Hematopoietic Stem Cell-based Gene Therapy

Clinical Impact and Current Challenges

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Comprehensive Center for The Cure of Sickle Cell and other Red Cell Disorders (CuRED)

Bone Marrow Failure and MDS Curative Therapies Team

Disclosure Statement

- bluebird bio: Consultancy

Indications for Stem Cell-Based Curative Therapy in Pediatrics

Malignant Diseases

- **AML**
- **MDS**
- **JMML**
- **CML:** rarely needed in TKI era
- **ALL:** SCT use ↓ since advent of CAR-T Cell Therapy

Non-Malignant Diseases

BMF/MDS Predisposition Syndromes

- Acquired Aplastic Anemia
- Amegakaryocytic Thrombocytopenia
- Diamond Blackfan Anemia
- Fanconi Anemia
- *GATA2* haploinsufficiency
- *MECOM* germline syndromes
- PNH
- *RUNX1* germline syndromes
- *SAMD9/SAMD9L* syndromes
- Severe Congenital Neutropenia
- Shwachman Diamond Syndrome
- Telomere Biology Diseases

Hemoglobin/Heme Disorders

- Beta Thalassemia Major
- Congenital Erythropoietic Porphyria
- Congenital Sideroblastic Anemia
- Pyruvate Kinase Deficiency
- Sickle Cell Disease

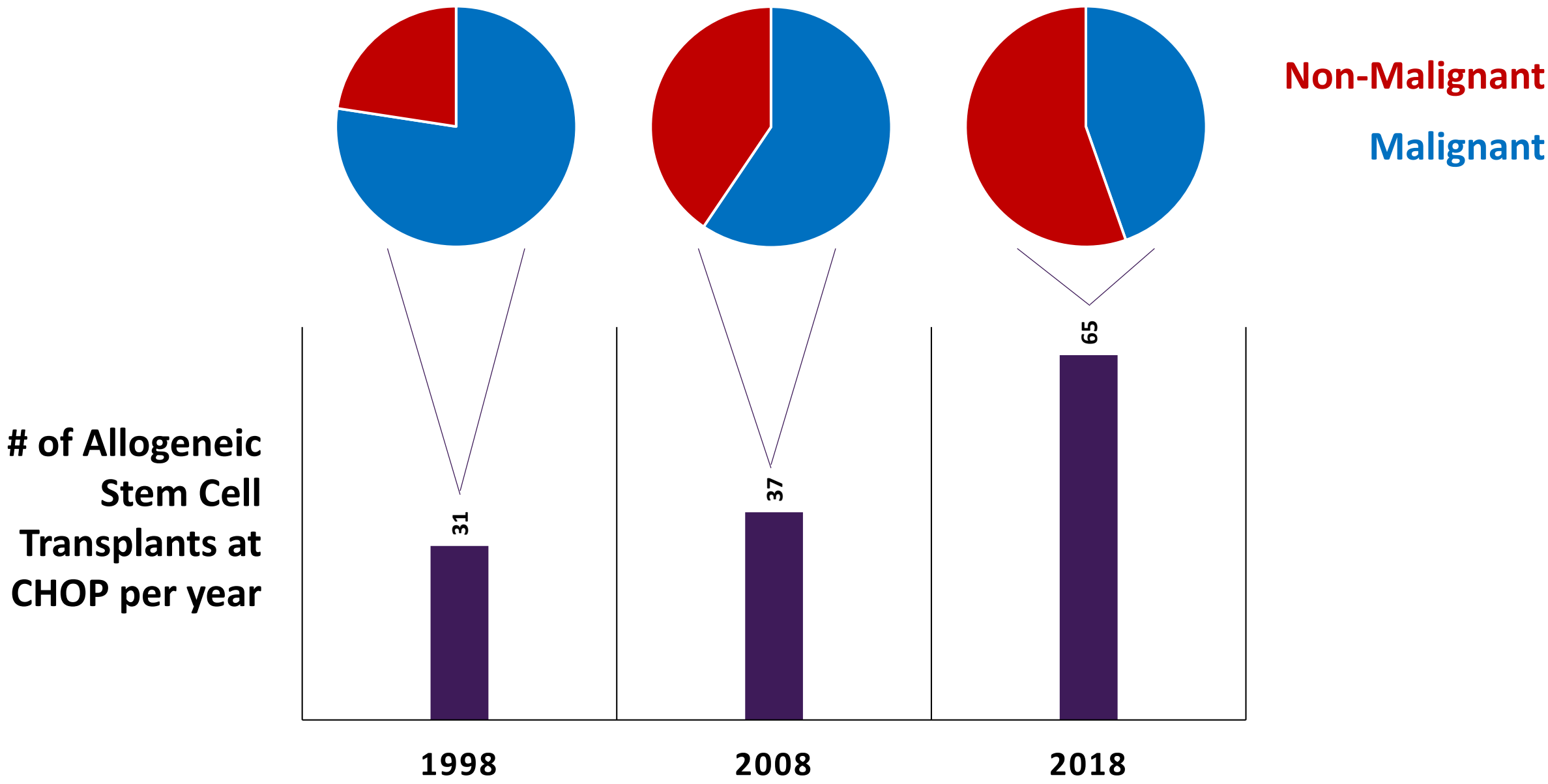
Immune Deficiency/Dysregulation

- Cartilage Hair Hypoplasia
- Chediak-Higashi syndrome
- Chronic Granulomatous Disease
- Hyper IgM Syndromes
- Hyper IgE Syndromes
- HLH-primary
- IL10R Deficiency
- Interferon-γ receptor deficiency
- IPEX
- Leukocyte adhesion deficiency
- LCH-Multicentric, refractory
- Omenn Syndrome
- SCID (X-linked, ADA, other)
- Wiskott-Aldrich Syndrome
- XIAP deficiency
- XLP

Inborn Errors of Metabolism

- Hurler's (MPS-I)
- Krabbe (Globoid Cell Leukodystrophy)
- Adrenoleukodystrophy
- Metachromatic Leukodystrophy
- Osteopetrosis

Pediatric BMT Programs: Increased Focus on Non-Malignant Diseases



Why Are More Patients/Families Pursuing Curative Stem Cell Therapy?

- Because optimal medical treatment and screening does not always prevent disease progression/complications
  - **Hemoglobinopathies:** acute and chronic pain, stroke, chronic lung disease
  - **Immune deficiencies:** life-threatening infections, permanent organ damage
  - **Neurologic/metabolic:** irreversible disease progression
- Because non-curative treatments can cause their own problems
  - **Hemoglobinopathes:** alloimmunization, transfusional iron overload, osteopenia
  - **Immune deficiencies:** Costs of care, antibiotic side effects
- Because curative technologies are now more widely available!!

Curative Therapy Options for Patients with Severe Non-Malignant Diseases

Historical

Matched Sibling Donor (MSD)- BMT*

Unrelated Donor BMT w/wo T cell depletion

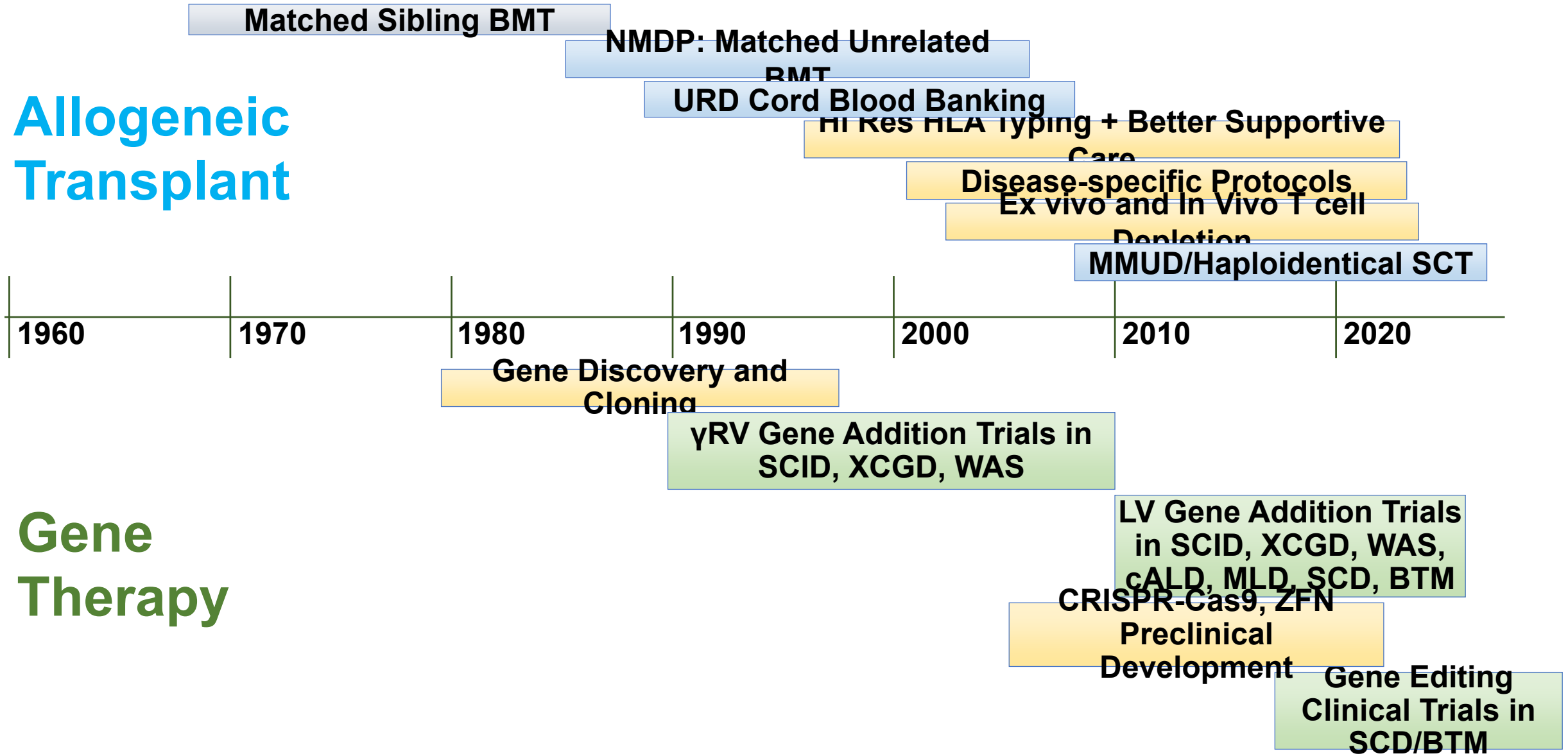
Haplo-BMT with PT-Cy or Ex vivo T cell Depletion

