

EMA: Scientific Symposium on Advanced Therapy Medicinal Product 'Contribution, evolution, revolution'

10 October 2024

*What challenges will arise from ATMPs
under development?*

The Academia Viewpoint

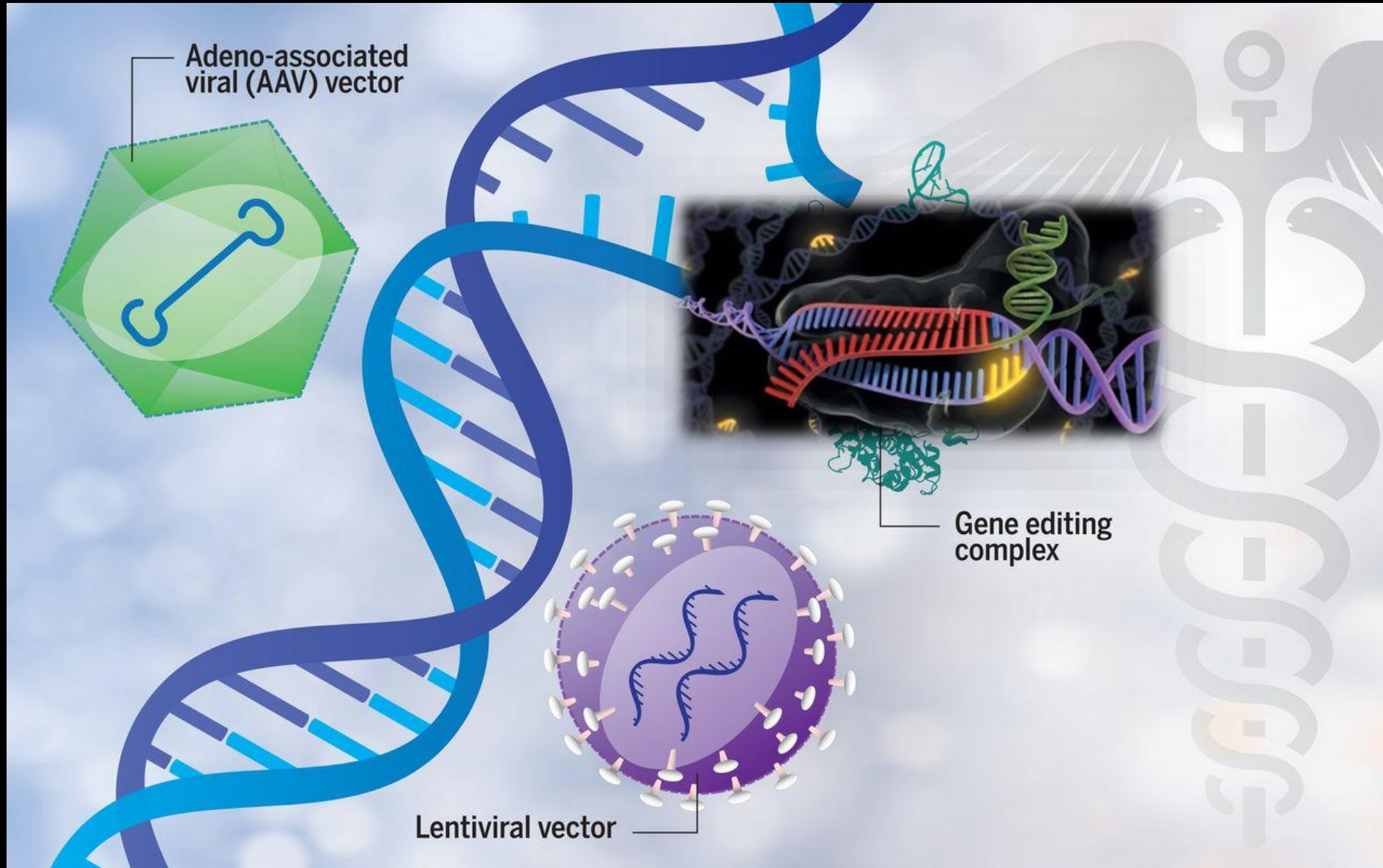
*Luigi Naldini, MD, PhD
Director, SR-Tiget*



Disclosures

- *Inventor of patents on*
 - *lentiviral vector technology*
 - *targeted genome editing*
 - *HSC manipulation / transplantation**owned & managed by Telethon Foundation & San Raffaele Hospital*
- *Consultant and SAB member of*
 - *Tessera Therapeutics*
 - *Tr1X*
 - *Nvelop Therapeutics*
- *Founder, equity holder, consultant and SAB member of*
 - *Genenta Science*
 - *Genespire*
 - *Chroma Medicine*

A New Medicine for the 3rd Millenium



A New Medicine for the 3rd Millenium



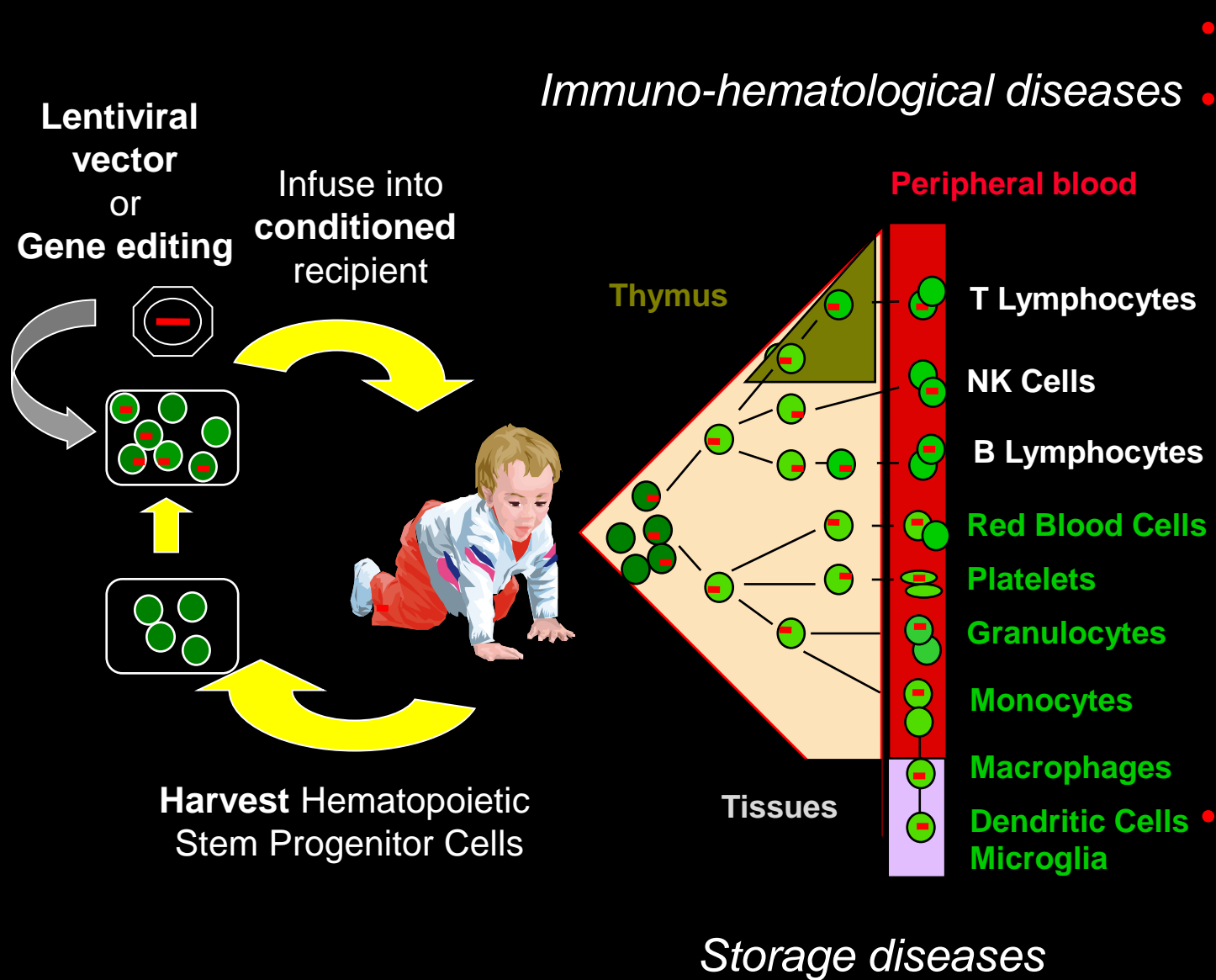
- *New technologies for transferring and editing genes (Gene Therapy)*
- *Effective strategies to isolate and transplant stem cells (Cell Therapy)*
- *Improved manipulation of biological weapons of immunity (Immunotherapy)*
- *Allow to design new therapies for severe to lethal diseases*



Ex vivo Gene Therapy: Major Clinical Achievements

- Hematopoietic Stem Cells (HSC)
 - *retro / lentiviral vectors*
 - Primary Immunodeficiencies
 - Lysosomal Storage Disorders
 - Hemoglobinopathies
 - Fanconi Anemia
 - *editing CRISPR/Cas9*
 - Hemoglobinopathies
- T-cells
 - *retro / lentiviral vectors*
 - Adoptive cancer immunotherapy (ALL, B lymphoma, MM) CAR & TCR
 - *editing ZFN / TALEN / CRISPR/Cas9*
 - HIV infection (CCR5 disruption)
 - Cancer Immunotherapy: MHC-I (B2M), Checkpoint Inhibitor Receptors disruption
- Keratinocytes
 - *retroviral vectors*
 - Epidermolysis bullosa

Hematopoietic Stem Cell Gene Therapy: Results Today



- *Safe with substantial & durable benefits*
- *Lentiviral Vectors*
 - >400 pts, up to 14 yr follow-up, 20 diseases*
 - Wiskott-Aldrich Syndrome, X-SCID, CGD
 - Artemis, LAD, RAG-1 deficiency ...
 - Adrenoleukodystrophy, Metachromatic Leukodystrophy, MPS-I, MPS-IIIA, Gaucher, Fabry's, Osteopetr, Cystinosis...
 - β -Thalassemia, Sickle Cell Disease, Fanconi's Anemia, PKD...
 - Approved: Zynteglo (Bthal), Skysona (ALD), Lyfgenia (SCD) Libmeldy / Lenmeldy (MLD)*
- *Gene Editing (BCL11A enh. disruption)*
 - >100 pts, up to 4 yr follow-up*
 - β -thalassemia, Sickle Cell Disease
 - Approved: Casgevy (CGD, Bthal)*

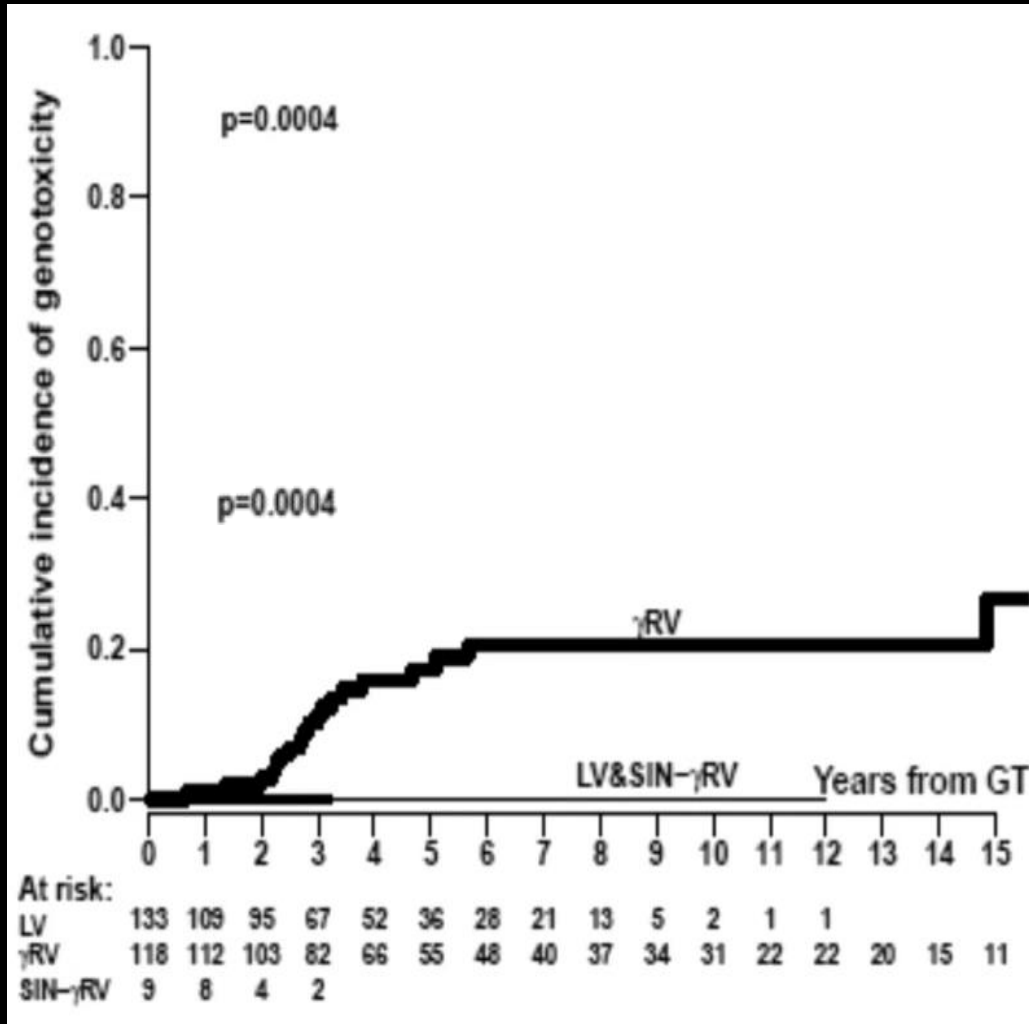
Major Properties of Lentiviral Vectors (LV)

