Decoding PI: The science of gene therapy and gene editing

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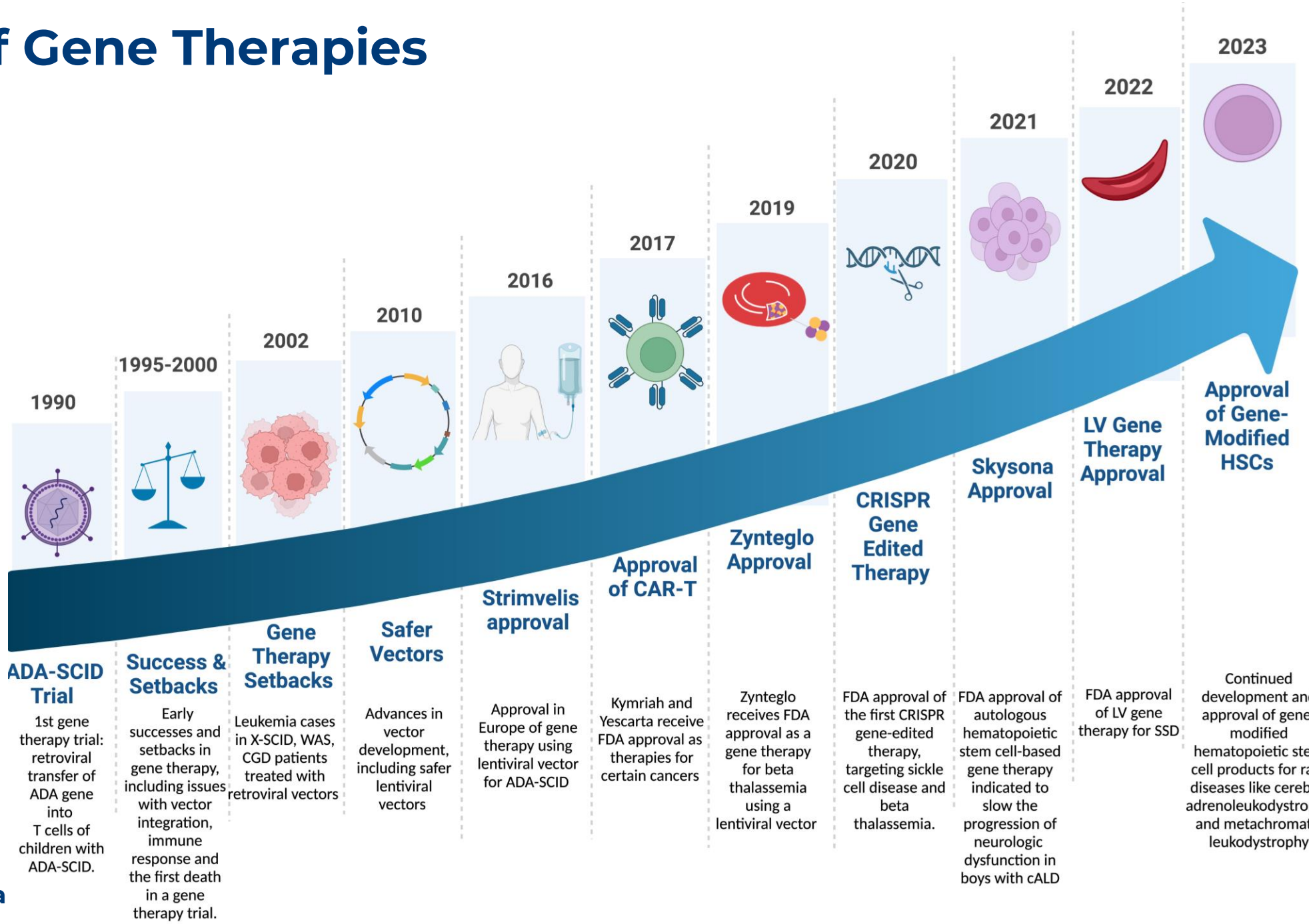
Disclosures

Support for the conduct of sponsored trials through Boston Children's Hospital from Atara Biotherapeutics, AlloVir and Jasper. Consulting for CellEvolve and Pierre Fabre

Atara Biotherapeutics holds the license & IND for the EBVCTL program developed at Memorial Sloan Kettering Cancer Center. IP related to this process with all rights assigned to MSKCC.

A History of Gene Therapies



Courtesy of A. Bertaina

Curative Therapy for Inborn Errors of Immunity

There are over 440 monogenic disorders associated with Inborn Errors of Immunity

While potentially curable with hematopoietic stem cell transplant, some patients can be predicted to have inferior outcomes and can be considered for alternative curative therapies including gene addition and gene editing therapies.

In each instance it is important to consider:

- The need for curative therapy
- Potential options for hematopoietic stem cell transplant.
- The outcome predicted with hematopoietic stem cell transplant

Gene Therapy alternative to Hematopoietic Stem Cell Transplantation

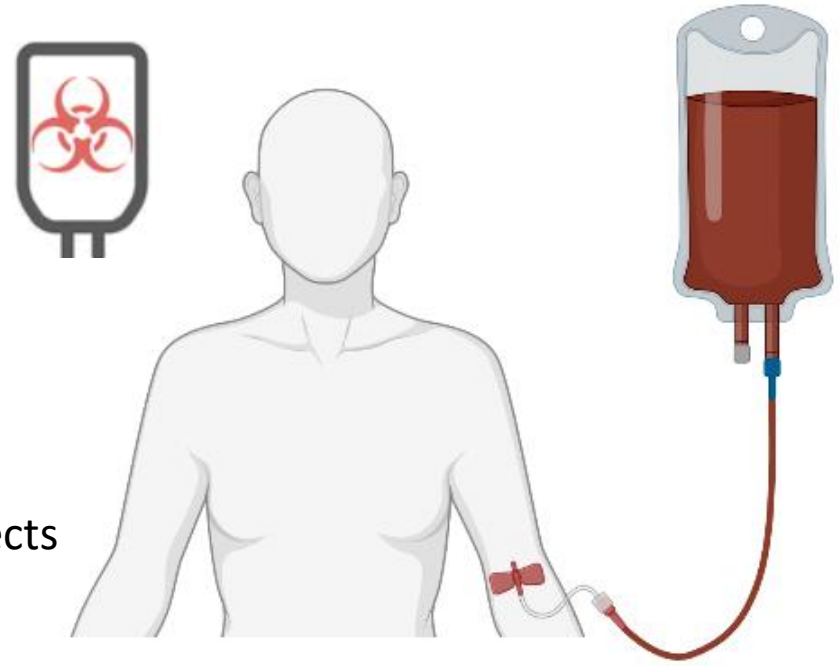
Gene Correction
Gene Addition
Ex Vivo

Manipulated
Non-Manipulated
Stem Cell Grafts

AUGMENT

Autologous
(self donor)

- Adequate Correction
- Conditioning intensity
- Genotoxicity
- Non-Genotoxic Side Effects
- Residual abnormality



REPLACE

Allogeneic
(non-self donor)

- Risk of Graft versus Host Disease
- Risk of Rejection
- Immune Compromise/Risk of Infection
- Non-Genotoxic Side Effects/TRM
- Adequate correction

Gene Therapy for Rare Disease

Desired attributes:

Safe, Available, Effective

How do we best develop, assess and use this modality in rare disease?

Inborn Errors of Immunity

Why IEI

Advantages

Hematopoietic disorder

Demonstration of level of correction needed

No benefit of alloreactivity

Established centers of excellence

Newborn screening for some of these disorders

Challenges

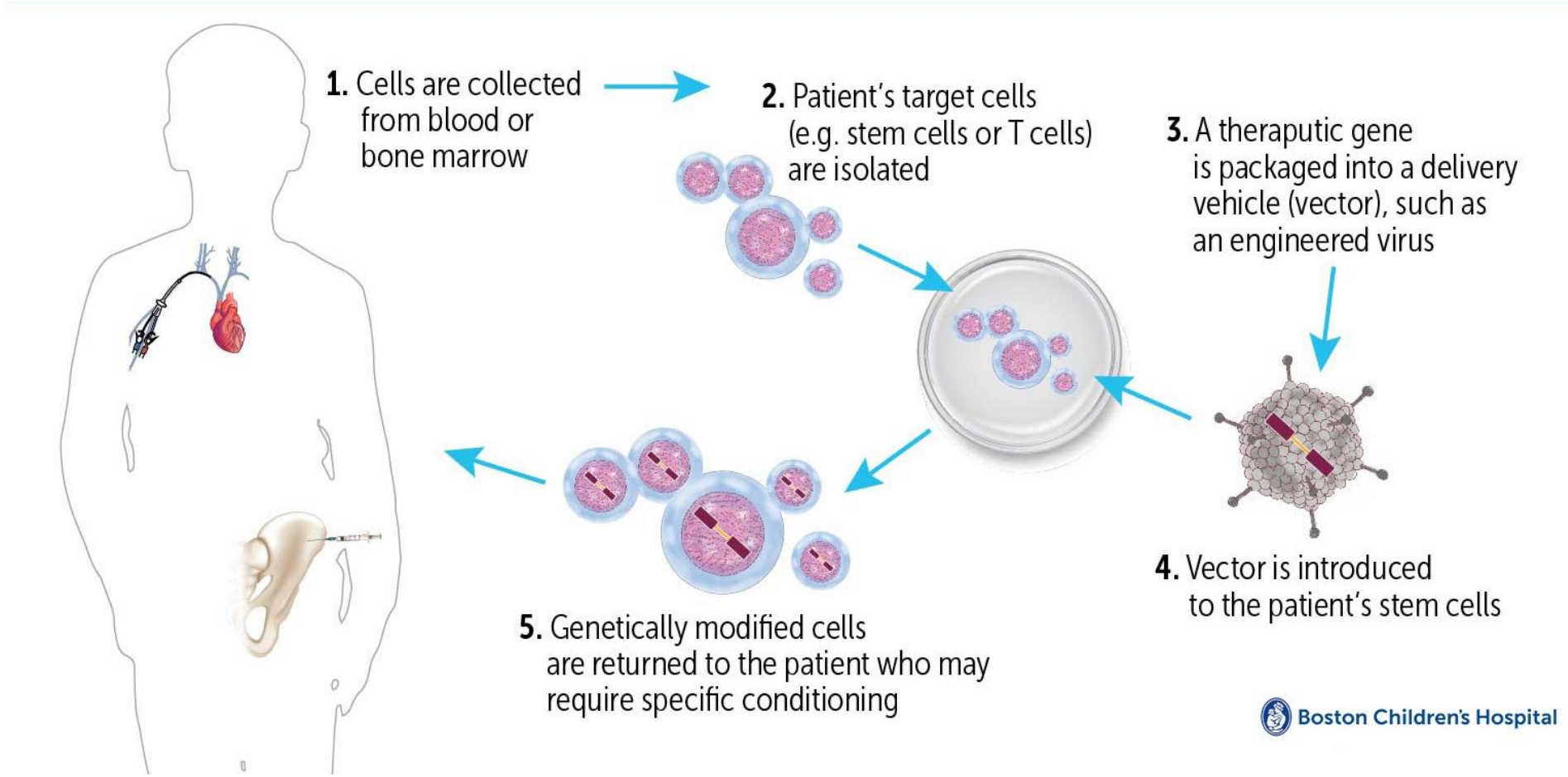
Rare disorders – unique referral patterns

