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

SAN RAFFAELE TELETHON INSTITUTE FOR GENE THERAPY
SR-Tiget

OSPEDALE SAN RAFFAELE

FONDAZIONE Telethon

Disclosures

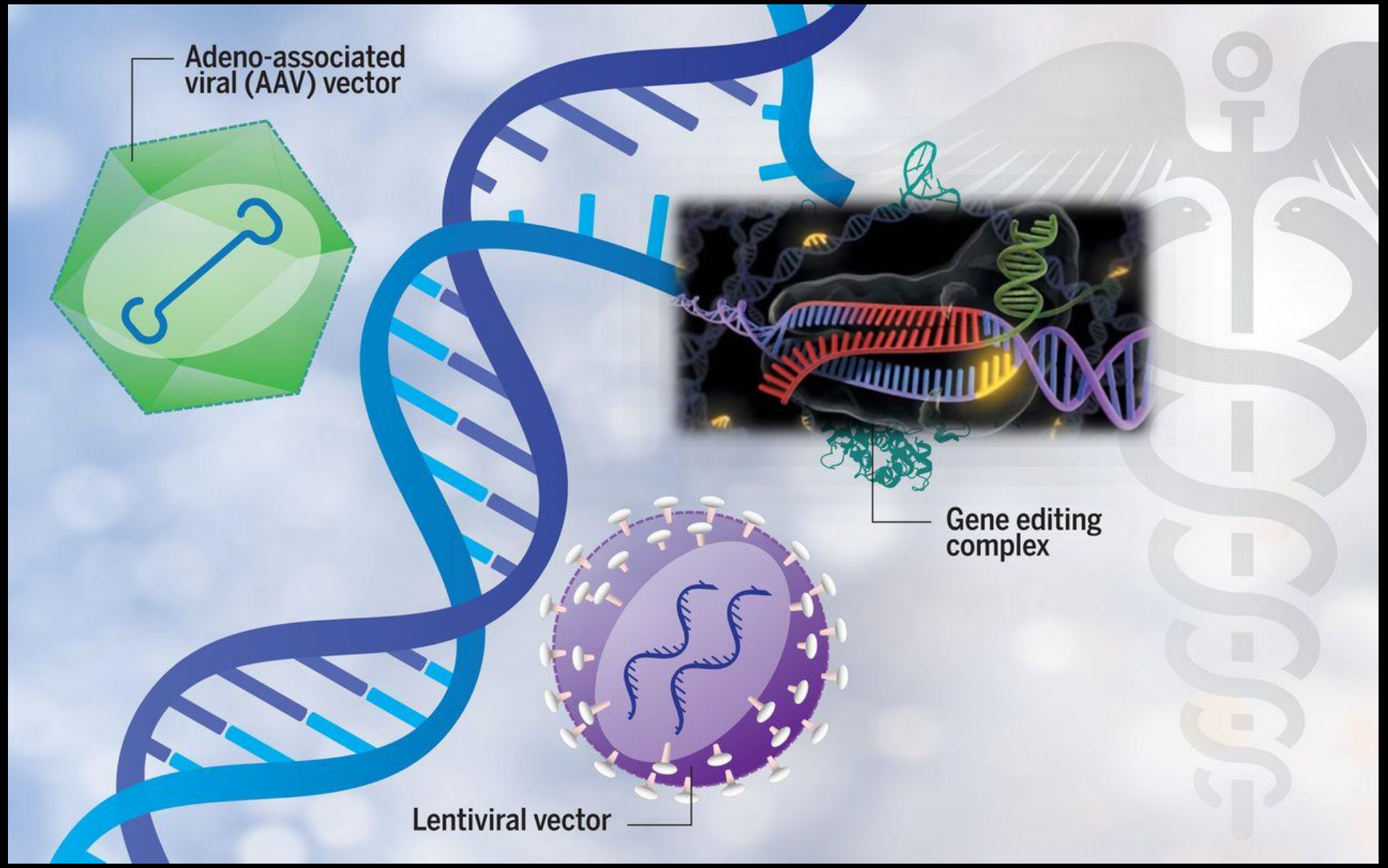
- *Inventor of patents on*
  - *lentiviral vector technology*
  - *targeted genome editing*
  - *HSC manipulation / transplantation*