HSC editing Gene Therapy

Lentivirus-based Gene Therapy

15-25% o patients with SCD *(Mentzer et al. AM J Pediatr Hematol Oncol, 1994)*

Milestones in Hematopoietic Stem Cell Based Curative Therapy



Active Stem Cell Based Gene Therapy Clinical Trials for Non-Malignant Diseases

Hemoglobinopathies

Product	Sponsor	Strategy	Diseases	Age (y)
Lentiglobin BB305	bluebird bio	LV Gene addition: β-globin	SCD, BTM	2-50
BCH_BB-LCR shRNA(miR)	Boston Children's	LV Gene addition: shRNA targeting BCL11a	SCD	3-40
Lenti/G-βAS3-FB	UCLA	LV Gene addition: β-globin	SCD	>18
GLOBE1	Multiple	LV Gene addition: β-globin	SCD, BTM	5-35
TNS9.3.55	Mem Sloan Kettering	LV Gene addition: β-globin	BTM	>18
CSL200	CSL Behring	LV Gene addition: γ-globin + shRNA734	SCD	18-45
CTX001	Vertex/CRISPR	CRISPR-CAS9 Gene editing: BCL11a	SCD, BTM	12-35
OTQ923/HIX 763	Novartis	CRISPR-CAS9 Gene editing: BCL11a	SCD	2-40
BIVV003	Bioverativ	ZFN Gene editing: BCL11a	SCD	18-40