Variability of disease manifestations

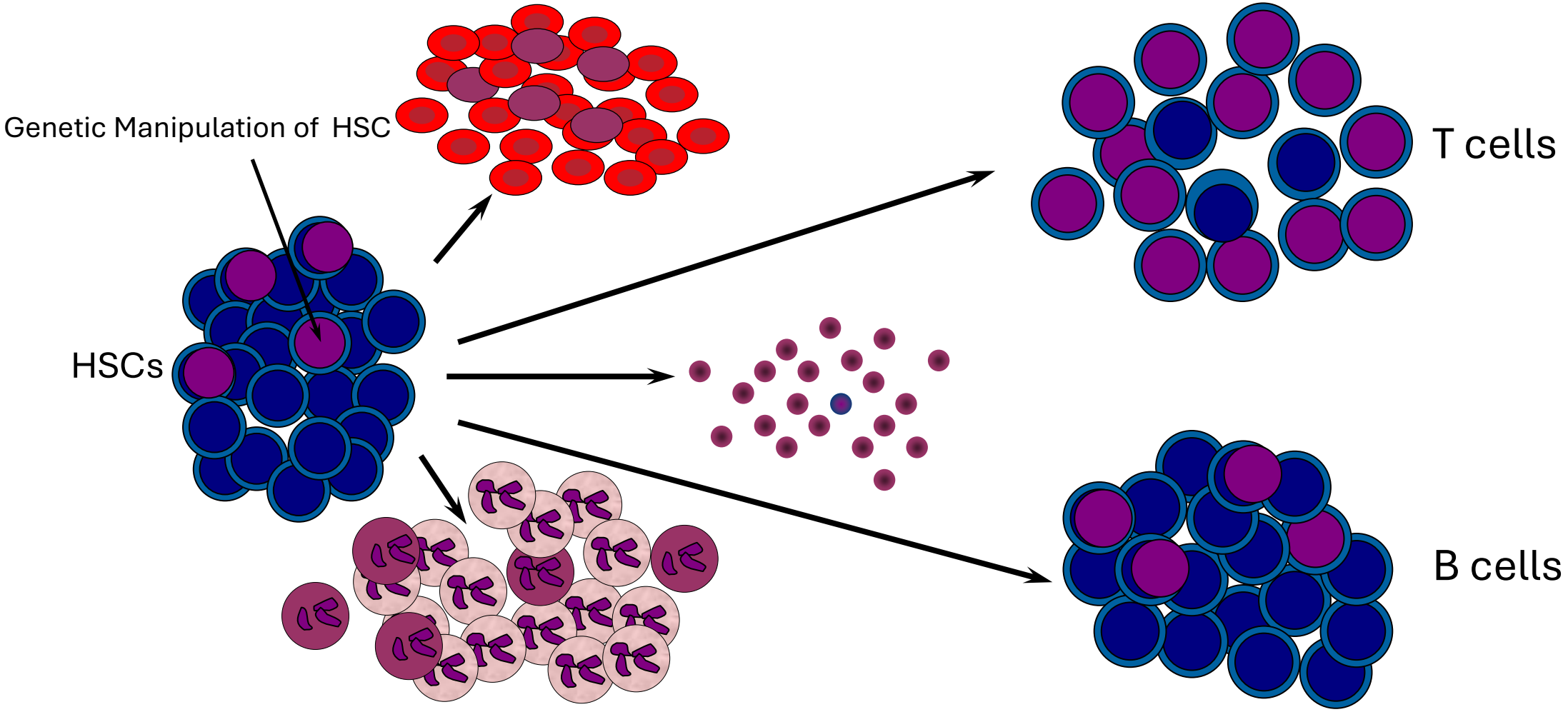
High-cost relative to patient volume

First area demonstrating genotoxicity

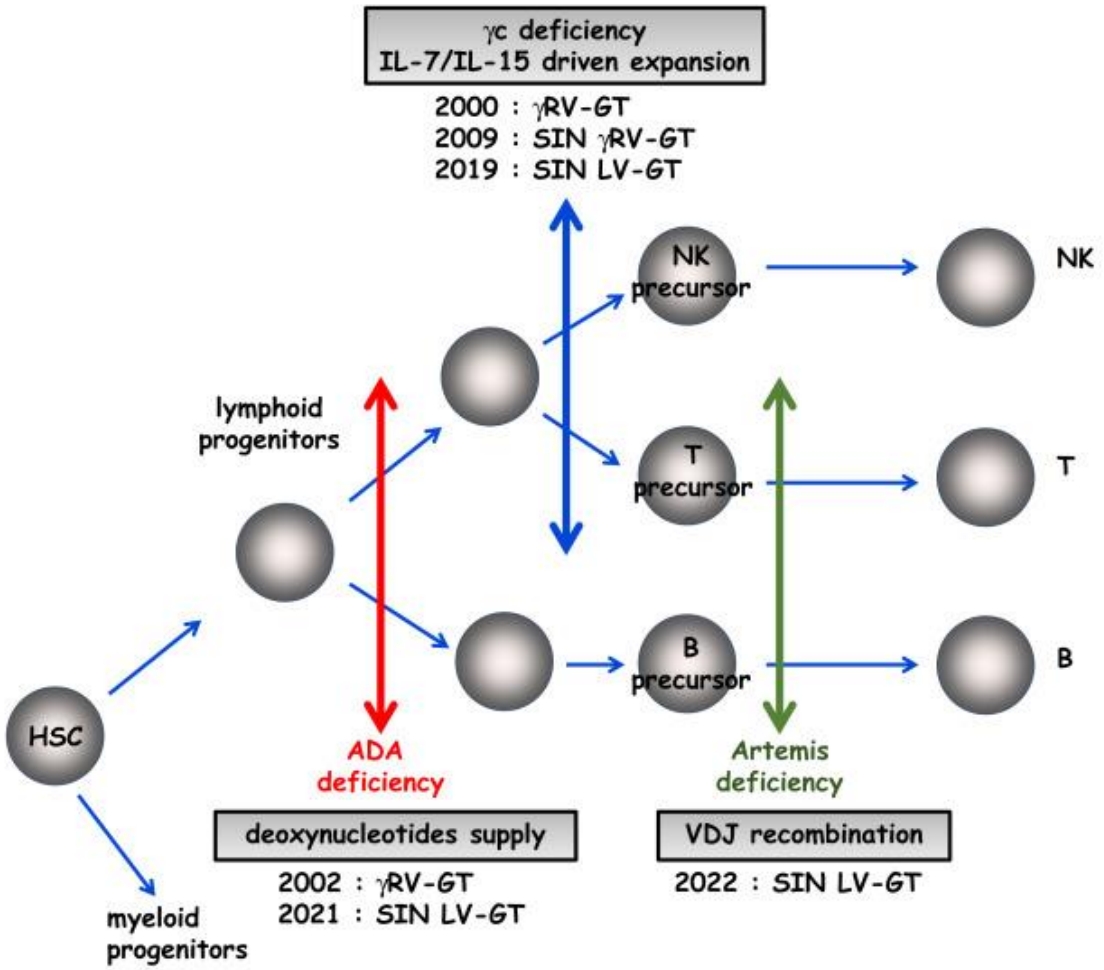
ex vivo Delivery of Gene Therapy



Stem Cell Based ex vivo Gene Therapy



Potential Targets of Gene Therapy



Fischer A and Neven B JACI 2023

Types of Toxicity

Genotoxic

Viral Integration at proto-oncogene sites

Silencing/disrupting of an essential gene

Non-Genotoxic

Effects of conditioning - exposure of HSC to eg Busulfan

On Target

Aberrant expression/over expression of the transgene

Off Target

Aberrant expression/over expression of a gene at the integration site

Early Gene Therapy for Rare Disease

Wiskott Aldrich Syndrome

First generation γRV (n=10) with major improvements in immune and platelet defects (n=9)

Insertional oncogenesis with LMO insertion-related leukemia in 7 of 9 participants.

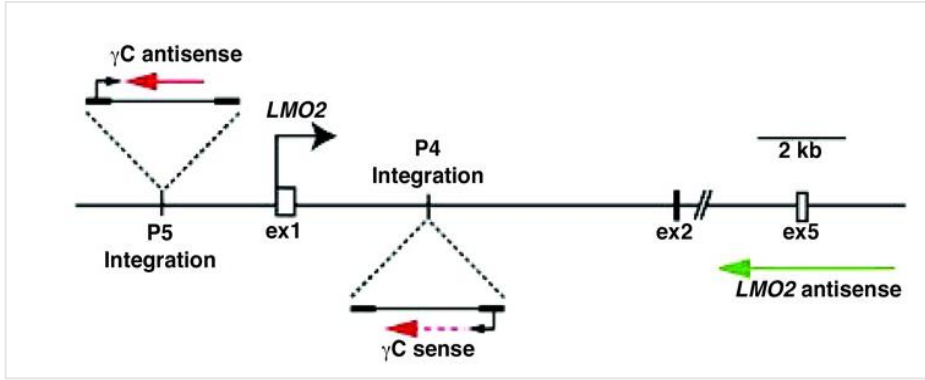
(Braun et al. Sci Transl Med 2014)

X-linked SCID (IL2Rg)

First generation gRV (n=20) with good immune reconstitution
No conditioning/no HSC engraftment
Insertional mutagenesis mediated leukemia (n=5)
(Haceyn-Bey et al.; NEJM 2010, Gaspar et al; Lancet 2004)

Chronic granulomatous disease

First generation gRV (n=5) with transient benefit
MDS/EVI1 related MDS in 3 of 5 participants
(Ott,Grez et al., Nat Med 2010)



Next-generation vectors in a self-inactivating (SIN) configuration

Deleting the enhancer elements in the U3 region of the LTR
Cellular internal promoter

Transition to lentiviral (LV) vectors

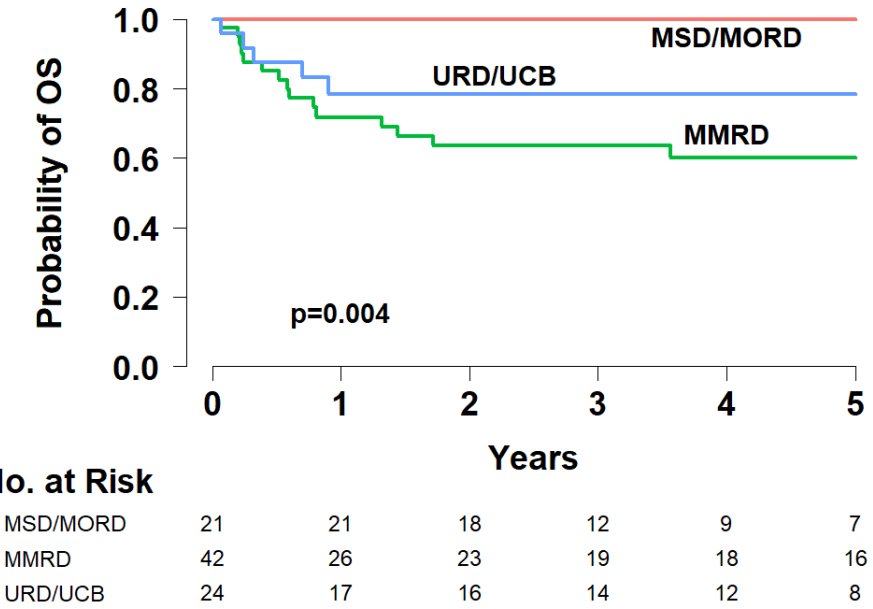
Decreased rate of integration near transcriptional start sites
Higher transduction efficiency of quiescent HSCs, enabling more polyclonal reconstitution.

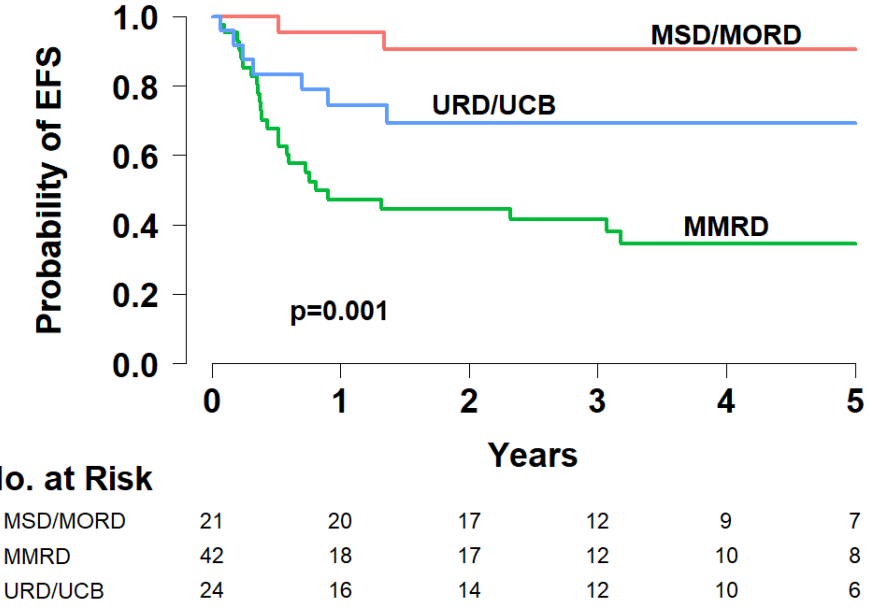
Curative Therapies for IEI – ADA SCID

Inferior Outcomes of Alternative Donor Transplant

Efficacy

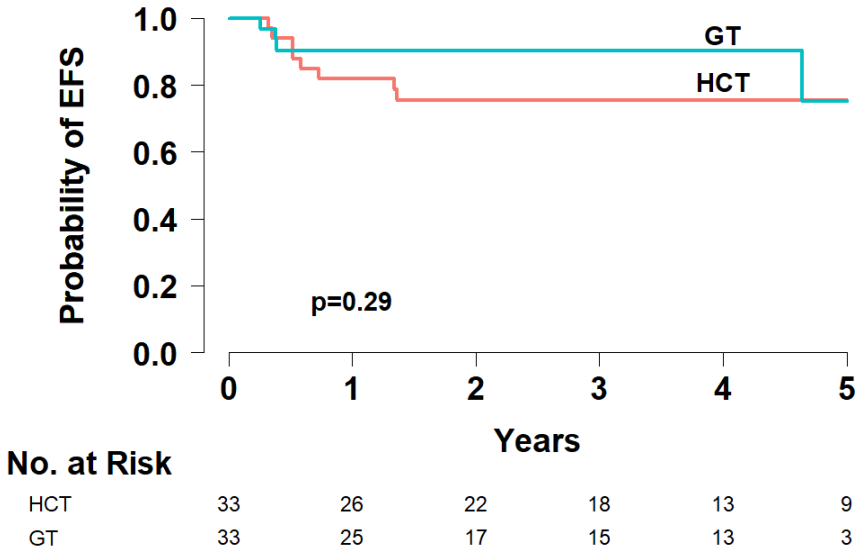
Toxicity



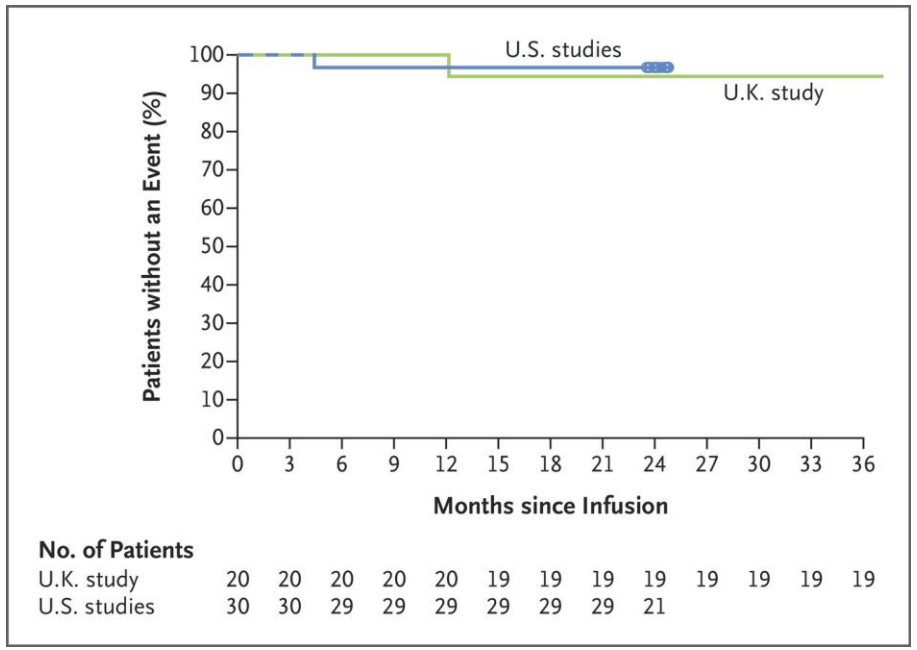


Cuvelier et al; Blood 2022. PMID: 35671392

Curative Therapies for IEI – ADA SCID
Gene Therapy as Alternative



Cuvelier et al; Blood 2022. PMID: 35671392



Kohn et al; NEJM 2021 PMID: 33974366

>50 treated patients worldwide (MILAN, UK, USA)

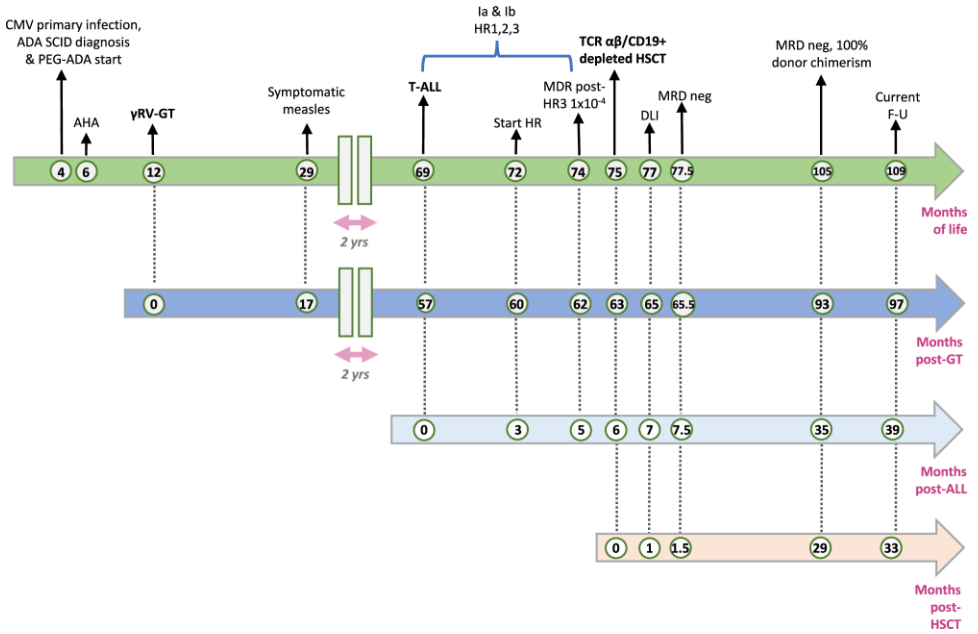
Limited vector-related events compared to other γRV-GT

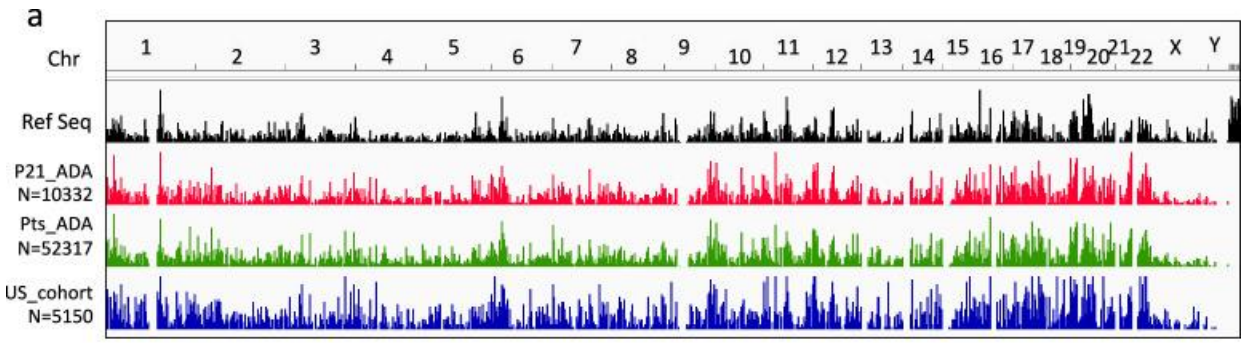
Toxicity of Gene Therapy - ADA

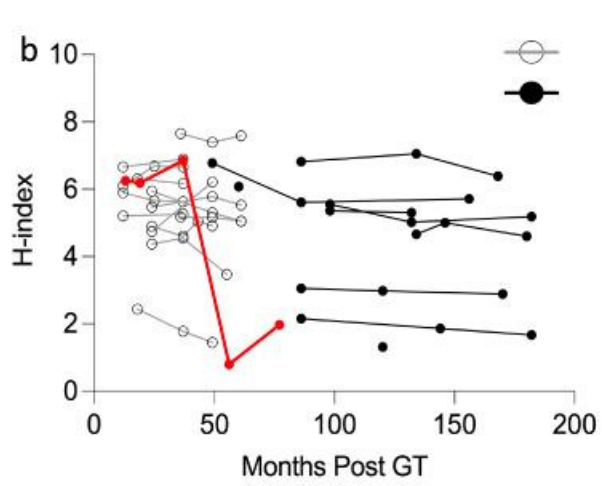
GT-related T-ALL 4.7 years after treatment.

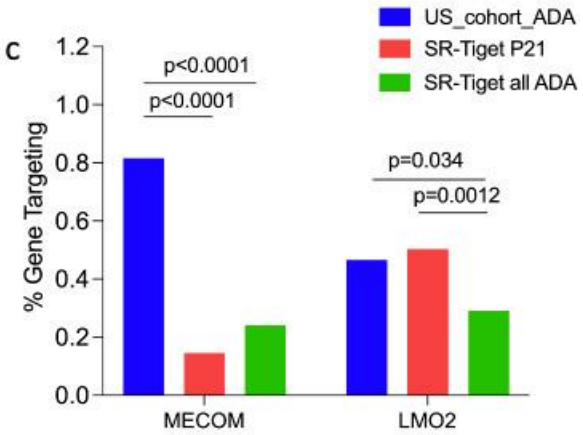
Vector insertion activating LMO2 proto-oncogene
Detected years prior to T-ALL in multiple lineages

Blast cells contain known and novel somatic mutations.









Cesana D et al; Nat Comm, 2024

Gene Therapy - ADA

In 2016, the European Commission granted market approval to GlaxoSmithKline (GSK) for *ex vivo* γ-retrovirus based gene therapy for the treatment of adenosine deaminase (ADA)-deficient SCID (Strimvelis™)

Strimvelis™ was the first *ex vivo* stem cell gene therapy to receive regulatory approval anywhere in the world.

In the US, Don Kohn developed a lentiviral based ADA gene therapy. In 2016, a pharmaceutical company licensed the therapy but halted its development in 2021 and in 2022 returned the license to UCLA.

Now an open trial with the goal of a commercial product.

Autologous Mobilized Peripheral Blood CD34+ HSPC Transduced with the Elongation Factor Alpha Short Promoter (EFS) - ADA Gene (EFS-ADA) Lentiviral Vector for ADA SCID NCT05432310

Curative Therapy for Artemis Deficient SCID

Inferior Outcomes with Alternative Donor Transplant

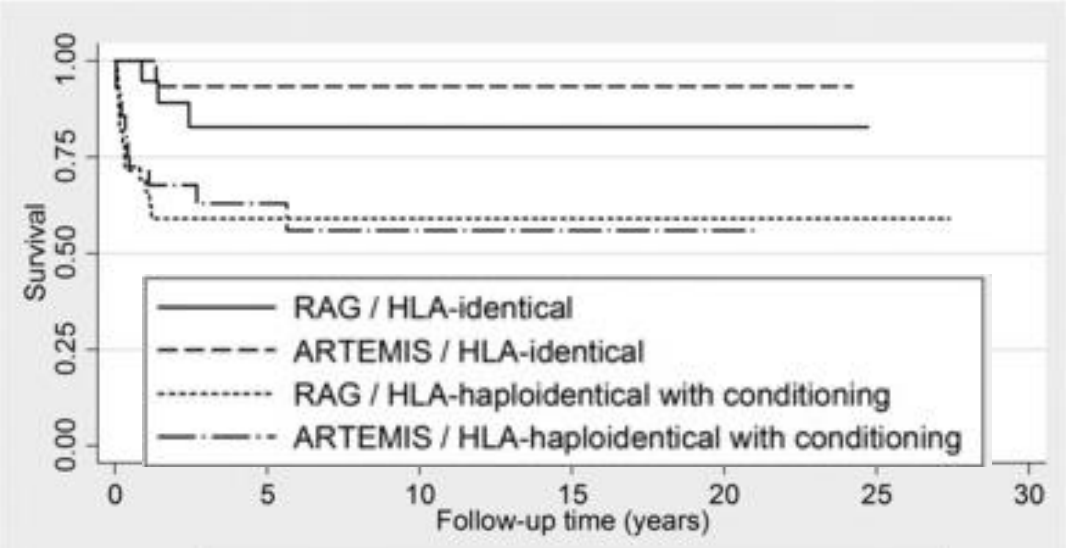
Artemis deficient SCID (T-B-NK+)

Results from autosomal mutations in DCLRE1C
Artemis plays a crucial role in repairing DNA breaks

Results in sensitivity to radiation & alkylating agents such as Busulfan

As a result transplant is typically performed with low dose or no conditioning

Poor immune reconstitution is more common.



ARTEMIS patients have similar overall survival to patients transplanted for RAG deficient SCID, but experience more late complications.

Schuetz C et al, Blood 2013

Curative Therapy for Artemis Deficient SCID – Gene Therapy

The NEW ENGLAND JOURNAL of MEDICINE

RESEARCH SUMMARY

Lentiviral Gene Therapy for Artemis-Deficient SCID

Cowan MJ et al. DOI: 10.1056/NEJMoa2206575

CLINICAL PROBLEM

Artemis-deficient severe combined immunodeficiency (ART-SCID), resulting from damaging variants in the gene *DCLRE1C*, accounts for 2 to 3% of all SCID cases. ART-SCID responds poorly to allogeneic hematopoietic-cell transplantation, which underscores the need for alternative treatments.

CLINICAL STUDY

Design: A phase 1–2, single-center, nonrandomized clinical study evaluated the effects of transfusion of autologous CD34+ bone marrow cells, transfected with a lentiviral vector containing *DCLRE1C* complementary DNA and its natural promoter, in infants with newly diagnosed ART-SCID.

Intervention: 10 infants first underwent bone marrow harvest for production of lentiviral *DCLRE1C*-corrected CD34+ cells. They then received conditioning with intravenous low-dose busulfan over a period of 2 days, followed 1 day later by infusion of the CD34+ cells. End points included safety and T-cell reconstitution.

