Recombinant DNA Advisory Committee Meeting, September 12, 2013

Unexpected false positive PCR test for HIV following a lentiviral gene therapy for X-linked severe combined immunodeficiency (X-SCID) (OBA Protocol #0901-964)

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Lentiviral gene transfer treatment for Older Children with X-SCID

- Background- IL2Rγ (γc) gene mutation results in profound cellular and humoral immune deficiency where the severe form is fatal in infancy without transplant or gene therapy
- Transplant from HLA-matched siblings results in best outcome
- Haploidentical transplants from parent most often without conditioning is life saving but most often results in incomplete immune reconstitution

LVXSCID-OC TRIAL

- Target population- XSCID patients who have received stem cell transplant(s) earlier, but achieved only partial correction; usually with limited or waning donor T cell function, and no B or NK function.
- Vector- 3rd generation, Self-inactivating, VSVg-pseudotyped, EF1 promoter, codon-optimized human γc (Cl20-4i-EF1a-hgcOPT)
- Vector production- stable lentivector producer cell line (St Jude Children's Research Hospital Vector Facility)

Subject #1

- 24yo Caucasian male, transplant from haplo-identical mother at 5 months with “boost” at 9 years of age.
- Indications for treatment- recurrent infections, lymphopenia with waning T cell function, IVIG dependent.
- Conditioning- busulfan 6mg/kg
- Hospital course to discharge- brief period of neutropenia and thrombocytopenia, no unexpected adverse events, discharged by 3 weeks post treatment

Cell Processing:

- Patient wt=49kg
- Viable patient CD34+ cells at culture initiation=5.6x10^8
- *Ex-vivo* culture 3 days, 2 exposures to vector
- At end of culture, total number of cells administered=1.02x10^9
- