Infusion of off-the-shelf *ex vivo* expanded cryopreserved progenitor cells to facilitate the engraftment of a single CCR5Δ32 homozygous or heterozygous cord blood unit in patients with HIV and hematological malignancies

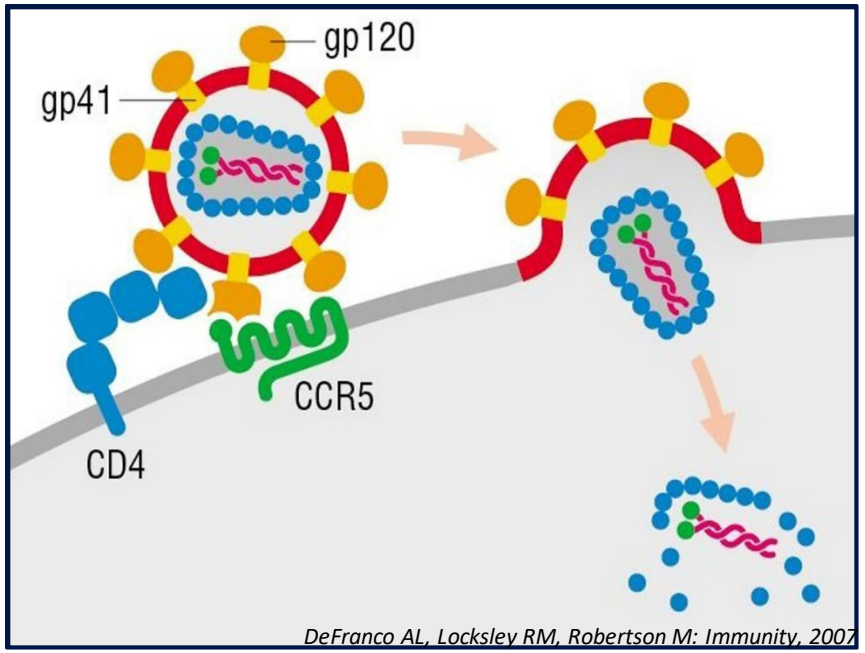
Background

- This is a Phase I/II prospective, open label, multi-site, non-randomized study in which patients with HIV & hematological malignancies will be enrolled.
- Objective will be to evaluate the safety of single unit homozygous or heterozygous CCR5 Delta32 cord blood transplant with a second non-matched off-the-shelf (OTS) *ex vivo* expanded cryopreserved UCB progenitor cells infused in patients with hematological malignancies and HIV infection in need of allogeneic stem cell transplant (alloSCT).
- AlloSCT has the potential for elimination of the HIV reservoir via GvH mechanism.
- Improbability of finding a CCR5Δ32 homozygous allogeneic marrow donor
  - *The feasibility to identify an HLA-matched homozygous CCR5Δ32 adult donor ranges only 0.1-0.4% for HIV infected hematology patients in need of alloSCT.*
- HLA mismatched UCB expands the likelihood of finding CCR5Δ32 homozygous donors. The prevalence of CCR5Δ32 homozygous units is estimated to be 0.8% with potentially over 5000 units carrying the mutation.

HIV entry into a host cell

- HIV strains can be broadly classified based on their co-receptor usage.
- Viruses that use the chemokine receptor **CCR5** are termed **R5 HIV**, those that use **CXCR4** are termed **X4 HIV**, and viruses that can use both co-receptors are called **R5X4 HIV.**
- Interestingly, despite identification at earlier time points and despite high levels of CXCR4 expression on circulating HIV target cells, X4 or even R5X4 HIV rarely predominate until late in infection.
- Focus of this study is the R5 HIV virus, which targets human CD4 T cells by CCR5 binding.

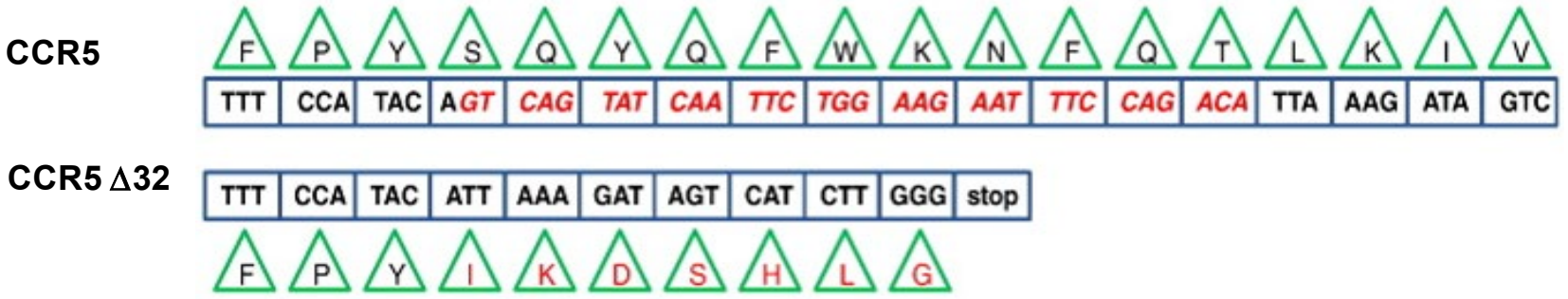
R5 HIV virus targeting CD4 T cells by binding to CCR5



DeFranco AL, Locksley RM, Robertson M: Immunity, 2007

Wild-type CCR5 and CCR5 Δ32

The CCR5 protein undergoes a 32 base pair deletion, producing a non-functional protein, known as CCR5 Delta32



CCR5 Δ32 homozygotes are resistant to HIV-1 and the heterozygotes have a slower disease progression

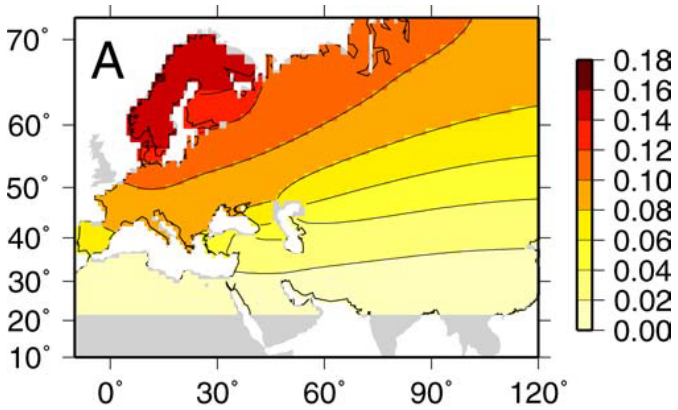
Barmania et.al, Applied and translational genomics, 2013

The “Berlin patient”

- Case of allogenic stem cell transplantation with HLA-matched adult donor homozygous for the CCR5 Δ32 deletion
- Led to a stable engraftment of an HIV resistant immune system
- ART was discontinued from 2007, with no evidence of viral rebound over a period of 13 years
- No traces of residual replication competent virus found to date

ART: Antiretroviral therapy

The Geographic Spread of the CCR5Δ32 Allele



The Δ32 mutation is found only in European, West Asian, and North African populations

The allele frequency exhibits a north–south decline with frequencies ranging from 16% in northern Europe to 6% in Italy and 4% in Greece

The broadest area of high frequency is located in north-eastern Europe, particularly the Baltic region, Sweden, Finland, Belarus, Estonia, and Lithuania

Probability of finding an adequately HLA-matched unit with a cell dose requirement of ≥1 × 10^7 TNC/kg is 85.6% for Caucasian pediatric patients and 82.1% for Caucasian adult patients

For members of minority ethnic groups, the projected probabilities are lower (e.g., 52.5% for Mexican American children, 34.1% for African American children, and 15.7% for Chinese American children)

Biol Blood Marrow transplant, 2013; 19(3):393-397

Screening for CCR5-D32/D32 Cord Blood Grafts

- Once a HIV infected hematology patient requiring alloSCT is identified, preliminary search for potential allogeneic donors is performed via NMDP/WMDA including UCB grafts (approximately 600K global current inventory)

- This will be followed by screening blood spots of the identified, suitably HLA-matched UCB for CCR5 D32 homo/heterozygous deletions

- The screening process involves CCR5 genotypic analysis using a nested PCR based assay system, on DNA preparations extracted from cord blood spots

Clinical Trial Plan

- The patient will receive an adequately HLA matched CCR5 Delta32 homozygous or heterozygous un-manipulated cord blood unit.
- This unit will be given with off-the-shelf, NOTCH-1 based *ex-vivo* expanded second UCB graft to support the patient during the first 90 days and to facilitate engraftment of the CCR5 Delta32 homo-/heterozygous graft.
- **Immune Reconstitution** is monitored post transplant to determine optimal timing of planned ART interruption.
- During ART interruption (expected approximately 12 months after UCB alloSCT), viral load is monitored closely to determine any evidence of rebound.

Study Design

Estimated Accrual: 10 patients

Patients with hematological malignancies and HIV in need of allogeneic HSCT at 5 different Centers