RESULTS

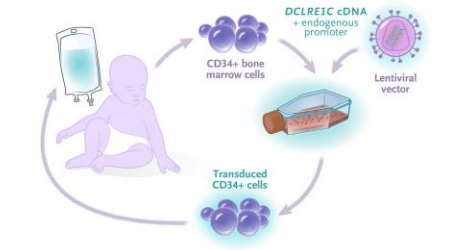
Safety: Busulfan toxicity manifested as transient blood cytopenias, of which 16 were grade 3 or 4. Autoimmune hemolytic anemia developed in four patients 4 to 11 months after infusion; all cases resolved with immune reconstitution.

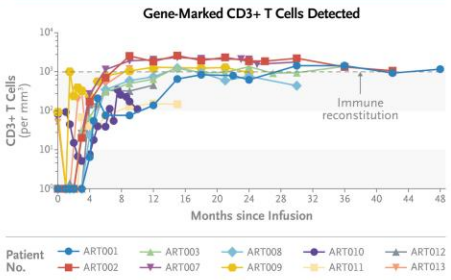
Immune Reconstitution: Gene-marked CD3+ T cells were detected at a median of 12 weeks in all 10 patients. Of 9 patients followed for at least 12 months, 4 had T-cell immune reconstitution at the 12-month mark. Of 6 patients followed for at least 24 months, 5 had T-cell reconstitution at a median of 12 months (range, 6 to 24 months). B cells were detected by flow cytometry and gene marking in all 10 patients, 4 of whom were able to stop immune globulin infusions.

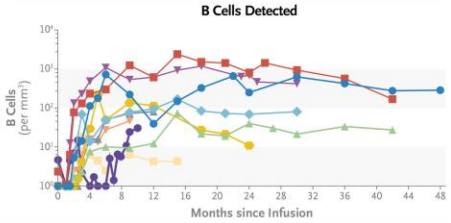
LIMITATIONS AND REMAINING QUESTIONS

- Larger studies of longer duration are needed to further assess the safety and efficacy of this approach.

Links: Full Article | NEJM Quick Take | Science behind the Study







CONCLUSIONS

Among infants with newly diagnosed Artemis-deficient severe combined immunodeficiency, infusion of autologous lentiviral gene-corrected CD34+ bone marrow cells after conditioning with low-dose busulfan resulted in gene-corrected, functional T cells and B cells and the expected grade 3 or 4 adverse events with chemotherapy.

Phase I/II trial of CD34+ BM HSCs transfected with a lentiviral vector containing *DCLRE1C* (NCT03538899)
Busulfan targeted to 20mg*h/L

A median of 31 months after infusion:
CD3+, CD4+, CD8+, CD4+, naïve and T regs increased over of 9mos
TREC were detected by 3 to 6 months
Lymphocyte proliferation normalized by 9 months
Five patients came off IVIG and four responded to vaccination

One patient required CMV specific T cells and a second infusion

Recruiting Gene Therapy Trials

NCT05071222 Phase I/II Hopitaux de Paris N=5

NCT03538899 Phase I/II UCSF Study N=25

Curative Therapy for X-Linked SCID

Several open and recruiting clinical trials in Stem Cell Transplant and Gene Therapy

Lentiviral Gene Therapy
NCT03217617 and NCT04286815 (China)

NCT03311503
Sponsor David Williams – multicenter
Phase I/II Trial of G2SCID lentiviral vector Gene Transfer for SCID-X1 with Low Dose Targeted Busulfan Conditioning

NCT01306019
Sponsor NIAID – dual center
Lentiviral Gene Transfer for Treatment of Children Older Than Two Years of Age With X-Linked Severe Combined Immunodeficiency (XSCID)

Prior transplant allowed.

Gene Therapy for X-Linked SCID

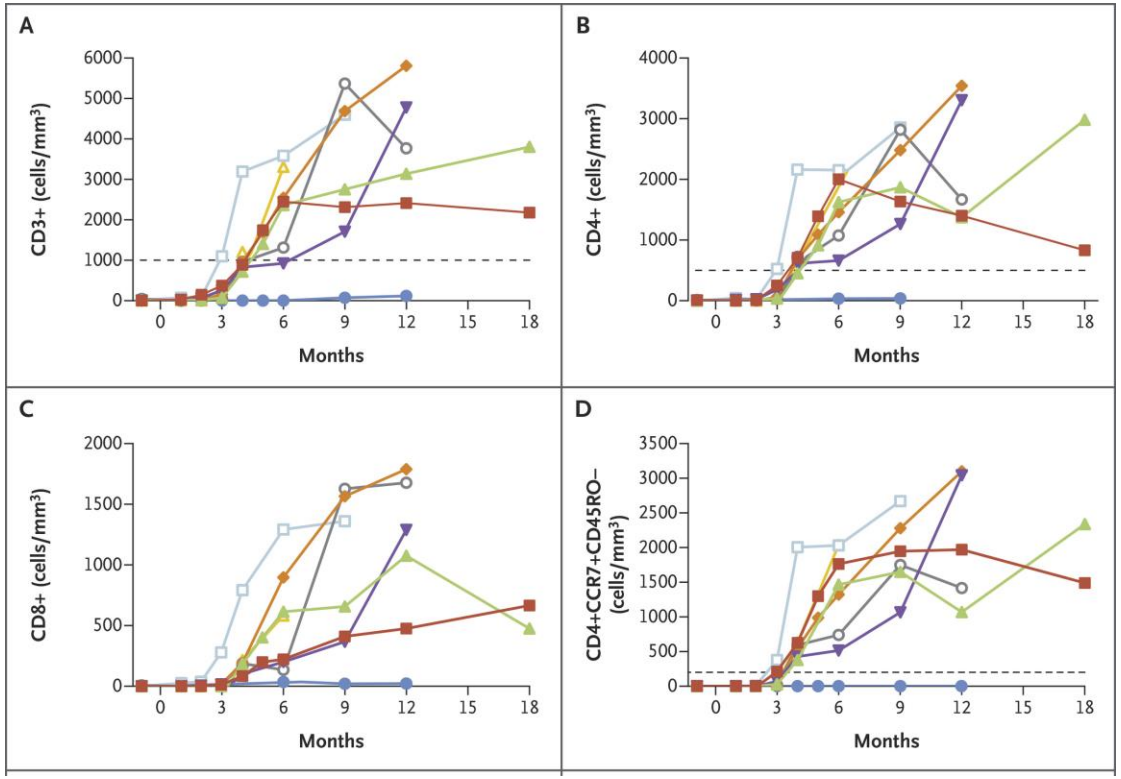
Phase I/II Dual center trial NCT01512888

Gene Transfer for X-Linked SCID in Newly Diagnosed Infants (LVXSCID-ND)

First 8 patients
Vector marking T, B, NK, myeloid and BM progenitors.
Initially no clonal dominance by integration site analysis

Immune Reconstitution
Previous infections cleared.
Normal CD3, CD4, Naïve CD4 and NK by 3-4 mo (n=7)
One reconstituted T and NK cells after a 2nd infusion

Normal IgM levels (n=7)
Off IVIG (n=4)
Responded to vaccine (n=3)



Lentiviral vector GT with low dose busulfan conditioning in infants with newly diagnosed X-SCID had low-grade acute toxicity, multilineage engraftment of transduced cells, reconstitution of functional T, B and cells during a median follow-up of 16 months.

E. Mamcarz, et al; NEJM 2019

Non-SCID Inborn Errors of Immunity
Curative Therapy for Wiskott Aldrich Syndrome

Boys with WAS can have residual or complete absence of WASp in cells leading to hetogeneity of clinical manifestations ranging from severe persistent thrombocytopenia, eczema, opportunistic infections and autoimmunity early after birth, to mild asymptomatic thrombocytopenia or neutropenia.

The majority of affected suffer inflammatory complications that impact significantly on quality of life, have an increased incidence of autoimmunity and are at risk of developing lymphoproliferative disorders and lymphoid malignancies.

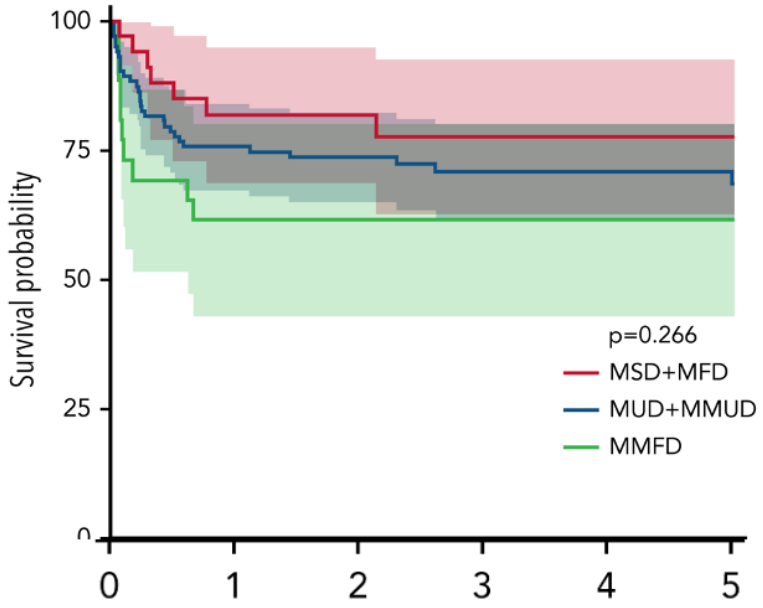
Without a definitive treatment, these boys usually do not survive beyond their second decade

Worth AJ, Expert Rev Clin Immunol 2015

Curative Therapies for IEI – Wiskott Aldrich Syndrome

Acceptable Outcomes of Alternative Donor Transplant

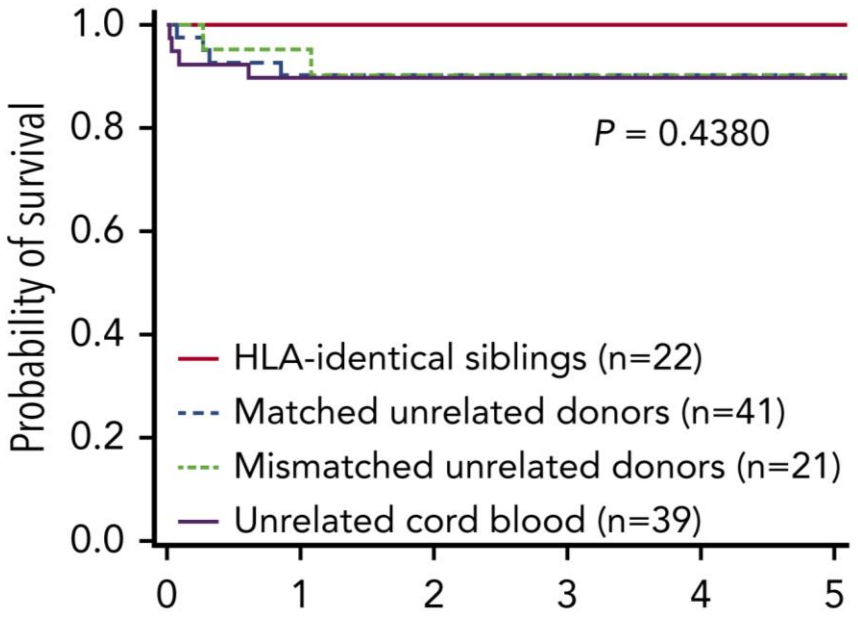
GRFS By Donor Type



MRD	34	25	20	11	7	5
M/mM URD	103	76	62	48	41	28
mMRD	26	15	11	9	6	6

Albert MH et al; Blood 2022. PMID: 35100336

OS By Donor Type



MRD	22	20	16	13	11	8
MUD	41	35	33	28	21	17
mURD	21	19	16	13.	11.	9

Burroughs LM et al; Blood 2023. PMID:

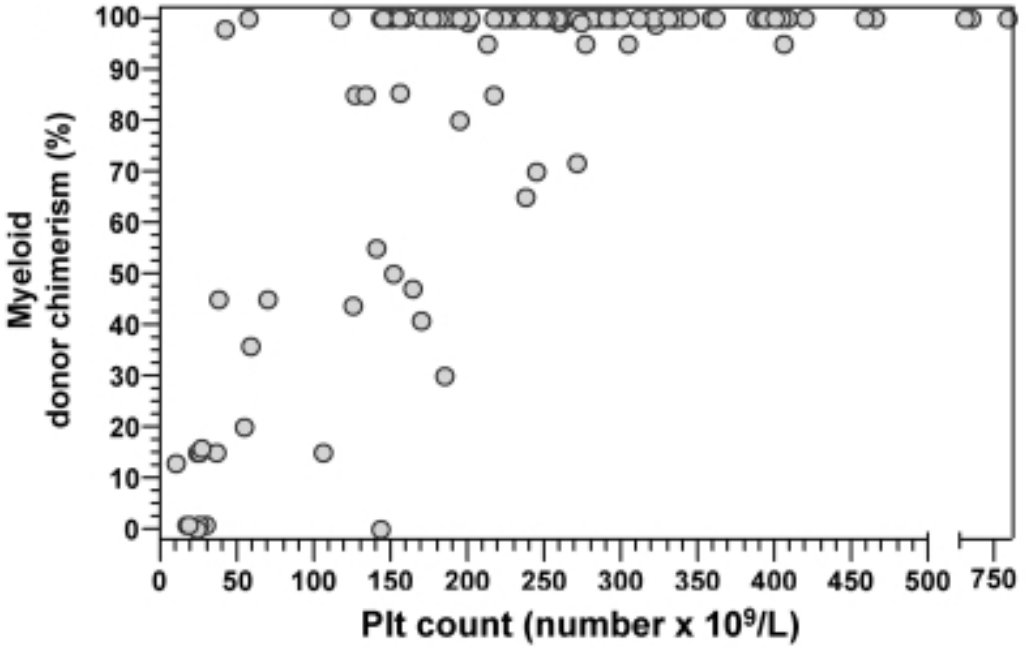
Chimerism and Transplant Outcomes for WAS