  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



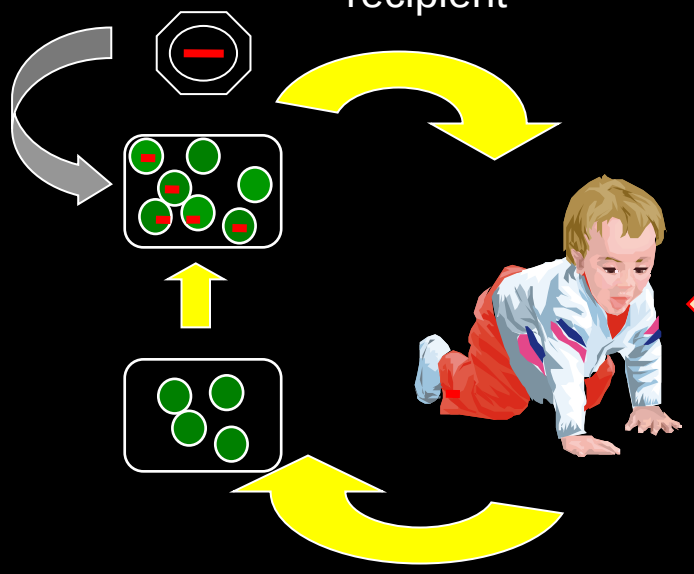
A New Medicine for the 3rd Millenium

- *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*

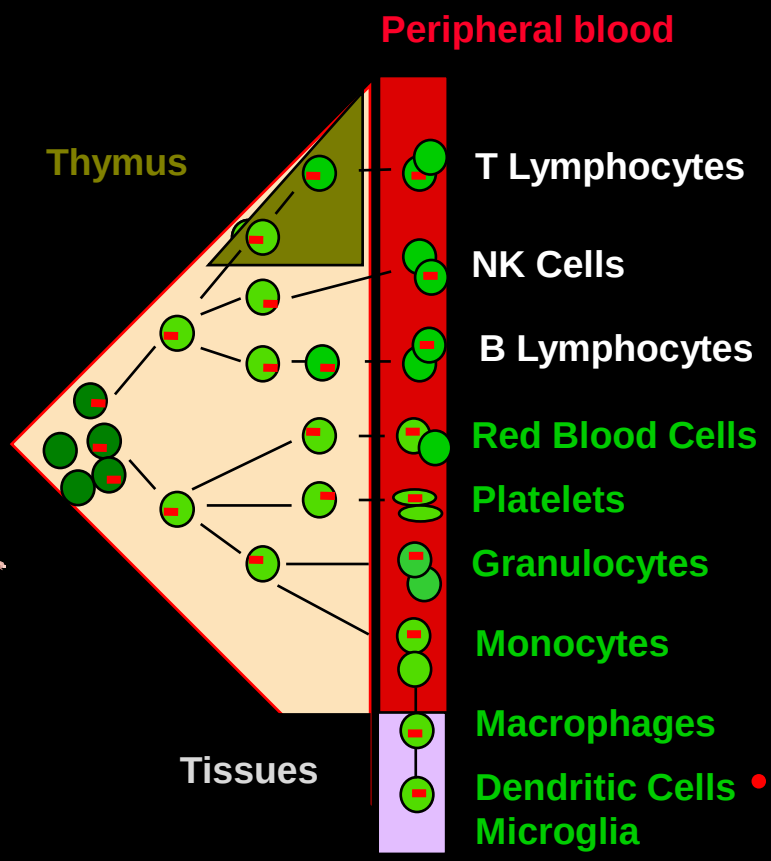
Ex vivo Gene Therapy: Major Clinical Achievements