- *Efficient and safe gene transfer*
 - Consistent safety and efficacy outcome upon >15 yr testing in *ex vivo* HSC gene therapies (>500 patients) and T-cells (>20,000)
 - Semi-random genome-wide integration
 - Stable maintenance, transmission to cell progeny
 - Stable transgene expression
 - **Insertional mutagenesis**
 - SIN vector and internal moderate promoter alleviate risk
- *Toxicity & Immunogenicity*
 - **Pathogenic parental virus** but virtually no risk of RCR contamination
 - Hybrid multi-split packaging system exploiting small fraction of viral genome
 - Extensive experience with thousands of lots tested

Safety Truck Record of Lentiviral Vector HSC GT

Stratified by vector type

Currently >400 patients



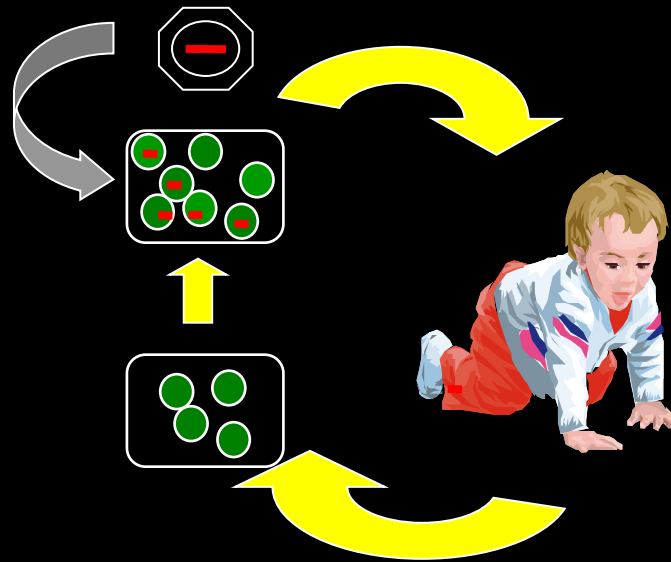
The Current Outlook for HSC Gene Therapy

- *May become preferred to allogenic HSC transplant in genetic diseases*
 - Available to every patient
 - Abrogates risk of graft vs host disease and rejection
 - Mixed chimerism sufficient for full benefit
 - Enhanced benefit by increased gene dosage
 - Growing applications expected (incl. beyond genetic diseases)
- *Outstanding challenges*
 - Delayed engraftment if process impacts HSC yield or fitness
 - Need for toxic conditioning but new biological strategies emerging
- *Concerns (long-term)*
 - Residual genotoxic risk of engineering
 - Long-term stability and clonal composition of engineered graft

Next Generation HSC Gene Therapy

Ex vivo Genetic Engineering (beyond lentiviral gene transfer)

- Alleviate residual concerns for genome-wide insertional mutagenesis
- Improve transgene expression
 - Consistency
 - Rescue physiological control



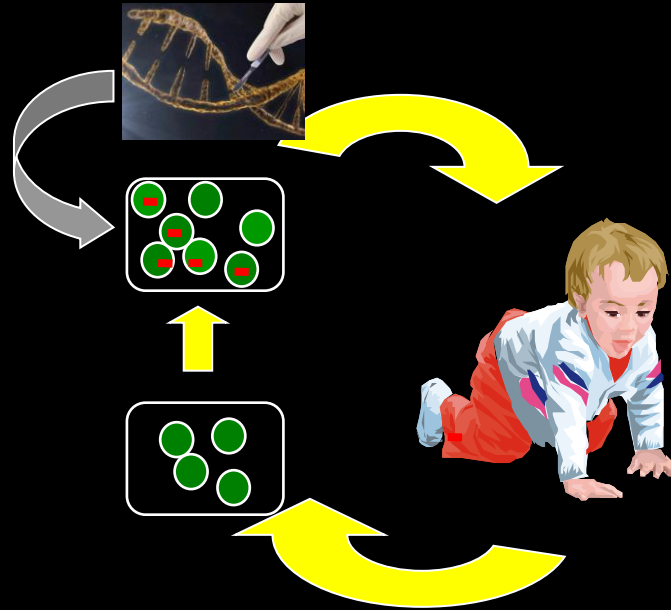
Infuse back into patient

Harvest HSPC

Next Generation HSC Gene Therapy

Ex vivo Genetic Engineering

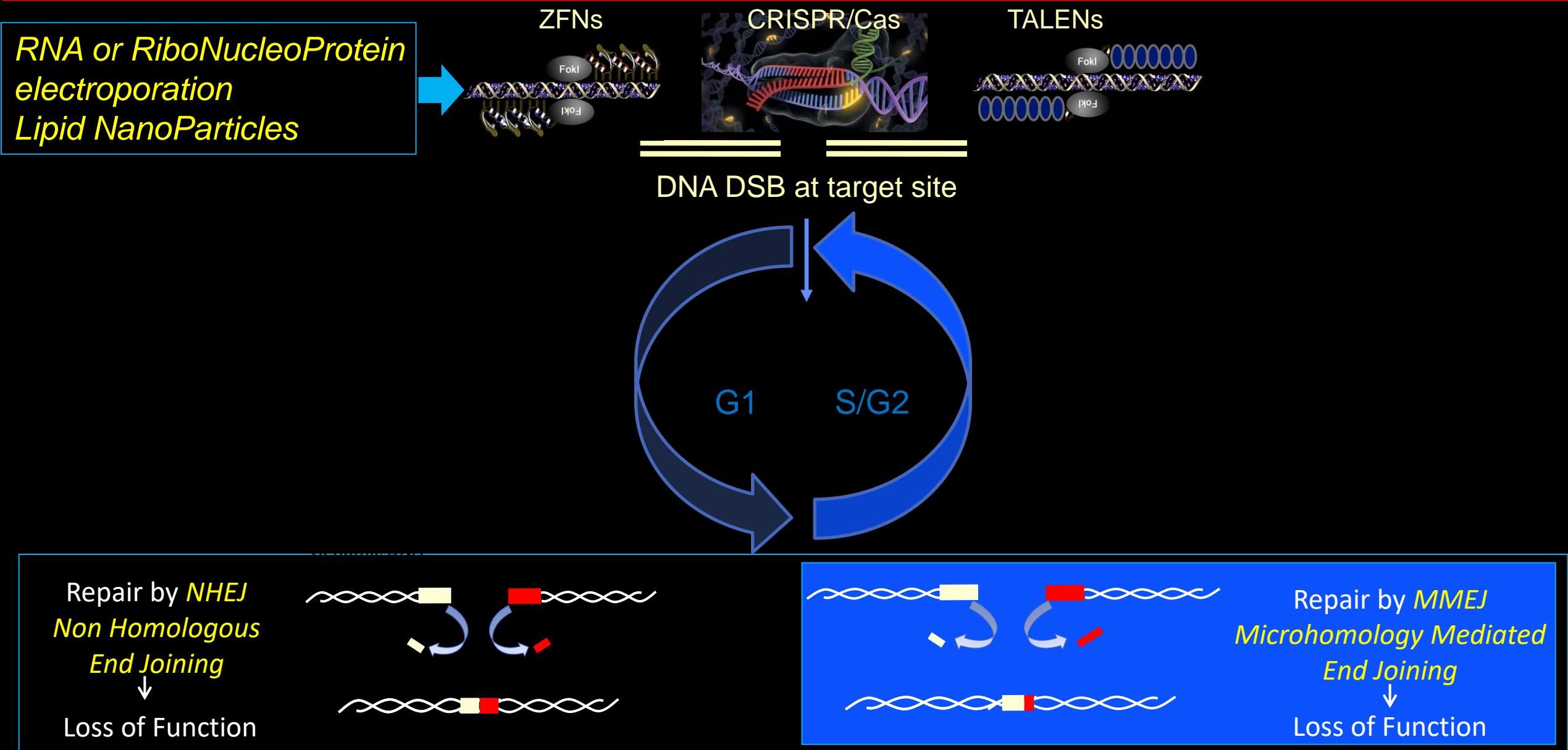
- *Improve precision by targeted gene editing*



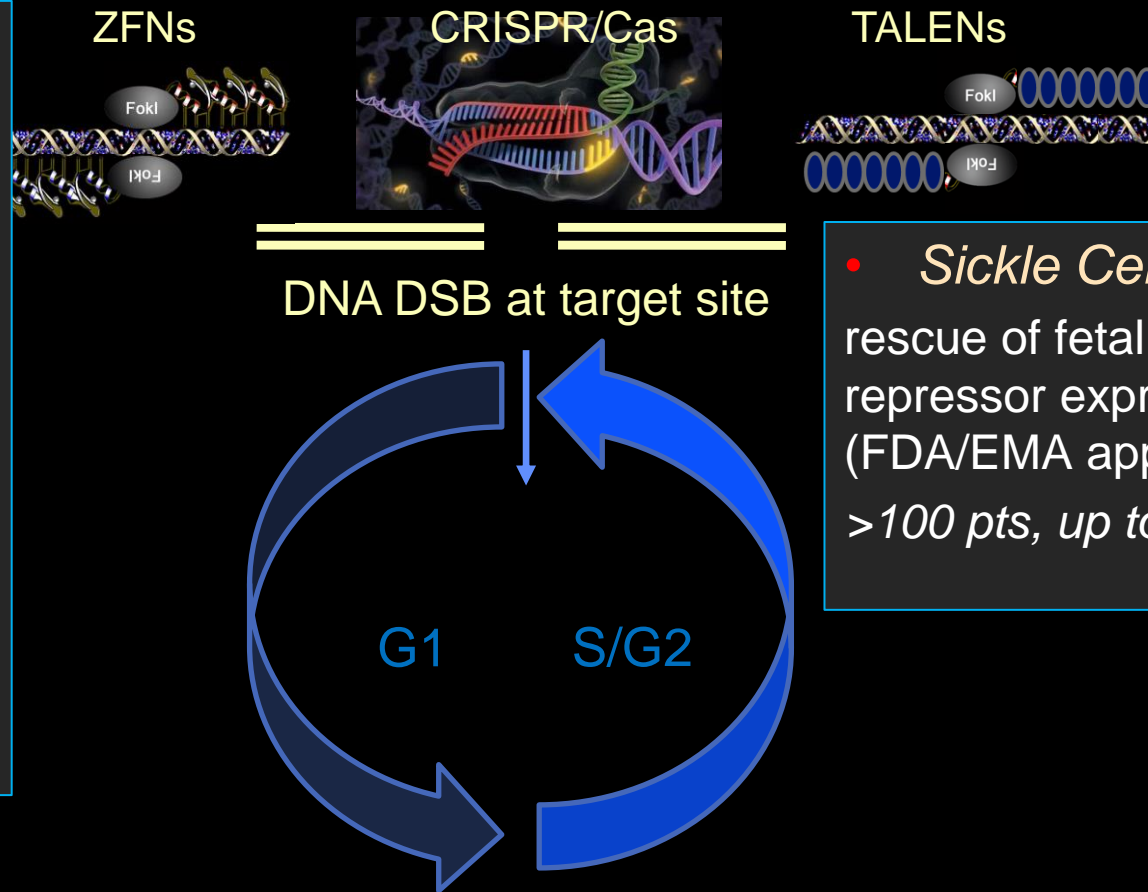
Infuse back into patient

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(Designer Endo-)Nuclease Mediated Targeted Gene Editing



Clinical Applications of NHEJ-Mediated Gene Disruption

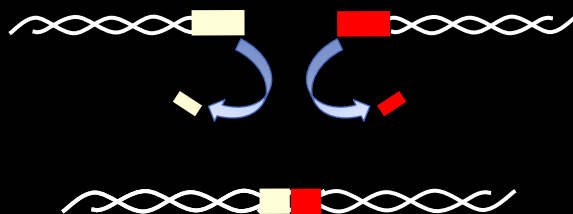


- *Unique application*
- *Achieved at high efficiency, also biallelic*
- *High target specificity by*
- choice among Cas variants, ZFNs, TALENs
- *Genotoxic risk*
- circumscribed to on & off nuclease targets
- deletions, translocations, loss of chromosome arm, LOH, chromotripsis

- *Sickle Cell disease, β -thalassemia*
rescue of fetal Hb by disrupting γ -globin repressor expression (erythroid enhancer) (FDA/EMA approved **Casgevy** by Vertex)
>100 pts, up to 4 yr follow-up

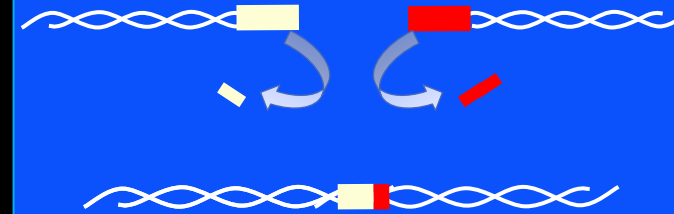
Repair by **NHEJ**
*Non Homologous
End Joining*

Loss of Function

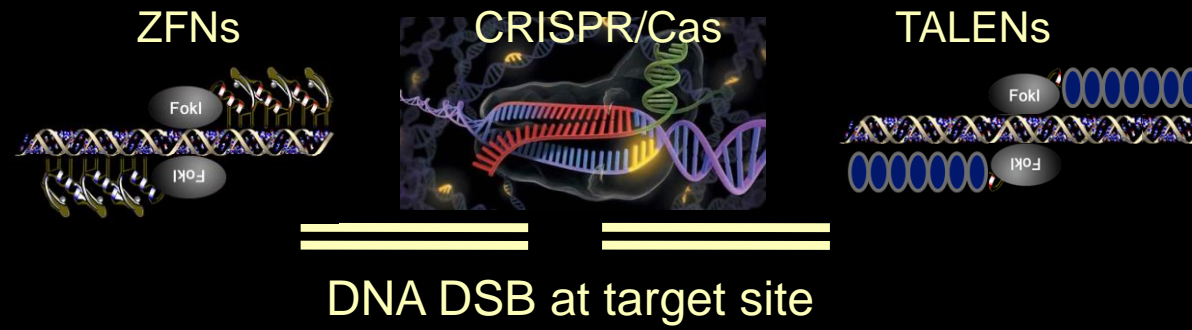


Repair by **MMEJ**
*Microhomology Mediated
End Joining*

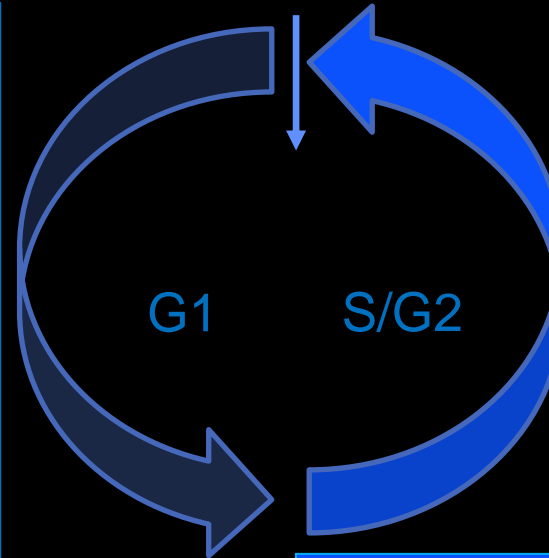
Loss of Function



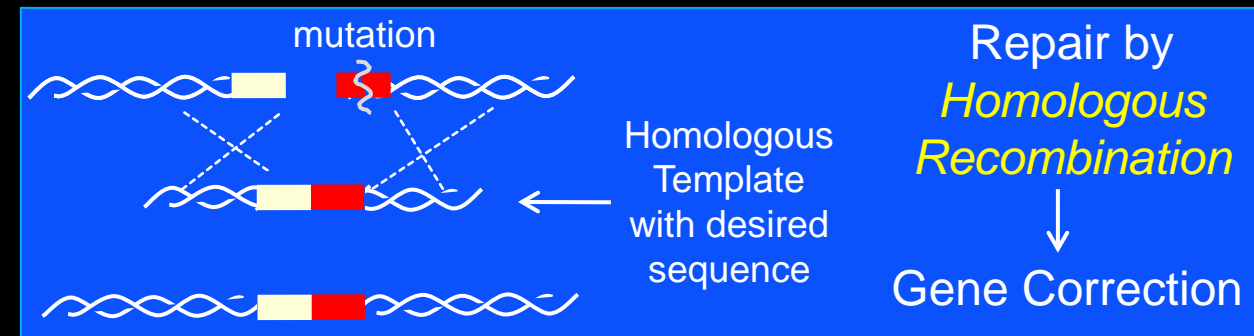
Targeted Gene Editing by Homology Directed Repair (HDR)



- Uniquely allows **long-range** gene editing*
- *in situ gene correction*
 - restoring function and
 - expression control
 - *Targeted integration of expression cassette into genomic safe harbor*
 - *Constrained in HSC by*
 - low efficiency of HDR
 - need for DNA template co-delivery
- Genovese et al., Nature 2014*



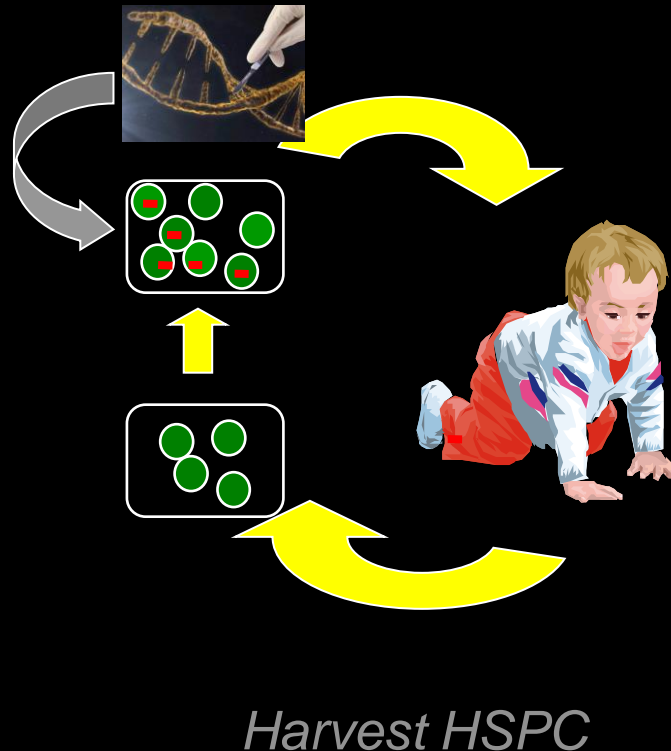
- Co-delivery of*
- *Nuclease by RNA/RNP electroporation*
 - *Donor template by AAV6, IDLV, oligonucleotide*



Next Generation HSC Gene Therapy

Ex vivo Genetic Engineering by Targeted gene editing

- Optimize efficiency
 - Inhibit (transiently) DDR
 - Delivery (electroporation vs. LNP)
 - Template choice/delivery
- Assess genetic outcome at target site
 - Occurrence of deletions & translocations
 - Heterogeneity of repair
- *Select for intended edit*
- *Purge unwanted outcomes*