Mixed chimerism
Associated with post-HCT autoimmunity

International Collaborative Study (n=194)
HSCT for WAS (1980-2009)

Low level donor HSCs (<10%)
reverses the WAS T cell phenotype,

High level myeloid chimerism (>50%)
required to correct the platelet defect



Daniele Moratto et al; Blood 2011

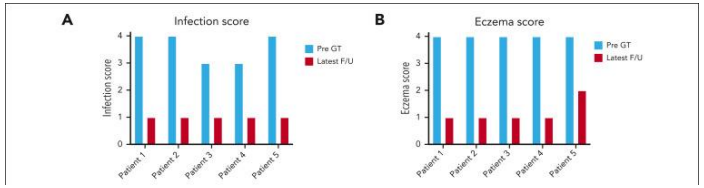
Curative Therapies for IEI – Wiskott Aldrich Syndrome

Gene Addition Therapy

Phase 1/2 clinical trials (NCT01410825, NCT01347346, and NCT 01347242) in 13 patients with WAS
Lentiviral vector-based trials with w1.6 WASp-WPRE-SIN-LV
SIN-LV w/ h*WAS* cDNA 1.6-kB fragment of the promoter

Busulfan Fludarabine conditioning

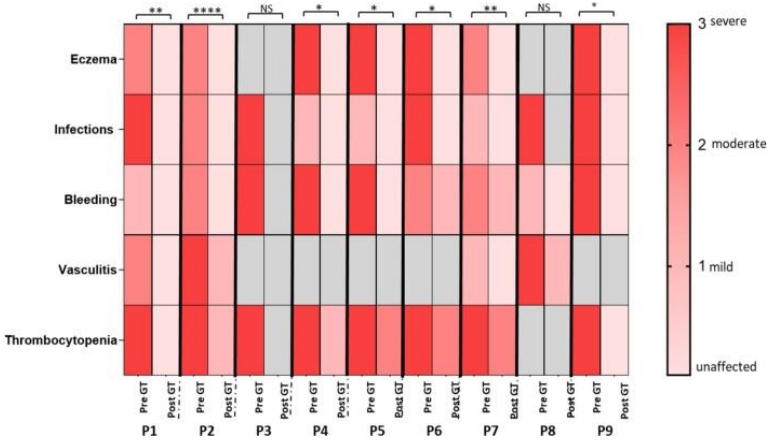
Primary outcomes
clinical, biological safety, efficacy and tolerability



Labrosse et al; Blood 2023

Interim analysis
All patients alive and well (median 7.6 years)
Sustained multilineage gene marking
Platelet count and myeloid cytoskeletal function with high VCN

Universal improvement of eczema, infections, and bleeding.
Despite sub-physiologic levels of WAS protein expression.
Two (less robust immune recovery) with flares in autoimmunity



Magnani et al; Nat Med 2022

Clinical and laboratory manifestations of WAS improved with GT with acceptable safety

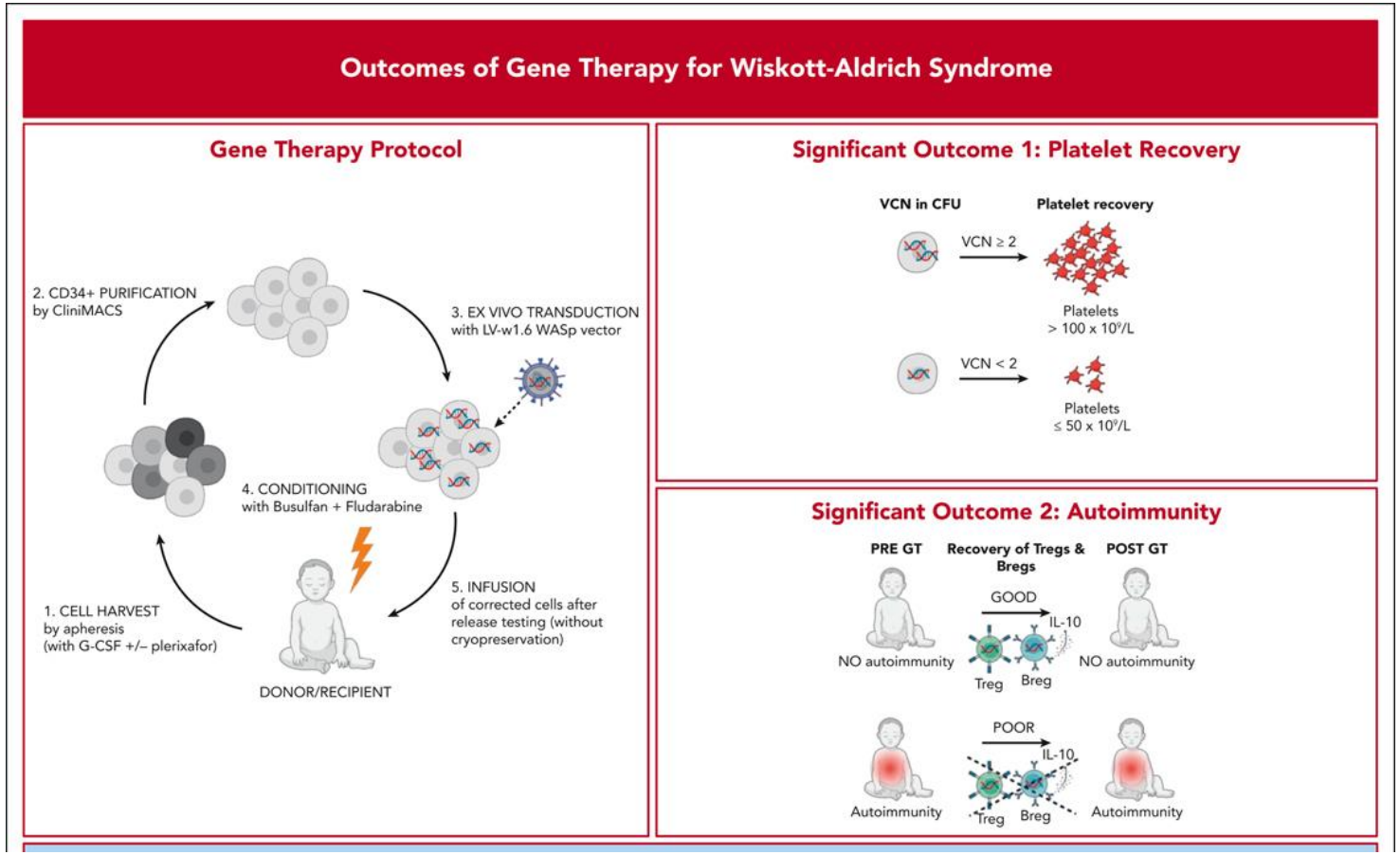
Gene Addition Therapy for WAS

Phase I/II Trial
NCT01410825 (n=5)

Higher expression/more potent promoter raises concern for genotoxicity

Lower level expression associated with incomplete correction of the platelet defect

<u>TiGET plans to open a trial of OTL-103 for treatment of boys with severe WAS</u>



Labrossa B, et al; Blood 2023

Gene Editing

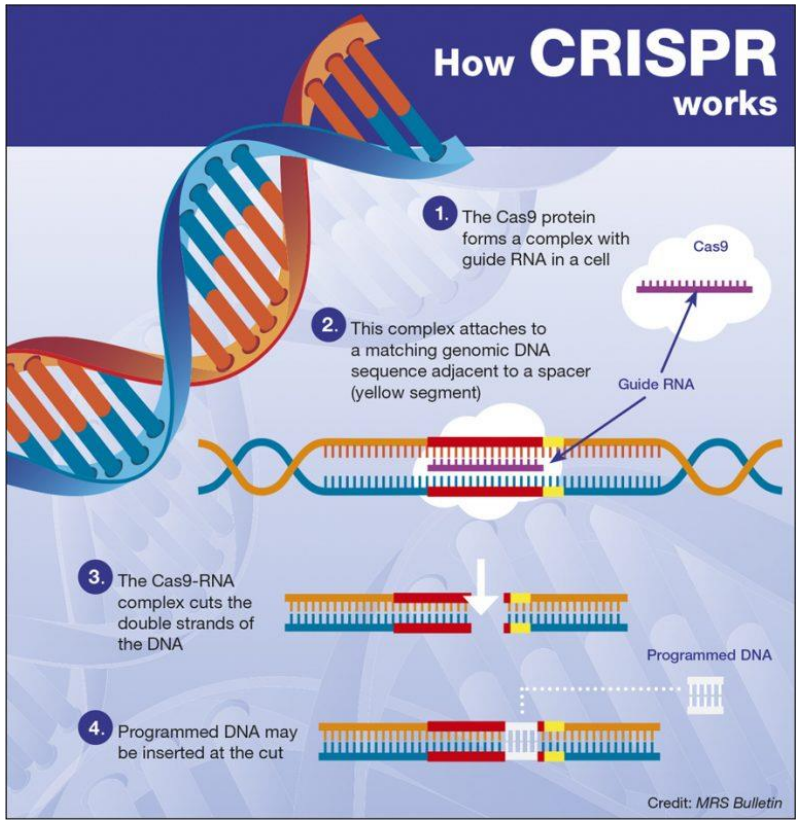
Genome editing is the idea of site specific modifications.

Requires two components,
nuclease capable of splicing the DNA
something to guide the specificity

The site can be disrupted (to stop expression of the gene)
The site can be repaired (to fix a mutation in the gene).

CRISPR/Cas9 has revolutionized the field of Gene Editing
The CRISPR/Cas9 complex includes

A short piece of RNA guides the complex to the desired site
The Cas9 enzyme creates a cut or nick in the gene



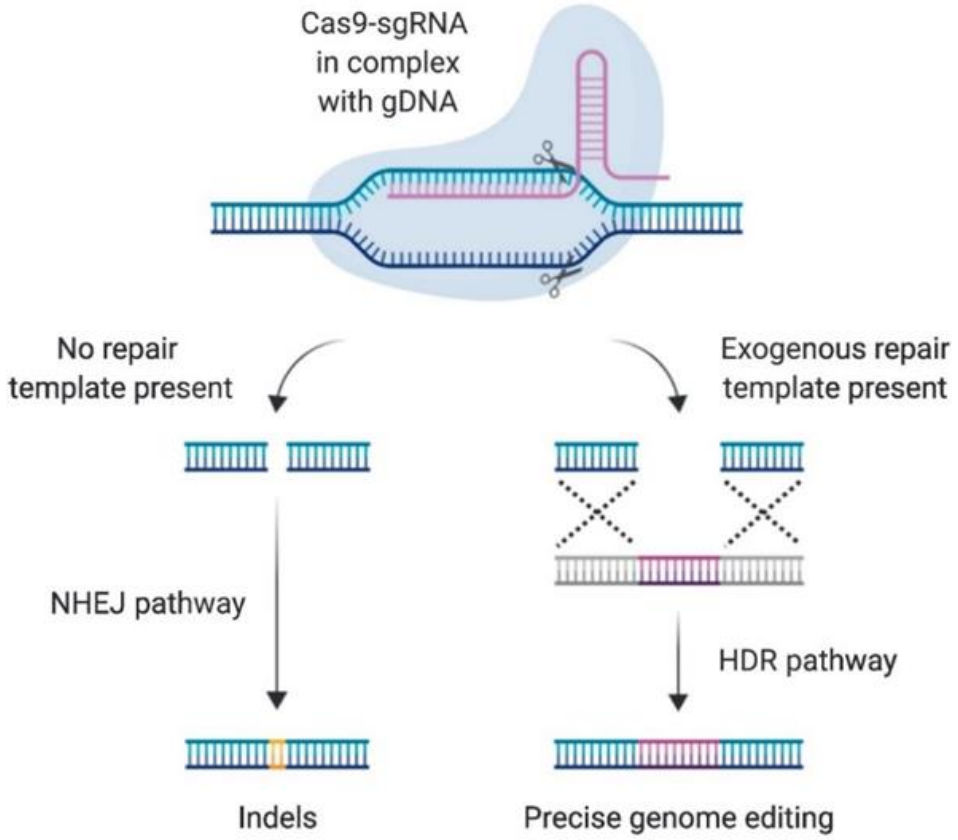
Gene Editing

Two ways to mediate site-specific gene correction or disruption

Breaks in both strands of the DNA (DSBs) at target loci

NHEJ fast and error-prone pathway
brings the ends together without a template
results in random insertions/deletions
disrupts the gene at the target site

HDR a DNA template that spans the break is
provided as part of the CRISPR package
potential to repair the gene at the target site



Davey, Front Line Genomics 2023

Curative Therapies for IEI – WAS
Gene Editing Therapy

Most IEIs are caused by loss of function mutations, the most frequently applied GE approach being explored relies on the activation of the HDR pathway for either correction of the mutations or site-specific addition of a correct copy of the faulty gene.