- 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



Immuno-hematological diseases



Storage diseases

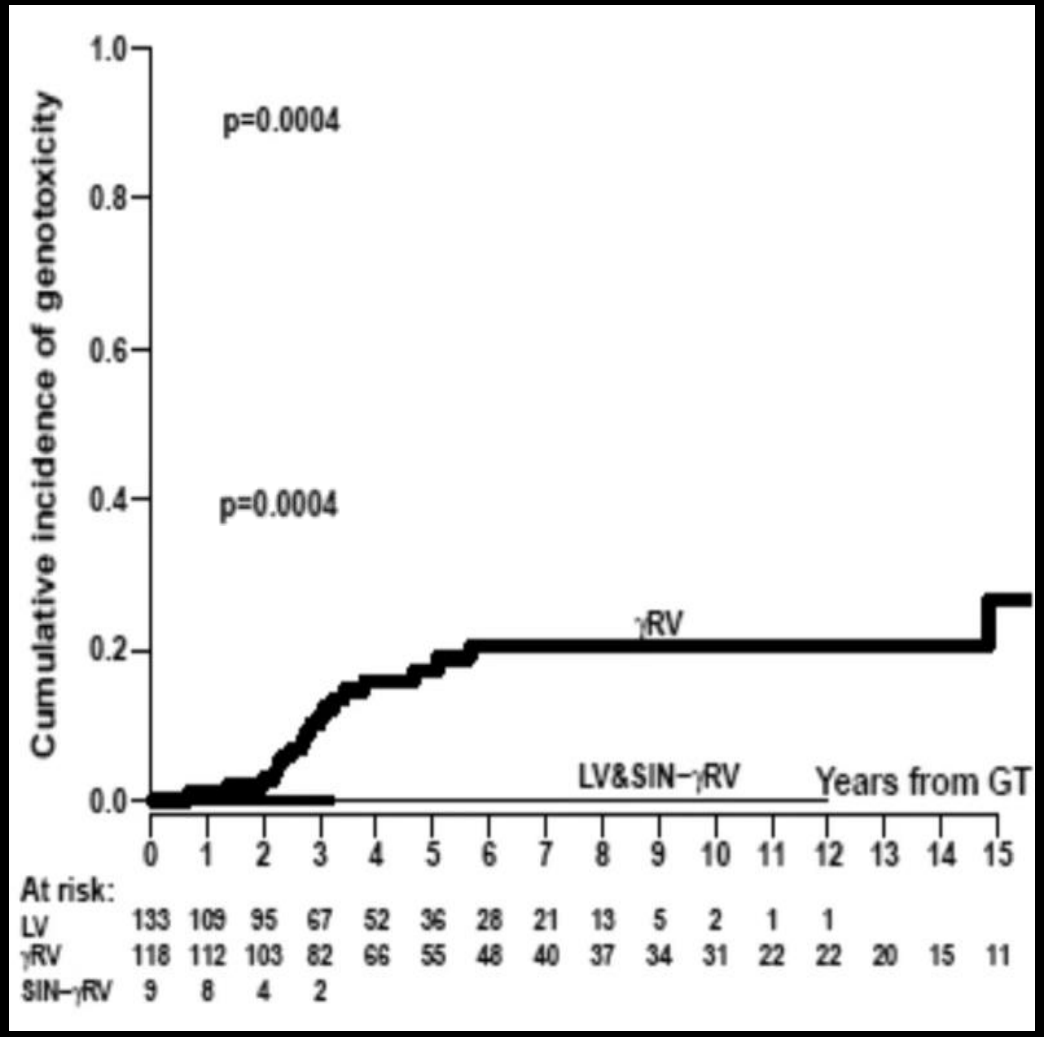
- *Safe with substantial & durable benefits*
- *Lentiviral Vectors*
  - *>400 pts, up to 14 yr follow-up, 20 diseases*
  - Wiskott-Aldrich Syndrome, X-SCID, CGD
  - Artemis, LAD, RAG-1 deficiency …
  - Adrenoleukodystrophy, Metachromatic Leukodystrophy, MPS-I, MPS-IIIA, Gaucher, Fabry's, Osteopetr, Cystinosis…
  - 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

