapeutic applications. While widely utilized, there is a lack of direct comparative studies assessing their efficacy, specificity, and long-term performance, hindering the selection and optimization of these technologies for clinical translation.

Methods: Transfection experiments were conducted in HeLa and HEK293 cells to compare the hyperactive SB transposase SB100X with CRISPR/Cas9 systems targeting ANTXR1, HBEGF, and PSEN2. For functional optimization of CRISPR/Cas9, a variety of sgRNA:donor ratios (1:1, 1:2, 2:1) were utilized. Efficacy was measured by quantification of transgene integration and the assessment of long-term expression via a colony-forming assay. Cell viability and toxicity were evaluated using a CCK-8 assay, while colony size, spatial distribution and morphology were analyzed using ImageJ. To assess the genome-wide specificity of the systems, a comparative analysis of existing NGS datasets was conducted.

Results: CRISPR/Cas9 demonstrated a clear short-term advantage, achieving ~10% higher transgene integration compared to SB100X, while also showing significantly higher transfection efficiency (~56%). The highest integration efficiency was observed at a 1:2 sgRNA:donor ratio. However, SB100X clearly outperformed CRISPR/Cas9 in long-term colony survival, resulting in 25-fold more colonies after selection. CRISPR/Cas9 colonies were more uniform, with higher circularity values and expressed lower immediate toxicity post-transfection.

Conclusion: The results provide valuable insights into the applicability of both systems, suggesting that SB100X offers advantages for sustained gene expression and robust cell survival, while CRISPR/Cas9 may be more appropriate for precise gene editing.

OR03

Using High-Throughput Genetic Screening to Improve Stable AAV Producer Cell Lines and to enhance viral vector production

J Mahlberg [1] T Bauer [1] V Simha [1] R Mahmuti [1] M Ruby [1]
1: Cytiva

Adeno-associated virus (AAV) vectors are key materials in many gene therapies, yet how recombinant AAV production is regulated within producer cells—including vector genome packaging efficiency and yield—still isn't well understood. To address this challenge, we developed a high-content, modular genetic screening platform that can evaluate hundreds of plasmid designs in a few weeks.

Our approach pairs combinatorial design with advanced analytics to evaluate how diverse genetic elements influence AAV production. Using this platform, we identified several design combinations that enhance vector genome titer and packaging efficiency, leading to marked increases in vector titers and fullness. These findings provide helpful insights into the molecular determinants of how AAV vectors are regulated and offer a useful tool to optimize AAV production processes to meet the growing demands of gene therapy candidates in the clinic.

OR04

Programmable genome disruption enables selective elimination of cancer cells using a novel CRISPR-Cas nuclease

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1: Akribion Therapeutics

CRISPR-Cas represent a groundbreaking tool in genetic engineering, and over the last decade, numerous Cas nucleases with novel properties have been identified for a variety of different applications.

Our metagenomics screen revealed a novel Cas12a2 nuclease variant, termed G-daseE, with an unexpected mode of action. In contrast to classical CRISPR systems, G-daseE induces collateral degradation of DNA and RNA upon gRNA-mediated recognition of a target RNA, which ultimately leads to cell death.

Here, we demonstrate the potential of G-daseE for therapeutic applications by selectively targeting and eliminating cancer cells. In cell culture studies with G-daseE ribonucleoprotein (RNP), we successfully achieved selective cell depletion by targeting the HPV oncogenes E6/E7 in cervical carcinoma cell lines, while sparing oncogene-negative cells.

Furthermore, we explored an additional, more therapeutically translational, delivery strategy using G-daseE-mRNA and synthetic gRNA. Recent advances in mRNA delivery technologies, such as lipid nanoparticle (LNPs), have shown great promise in cancer therapy. We designed and tested different G-daseE mRNA constructs by varying stabilizing regulatory elements, codon optimizations, and nucleotide modifications to achieve optimal mRNA stability and translational efficiency. Using the optimal mRNA variant, we successfully induced programmed cell death in human cancer cells – an important step towards the development of LNP-based systems for in-vivo applications.

Overall, we have shown that G-daseE can be delivered as mRNA to induce selective cell elimination upon recognition of marker RNAs, providing an innovative strategy for targeted cancer therapies.

OR05

‘One-pot’ PASTA - Advanced T cell Engineering through Precise Integration of Super-Large Exogenous DNA

J Kath [1] I Kassing [1 2] A Nituslescu [1] L Hartmann [1] Y Pu [1] V Glaser [1] M Stein [1] D Ibrahim [1 3] DL Wagner [1 2]
1: Charité University Medicine 2: Baylor College of Medicine 3: Max Planck Institute for Molecular Genetics, Berlin

Adoptive T-cell therapy using gene-modified cells expressing target-specific receptors has emerged as a powerful treatment modality for malignancies and autoimmune diseases. However,

current gene transfer methods, such as lentiviral transduction and homology-directed DNA repair (HDR), are limited in their ability to integrate large DNA sequences, restricting the potential of engineered T-cells.

To address these challenges, we developed PASTA (programmable and site-specific transgene addition), a method enabling efficient, site-specific integration of large transgene cassettes. PASTA functions in two phases: Phase 1 integrates a small DNA landing pad into the genome using CRISPR-Cas-mediated HDR. Phase 2 employs a large serine integrase (e.g., bxb1) to insert a circular DNA vector at the landing pad. This 'one-pot' process is initiated by a single transfection, simplifying the procedure and making it suitable for clinical application.

'One-pot' PASTA facilitates the precise incorporation of multicistronic constructs, offering a simpler and more flexible alternative to approaches like PASSIGE and PASTE. Leveraging serine integrases, PASTA enhances T-cell engineering capabilities, enabling co-expression of therapeutic transgenes such as antigen receptors, safety markers, and cytokines, thereby boosting therapeutic efficacy and safety.

Beyond T-cells, 'one-pot' PASTA holds promise for treating genetic disorders involving large deletions, providing new treatment strategies for unmet clinical needs.

OR06

Gene editing for pre-leukemia bone failure syndrome, severe congenital neutropenia, – current advances and critical considerations

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1: University Hospital Tuebingen

Patients with severe congenital neutropenia (CN), an inherited pre-leukemia bone marrow failure syndrome, suffer from severe bacterial infections that usually begin shortly after birth. In addition to impaired granulopoiesis, patients with CN are at risk of myelodysplastic syndrome and acute myeloid leukemia. Most CN patients respond to daily subcutaneous injections of rhG-CSF. However, some do not, and some continue to have frequent infections despite rhG-CSF therapy. Many patients, particularly in adolescence and adulthood, experience bone pain with rhG-CSF, leading to treatment discontinuation and high risk of severe infections. The only curative treatment for CN is allogeneic hematopoietic stem cell transplantation, which is associated with severe side effects. Recent efforts by our group and others have led to the establishment of gene editing approaches aimed at correcting CN-associated mutations (e.g. in the *ELANE* and *HAX1* genes) or inhibiting mutated genes in patients' hematopoietic stem and progenitor cells ex vivo using CRISPR/Cas gene editing. Our newly established inhibition of *ELANE* mRNA expression by targeting its promoter with two Cas9 nickases is also applicable to other bone marrow failure syndromes. At the current stage of clinical translation, safety criteria, desirable therapeutic thresholds, patient selection criteria and ethical considerations are important for gene therapies using CRISPR editors in patients with CN and other pre-leukemic bone marrow syndromes. We have proposed a guideline for this, which needs to be further discussed and elaborated with experts in the field.

OR07

CRISPR-edited allogeneic BCMA-CAR NK cells to overcome loss-of-target in multiple myeloma

T Bexte [1 2] L Krähl [1] M Baulig [1] A Moter [1] S Friedel [3] Z Ivics [4] T Cathomen [5 6] M Hudecek [3 8] E Ullrich [1 9]
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Background: CAR immune cell therapies targeting B cell maturation antigen (BCMA) show promise for the treatment of Multiple Myeloma (MM). But tumor escape through BCMA loss limits their effectiveness. Natural killer (NK) cells offer potent killing capacity and a favorable safety profile as allogenic products in early clinical trials.

Here, we aim to overcome BCMA-target loss by enhancing the intrinsic anti-MM killing capacity of BCMA-CAR NK-cells through CRISPR-editing of inhibitory-immune-checkpoints. We present for the frist time, full non-viral protocol for CRISPR/Cas9-edited, SleepingBeauty (SB) BCMA-CAR NK-cells.

Methods: Primary NK cells were genetically modified using SB/minicircle-technology in parallel with CRISPR/Cas9-knockout of *KLRC1*. Genomic (TIDE) and phenotypical (flow cytometry) analysis were performed and anti-tumor functionality was addressed.

Results: BCMA-CAR NK-cells exhibit a similar expansion rate compared to non-transfected (NT) NK-cells. Long-lasting BCMA-CAR expression was monitored over four weeks. Significantly enhanced cytotoxicity of BCMA-CAR NK-cells compared to NT-NK cells was observed in end-point co-incubation and long-term live-cell imaging.

To model BCMA-target escape, we generated a MM1.S BCMA-knockout cell line, and confirmed loss of CAR-dependent anti-tumor capacity. To overcome tumor escape, NT and BCMA-CAR NK-cells were modified using CRISPR/Cas9-deletion of the *KLRC1* gene (encoding for NKG2A), in an efficient one-step nucleofection process. The fully virus-free engineered NKG2A-KO-BCMA-CAR NK-cells exhibited improved cytotoxic capacity compared to NT, CAR, or NKG2A-KO NK-cells against MM.

Conclusion: Advanced engineering of CAR-NK cells has the potential to overcome limitations of current immune cell therapy in MM. Our CAR-FACTORY-consortium, funded by German-Cancer-Aid, will support GMP manufacturing for upcoming phase I/II clinical studies.

OR08

AAV-mediated gene therapy in a porcine model of Duchenne Muscular Dystrophy

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1: Technische Universität München

Duchenne Muscular Dystrophy (DMD) is the most frequent hereditary childhood myopathy leading to progressive muscle degeneration followed by respiratory and cardiac failure and, ultimately, premature death. DMD is usually caused by frameshift mutations in the Dystrophin gene which encodes a 427 kDa protein.

As previously demonstrated, dual-AAV-delivered, split-CRISPR-Cas9-mediated excision of exon 51 can reframe the dystrophin gene in the DMDΔ52 pig model and ameliorate the muscular phenotype. However, in this study, we aim to achieve exon skipping by developing a base editor (BE) delivered as single- or dual-AAV-system targeting the splice acceptor site (SAS) of either exon 51 or 53 with the benefit of avoiding double strand breaks.

Screening of various deaminase and Cas9 combinations, together with suitable sgRNAs was performed in primary kidney fibroblasts of a DMD pig. Finally, editing efficacies up to 17% could be observed using a dual-AAV-system containing the ABE8e-BE and spRY-Cas9 targeting the SAS of exon 53. The two most promising dual-AAV-constructs were packed as AAVs and used to transduce *ex vivo* heart slices, followed by transduction and editing analysis. Furthermore, AAVs containing orthologous spacers were applied to human iPSC-derived DMDΔ52 cardiomyocytes resulting in an editing efficacy of 9.2%. and an exon skip rate of 10.4%.

Finally, in vivo application of the AAVs intracoronarily to DMDΔ52 pigs is scheduled, followed by electrophysiological, molecular and histological analyses.

This study aims to demonstrate that base editing may critically improve efficacy and safety of gene editing in DMD, a rapidly progressing disease with few effective alternate options.

OR09

An enhanced Single-Strand Break-Based Strategy for safer and more efficient Genome Editing

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Precise genome editing requires recombination between a DNA repair template and chromosomal sequences, typically initiated by DNA double-strand breaks (DSBs). However, DSBs are highly genotoxic and pose significant safety concerns. Emerging technologies such as base and prime editing reduce genotoxic risk by relying on single-strand breaks (SSBs). Despite their promise,

base editing has been linked to unintended DSB byproducts, while prime editing remains limited to small insertions or edits. Designer nickases have been explored as low-genotoxic tools for genome editing, yet the inherent inefficiency of SSB-mediated editing has driven efforts to enhance its performance. Strategies such as double nicks or coordinated paired nicks—targeting both the chromosomal site and the repair template—have shown potential for increased editing efficiency. Building on this concept, we developed a novel approach using Cas9 nickase (nCas9) fused to DNA repair pathway effectors. These fusions significantly improve the integration of a GFP expression cassette at a genomic locus via the in trans paired nicks strategy, achieving a 3-fold increase in efficiency compared to unmodified nCas9. Ongoing work involves testing additional nCas9 variants and repair effectors to optimize SSB resolution through homology-directed repair. These DSB-free strategies offer a safer alternative to traditional genome editing, mitigating risks associated with non-homologous end joining (NHEJ)-mediated repair. This approach holds particular promise for clinical applications, especially in targeting coding regions implicated in dominant genetic disorders.

Poster Presentations

P01

CAR-NK cell therapies for treatment of severe autoimmune diseases

M Arriens [1 2] A Eugster [4] E Bonifacio [4] S Bornstein [5] S Michen [6] A Temme [6] T Plötz [7] A Aigner [7] C Günther [8] K Hölig [1 2] SR Künzel [1 2] T Tonn [1 2 3] J Eitler [1 2 3]
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Autoimmunity affects 8-10% of the world's population, and in many cases, the standard of care is expensive and non-curative. Recently, CD19-specific chimeric antigen receptor (CAR) T cells have shown encouraging clinical efficacy in the treatment of selected B-cell-driven autoimmune diseases. However, autologous CAR-T cell therapy is expensive, logistically complex and has been associated with potentially serious side effects in the treatment of hematologic malignancies. Alternatively, natural killer (NK) cells have been shown to be potent but safe cytotoxic cells in hematologic oncology settings and can be used allogeneically as an off-the-shelf product.

Here, we developed CAR-NK cells targeting autoimmunity. We lentivirally transduced the clinically used NK-92 NK cell line or peripheral blood-isolated primary NK cells with a CAR

targeting the pan-B cell CD19 molecule (CD19.CAR) or with a CAR specifically binding B cells that are autoreactive against the DSG3 protein (DSG3.CAR).

CD19.CAR NK-92 and CD19.CAR primary NK cells showed stable CAR expression and high cytotoxicity against the CD19-positive B cell line NALM-6. In addition, CD19.CAR NK-92 cells selectively depleted B cells from autoimmune patients in vitro, while leaving other cell populations unaffected. DSG3.CAR NK-92 cells also exhibited strong and stable CAR expression and demonstrated functionality in a CD107a-based degranulation assay after stimulation with immobilized anti-DSG3 antibodies.

Thus, our data demonstrate that CAR-NK cells can selectively eliminate B cells and have a great potential for curative, affordable and safe treatment of patients with B cell-driven autoimmune diseases.

P02

Distinct preferences of CD8$^+$ and CD4$^+$ CAR-T cells for transcription factor enhancement

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We conducted a systematic analysis to determine the effect of specific AP-1 family members on distinct CD8$^+$ and CD4$^+$ CAR-T cell functions. Herein, the identity of specific overexpressed transcription factors (TFs) are blinded as TF-A, TF-B, etc. and will be disclosed at the time of the presentation.

We identify TF-C as a lead candidate for CD8$^+$ CAR-T cell modification providing improved specific proliferation, reduced activation-induced cell death, diminished exhaustion-marker expression, and a memory-like phenotype. TF-C attenuated IL-2 secretory capacity compared to conventional CAR-T cells. Conversely, TF-A/CAR-T cells consistently exhibited the highest levels of IL-2 and IFN-γ secretion accompanied with the best Ag^{low} tumor control. Importantly, none of the tested TFs permitted antigen-independent proliferation. Transcriptional analysis of CD8$^+$ TF-C/CAR-T cells revealed a signature of lower activation, reduced exhaustion, and a unique chemokine receptor profile compared to conventional CAR-T cells. The characteristics induced by TF-C significantly minimised lung sequestration compared to conventional CAR-T cells due to increased LFA-1 recycling via βII-spectrin *in vivo*.

TF-C-modification proved detrimental to the potential of CD4$^+$ CAR-T cells to elicit effector functions, highlighting distinct preferences of T cell subsets for TF-modification. However, CD8$^+$ TF-C/CAR-T cell proliferation was synergistically enhanced in the presence of CD4$^+$ TF-A/CAR-T cells both *in vitro* and *in vivo*.

To conclude, overexpression of TF-C endows CD8$^+$ CAR-T cells with favourable attributes facilitating engraftment, persistence, homing and exhaustion resistance. Our data pinpoint a blend of CD4$^+$ TF-A/CAR and CD8$^+$ TF-C/CAR-T cells augmenting the intrinsic properties of CAR-T cells conferring superior engraftment and augmented antitumor efficacy *in vivo*.

P03

Zebrafish as a novel *in vivo* model to assess CAR-NK cell efficacy against metastatic breast cancer

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NK cells are attractive effectors for adoptive immunotherapy of cancer. Results from first-in-human studies with chimeric antigen receptor (CAR)-engineered primary NK cells and NK-92 cells are encouraging in terms of efficacy and safety. To further improve treatment strategies and to test the efficacy of CAR-NK cells in a personalized manner, high-throughput preclinical screening assays using patient-derived tumor samples are needed. Here, we established a flexible Danio rerio (zebrafish) larvae *in vivo* xenograft model and tested the efficacy of PD-L1-targeting CAR NK-92 cells (PD-L1.CAR NK-92) against the PD-L1-expressing breast cancer cell line MDA-MB-231. We have shown that MDA-MB-231 GFP cells injected into zebrafish larvae at 2 days post fertilization (dpf) are viable and migrate to peripheral parts of the zebrafish body, including the tail region. PD-L1.CAR NK-92 cells injected 2.5 hours later could also migrate to the zebrafish periphery and eliminate cancer cells throughout the body as early as 24 hours, in contrast to parental NK-92 or uninjected controls. Residual cancer cells were further eliminated at later time points, with 48 hours post-injection (hpi) being the best time point for analysis. Confocal live-cell imaging *in vivo* demonstrated real-time interaction of PD-L1.CAR NK-92 and MDA-MB-231 cells, resulting in cytotoxicity. We further demonstrated that transgenic zebrafish models with labelled blood vessels can be used to study CAR-NK migration through the vasculature. Our data thus suggest that zebrafish larvae can be used for rapid assessment of CAR-NK cell potency *in vivo* to predict patient response to therapy.

P04

Development of a Broad-Spectrum siRNA Therapy Against Human Coronaviruses

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The emergence of highly pathogenic coronaviruses (CoVs) like SARS-CoV, MERS-CoV, and SARS-CoV-2 has underscored the critical need for effective antiviral therapies. Traditional antiviral strategies employing small-molecule inhibitors have faced challenges due to the limited availability of specific targets and low efficacy against diverse viral strains. In contrast, RNA interference (RNAi) offers a promising alternative by directly targeting and destroying viral RNA as well as exploiting the conserved nature of viral RNA sequences essential for replication. In this study, we present the development of a broad-spectrum small interfering RNA (siRNA) therapeutic targeting multiple human coronaviruses.

Through comprehensive bioinformatic analysis, we identified highly conserved genomic regions across human coronaviruses. Employing an innovative high-throughput virus infection model based on a recombinant SARS-CoV-2 strain expressing GFP, we screened 347 siRNAs and identified two potent siRNAs with picomolar IC50 values. These siRNAs demonstrated remarkable antiviral efficacy against SARS-CoV-2, SARS-CoV-1, MERS-CoV, HCoV-HKU1, HCoV-NL63, and HCoV-OC43 targets in a Dual-Luciferase reporter system. In an *in vitro* infection model, the two selected siRNAs exhibited significant antiviral activity, reducing viral genomic RNA by more than 1000-fold for HCoV-OC43, SARS-CoV-2 and SARS-CoV-1. In a next step, the efficacy of the two siRNAs will be further assessed in an air-liquid interface (ALI) HCoV-OC43 infection model, utilizing biodegradable polymer nanoparticles for siRNA delivery.

This study advances the development of broad-spectrum antiviral therapies against coronaviruses. By harnessing siRNA-based RNA interference, our findings contribute to global efforts in pandemic preparedness and response to emerging viral threats.

P05

IRON: IRES launched RNA replicon for versatile and efficient gene expression

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Self-amplifying RNA (saRNA) represents a promising advancement in RNA-based vaccines and therapeutics development due to its ability to autonomously multiply within cells, reducing the required initial dosage. Traditional saRNA systems, derived from alphaviruses, employ a bicistronic mRNA with a replicase as the first open reading frame (ORF), a gene of interest (GOI) as the second ORF and a subgenomic promoter in between. Replication initiation relies on cap-dependent translation of the replicase, necessitating *in vitro* capping, which is both complex and costly. To remove the constraints imposed by cap-dependent translation of the replicase, we engineered a novel RNA replicon with replicase translation independent of the 5'-terminal cap. In short, we placed the replicase under the control of an internal ribosome entry site (IRES) thereby the initiation of translation depends on the molecular properties of the IRES to direct the ribosome to the translational start site. This IRES-launched replicon demonstrated expression levels comparable to 5'-capped saRNA in human primary fibroblasts. Additionally, our design facilitated the insertion of a second GOI upstream of the IRES, translated under the control of a 5' cap added in cells by the replicase. We explored this site by inserting viral immune evasion genes to boost replicon expression by inhibiting cell-autonomous innate immunity. This strategy resulted in a seven-fold increase in expression, highlighting the benefit of application of viral immune evasion genes.

In conclusion, IRES-launched RNA replicons offer significant advantages, including the use of uncapped synthetic saRNA without activity loss, streamlined synthesis processes, and reduced costs.

P06

RNAvolution in CAR-T cell therapy - Optimising RNA to generate transient CAR-T cells

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Chimeric Antigen Receptor (CAR)-T cell therapy has emerged as one of the most prominent cancer immunotherapies. To date, all marketed CAR-T cell products use viral vectors for permanent genomic integration of the CAR gene. However, this approach faces significant limitations. For example, it poses the risk of insertional mutagenesis, can lead to long-term side effects by on-target-off-tumor cytotoxicity, and complex manufacturing requirements contribute substantially to high therapy costs.

We are addressing these shortcomings by using RNA instead of viral vectors for CAR-T cell generation. As demonstrated by the recent breakthrough of mRNA vaccines, mRNA therapeutics can be considered safe and feasible for large-scale production. Nevertheless, the labile nature of mRNA presents a challenge in CAR-T cell therapy, as it leads to a rapidly declining CAR expressing and therefore a short-lived CAR-T cell efficacy. To address this challenge, we systematically compared innovative RNA formats, namely self-amplifying, trans-amplifying, and circular RNA, with a standard optimised mRNA in terms of transgene expression in T cells. To this end, the different RNAs were produced *in vitro*, optimised, and transfected into Jurkat T cells and primary T cells. Protein expression and cell viability were analysed using flow cytometry. The best RNA candidates will be used for CAR-T cell generation, with a focus on further improving RNA stability, transfection, and CAR-T cell efficacy. Ultimately, we pursue to pave the way for a safer, more cost-effective, and therefore more widely accessible alternative to the current CAR-T cell products.

P07

An all-in-one approach for mutations in STAT3 causing Hyper-IgE-syndrome

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Hyper-IgE-syndrome is a rare primary immunodeficiency characterized by recurrent cutaneous and pulmonary abscesses, elevated IgE serum levels, absence of Th17 cells and a deteriorating quality of life. The disease-causing mutations in patients are usually heterozygous and mainly found in the DNA-binding or the SH2-dimerization domains of *STAT3*. Mutations in these domains interfere with STAT3's function as a transcription factor and impede the activation of downstream target gene expression, such as *SOCS3*. In a proof-of-concept study, we have demonstrated that base editing can lead to a functional rescue of patient T cells. However, this approach is restricted to the underlying point mutation and only beneficial for a few patients. Thus, the present study explored a more universal approach based on the introduction of a

corrective *STAT3* cDNA at the endogenous locus. To investigate the impact of the integration site, we designed nucleases targeting either intron 7 or exon 8 of *STAT3* and integrated a GFP cassette into these sites. Treated K562 cells showed stable GFP expression over time, with a maximum of 10% GFP-positive cells when targeting the intronic site and 30% when integrating into exon 8. Further, we evaluated performance of two different repair template designs, comprising a *STAT3* cDNA with either one or six introns. Based on the expression levels of STAT3 and the activation of SOCS3 upon cell stimulation, our preliminary data suggest beneficial effects of integrating a *STAT3* cDNA separated by multiple introns. Overall, this all-in-one approach could correct over 90% of all mutations in HIES patients.

P08

Generation of mRNA-based therapeutic vaccines against chronic HBV infection

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Despite the availability of the prophylactic vaccine, hepatitis B virus (HBV) infection remains a major global public health concern, with over 250 million people chronically infected and 1.1 million deaths each year. Scarce and impaired virus-specific CD8+ T-cell responses mark a chronic HBV infection. Inducing HBV-specific immunity by therapeutic vaccination has the potential to break HBV-specific immune tolerance and cure chronic infection. Recent advancements in mRNA vaccine technology suggest this could be a promising approach for therapeutic vaccination. The main objective of this study is the development and characterization of a potent mRNA vaccine encoding major HBV epitopes. These epitopes were selected based on the most prevalent genotypes, A-E, covering 95% of globally circulating HBV strains. We characterized various structural elements of mRNA to assess their impact on stability, translatability, and half-life in an in-vitro system. We demonstrated that incorporating untranslated regions (5' and 3' UTRs), 5' capping, Poly-A tailing, and chemical modifications significantly enhanced the potency of the mRNA generated. In the next steps, we aim to evaluate the impact of these modifications on reactogenicity against our candidate mRNAs. Consequently, we aim to develop and characterize various delivery formulations that effectively deliver our mRNA vaccine *in vivo*. Our ultimate goal is to assess our mRNA vaccine's ability to elicit strong and multi-specific T-cell responses, resulting in robust antiviral effects in a mouse model of persistent HBV infection.

P09

A base editor mediated knock-in (BEKI) system for the non-viral generation of multiplex gene edited CAR T cells

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The CRISPR-Cas system enables the insertion of DNA transgenes at specific genomic sites through homology-directed repair (HDR). Gene editing has been utilized to redirect T cells towards tumor cells by introducing a chimeric antigen receptor (CAR). Additional genetic modifications (knock-out, epitope editing) can enhance the ability of CAR T cells to resist exhaustion and immunosuppressive environments. Base editing (BE) is a precise tool for gene disruption via splice site inactivation or stop codon introduction, minimizing genotoxicity associated with DNA double-strand breaks.

In this study, we propose a system for base editing mediated knock-in (BEKI) that utilizes the nCas9 domain of the base editor to introduce paired nicks on opposite DNA strands, promoting HDR-mediated transgene insertion. Across different base editors, we demonstrate precise CAR knock-in into a variety of genes (such as *TRAC*, *CD3ζ* and *B2M).* We found that guide RNA orientation, specific base changes within the editing window and nick positioning impacted the BEKI efficiency. Furthermore, pharmacological HDR enhancement improved CAR knock-in, leading to CAR^+ rates exceeding 50%.

Due to the favourable safety profile of base editors, the BEKI system is well-suited for clinical applications requiring transgene knock-in with simultaneous (multiplex) gene knock-outs. Using BEKI, we generated quintuple gene-edited CAR T cells to enhance cytokine secretion, mediate selective drug resistance and allow off-the shelf use. Multiplex BEKI engineered CAR T cells did not show chromosomal translocations between the targeted loci. This streamlined approach, using a single gene editing tool for multiplex editing, offers significant potential for clinical translation.

P10

UCAST: Universal detection of off-target effects of base editors

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Genome editing poses inherent risks of genotoxicity that require thorough evaluation prior to clinical application. CAST-Seq, a genome-wide method for detecting CRISPR-Cas nuclease-induced chromosomal aberrations, has demonstrated high sensitivity but is less effective in identifying off-target activities of base editors (BEs), which are not designed to create DNA double-strand breaks (DSBs). To address this limitation, we developed Universal CAST-Seq (UCAST), a method that uses a DSB introduced by an auxiliary nuclease as an “anchor” to detect off-target effects associated with BEs. To prevent guide RNA cross-contamination, we selected a CCR5-targeting SaCas9 nuclease as the anchor nuclease. After validating UCAST using CRISPR-Cas9 nucleases with known off-target profiles, we applied it to assess the specificity of various BEs targeting the EMX1 locus in primary T cells. Amplicon sequencing of 39 off-target sites identified by UCAST revealed base editing at 3 off-target sites with TadCBEd, 10 with ABE8e, and 32 with evoCDA1-BE4max. Notably, only 1 of these sites showed off-target activity when cells were edited with the EMX1-targeting Cas9 nuclease. These results underscore that (i) off-

target mutagenesis by BEs is challenging to predict, (ii) BEs can induce DSBs leading to chromosomal translocations, and (iii) Cas9 nucleases are unsuitable as surrogates for off-target detection of BEs. In conclusion, UCAST offers a robust, cost-effective, and time-efficient platform for genome-wide detection of off-target effects without requiring primer optimization. This makes UCAST especially valuable for early-stage safety screening of next-generation genome-editing tools.

P11

An *ex vivo* CRISPR/Cas9 based homology-directed repair gene therapy for p.W44X mutations in the *HAX1* gene

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Homozygous mutations in *HAX1* cause severe congenital neutropenia (*HAX1*-CN), characterized by fewer than 500 neutrophils/µl, a high risk of severe infections, and a 20% likelihood of developing MDS/AML. Currently, the only curative option is allogeneic bone marrow transplantation, which carries significant comorbidities. We aim to develop an *ex vivo* autologous hematopoietic stem cell gene therapy to correct the most frequent *HAX1* mutation, p.W44X, found in ~80% of *HAX1*-CN patients.

We designed a single guide RNA targeting p.W44X and a correction template delivered via an AAV6 vector. Patient-derived hematopoietic stem and progenitor cells (HSPCs) were cultured for two days, electroporated with CRISPR/Cas9, then transduced with the AAV6 repair vector. This approach achieved ~65.8% gene correction, restoring HAX1 protein expression (Western blot).

Gene-corrected cells showed a 2.5-fold increase in neutrophil production. Corrected neutrophils performed essential functions: bacterial phagocytosis, chemotaxis, and reactive oxygen species generation. Restoration of HAX1 also rescued apoptosis resistance, as confirmed by live-cell imaging of caspase 3/7 activity. GUIDE-Seq and CAST-Seq revealed eight potential off-target sites, to be validated by targeted sequencing.

This efficient, selection-free strategy shows promise for curing *HAX1*-CN. Ongoing studies in immunodeficient mice will assess long-term safety and efficacy. These findings pave the way for future clinical translation, suggesting that congenital neutropenia could become routinely curable through gene therapy.

P12

AAV-Mediated Prime Editing for the correction of Familial Mutations Associated with Cardiomyopathy

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The phospholamban (PLN) gene regulates calcium homeostasis in cardiomyocytes by inhibiting the sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a). A PLN founder mutation identified in Greek and Dutch populations is linked to severe dilated cardiomyopathy (DCM) and arrhythmogenic cardiomyopathy (ACM). Patients often exhibit low-voltage ECGs, ventricular arrhythmias, and progressive heart failure, typically by their fourth decade, though some carriers remain asymptomatic. This mutation disrupts calcium handling, induces metabolic dysfunction, and promotes protein aggregation in cardiomyocytes. Current treatments manage symptoms, prevent sudden cardiac death, or involve heart transplantation in advanced cases. This study aimed to develop an AAV-based prime editing (PE) strategy to correct the PLN mutation in human iPSC-derived cardiomyocytes and humanized PLN mice. Screening four pegRNAs with various nicking gRNAs and Cas9 variants yielded correction efficiencies up to 30%, identifying the most promising PE tools. A dual AAV-PE system incorporating optimized components achieved 10-13% mutation correction in patient-derived cardiomyocytes.In vivo, AAV9-PE vectors were administered to humanized PLN mice and evaluated after five weeks using a stress-induced arrhythmia protocol. The intervention reduced pathogenic PLN expression and significantly decreased arrhythmic events compared to controls. These findings demonstrate the potential of dual AAV-PE for targeted PLN mutation correction and associated cardiac dysfunction mitigation. Ongoing studies in humanized porcine PLN models aim to expand its translational applications.

P13

Enhancing CRISPR/Cas9 Gene Therapy: Chemical Modulators for Improved Safety and Precision

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The CRISPR/Cas9 system is a transformative gene editing technology with immense therapeutic potential for a broad spectrum of genetic disorders. Despite its promising capabilities, especially in ongoing cancer clinical trials, the safety concerns associated with off-target effects and the prohibitive costs—surpassing $2 million USD per treatment—remain significant hurdles. Addressing these challenges, our study involved an extensive chemical screening through which we identified a series of small-molecule Cas9 modulators. These modulators significantly enhance Cas9 specificity and efficiency, thus minimizing off-target risks. Our findings advocate for the integration of these chemical modulators into both genome and epigenome editing therapies, proposing a path toward more efficacious, safer, and economically viable gene therapies. Such innovations are crucial for expediting the clinical adoption of gene therapy.

P14

Modulating C-to-T editing with TALE base editors

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TALE base editors (TALEB) are fusions of a transcription activator-like effector domain (TALE), split-DddA deaminase halves coupled to an uracil glycosylase inhibitor (UGI). By converting a cytosine (C) to a thymine (T) via the formation of an uracil intermediate, TALEB function as a C-to-T class of base editor able to edit double strand DNA.

Taking advantage of a highly precise and efficient TALEN®-mediated ssODN knock-in in primary T cells, we applied a strategy that allowed the comprehensive characterization of C-to-T conversion efficiencies, how target composition, and spacer variations, affect TALEB activity/efficiency within the editing window.

Notably, we highlighted that the composition of bases surrounding the target TC may strongly influence the editing efficiencies. We also demonstrated that by varying the TALEB scaffold, we could not only relax target sequence limitations, but with thoughtful consideration modulate bystander editing within the editing window, overall allowing TALEB to be fine-tuned for a desired gene editing outcome.

We then thought to further assess characteristics of nuclear genome editing by applying a range of different techniques. First focusing on on-target editing, we also explored the possibility, and risk, associated with genome wide TALE dependent/independent binding and editing. Using an experimental model, we demonstrated that a single TALEB arm does not lead to detectable editing (detection limit: 0.1-0.2%) which we then validated with hybrid capture assays.

Altogether, the datasets obtained in this study enhanced our understanding of TALEB and permits the design of extremely efficient and specific TALEB, compatible with the development of future therapeutic applications.

P15

EDSpliCE: AAV-deliverable enhanced deletion RNA-guided nucleases for therapeutic splicing modulation in *USH2A*

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Retinitis pigmentosa (RP), a leading cause of progressive vision loss, affects over one million people worldwide. Pathogenic variants in the *USH2A* gene are common in both autosomal recessive and syndromic RP forms. The deep intronic variant *USH2A*:c.7595-2144A>G, associated

with Usher syndrome (RP combined with hearing loss), disrupts mRNA splicing, resulting in defective USH2A protein production.

As a safe therapeutic approach for splicing modulation in this and other genes, we developed the Enhanced Deletion Splicing Correction Editing (EDSpliCE) platform, utilizing Engineered Enhanced Deletion synthetic RNA-Guided Nucleases (EDsRGNs). These small chimeric proteins combine the synthetic RNA-guided endonuclease sRGN3.1 with the human exonuclease TREX2. EDsRGNs create larger deletions at target sites with a single gRNA. While disrupting the disease-related sequence, this nuclease engineering aims to incorporate crucial safety features.

Using minigene splicing rescue assays in HEK293T cells, up to 90% correct *USH2A* splicing was achieved. Delivery via adeno-associated viruses (AAVs) confirmed efficient transduction and splicing rescue. Interchromosomal junction PCR showed nearly undetectable chromosomal translocations with EDsRGNs as opposed to sRGN3.1. Next-generation sequencing (NGS) revealed that EDsRGNs induced enhanced deletions, predominantly in one direction of the cut site. γH2AX immunofluorescence demonstrated the transient occurrence of DSBs induced by EDsRGNs. GUIDE-Seq analysis showed low off-target editing activity.

In sum, EDSpliCE successfully rescued *USH2A* splicing *in vitro* while showing improved safety attributes. EDsRGNs are compatible with all-in-one AAVs, enabling efficient delivery into various cell types. Ongoing work includes optimizing AAV vectors, validating off-targets, and protein rescue experiments in patient-derived photoreceptor precursor cells and retinal organoids expressing 3xFLAG-tagged endogenous USH2A.

P16

Design and test of a prime editor and LNPs for the CFTR F508 deletion in lung cells

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Cystic Fibrosis (CF) is one of the most prevalent inherited disorders, characterized by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (*CFTR*) gene. In human lungs, dysfunctional CFTR leads to a decrease in mucociliary clearance leading to infections and contributing to high mortality. Approved treatments require constant application to alleviate symptoms but do not cure the genetic cause and are often extremely expensive. We present an approach towards correcting the prevalent p.F508del mutation using prime editing delivered via RNA in lipid nanoparticles (LNPs). The LNPs comprised ionizable lipids for endosomal escape, DOTAP for improved lung specificity, PEGylated lipids, and an RNA complexed with chitosan for increased stability. Physicochemical characterization (DLS, cryo-EM, zeta potential) confirmed optimal hydrodynamic size (~100 nm) and stability. Upon testing various editing options, a PE6c prime editor was chosen, in which prime editing guide RNAs (pegRNA) were equipped with silent edits and a protective 3' motif. Correction of a fluorescent reporter plasmid mimicking F508del was achieved in HEK-293 and CFBE41o- bronchial epithelial cells as observed by flow cytometry and microscopy.

P17

V_HH-based CLEC12A-CAR-NK cells based on llama nanobodies for treatment of Acute Myeloid Leukemia

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Acute myeloid leukemia (AML) faces challenges due to the disease heterogeneity and a lack of tumor-specific antigens. CLEC12A (*C-type lectin-like molecule-1*) is a promising target for chimeric antigen receptor (CAR)-based immunotherapy, as it is expressed on leukemic blasts and leukemia-initiating cells in up to 92% of AML patients while being absent on hematopoietic stem cells. Furthermore, CAR-engineered natural killer (NK) cells offer an “off-the-shelf” therapeutic option, leveraging CAR-mediated cytotoxicity and innate receptor activity.

This study aimed to improve the anti-leukemic activity of CLEC12A-CAR-NK cells through the selection and implementation of a novel V_HH binding domain. Llama immunization using VLPs followed by yeast surface display (YSD) screening successfully identified AC12VHH4, a high-affinity CLEC12A-specific V_HH domain. Following implementation into the CAR and NK cell transduction, V_HH-based CLEC12A-CAR NK cells exhibited superior tumor cell lysis compared to their scFv-based counterparts and non-transduced NK cells. Enhanced anti-leukemic activity was particularly prominent at lower effector-to-target (E:T) cell ratios. In the OCI-AML2 *in vivo* xenograft model treatment with V_HH-based CLEC12A-CAR NK cells inhibited leukemia progression and improved survival. Use of the novel V_HH domain AC12VHH4 enables potent tumor cell elimination by CLEC12A-CAR NK cells, particularly at low E:T ratios. Thereby, the V_HH identified in this study constitutes a more effective binding domain for the generation of CLEC12A-CAR NK cells than a scFv domain derived from therapeutic antibody Tepoditamab. Importantly, V_HH-CLEC12A CAR-NK cells also demonstrated potent *in vivo* efficacy in an OCI-AML2 xenograft model, highlighting the potential impact of this new CAR-based therapeutic concept for AML immunotherapy.

P18

Peptide-assisted tethering of critical DNA repair effectors to Cas9 enables precise genome editing in Fanconi anemia

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The CRISPR-Cas9 can be exploited to induce DNA breaks, enabling correction of disease-causing mutation via the homology-directed repair (HDR) pathway. However, HDR efficiency is often limited by competition with the dominant non-homologous end-joining (NHEJ) pathway. Existing strategies to enhance HDR have yielded inconsistent results across cell types due to variations in

endogenous DNA repair factor and to the different repair templates used, making universal approaches impractical. To address this challenge, we introduce the CRISPR Peptide Assisted Localization (PAL) toolkit, a modular system that customizes repair factor recruitment to specific editing conditions. CRISPR-PAL comprises a Cas9 nuclease fused to a synthetic peptide complementary to a second peptide that can be fused to a library of DNA repair effectors. We used CRISPR-PAL to identify optimal combinations of effectors tailored to specific cell types and repair templates, achieving up to a 3.1-fold increase in HDR compared to unmodified Cas9. To demonstrate its potential in challenging repair scenarios, we applied CRISPR-PAL to Fanconi anemia (FA), a disorder characterized by defective DNA repair. In *FANCC* mutant lymphoblastoid cells (LCLs), we used the CRISPR-PAL system to complement the missing *FANCC* effector, achieving over a 4-fold increase in precision score compared to unmodified Cas9 or to a direct Cas9-FANCC fusion. These results highlight CRISPR-PAL as a versatile and powerful strategy for enhancing HDR efficiency and correcting DNA repair deficiencies. By enabling precise, customizable recruitment of repair factors, CRISPR-PAL opens new avenues for addressing complex genetic disorders like FA.

P19

CRISPR Therapeutic Strategy for Stargardt Disease: Correcting Exonic *ABCA4*:c.768G>T Mis-splicing

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Bi-allelic pathogenic variants in ***ABCA4*** cause **Stargardt disease** (STGD1), an autosomal recessive retinal disorder leading to progressive central vision loss, typically beginning in childhood. The disease results from the accumulation of cytotoxic products in photoreceptors due to dysfunctional ABCA4 protein. Among the diverse mutations in *ABCA4*, the **exonic c.768G>T variant** is significant and frequent, causing mis-splicing that retains a 35-nucleotide elongation of exon 6 in mature mRNA. This mis-splicing arises from a weakened canonical donor splice site and a strong cryptic splice donor site downstream, leading to a frameshift and a truncated protein.

CRISPR/Cas-mediated genome editing has shown promise in correcting splicing defects. However, no strategies have been established for variants near exon/intron boundaries. To address this, we employed our **EDSpliCE** (**E**nhanced-**D**eletion **S**plicing **C**orrection **E**diting) platform to correct the c.768G>T splicing defect. We cloned a mutant minigene plasmid with the *ABCA4*:c.768G>T variant and confirmed pathogenic splicing through a minigene assay in HEK293T cells. The validated minigene was co-transfected with individual sgRNAs and Cas9-ortholog EDSpliCE. Correctly spliced transcript percentages were analyzed via PCR and chip electrophoresis, with editing efficiency assessed by targeted high-throughput sequencing. Then, to explore top sgRNAs for correcting splicing defects in photoreceptor precursor cells, an isogenic *ABCA4*:c.768G>T iPSC line was created using CRISPR/Cas9 RNP and a donor template.

Our findings indicate that EDSpliCE can **effectively correct splicing defects** at exon/intron boundaries, achieving up to **85% correction of the c.768G>T defect**, outperforming traditional Cas9 methods. This positions EDSpliCE as a promising therapeutic approach for addressing splicing variants like *ABCA4*c.768G>T in STGD1.

P20

Efficient Single-Base Substitution with HDR-Cas9: A Step Towards Sickle Cell Disease Cure

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Sickle cell disease (SCD) results from a point mutation in the β-globin gene that leads to the production of hemoglobin S. This abnormal form of hemoglobin causes red blood cells to take on a sickle shape, reducing their flexibility and increasing the likelihood of hemolysis and vascular occlusion. The mutation is responsible for the key symptoms of SCD, such as chronic anemia, vaso-occlusive crises and multi-organ complications. A potential cure for SCD involves correcting the β-globin gene mutation through targeted genome editing. In our research, we compared our proprietary HDR-Cas9 fusion (which combines Cas9 with CtIP and dnRNF168) to unmodified Cas9. We used highly efficient gRNAs targeting the HBB locus in K562 cells and identified G10/R-02 as the most effective guide for introducing single base substitutions using a non-symmetric single-stranded repair template. Using the G10/R-02 gRNA, we tested different repair template designs while simulating the SCD mutation. Our results showed that HDR-Cas9 exhibited superior precision in making single base substitutions compared to unmodified Cas9 across all repair templates tested. The most remarkable results were achieved using a double-stranded repair template that included Cas9 target sites for improved nuclear shuttling, which resulted in approximately 2.4 fold of error-free editing. These results validate the efficacy of our HDR-Cas9 fusion and highlight the potential benefits of enhancing HDR factors at the target site to improve precision in genome editing, especially at the single base level.

P21

Developing Designer-Recombinase induced Gene Replacement (DRiGR) with clinical implications

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Genome engineering systems capable of producing large DNA edits are required for numerous applications. We explored the possibility of utilizing site-specific recombinases (SSRs) for this purpose, employing a panel of different enzymes from different recombinase families. SSRs have been demonstrated to achieve gene-sized DNA replacement via recombinase-mediated cassette exchange (RMCE), which precisely exchanges a region of genomic DNA flanked by heterospecific target sites with a similarly flanked DNA donor. However, RMCE with a single recombinase generally displayed insufficient efficacy, which can be elevated via dual-RMCE (dRMCE) with two distinctive recombinases. We will present optimal conditions of dRMCE, showcasing its therapeutic potential. By elucidating the optimal conditions of dRMCE, we drastically enhanced its efficacy to therapeutically meaningful levels. This study, along with directed-evolution and on-going research to re-direct the specificity of SSRs to defined genomic targets, can ultimately offer designer-recombinase induced gene replacement (DRiGR) as a powerful genome engineering system to correct for mutations spanned across a large genomic region, without the necessity of integrating wild type SSR target sites. The presented tools represent promising technology that

we believe has potential for subsequent pre-clinical studies to prepare DRiGR for clinical applications.