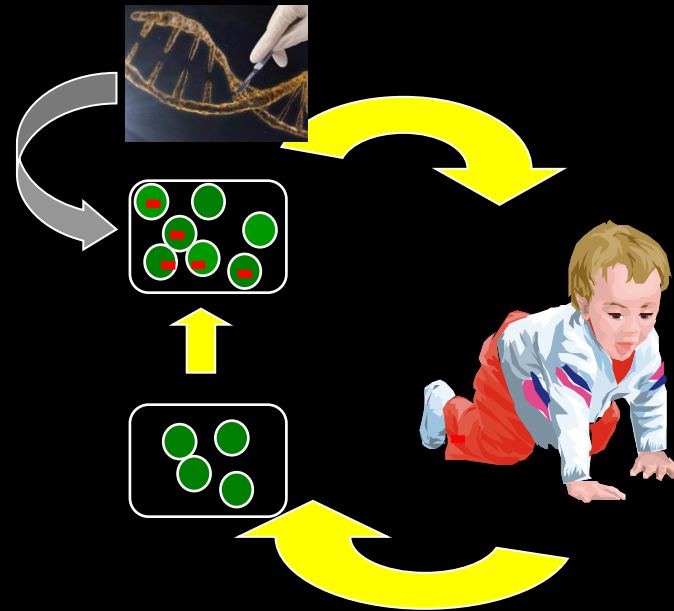
Infuse back into patient

Harvest HSPC

Next Generation HSC Gene Therapy

Ex vivo Genetic Engineering

- *Improve precision by targeted gene editing*
- *Editing without DNA Double Strand Break ?*

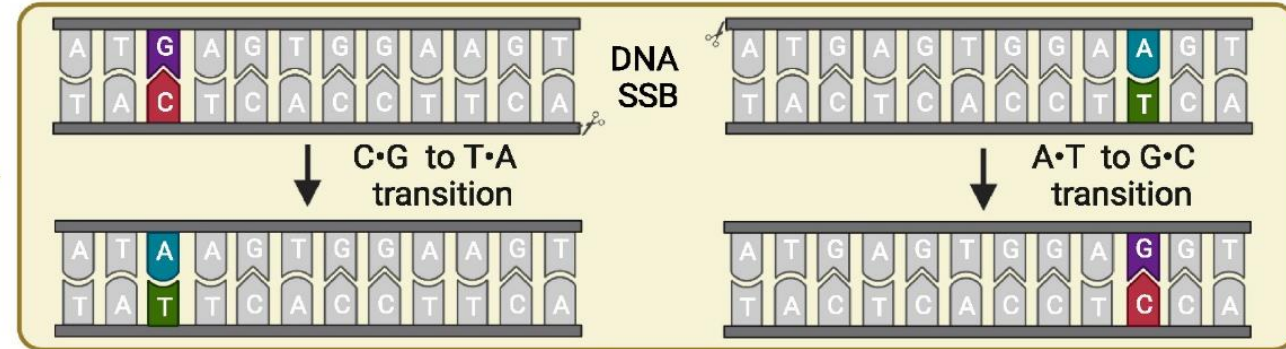
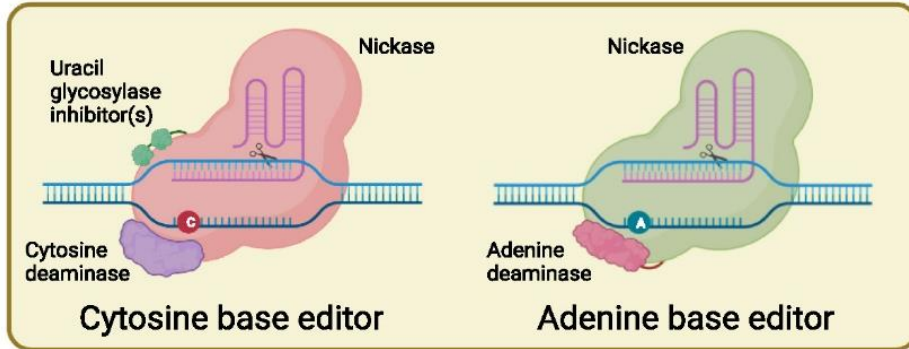


Infuse back into patient

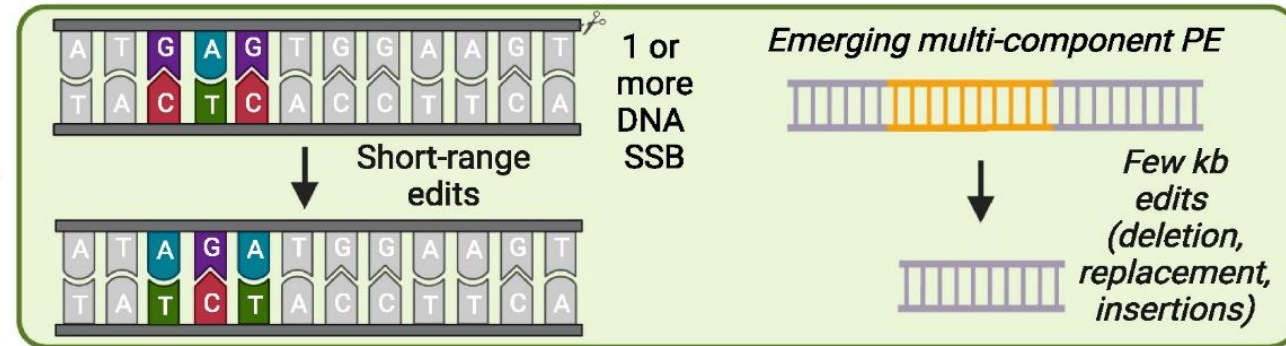
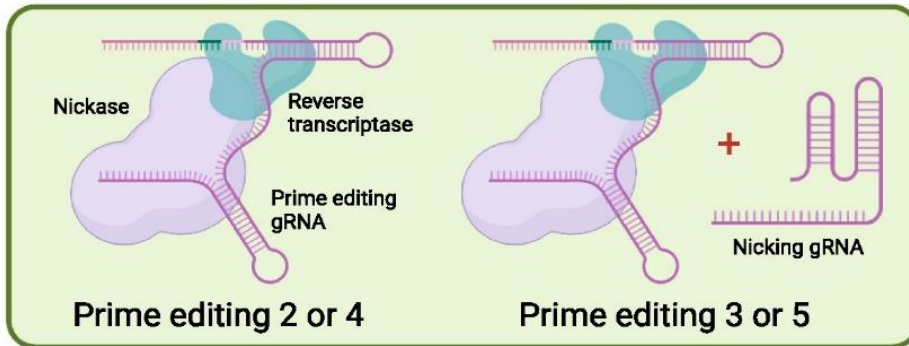
Harvest HSPC