Tucci et al. Nat Commun, 2022

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

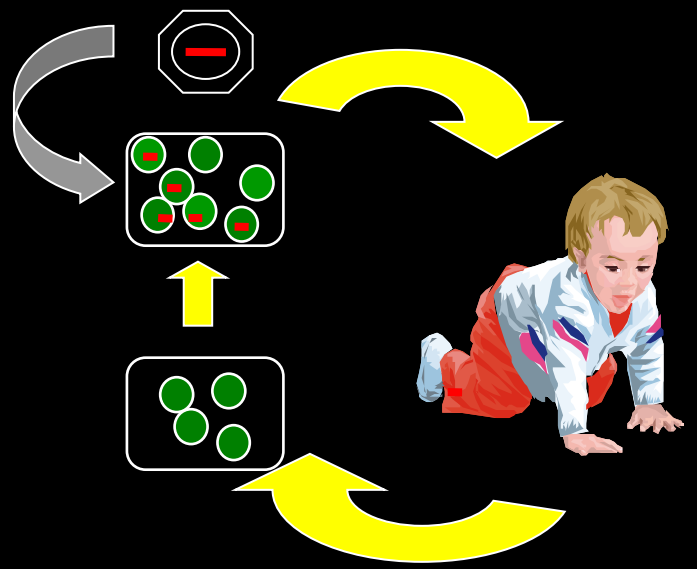
- *Concerns (long-term)*
  - Residual genotoxic risk of engineeering
  - Long-term stability and clonal composition of engineered graft

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



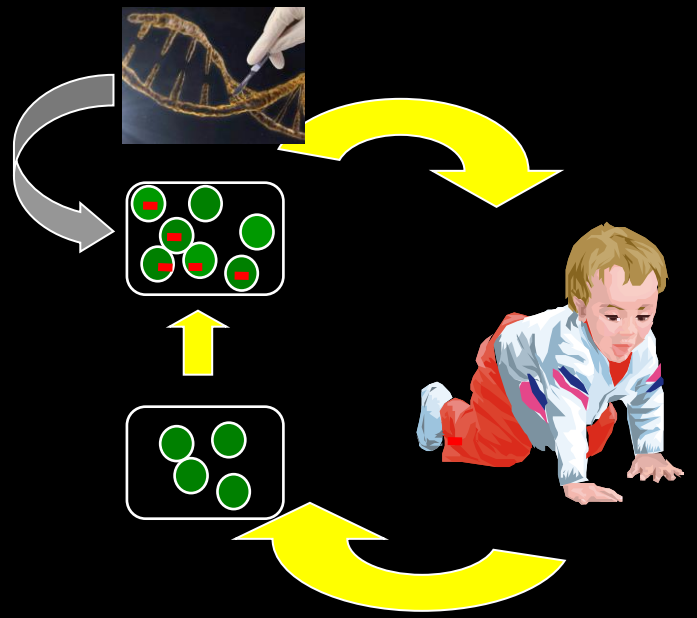
Infuse back into patient

Harvest HSPC

Next Generation HSC Gene Therapy

Ex vivo Genetic Engineering

- *Improve precision by targeted gene editing*



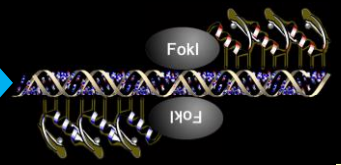
Infuse back into patient

Harvest HSPC

(Designer Endo-)Nuclease Mediated Targeted Gene Editing

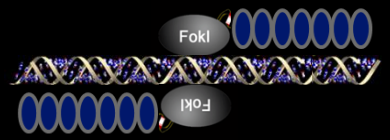
RNA or RiboNucleoProtein
electroporation
Lipid NanoParticles

ZFNs

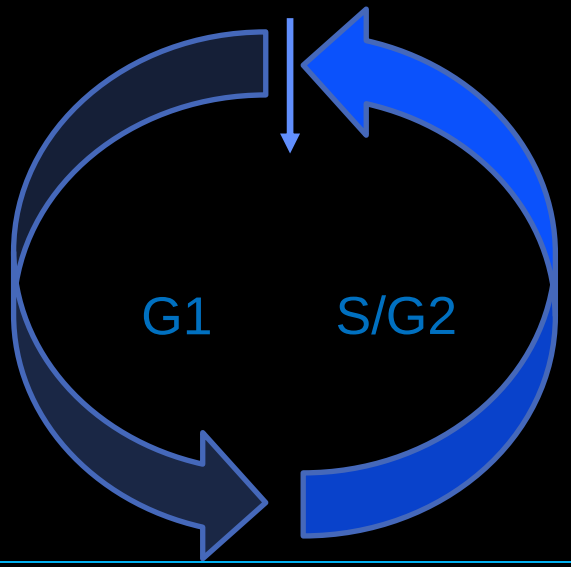


CRISPR/Cas

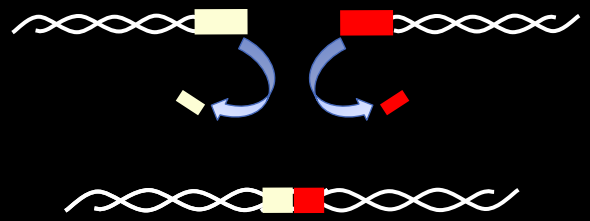
TALENs

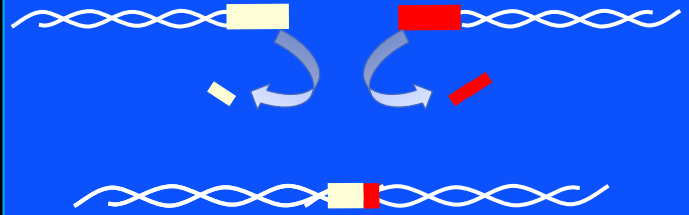


DNA DSB at target site



Repair by *NHEJ*
Non Homologous
End Joining
↓
Loss of Function



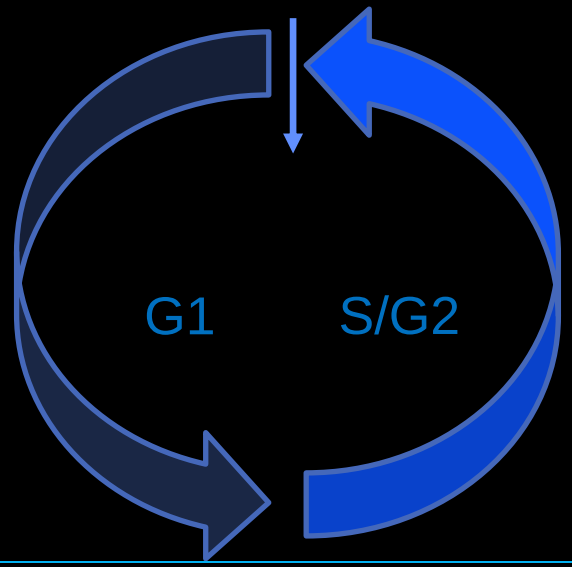


Repair by *MMEJ*
Microhomology Mediated
End Joining
↓
Loss of Function

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

DNA DSB at target site



- *Sickle Cell disease, β-thalassemia*

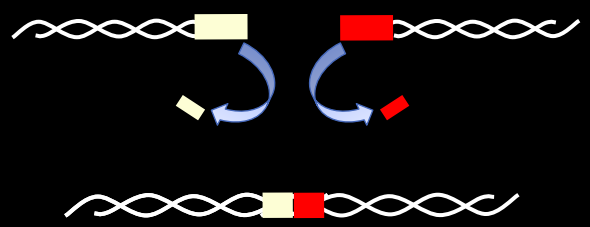
rescue of fetal Hb by disrupting γ-globin repressor expression (erythroid enhancer) (FDA/EMA approved Casgevy by Vertex)

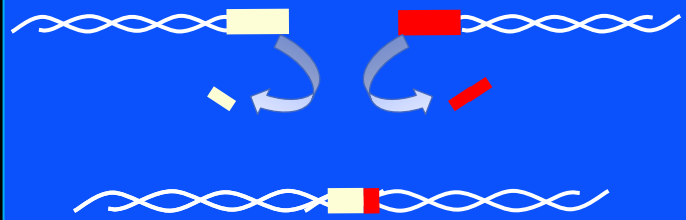
>100 pts, up to 4 yr follow-up

Repair by *NHEJ Non Homologous End Joining*

↓

Loss of Function





Repair by *MMEJ Microhomology Mediated End Joining*

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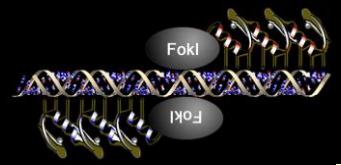
Loss of Function