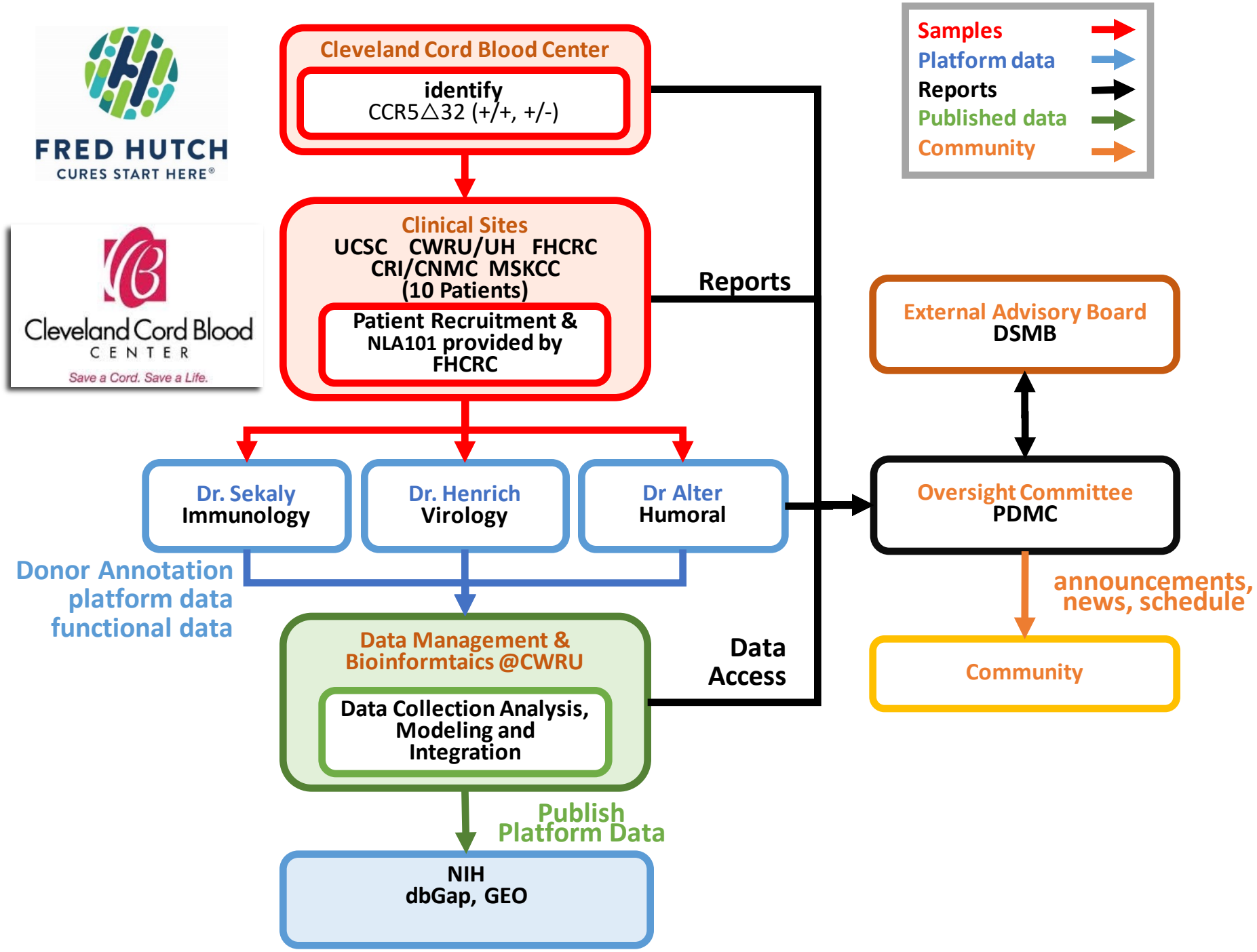
CB searches should identify at least 15-25 potential grafts matched at 4 of 6 HLA loci or better with a **MINIMUM of 2.5 x 10^7 TNC/kg** and with a **MINIMUM of 1.7 x 10^5 CD34/kg**. CB searches results will be communicated to Cleveland Cord Blood Bank.

Cleveland Cord Blood bank will request blood spots or segments of the 15-25 potential CB units: Screening for CCR5Δ32 homozygous or heterozygous mutation.

If a CCR5Δ32 homozygous or heterozygous cord blood unit is identified then the patient will be enrolled. Screening failure log will be maintained

Study Sites:

- Fred Hutch/SCCA (Filippo Milano, PI)
- Case Western/University Hospitals (Rafick-Pierre Sékaly & Marcos de Lima, PI)
- Children's Research Institute/Children's National Medical Center (Blachy Davila-Saldana, PI)
- Memorial-Sloan Kettering Cancer Center (Juliet Barker, PI)
- University of California-San Francisco (Timothy Henrich, PI)
- Cleveland Cord Blood Center (Mary Laughlin, PI)


Samples
Platform data
Reports
Published data
Community
Cleveland Cord Blood Center
identify
CCR5△32 (+/+, +/-)
Clinical Sites
UCSC CWRU/UH FHCRC
CRI/CNMC MSKCC
(10 Patients)
Patient Recruitment &
NLA101 provided by
FHCRC
Reports
External Advisory Board
DSMB
Dr. Sekaly
Immunology
Dr. Henrich
Virology
Dr Alter
Humoral
Oversight Committee
PDMC
Donor Annotation
platform data
functional data
announcements,
news, schedule
Data Management &
Bioinformtaics @CWRU
Data Collection Analysis,
Modeling and
Integration
Data
Access
Community
Publish
Platform Data
NIH
dbGap, GEO

Collaborators for the Phase I/II Clinical trial

Fred Hutchinson Cancer Research Center	Filippo Milano, MD, PHD Associate Director Cord Blood Transplantation
Cleveland Cord Blood Center	Mary Laughlin, MD
Case Western Reserve University	Rafick-Pierre Sekaly, PhD Co-director, CFAR Systems Biology Core
University of California San Francisco	Tim Henrich, MD
Memorial Sloan Kettering	Juliet Barker, MD Director Cord Blood Transplantation
U. Washington Children's	Blachy Davila-Saldana, MD