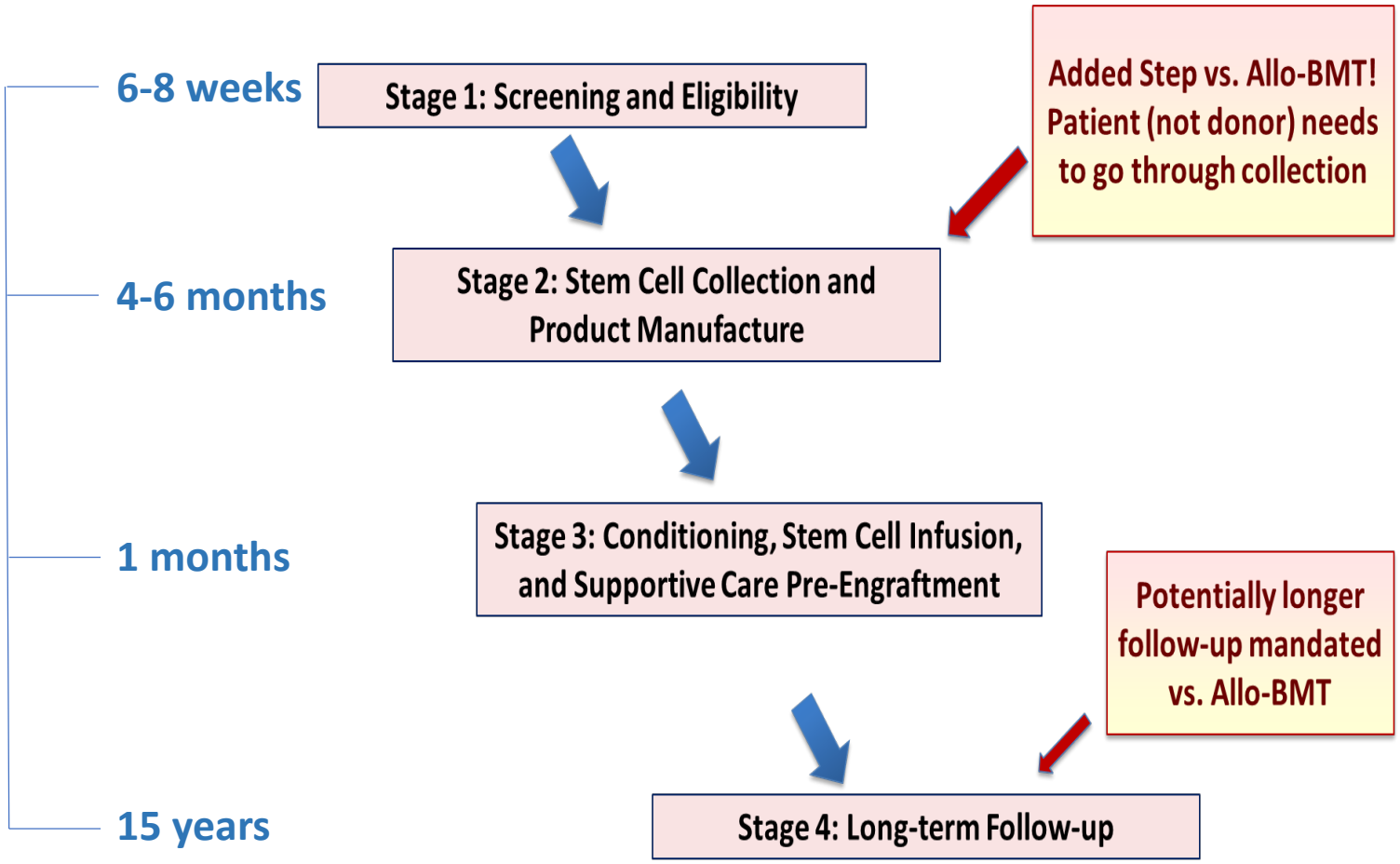
Primary Immune Deficiencies

Product	Sponsor	Strategy	Diseases	Age (y)
OTL-101	Orchard	LV Gene addition: ADA	ADA-SCID	< 18
AProArt	UCSF	LV Gene addition: DCLRE1C	Artemis-SCID	> 2mth
SIN-LV-RAG1	Leiden Univ	LV Gene addition: RAG1	RAG-1 SCID	< 2
G2SCID	Boston Child	LV Gene addition: IL2RG	X-linked SCID	≤ 5
CL20-i4-EF1α-hγc-OPT	Multiple (Mustang)	LV Gene addition: IL2RG	X-linked SCID	varies
OTL-103	Orchard	LV Gene addition: WAS	Wiskott-Aldrich	> 5
G1XCGD	Genethon	LV Gene addition: CYBB	X-linked CGD	> 2

Neurologic, Metabolic, and BMF Disorders

Product	Sponsor	Strategy	Diseases	Age (y)
OTL-200	Orchard	LV Gene addition: Arylsulfatase A	MLD	0-7
Lenti-D	bluebird bio	LV Gene addition: ABCD1	cALD	0-17
IDUA	IRCCS San Raffaele	LV Gene addition: α-L-iduronidase	MPS-1 (Hurler's)	0-11
RP-L401	Rocket	LV Gene addition: TCIRG1	Infantile Osteopetrosis	> 1mth
RP-L102	Rocket	LV Gene addition: FANCA	Fanconi Anemia	> 1

Timeline of Gene Therapy

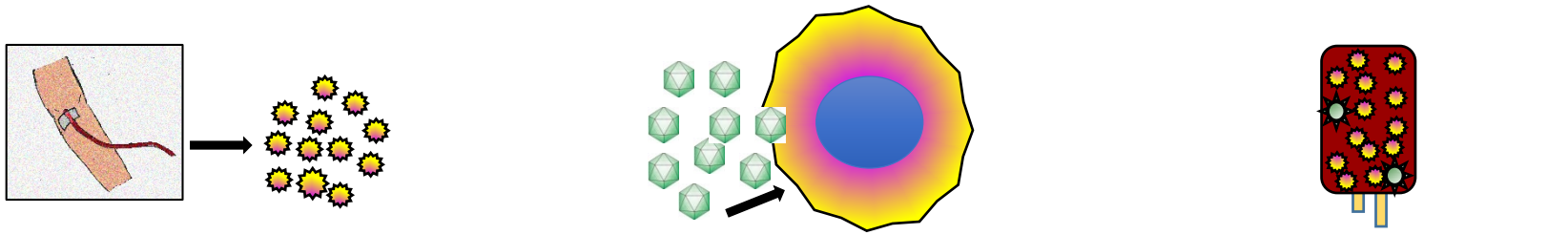


- **Stem Cell Collection:**
  - Mobilized PSC preferred
    - Plerixafor only for Sickle Cell
  - BM Harvest necessary in young infants SCID)
    - HSC dose may be lower

- **Conditioning:**
  - Myelablation still preferred
    - Busulfan in most studies
  - Non-genotoxic agents would be preferable
    - Efficacy in Gene Therapy vs allo-BMT?

***Shortening time to treatment a major challenge in many diseases (SCID, cALD)**

Cell Manufacturing: Lentivirus Based Gene Addition



Collect stem cells from patient with genetic defect in blood/immunity → GMP* Ex vivo lentiviral transduction → production of gene of interest → Infusion of genetically modified HSC back into patient

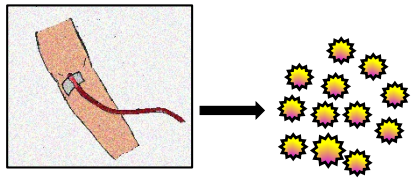
Condition Patient with Stem Cell-Directed Chemotherapy

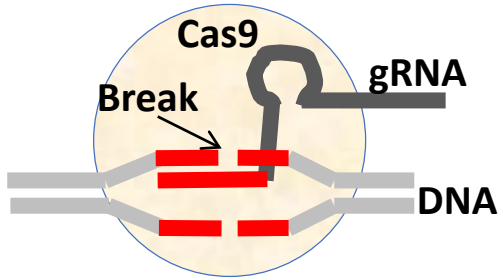
*GMP → Good Manufacturing Practice regulations per Code of Federal Regulations (CFR)

Options for Gene Addition

1. Add a normal copy of the gene associated with the disorder (β-globin in SCD)
2. Add a compensatory gene that fixes phenotype (γ-globin in SCD)
3. Add a Short hairpin(sh)RNA (miR) that impacts gene expression to fix phenotype (shRNA targeting *BCL11A* enhancer in SCD)

Cell Manufacturing: Gene Editing





Collect stem cells from patient with genetic defect in beta globin

Electroporation of Guided Nuclease constructs to induce strand breaks at site of interest → gene correction or disruption

Infusion of genetically corrected HSC back into patient

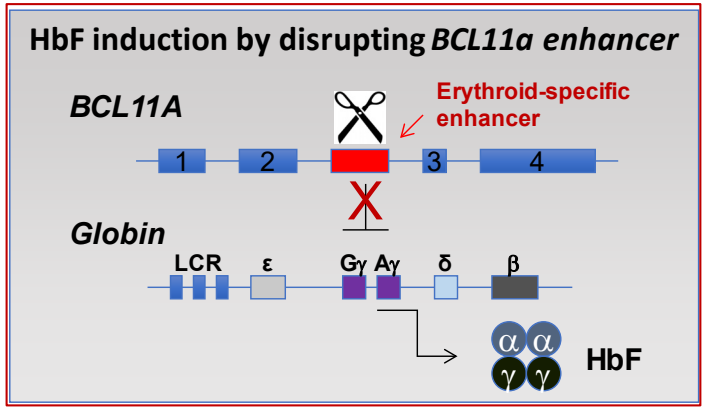
This is not embryonic gene editing!!

Patient Receives Stem Cell-Directed Chemotherapy

Dr. Mattew Porteus "Gene Therapy Techniques"

Goals of gene editing

1. Gene correction: challenging because needs DNA repair by homologous recombination, which is inefficient (soon to be in trials)
2. Gene disruption by error-prone non-homologous end joining (NHEJ) creation of insertions/deletions (efficient, currently in trials)



Allogeneic HSCT versus Gene Therapy: How to Decide?

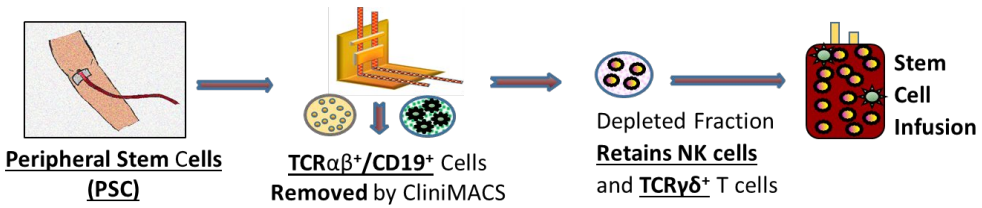
- **If HLA matched sibling available, MSD-BMT is typically preferred**
  - Exception: MSD who are carriers of some genetic conditions
- **For patients lacking MSD:**