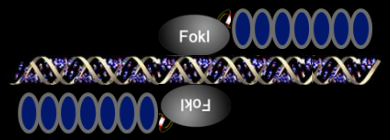
Targeted Gene Editing by Homology Directed Repair (HDR)

ZFNs

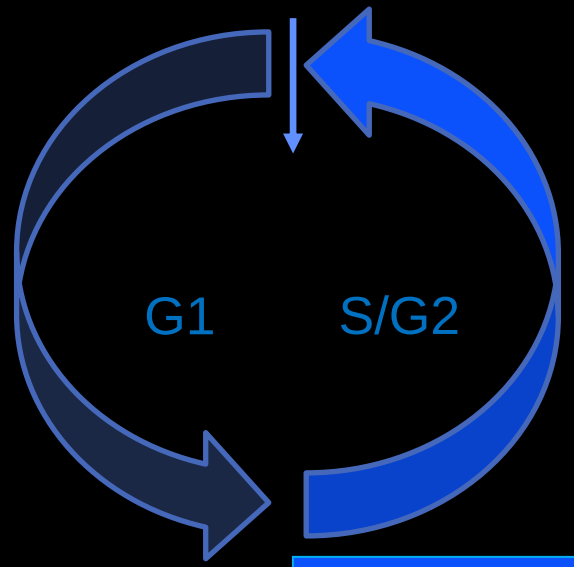


CRISPR/Cas

TALENs



DNA DSB at target site



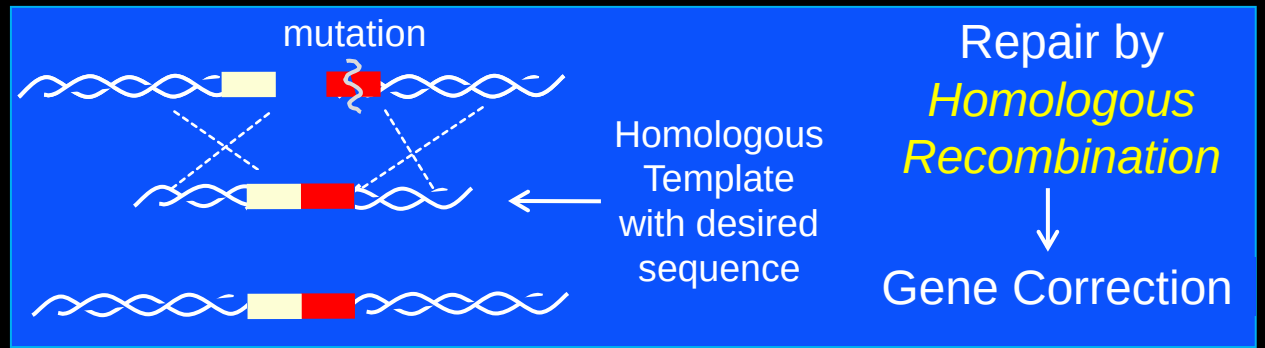
Uniquely allows long-range gene editing

- *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

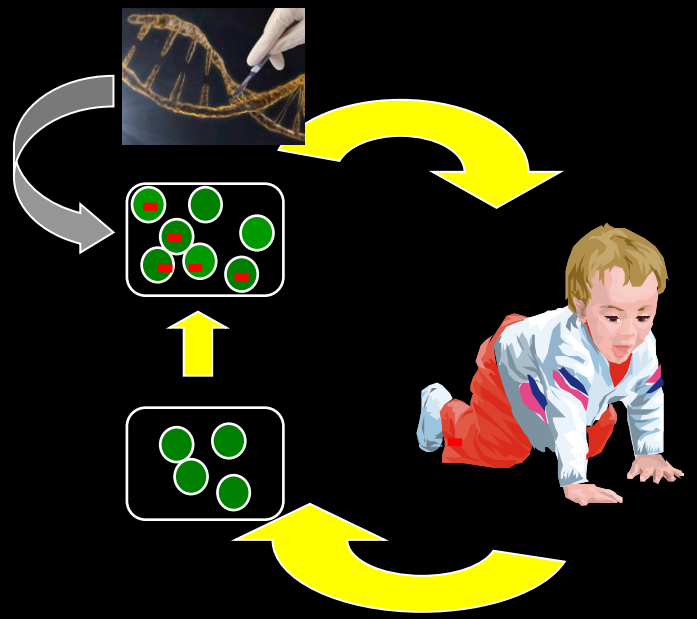
- *Nuclease by RNA/RNP electroporation*