P22

A base editing platform for the correction of cancer driver mutations unmasks conserved p53 transcription programs

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Understanding the role of cancer hotspot mutations is essential for unraveling mechanisms of tumorigenesis and identifying therapeutic vulnerabilities. Correcting cancer mutations with base editing is a novel, yet promising approach for investigating the biology of driver mutations. Here, we look forward like to present a versatile platform to investigate the functional impact of cancer hotspot mutations through adenine base editing in combination with transcriptomic profiling. Using this approach, we corrected TP53 hotspot mutations in cancer cell lines derived from diverse tissues, followed by mRNA sequencing to evaluate transcriptional changes. Remarkably, correcting these mutations not only revealed the dependency on mutant allele expression, but also restored highly conserved tumor-suppressive transcriptional programs, irrespective of tissue origin, or co-occurring mutations, highlighting a shared p53-dependent regulatory network. Our findings demonstrate the utility of this base editing platform to systematically interrogate the functional consequences of cancer-associated mutations and their downstream effects on gene expression. This work establishes a robust framework for studying the transcriptional dynamics of cancer hotspot mutations and sheds light on the conserved biological processes reinstated by p53 correction, offering potential avenues for future targeted therapies.

P23

Towards a prime-boost vaccine platform based on different adenovirus types

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During the SARS-CoV-2 pandemic different vaccination approaches have been pursued, one of which based on the use of adenovirus (Ad) vectors for vaccine antigen expression. Next to very good immunization properties, additional advantages of Ad vectors included scalability during production, low production costs and stability of the vaccine. The COVID-19 vaccines developed by Oxford University/AstraZeneca and Johnson & Johnson were based on adenoviruses of chimpanzee (Y25) or human (hAdV-26) origin, respectively, both expressing the SARS-CoV-2 spike (S) protein as antigen. Since a previous exposure to one Ad type during prime vaccination will negatively impact on the immune response during boost vaccination with the same Ad type, we wish to expand the choice of Ad vector types that could be used in prime-boost vaccine regimen. Therefore, we characterized twelve potential alternative human adenovirus (hAdV) types

from species B and D in different *in vitro* assays, including (i) production yield, (ii) target cell binding, and (iii) seroprevalence. hAdV-11, -34, -37, -43, -48 and -50 were identified to be producible to high titers, demonstrated good target cell binding and had a low seroprevalence. The full-length genomes of this sub-selection were introduced into bacterial artificial chromosomes and the E1 genes were replaced by an eGFP-firefly luciferase reporter gene to yield replication-deficient Ad vectors. After further characterization and selection, the E3 genes will be deleted to increase vector stability, DNA transport capacity and vector immunogenicity. Additionally, the Spike-protein will be introduced as the vaccine antigen and vaccination studies will be performed in mice.

P24

AAV's next top model: Influence of animal species on directed evolution of myotropic AAV capsids

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A hallmark of directed evolution of AAV capsids for gene therapy applications is their ex or in vivo selection from diverse libraries, which are typically composed of hundreds of different variants (in the case of pre-selected, barcoded capsids) or up to billions (in the case of e.g. shuffled capsids). Recently, though, it has become increasingly apparent that the type of selection model can significantly affect and bias the outcome, evidenced by capsids that do not translate from cultured cells to animals or humans, or that even perform differently between various strains from the same species, such as mice or monkeys.

Here, we have systematically investigated and confirmed this phenomenon, by analyzing and comparing the results of iterative or single-round AAV capsid screening ex vivo in cultured murine muscle cells, or in vivo in the musculature of mice or of monkeys.

To this end, we initially screened a shuffled capsid library composed of AAV serotypes 1, 6, 8 and 9 upon systemic delivery in mice or monkeys, with a focus on the striated musculature as our major target of interest in this study. In mice, we observed a gradual loss of AAV1, 6 and 8 together with a concurrent enrichment of AAV9, which eventually contributed to about 72% of all capsid sequences at the end of the selection. In striking contrast, AAV9 but also AAV8 were diminished in macaques, while AAV1 and 6 became enriched and ultimately made up roughly 57% of all capsid sequences.

Based on these data, we next prepared a barcoded pool of 54 capsids enriched in the previous study and including three clusters of similar sequences, i.e., the AAV1/6-derived clusters 1 and 2 as well as the AAV8-derived cluster 3. Moreover, we added the parental AAV1, 6, 8 and 9 as benchmarks, as well as the AAV9-based peptide display variant AAVMYO that exhibits a pronounced muscle tropism in rodents.

Also in this secondary screen, we noted substantial differences in the composition and phylogeny of the capsids that performed best in each of three selection systems, i.e., murine C2C12 myoblasts or intravenously injected NMRI mice or macaques. Most notably, AAV9 and AAVMYO outperformed all other variants on the RNA level in the mouse musculature, where they were enriched 7.1- or 30.7-fold over the input library, respectively. In contrast, AAV6 and two

derivatives in cluster 2 with ~97% identity to AAV6 performed much better in the macaques, where they were ~5-fold enriched over the input library. The lead benchmark in mice, i.e., AAV9, was enriched 1.8-fold in the macaque model and thus 3.9-fold less efficiently than in the mouse model.

Collectively, this work enables us to draw two major conclusions, the first being that AAV6 might represent a better scaffold than the commonly used AAV9 for capsid evolution in the primate musculature. Secondly, we validate the concern that the outcome of AAV library screenings is critically affected by the selection model, underscoring the need for concerted efforts across academia and industry to find solutions to this problem.

P25

CAPSULON: The Next Step in Efficient and Safe siRNA Delivery for Clinical Applications and Gene Therapy

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Small interfering RNA (siRNA) has emerged as a powerful tool for achieving precise and targeted gene silencing, enabling the regulation of gene expression with exceptional specificity. However, the clinical translation of siRNA therapies remains limited by the challenge of safe and efficient delivery. Conventional RNA interference (RNAi) delivery methods, including polymer-, lipid-, and nanoparticle-based systems, are often impeded by drawbacks such as immunogenicity, toxicity, scalability issues, and high production costs.

CAPSULON polymer nanocapsules present a transformative approach to overcoming these limitations. Synthesized from biocompatible polymers, CAPSULON nanocapsules encapsulate nucleic acids with high efficiency, ensuring enhanced stability, controlled release, and reduced immunogenicity. This innovative system also facilitates cellular uptake, minimizing off-target effects and maximizing therapeutic efficacy.

This study introduces CAPSULON nanocapsules as a next-generation delivery platform that leverages the principles of natural extracellular vesicles (EVs) for the safe, efficient, and non-toxic transfection of biological molecules, including siRNA. Notably, CAPSULON technology achieves effective delivery even at minimal siRNA concentrations, providing researchers with unprecedented flexibility in optimizing dosage and release kinetics for their experimental needs.

In conclusion, polymer nanocapsules such as CAPSULON represent a highly promising, non-viral gene delivery strategy with the potential to redefine therapeutic approaches in gene therapy and personalized medicine.

P26

Bypassing ex vivo CAR T cell manufacturing: leveraging LNPs for in vivo delivery

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Chimeric Antigen Receptor (CAR) T cell therapy has emerged as a groundbreaking treatment for various cancers. Its applications are now spreading beyond oncology into areas such as autoimmune diseases. However, its widespread use is hindered by the current *ex vivo* production method, which is labor-intensive, logistically complex, time-consuming (2-7 weeks), and expensive with list prices approximately 320,000 EUR in Europe.

To address these challenges, our project explores mRNA-loaded lipid nanoparticles (LNPs) for *in vivo* CAR gene delivery to T cells. A key hurdle in this approach is enhancing specificity to T cells. We are engineering targeted LNPs (tLNPs) by conjugating T cell targeting molecules to functionalized lipids, aiming to achieve T-cell specificity through receptor-mediated endosomal uptake.

In vivo delivery has the potential to transform CAR T cell therapy into an off-the-shelf product, promoting its widespread use and accessibility across a broader range of diseases. Additionally, the transient expression of CARs would enable safer targeting of antigens previously excluded due to on-target-off-tumor toxicities, broadening the spectrum of treatable conditions and antigens.

P27

Versatile lipid nanoparticle platform for efficient CRISPR-Cas9 gene editing in primary T cells and CD34+ hematopoietic stem cells

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Introduction: Lipid nanoparticles (LNPs) are a promising nonviral alternative for delivering CRISPR-Cas9 components to difficult-to-transfect cell types like human primary T cells and CD34+ hematopoietic stem and progenitor cells (HSCs). We developed a library of novel ionizable lipids and LNP compositions for *ex vivo* applications. This study presents the screening and optimization of Cas9 mRNA and sgRNA delivery for gene knockouts in T cells and HSCs and explores protocols for scale-up and clinical translation.

Methods: Novel LNPs were formulated using the NxGen™ mixing platform and tested on human primary T cells or CD34+ HSCs from peripheral blood. Various RNAs, including GFP, αCD19-CAR

mRNA, Cas9 mRNA, and sgRNAs targeting TCR/CD52 (T cells) and CD33/CD45 (HSCs) were encapsulated into LNPs. Culture media, supplements, and transfection kinetics were optimized for gene delivery efficiency. Viability, proliferation, and colony-forming potential were measured post-editing.

Results: We achieved up to 90% TCR−/CD52−double knockout in T cells with > 90% viability. In HSCs, 80% and 90% knockouts (CD45 and CD33, respectively) were achieved, with > 95% viability and strong proliferative capacity. Colony-forming unit assays showed no significant changes in multilineage differentiation. Media optimization was crucial, with some commercial media yielding higher editing efficiencies. Treatment kinetics and media supplements significantly impacted editing efficiency in long-term HSCs. We successfully scaled-up LNPs and optimized conditions from well-plates to G-Rex bioreactors.

Conclusions: We present a versatile and scalable LNP platform with minimal cytotoxicity for efficient gene delivery and editing in difficult-to-transfect cell types. The method has potential for clinical translation in T cells and HSCs.

P28

Genome-Scale CRISPR/Cas9 screening in tumor cells to identify mechanisms for overcoming cancer resistance in NK cell-based immunotherapy

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Natural killer (NK) cells can rapidly identify and destroy malignant cells, making them a promising tool for cancer immunotherapy. Enhancing NK cell efficacy by expressing chimeric antigen receptors (CARs) or Fc receptors (CD16) in combination with monoclonal antibodies (ADCC) has shown significant potential. However, resistance to NK cell cytotoxicity, especially in solid tumors, remains a challenge.

To dissect the mechanisms of cancer cell resistance, we performed six genome-wide CRISPR-Cas9 knockout screens using leukemia, breast and pancreatic cancer cell lines in combination with the clinically relevant NK cell line NK-92 or its derivatives genetically engineered to express CAR specific for ErbB2 or high-affinity Fc receptors, in combination with therapeutic antibodies. Genomic DNA from surviving cancer cells was sequenced, and data was analyzed using Model-based Analysis of Genome-wide CRISPR/Cas9 Knockout (MAGeCK).

Molecular pathway analysis of the screening data revealed that cancer cells employ diverse and heterogeneous mechanisms to evade NK cell cytotoxicity. Each screen identified at least five genes with high-ranking scores for NK cell resistance or susceptibility. Validation of the top-ranked genes with high-throughput live-cell imaging cytotoxicity assays identified genes strongly responsible for the resistance to NK cell cytotoxicity. Among these, ICAM-1 was identified as a critical checkpoint required for natural and trastuzumab-mediated NK cell cytotoxicity, whereas anti-ErbB2-CAR targeting was able to overcome this resistance.

These findings highlight multiple clinically relevant resistance mechanisms to NK cell cytotoxicity, and provide valuable insights to enhance NK cell-based therapies for resistant cancers.

P29

Shortened Half-Life Sleeping Beauty Transposase for Biosafety-Enhanced CAR-T Cell Production

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Chimeric antigen receptor (CAR) T-cell therapy represents a promising anti-cancer immunotherapy approach. It involves genetically modifying a patient's own T-cells to create a personalized 'living anti-cancer medicine' that is capable of targeting and destroying cancer cells. It is of utmost importance to provide genomically stable CAR-T cell products, and to achieve this goal, the Sleeping Beauty (SB) transposon system is emerging as an efficient and promising gene delivery tool.

The hyperactive SB100X transposase, paired with the pT2 transposon optimised for its activity, represents an alternative approach to CAR-T cell production that is comparable in efficacy to retroviral and lentiviral delivery methods. To enhance biosafety, it is desirable to limit recombinase activity to a brief window after delivery, in order to minimise the risk of genomic toxicity. Nevertheless, the half-life of SB100X, which exceeds 30 hours, represents a considerable challenge. During this period, the transposase may potentially induce further undesired integrations or transposon remobilization. Targeting the transposase for processing by cellular degradation machinery may prove an effective solution to this issue.

To address this, the SB100X gene was modified to include an N-terminal nuclear export signal (NES) and a chaperone-mediated autophagy (CMA) signal. These modifications facilitate nuclear-to-cytoplasmic export and lysosomal degradation, resulting in a shortened half-life for the SB transposase variant.

This engineered enzyme exhibits high gene integration efficiency while demonstrating significantly increased degradation rates and nuclear export. These advancements, validated in primary T cells and various human cell lines, established an improved transposase for CAR-T cell production without compromising performance.

P30

FindMe2care – a contact platform for patients with genetically confirmed diagnoses

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FindMe2care was initiated in January 2024 as a platform to contact and possibly recruit patients with genetically confirmed diagnoses for research projects, specialized disease registries and clinical trials. It is a non-profit initiative that academic and non-academic genetic laboratories in Germany can join.

Patients who receive their diagnosis of a monogenic disease are provided with an individual QR code encrypting the details of their genetic report. This QR code can be used by a lay person to self-register on the platform without transmission errors. Upon successful registration, users receive diagnosis-specific information or inquiries from external entities. Such inquiries can be submitted by any interested entity (e.g. basic or clinical researchers) and will undergo evaluation by an independent scientific advisory board before being forwarded to patients.

Within the first 11 months of operations, 271 patients with 187 unique OMIM diagnoses registered using QR-codes provided by 4 participating genetic laboratories. 10 more genetic laboratories across Germany are committed to enrich their reports with FindMe2care-QR-Codes starting in the near future. FindMe2care received phenotypic and genetic patient data of excellent quality in the form of PhenoPackets, fulfilling the requirements of international research networks. To date, invitations to 3 specific disease registries, 2 studies and 1 patient organization were forwarded to eligible patients and will be provided prospectively to any new eligible patient registering on the platform.

FindMe2care acts as a data trustee for patients who wish to participate in basic or clinical research and benefit from personalized treatments, helping to improve care for genetic disorders.