	Allo HSCT	Gene Therapy
Myeloablation Risks	Present	Present
Speed of Engraftment	Faster	Slower, particularly platelets
Alloimmunity Risk	Present	None
Infection Risk	Higher	Lower
Medication Burden	Higher	Lower
Degree of Phenotype Correction	Typically complete (if full chimerism achieved)	Often partial correction (but this may be ok for many diseases)
Time When Efficacy Can Be Determined	Early (by a month?)	Late (6-9 months or more)

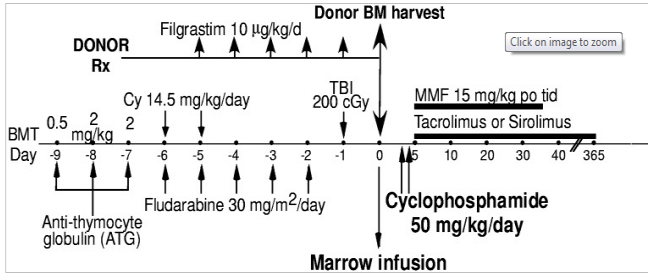
- **Biggest Problem for Gene Therapy: Access**
  - Trial slots, post-approval capacity, insurance?

Allogeneic HSCT versus Gene Therapy: How to Decide?

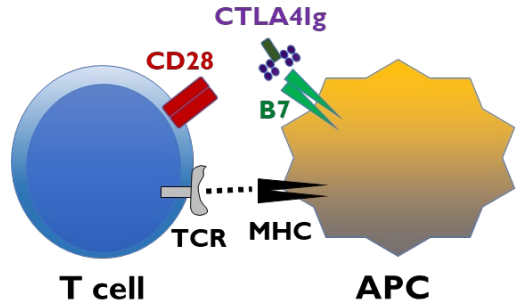
GVHD risk should really no longer be the differentiator….



TCRαβ⁺ T cell with CD19⁺ Depletion



Johns Hopkins PT-Cy (Bolanos-Meade, Blood 2012)



Abatacept for URD-SCT in Pediatric SCD

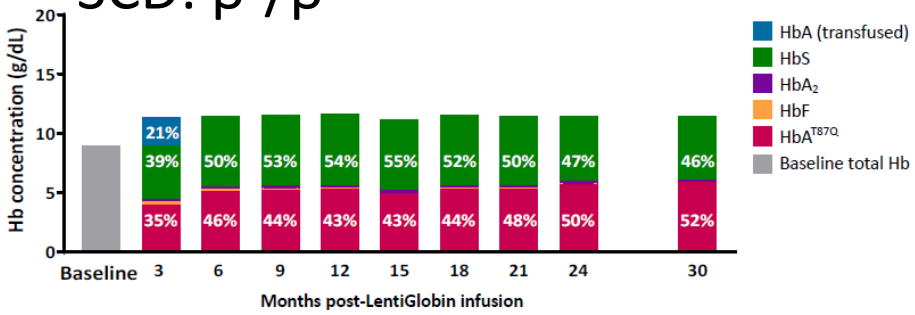
….but depth of impaired immunity post-treatment may be

- No need for serotherapy, fludarabine, cyclophosphamide in Gene Therapy conditioning
- No post-transplant immune prophylaxis (no rejection risk)
- Risk of virus reactivation post-gene therapy minimal (except in PID!)

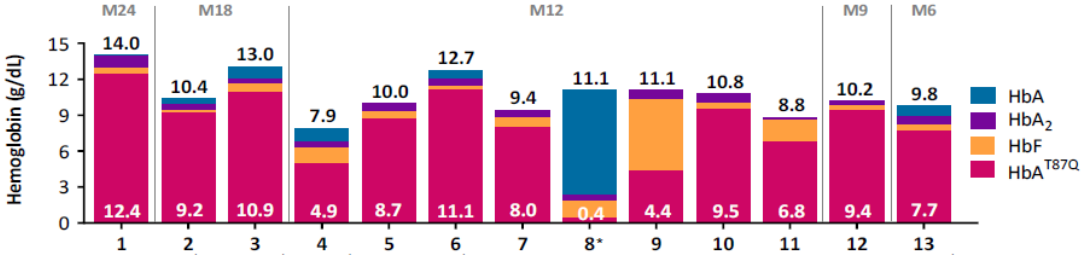
Gene Therapy for Hemoglobinopathies: Dr. Janet Kwiatkowski

bluebird bio Lentiglobin Studies

SCD: β^S/β^S

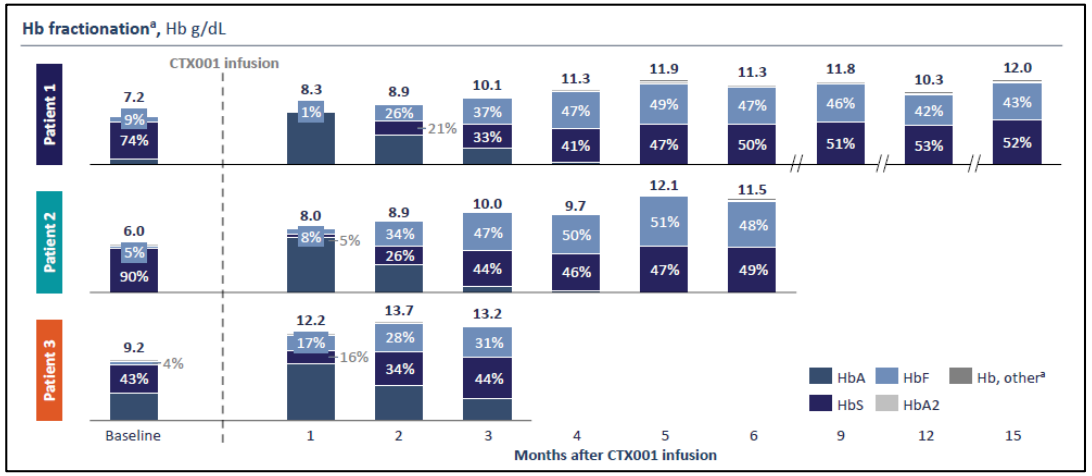


Thalassemia: β^0/β^0



Thompson et al., 2020 ASH Annual Meeting, Yannaki et al. 2020 EHA Annual Congress, Courtesy of bluebird bio, inc.)

CRISPR/Vertex: CTX001-121



Frangoul et al., 2020 ASH Annual Meeting

Challenges:

- **Thalassemia:** What Hgb level is enough to prevent ineffective erythropoiesis
- **SCD:** How low does the HbS level need to be to prevent symptoms?
- **Both:** Clonal evolution and MDS/AML risk

Gene Therapy for Primary Immune Deficiency Syndromes: Dr. Sung-Yun Pai

Clinical efficacy of gene-modified stem cells in adenosine deaminase–deficient immunodeficiency

Kit L. Shaw,[1] Elizabeth Garabedian,[2] Suparna Mishra,[1] Provaboti Barman,[1] Alejandra Davila,[1] Denise Carbonaro,[1] Sally Shupien,[3] Christopher Silvin,[2] Sabine Geiger,[1] Barbara Nowicki,[4] E. Monika Smogorzewska,[5] Berkley Brown,[3] Xiaoyan Wang,[6] Satiro de Oliveira,[1,3] Yeong Choi,[1] Alan Ikeda,[7] Dayna Terrazas,[3] Pei-Yu Fu,[1] Allen Yu,[1] Beatriz Campo Fernandez,[1] Aaron G. Jaya Suzan David

Letter | Published: 27 January 2020

Lentiviral gene therapy for X-linked chronic granulomatous disease

Donald B. Kohn , Claire Booth, Elizabeth M. Kang, Sung-Yun Pai, Kit L. Shaw, Giorgia Santilli, Myriam Armant, Karen F. Buckland, Uimook Choi, Suk See De Ravin, Morna J. Dorsey, Caroline Y. Kuo, Diego Leon-Rico, Christine Rivat, Natalia Izotova, Kimberly Gilmour, Katie Snell, Jinhua Xu-Bayford Dip, Jinan Darwish, Emma C. Morris, Dayna Terrazas, John K. Gaspar,

Lentiviral haemopoietic stem/progenitor cell gene therapy for treatment of Wiskott-Aldrich syndrome: interim results of a non-randomised, open-label, phase 1/2 clinical study

Francesca Ferrua, Maria Pia Cicalese*, Stefania Galimberti, Stefania Giannelli, Francesca Dionisio, Federica Barzaghi, Maddalena Migliavacca, Maria Ester Bernardo, Valeria Calbi, Andrea Angelo Assanelli, Marcella Facchini, Claudia Fossati, Elena Albertazzi, Samantha Scaramuzza, Immacolata Brigida, Serena Scala, Luca Basso-Ricci, Roberta Pajno, Miriam Casiraghi, Daniele Canarutto, Federica Andrea Salerio, Michael H Albert, Antonella Bartoli, Herma Koenraad van Rossem, Gi*