- *Donor template by AAV6, IDLV, oligonucleotide*



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*



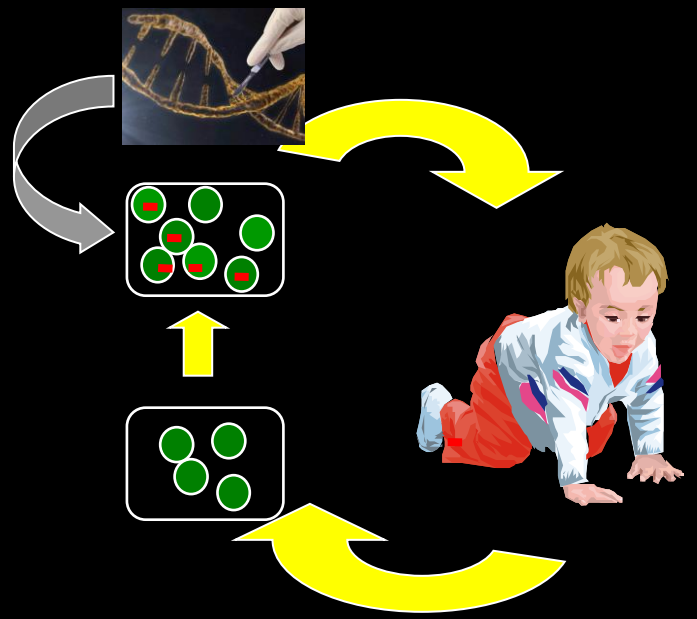
Harvest HSPC

Infuse back into patient

Next Generation HSC Gene Therapy

Ex vivo Genetic Engineering

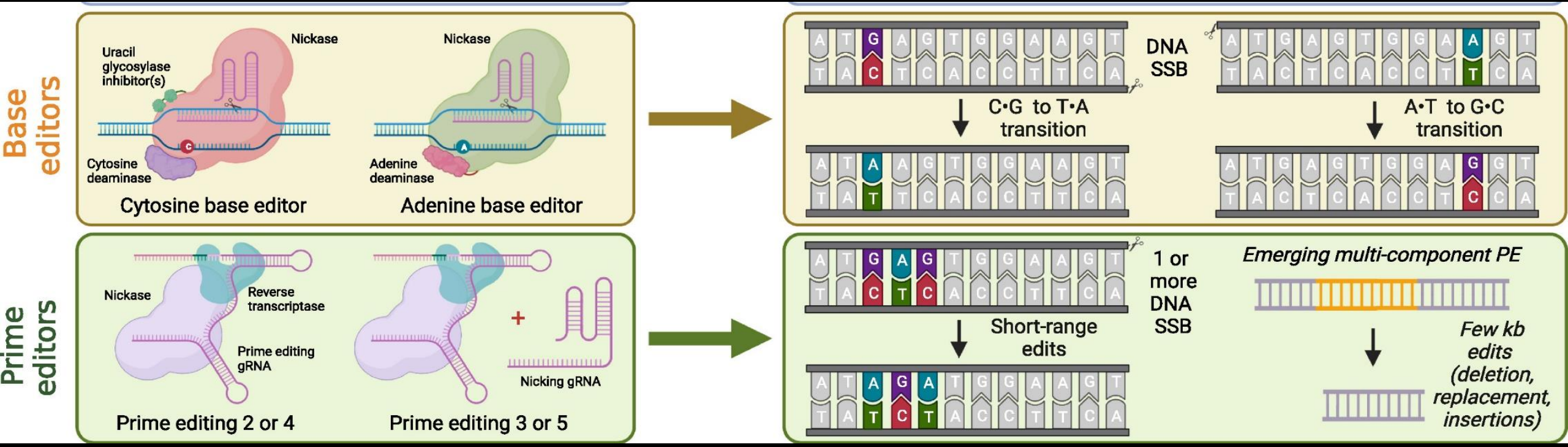
- *Improve precision by targeted gene editing*
- *Editing without DNA Double Strand Break ?*



Infuse back into patient

Harvest HSPC

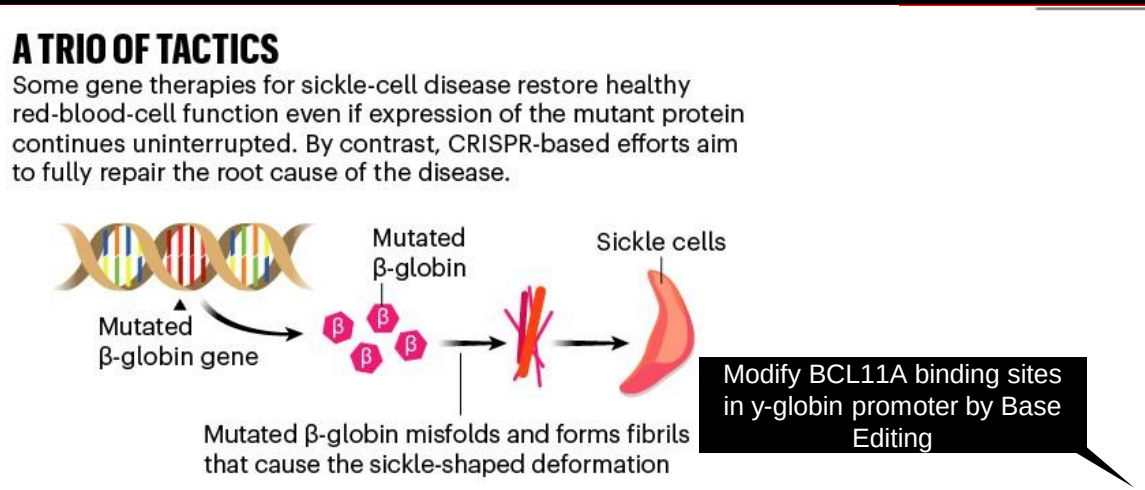
Nickase-mediated Targeted Gene Editing



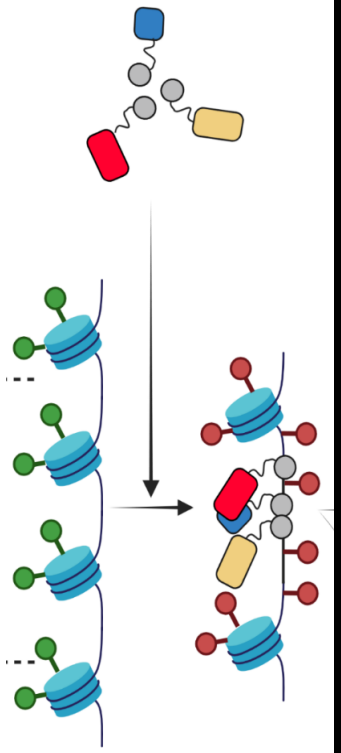
Summary: Nickase-Based *Base* and *Prime Editing*

- *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

HSC GT for SCD / β-Thalassemia: Multiple Options



Modify BCL11A binding sites in y-globin promoter by Base Editing



Silence BCL11A by epigeneitic editing

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

The HSC Genetic Engineer's Toolbox

1995 00 2005 2010 2015 2020

- ***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 constrains - *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

Cell Stem Cell

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

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 form 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 understanc
  - Requires novel *ad-hoc*

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)

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	Potential solution
High production costs	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