The first approach, using single strand oligo DNAs (ssODN) as homology templates, has been shown to be the most efficient and straightforward between the two, however it requires expensive and time-consuming tailoring of the GE platform to each single patient, given that most PIDs are caused by a wide range of mutations spread across the faulty genes.

As such, targeted gene insertion is considered the preferred methodology to tackle IEI, providing a one size-fits all approach that could be applied to all the patients affected by a specific disease

However, low level engraftment of gene edited HSCs has been observed especially when performing HDR mediated correction

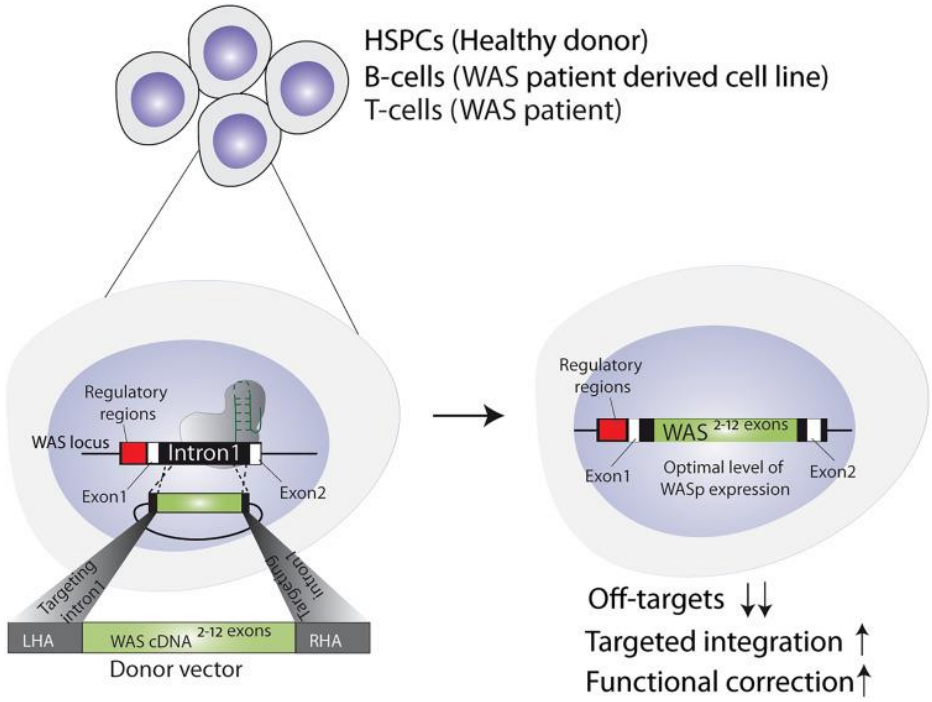
Gene Editing for WAS

HDR can be harnessed to

1. Insert a gene in its own genomic locus, resulting in its tight, physiological expression regulated the way the normal gene is regulated.
2. Into ‘safe harbor’
3. Into a highly transcribed area to achieve strong and/or tissue specific expression

WAS cDNA into the endogenous locus

- Wild Type HSC and HSPC -> 50% frequency
- WAS T cells -> 10% resolved functional defects



Naseem, Cavazza Mol Ther Methods 2024

Pille M, Mol Ther Methods 2024

Gene Addition Therapy vs Gene Editing for WAS

.

LV based Gene Therapy

More established

Safety/Efficacy in Phase I/II Trials

Requires high level gene marking

Incomplete reversal of phenotype

Risk of under and overexpression

Gene Editing

High rates of gene correction in pre-clinical models

Unclear if sustained/true HSCs

Unknown toxicities

Curative Therapies for IEI – CGD

Transplant

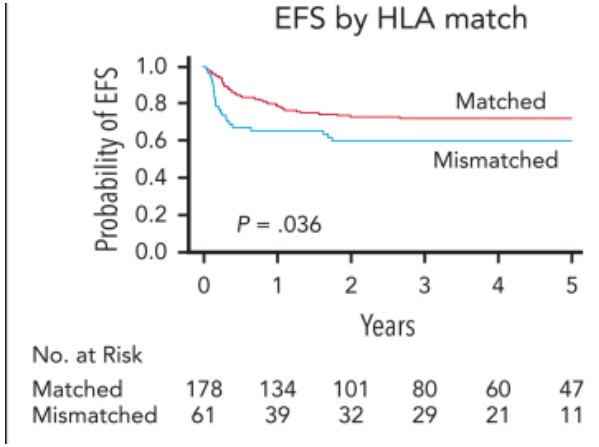
PIDTC study (n=391) compared HCT to non-HCT cohorts (1996-2018)
HCT cohort with more infections, lung and liver disease, steroid use

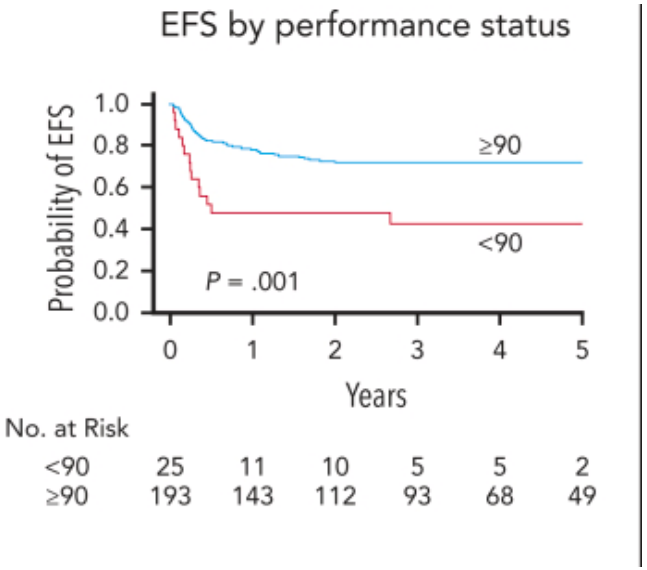
Similar OS and EFS for both cohorts
3yr OS 82% 3yr EFS 69%

HCT recipients improved (over baseline and over non-HCT)
growth and nutrition
infection and inflammatory disease

In a multivariate analysis of HCT
Age, genotype, and oxidase status did not affect outcomes
Lansky/Karnofsky score <90
HLA-mismatched donors
Significant incidence of Graft Failure/2nd HCT (17.6%)

HCT leads to durable resolution of CGD symptoms
HCT lowers the burden of the disease





Leiding JW et al, Blood 2023

Curative Therapies for IEI – Autosomal Recessive CGD

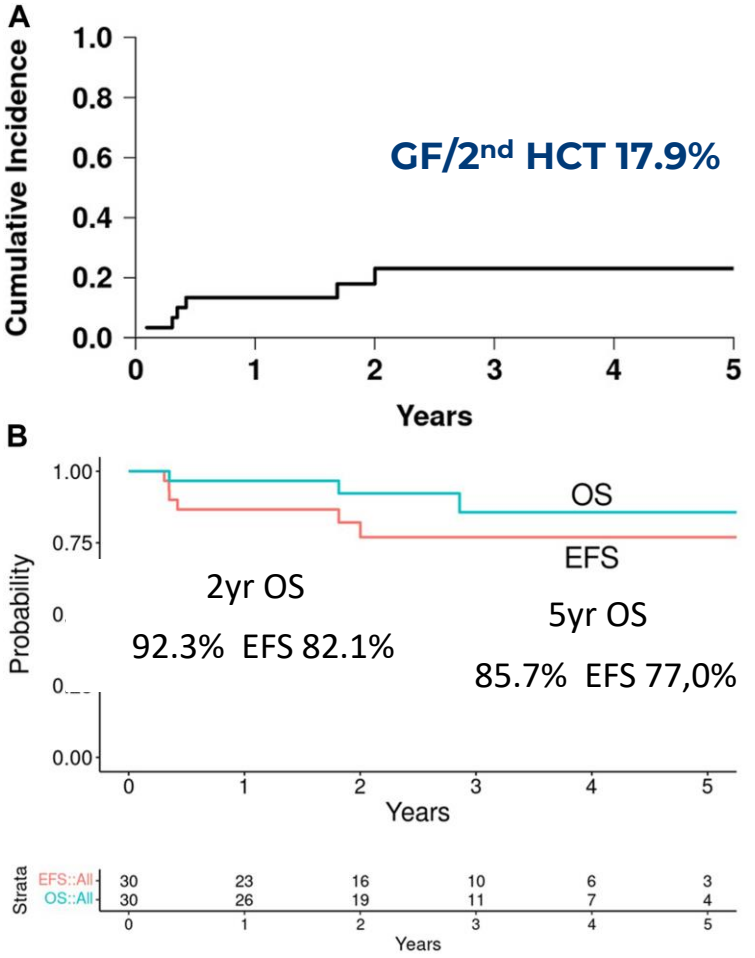
Transplant

PIDTC Study
p47phox CGD (n=37) s/p allogeneic HCT
- Median age 9.1 years (1.5-23.6)
- Inflammatory disorders 36.7%
- RIC/Reduced Toxicity conditioning (90%)

Related HLA matched (40%) mismatched (10%)
Unrelated HLA matched (37%) mismatched (13%)

Infections per person year
0.38 -> 0.06 (p=0.38)
Inflammatory bowel disease & steroids decreased

Full donor myeloid chimerism
- 1yr 93.8% and 2yr 87.5%



Grunebaum E et al, JACI, 2024

Curative Therapies for IEI – CGD

Gene Addition

X-CGD (n=9) lentiviral based gene therapy GX1CGD (NCT02234934 and NCT01855685).

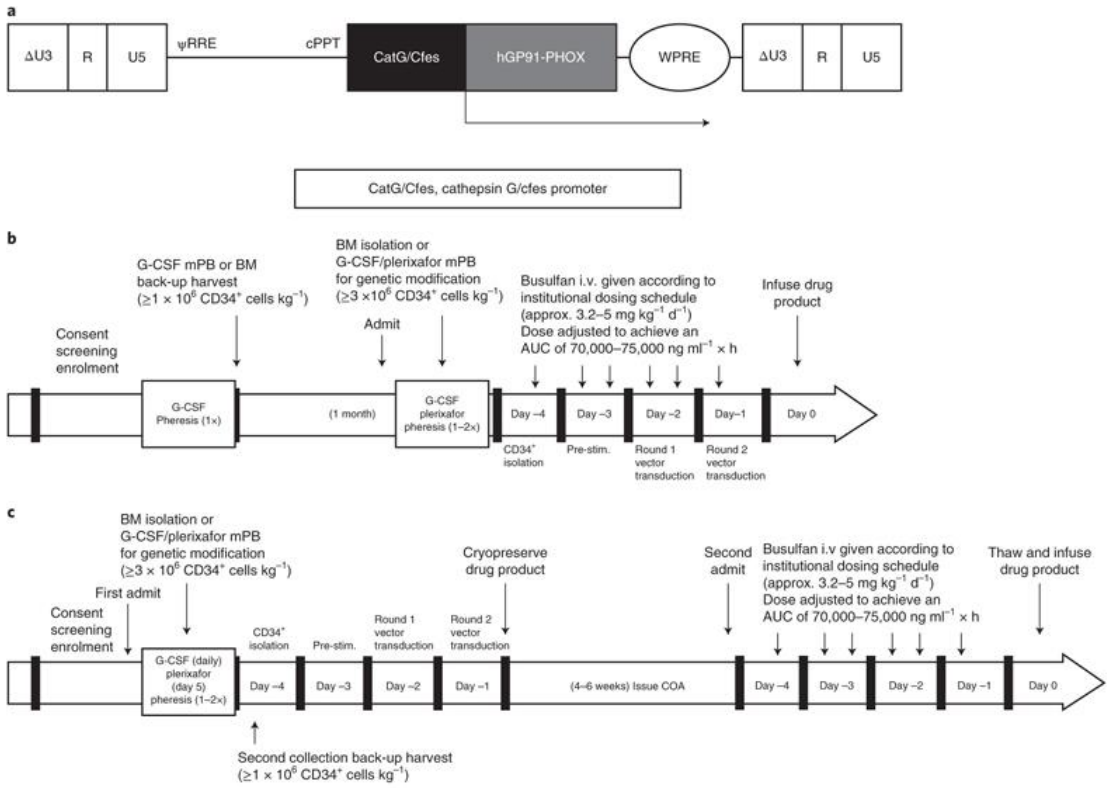
Two patients died early

6 w/ stable VCNs (0.4-1.8) and DHR (16-46%)
No new CGD-related infections
Off antibiotics