Nickase-mediated Targeted Gene Editing

Base editors



Prime editors



Summary: Nickase-Based *Base* and *Prime Editing*

- *Single base or short sequence edits (mutation specific correction)*
 - RNA engineering improves efficiency, precision & dampens detrimental responses
 - reduces (vs. Nuclease) but does not abolish DNA DSB and adverse consequences
 - variably affected by deaminase type & expression level and
 - *interaction with endogenous repair pathways (BER)*
 - *detectable impact of BE on exome mutational landscape by ultra-deep WES*
 - *likely through inhibition / saturation of BER (CBE)*
 - *engagement of alternative error-prone DNA repair*
- development of improved versions may alleviate some of above concerns

nature biotechnology

Article

<https://doi.org/10.1038/s41587-023-01915-4>

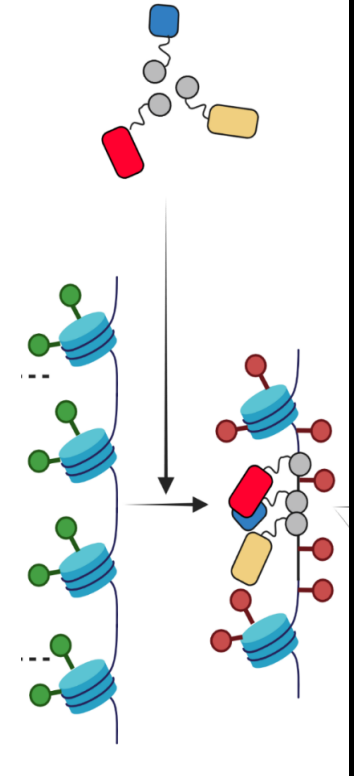
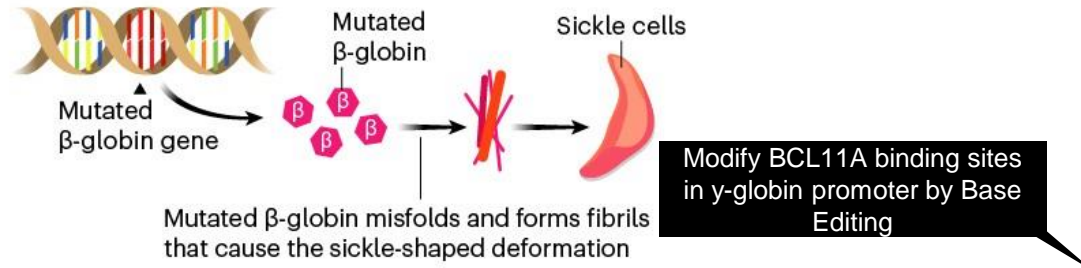


**Genotoxic effects of base and prime editing
in human hematopoietic stem cells**

HSC GT for SCD / β -Thalassemia: Multiple Options

A TRIO OF TACTICS

Some gene therapies for sickle-cell disease restore healthy red-blood-cell function even if expression of the mutant protein continues uninterrupted. By contrast, CRISPR-based efforts aim to fully repair the root cause of the disease.



Silence BCL11A by epigenetic editing

Modified from
Nature **596**, S2-S4 (2021)
<https://doi.org/10.1038/d41586-021-02138-w>

The HSC Genetic Engineer's Toolbox



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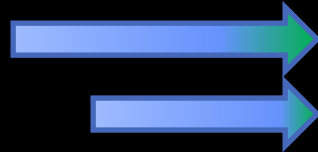
- **Gene Replacement**

- Lentiviral Vectors



- **Gene Editing**

- ZFNs, TALENs, MegaNs
- CRISPR/Cas



- **Emerging Break-free Editors**

- Base Editors
- Prime Editor



- **Epigenetic Editing**



Choosing the Right Engineering Tool

Lentiviral Vectors

Efficient *Gene Replacement*

Preserve long-term
engraftment of HSC & T cells

Genome-wide insertion

Variable expression level

Solid clinical track record

*Insertional genotoxicity
minimized by vector design*

Nuclease-based Editing

Efficient *Gene Disruption*

Allows - with constraints -
Long Edit / Gene Correction

Impacts graft clonality

Early clinical stage

*Off-target activity
Large genomic rearrangements*

Nickase-based (B & P) Editors

Efficient *Mutation Correction*

and Gene Inactivation

Low DNA DSB burden

Just in the clinic (BE)

Bystander edit

*Sequence-independent
off-target activity*

Epigenetic Editing

Stable Gene Silencing

DNA DSB-free

R&D stage



Review

Genetic engineering meets hematopoietic stem cell
biology for next-generation gene therapy

Cell Stem Cell

Ferrari,...& Naldini, 2023

Advanced Therapy Medicinal Products (ATMPs)

- *Transformative Medicines*
 - Complex “live” medicines made with genes, modified viruses, cells and/or designer DNA-modifying enzymes...
 - “*Once and done*” treatments with potential for “cure” of otherwise severe-lethal diseases
 - Life-long benefits but also possible delayed adverse effects
 - Fully personalized if made with own patient’s cells
- *Disruptive procedures*
 - Complexity & high costs of manufacturing and supply chain
 - High market values can be claimed, but spread over decades ahead
 - Staff and structure at point-of-care actively involved in the process
 - Challenging to provide fair, equitable and broad access

Addressing the Current Challenges of ATMP Development

Research is heading towards

- *in vivo applications*
 - Easing burden of ex vivo manufacturing & conditioning
 - *Poor control on target cells, product profile & biodistribution*
 - *Higher risk of immunogenicity*
- *Allogenic hypo-immune (universal donor) cells*
 - Off-the-shelf product
 - Readily available to patients with short life expectancy, poor harvest
 - *Constrained batch size, pooled donors, heterogeneity*
 - *Challenge to maintain biological potency and*
 - *genome integrity upon extensive genetic engineering and culture*

Adopting a “Platform” Approach throughout ATMP Development

- *ATMPs are complex bio-entities comprising shared elements among each class*
 - Vector genetic backbone, manufacturing process & particle structure/composition
 - Gene Editor molecule, assembly & delivery process
- *Many pharmacological properties depend on class-specific elements without being influenced by transgene/guideRNA specific sequence*
 - stability, biodistribution, genotoxicity
- *Increased knowledge on underlying biology and extensive clinical experience with most common vectors should relieve need for iterative studies*
 - benefit/risk evaluation of the first-in-class is no longer an appropriate benchmark
 - adapted/updated evaluation needed after hundreds patients treated with similar products across different sponsors/diseases and far more data is available on which to assess similar products
- *Establish & enable open/confidential access to Class “Master Data Files”*
- *Design space in the CMC process*
- *Realize limited scope of testing each new product*
 - In animal recipients (especially for human cell products)
 - Using healthy donor vs. disease/patient specific cells
 - Mostly providing class- vs. product-specific information

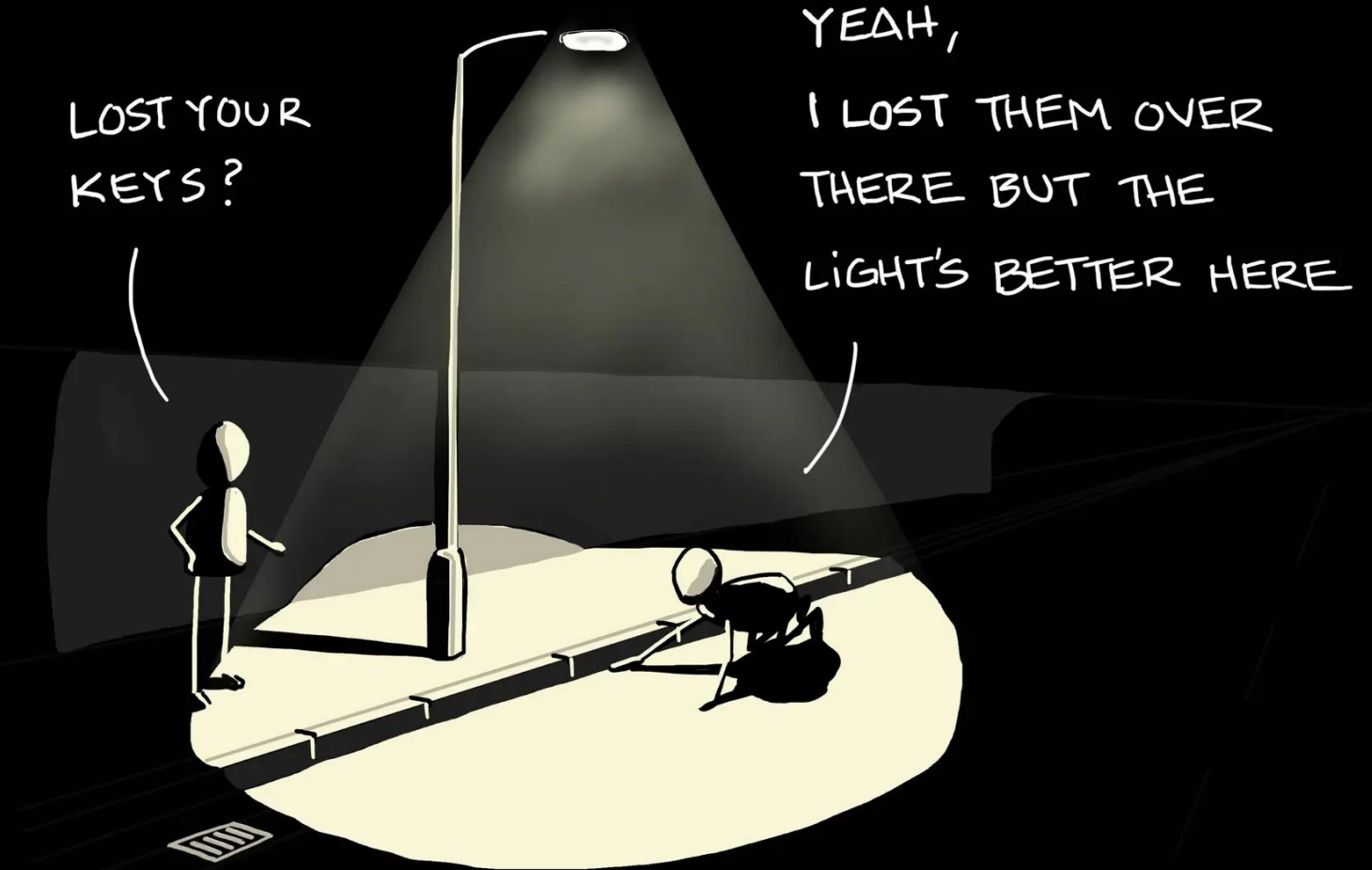
Genotoxicity of Gene Transfer Strategies

- *We have learned much more from clinical than pre-clinical testing*
 - Better start clinical testing earlier and address emerging risks
- *Genotoxicity becoming better understood as emerging from interplay of*
 - Vector design (promoter, transcriptional signals)
 - CMC process & yield
 - Disease background and prior drug exposure
- *Emerging tumorigenesis in HSC gene therapy for SCD and in CART*
 - Mostly ascribed to preexisting mutations in harvested cells vs. vector/treatment.
 - Patient-specific risk factors emerging with broader clinical use
 - May instruct safer deployment (pre-screening for at-risk mutations).
 - No standard pre-clinical test would have captured this

Addressing

- *Plethora of emerging r*
- *Human genome seque*
regional differences in
 - Limits off-target predicti
 - Animal models useless
 - Unavoidable level of un
 - Fair to address patient-
- *Complexity of interacti*
 - Need better understand
 - Requires novel *ad-hoc*

LOOKING UNDER THE LAMPOST



Addressing Genotoxic Risk of Gene Editing

- *Plethora of emerging new tools in constant evolution*
- *Human genome sequence variability and unbalanced representation of regional differences in available database*
 - Limits off-target prediction by testing a generic / individual donor human genome
 - Animal models useless except for target sequence-independent effects
 - Unavoidable level of uncertainty for each new recipient
 - Fair to address patient-specific emerging toxicity in the clinic
- *Complexity of interaction with cellular DNA and repair machinery*
 - Need better understanding of biology
 - Requires novel *ad-hoc* testing (avoid the “looking under the lamppost paradox”)
 - Long-term clinical follow-up needed
- *Hit-and-run process makes difficult to trace eventual adverse events*

A Perfect Storm (for Rare Disease Gene Therapies)

- *They paved the way*
 - Validated rationale of cure at the genetic bases of disease
 - Provided proof of safety and efficacy of all major platforms
- *First to reach the market*
 - Claiming highest price of any other medicine
 - Yet failed to achieve sustainable deployment by pharma industry
- *Pharma disinvesting from rare to privilege common diseases*
 - CMC / supply chains not developed to enable economy of scale
- *Many orphan diseases potentially amenable to treatment*
 - sometimes undertaken by startups with limited resources

Ensuring a Future for Gene Therapy for Rare Diseases

Nature Medicine volume 28, pages1985–1988 (2022)

 Check for updates

comment

Ensuring a future for gene therapy for rare diseases

Alessandro Aiuti, Francesca Pasinelli and Luigi Naldini



Non-Profit ATMP Manufacturing & Supply

- *Is “Hospital Exemption” a solution?*
- *CMC may be designed to contain development / manufacturing costs*
 - Meeting safety but not commercial manufacturing standards
 - platform approach, master data files, shared tools, safe harbors
 - Primary goal is not competition with industry on approved ATMP
- *Supported by public sponsored schemes*
 - Leveraging on harmonized & centralized procedures & point-of-cares
- *Provide education and training of specialized personnel*
- *Foster innovation*
 - May ease introducing “platform” improvements in vector design & process
 - Also on approved products when regulatory requirements for changes are too costly or perceived risky

Ensuring a Future for Gene Therapy for Rare Diseases

Critical issue

High production costs

Potential solution

Technological innovation, such as stable vector packaging cell lines and large-scale bioreactor production, closed system and optimized transduction, shortened manipulation and minimal batch testing prioritizing molecular methods