ORIGINAL ARTICLE

Lentiviral Gene Therapy Combined with Low-Dose Busulfan in Infants with SCID-X1

E. Mamcarz, S. Zhou, T. Lockey, H. Abdelsamed, S.J. Cross, G. Kang, Z. Ma, J. Condori, J. Dowdy, B. Triplett, C. Li, G. Maron, J.C. Aldave Becerra, J.A. Church, E. Dokmeci, J.T. Love, A.C. da Matta Ain, H. van der Watt, X. Tang, W. Janssen, B.Y. Ryu, S.S. De Ravin, M.J. Weiss, B. Youngblood, J.R. Long-Boyle, S. Gottschalk, M.M. Meagher, H.L. Malech, J.M. Puck, M.J. Cowan, and B.P. Sorrentino*

OCCURRENCE OF LEUKAEMIA FOLLOWING GENE THERAPY OF X-LINKED SCID

Donald B. Kohn, Michel Sadelain† and Joseph C. Glorioso§*

Recombinant viral vectors have allowed gene transfer to be developed as a promising approach to the treatment of genetic diseases. Recently, gene therapy of children with X-linked severe combined immune deficiency resulted in impressive levels of immune reconstitution — a triumph that was later overshac causes of this cancer, a minimizing risk to the p

REVIEW ARTICLE

MECHANISMS OF DISEASE

Activation of the T-Cell Oncogene *LMO2* after Gene Therapy for X-Linked Severe Combined Immunodeficiency

Matthew P. McCormack, Ph.D., and Terence H. Rabbitts, Ph.D.

GENE THERAPY

Gene Therapy for Wiskott-Aldrich Syndrome—Long-Term Efficacy and Genotoxicity

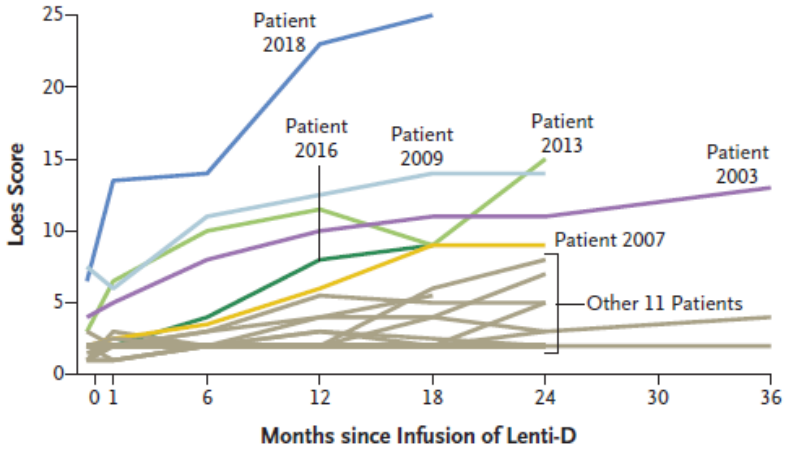
Christian Jörg Braun,[1]* Kaan Boztug,[2]*† Anna Paruzynski,[3]* Maximilian Witzel,[1]* Adrian Schwarzer,[2,4] Michael Rothe,[4] Ute Modlich,[4] Rita Beier,[2] Gudrun Göhring,[5] Doris Steinemann,[5] Raffaele Fronza,[3] Claudia Regina Ball,[3,6] Reinhard Haemmerle,[4] Sonja Naundorf,[7] Klaus Kühlcke,[7] Martina Rose,[8] Chris Fraser,[9] Liesl Mathias,[10] Rudolf Ferrari,[11] Miguel R. Abboud,[12] Waleed Al-Herz,[13] Irina Kondratenko,[14] László Maródi,[15] Hanno Glimm,[3,6] Brigitte Schlegelberger,[5] Axel Schambach,[4] Michael Heinrich Albert,[1] Manfred Schmidt,[3]* Christof von Kalle,[3,6]* Christoph Klein[1]*‡

Gene Therapy for Leukodystrophies: Dr. Amy Waldman

STARBEAM Study (ALD-102)

Figure S1. Lenti-D lentiviral vector encoding ALD protein

- Bluebird Bio led, multicenter study (Boston, Minnesota, GOS, Paris)
- Stabilization of imaging findings in most



Eichler…Williams NEJM, 2017 (courtesy of bluebird bio)

TIGET-MLD

- San Raffaele, Milan (Orchard)
- Ad hoc analysis in 2016
- 8/9 subjects showed prevention of disease onset or no progression.

Challenges:

- ➢ Biomarkers of clinical activity
- ➢ Early identification of patients requiring therapy
- ➢ Elimination of neurotoxic conditioning

Current and Future Challenges for Gene Therapy

- **Availability**
  - **Current:** limited trial slots
  - **Future:** limited manufacturing capacity?
- **Exportability**
  - Conditioning/infusion straightforward
  - Collection requires specialized expertise
- **Long Term Follow-up**
  - Coordinating network of centers likely needed
- **In The Future…**
  - Head to head comparisons with Allo-BMT
  - Elimination of alkylating agent conditioning?