1 patient w/initial high levels declined
c/w low frequency of LT-HSCs engrafted

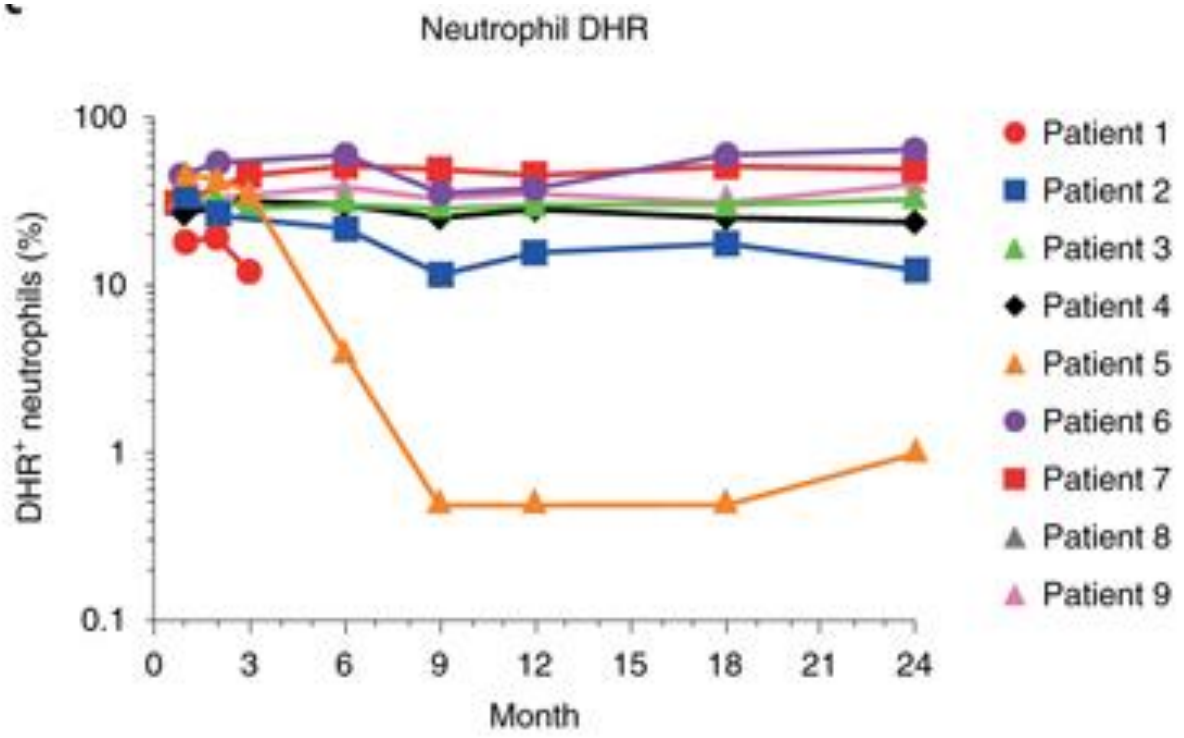
No clonal dysregulation

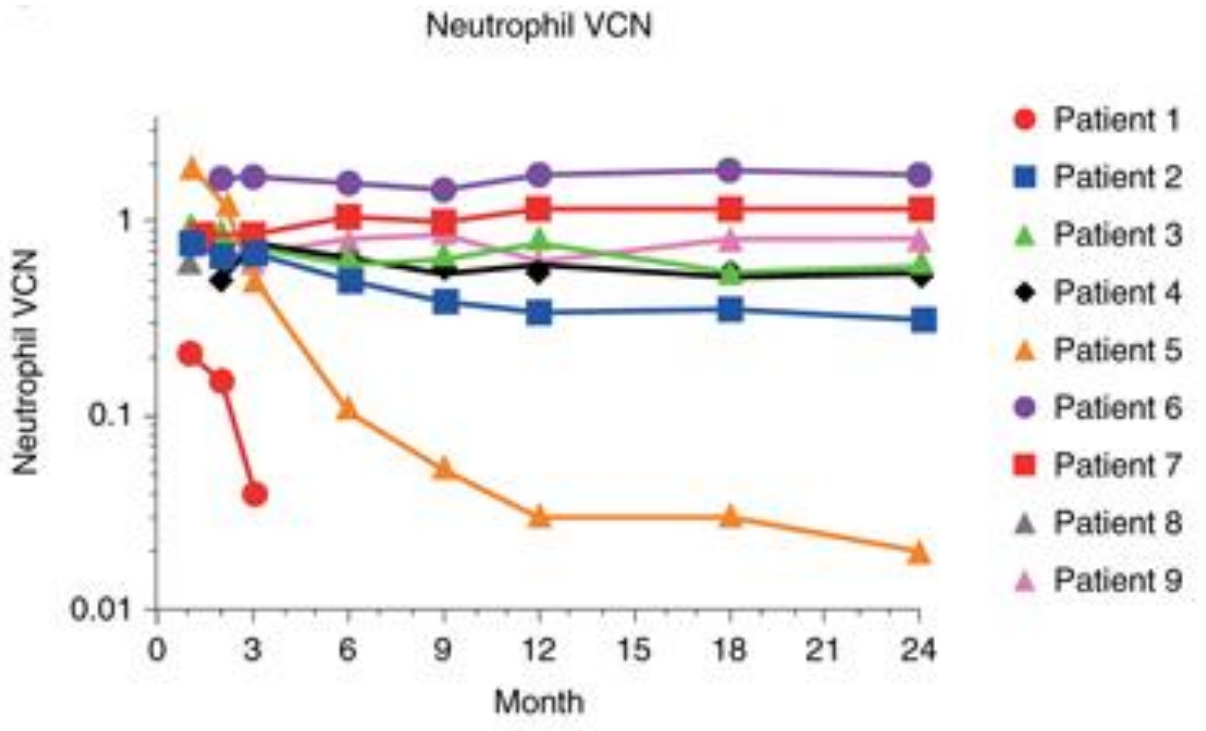
The primary objective was met in six of the nine patients at 12 months follow-up



Don Kohn et al, Nature Medicine, 2020

Curative Therapies for IEI – CGD





Four additional patients treated
No CGD related infections
1 sustained high levels of DHR+ neutrophils (77.2%)
3 with initial high levels of recovery declined

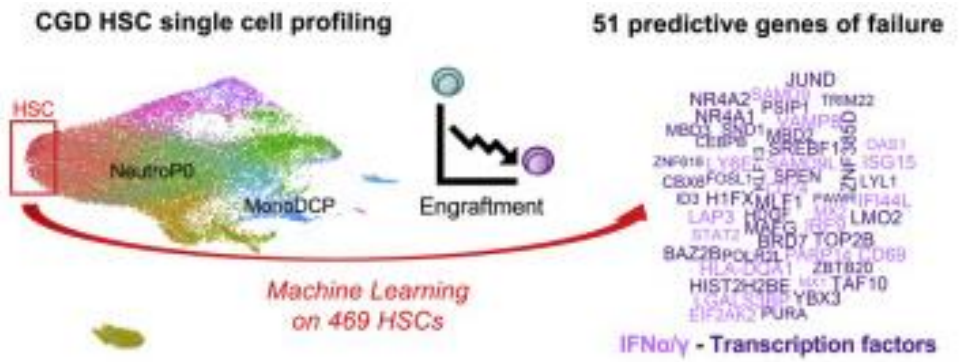
Don Kohn et al, Nature Medicine, 2020

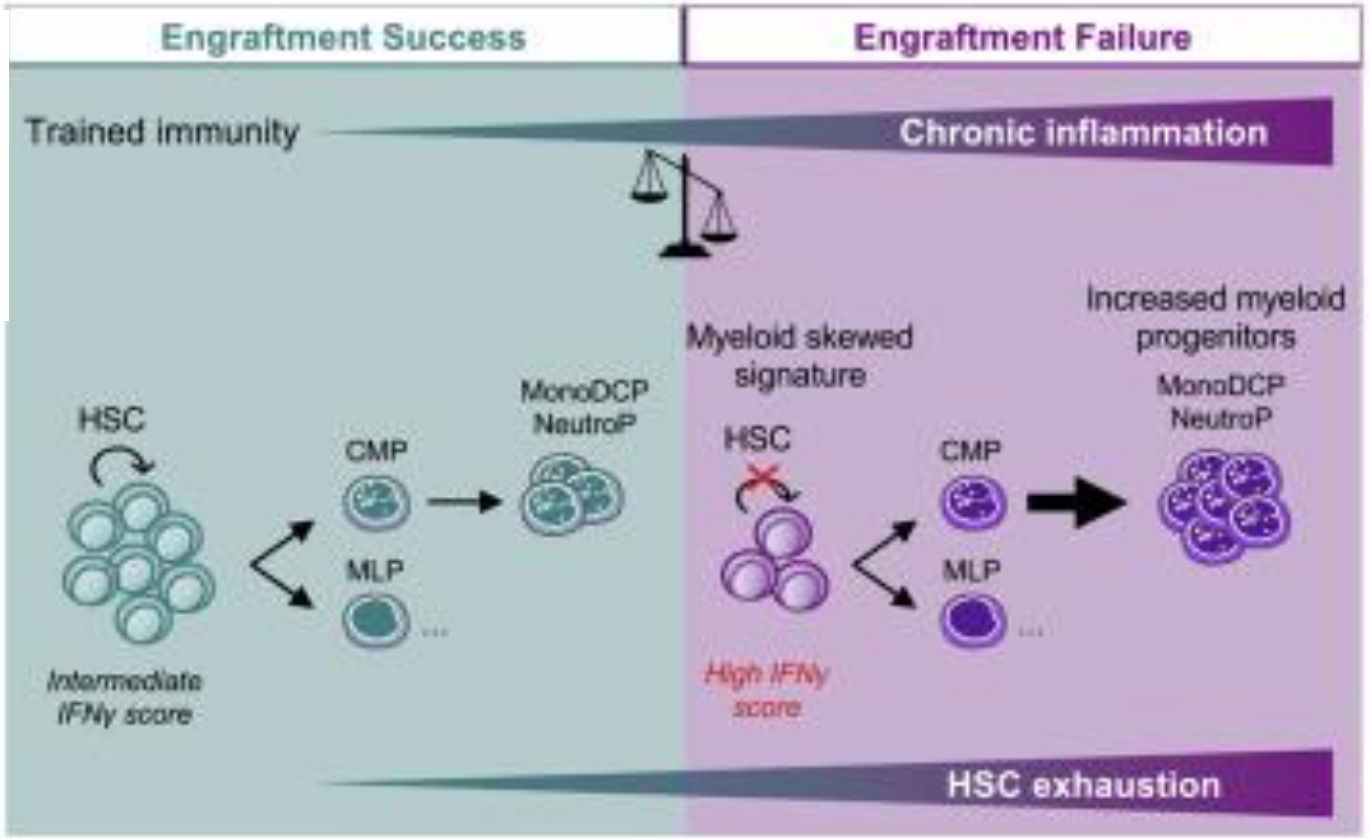
Curative Therapies for IEI – CGD

Gene Therapy

French Trial (n=4)
Defect in engraftment of gene corrected HSCs (n=2)

Correlated upregulation of the IFN pathway
Set of biomarkers predictive of GT failure





Steicy Sobrino et al, Cell Reports Medicine 2023

Curative Therapies for IEI – CGD

Gene Addition Therapy – AR p47 CGD

Two enrolling trials

NCT06253507
Phase I/II lentiviral pCCLCHIM-p47
high dose busulfan.
Sponsor: NIH
Must weigh 15 kg. and > 3 yo

NCT05207657
Phase I/II lentiviral pCHIM-p47
Sponsor: Great Ormond Street
Must be >23 months of age

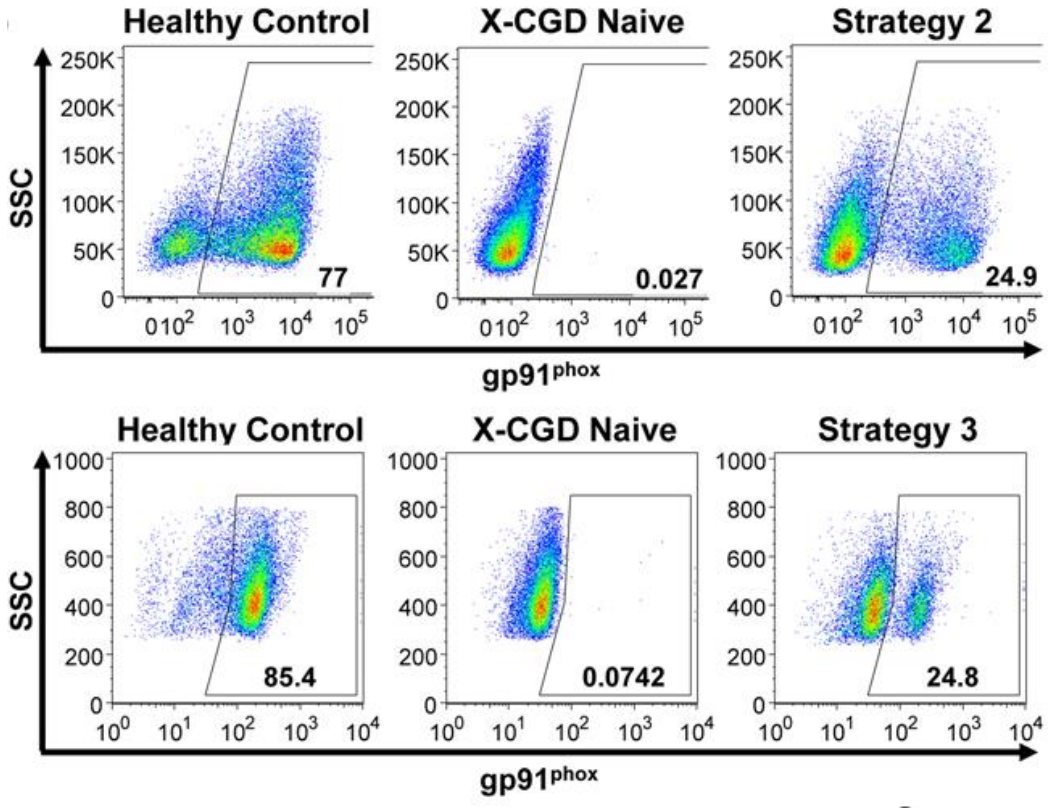
Curative Therapies for IEI – CGD
Gene Editing

Pre-Clinical

CRISPR/Cas9 CYBB gene of $CD34^+$ HSPCs in X-CGD repair of >20% of HSPCs

Functional mature myeloid and lymphoid cells

No events outside of the CYBB gene locus



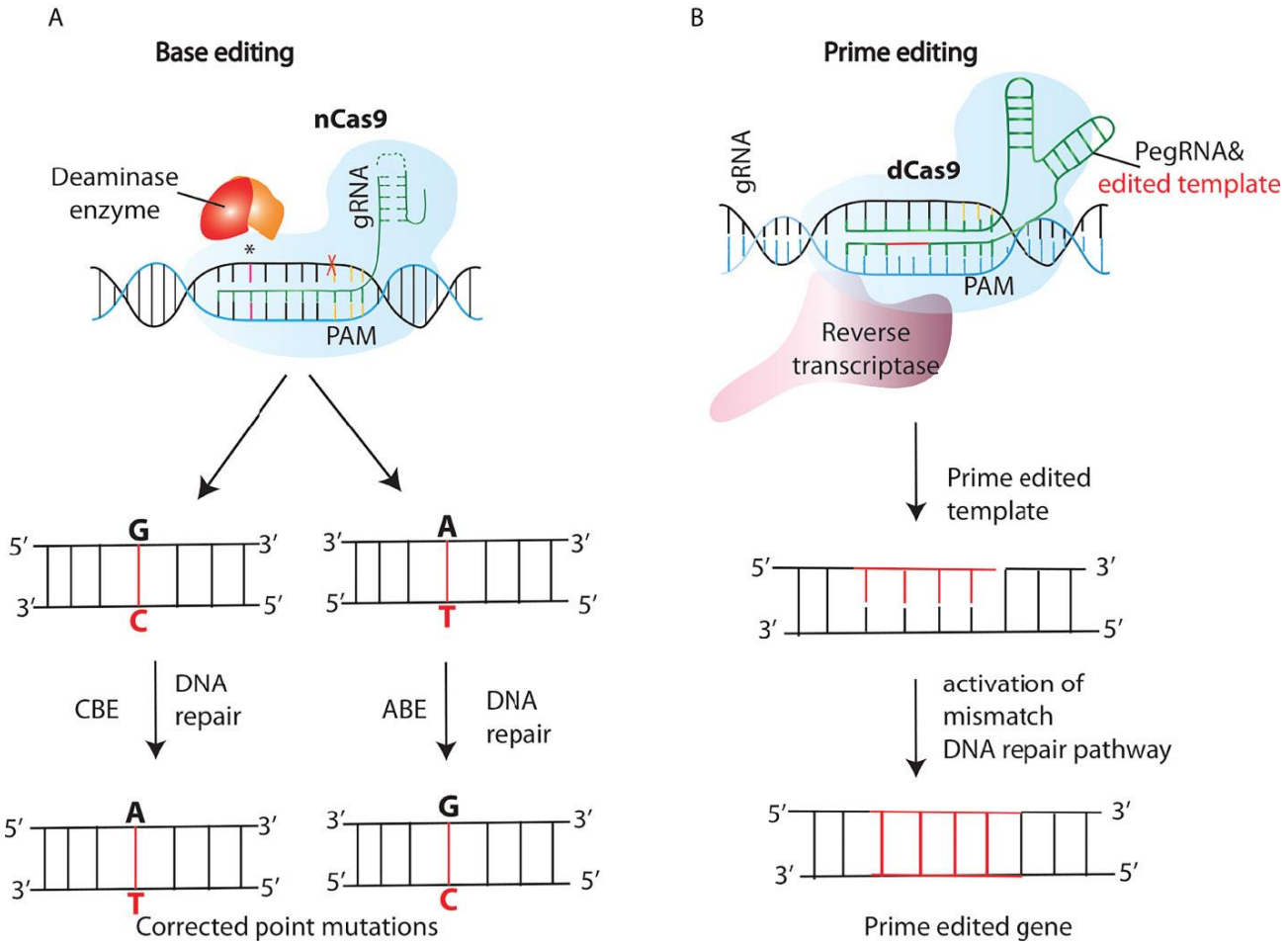
De Ravin SS et al, STM 2017

Alternative Gene Editing

Base Editors (BEs)
Change one target DNA base

This is a powerful way to correct many of the most common mutations without creating DSBs

Thus this approach is both more accurate and less risky.

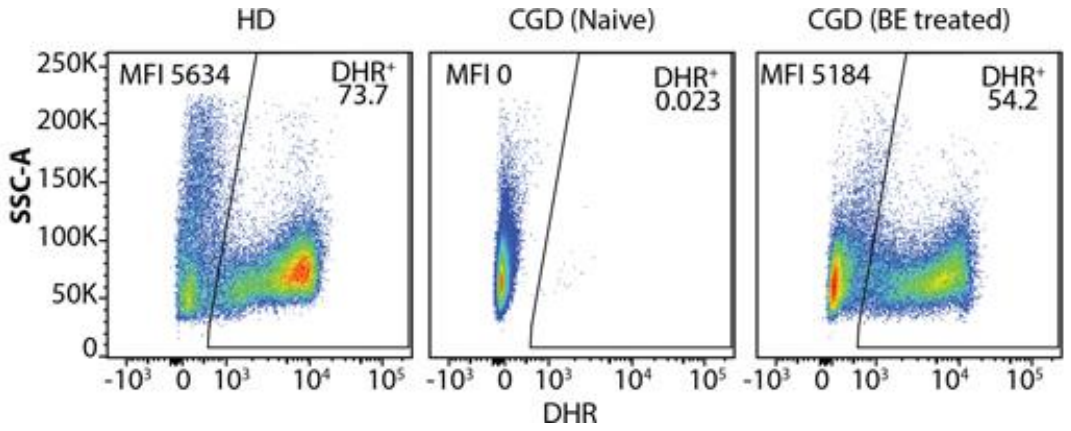


Naseem, Steinberg, Cavazza Front Immunol 2022

Curative Therapies for IEI – CGD

Gene Editing X-linked CGD

X-CGD *CYBB* c.676C>T, ABE8e-SpRY



NADPH oxidase release of reactive oxidative species (ROS) upon stimulation reduces dihydrorhodamine to rhodamine

Up to 70% correction (3.5x higher than CRISPR

Minimal off-target or bystander edits.

Bzhilyanskaya V et al, STM 2024

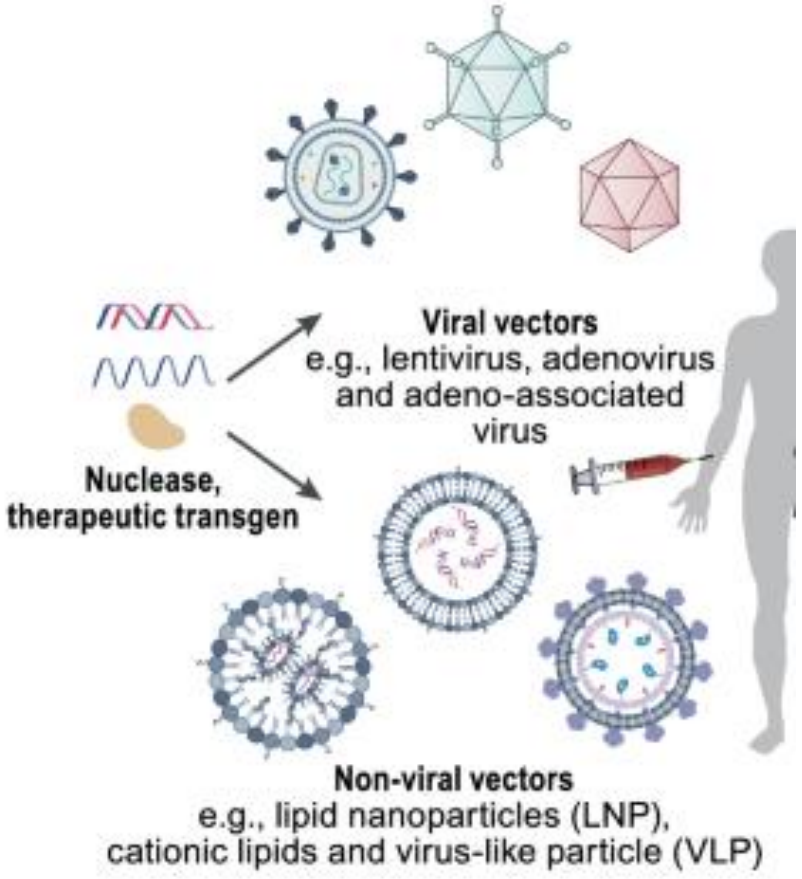
These drug-enabling studies demonstrated efficient and precise correction of the X-CGD mutation supporting a first-in-human clinical trials.

NCT06325709
Phase 1/2 trial of Based Edited HSPCs for X-CGD (CYBB c.676C>T) after Busulfan conditioning
Sponsor: NIAID
Eligibility >18yo

NCT06559176
Phase I/2 multicenter trial for Autosomal recessive p47phox CGD (delGT mutation)
PM 359
Sponsor: Prime Medicine

Curative Therapies for IEI

in vivo Gene Therapy



in vivo Gene Therapy for X-SCID

Two SCID-X1 neonatal canines treated with this approach achieved long-term immune reconstitution with no prior conditioning.

Corrected polyclonal $CD3^+$ T cells to 16 months, T cell functionality were within normal range.

This approach is translatable to a clinical setting, thus providing for a highly portable and accessible gene therapy platform for SCID-X1.

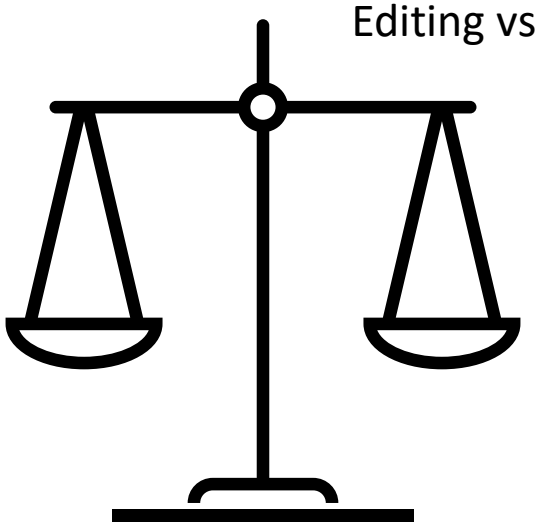
Rajawat YS human gene ther 2021

HCT versus Gene Therapy

Finding Equipoise/Assessing Success

Rare Disease

Transplant	Gene Therapy
Hard to compile data	Continued low volume
	Rapidly evolving
	Disease specific risks
	Editing vs Addition



Common Disease

Transplant	Gene Therapy
Large data sets	Cost savings over time
Evolving risks of TRM	Risk of genotoxicity

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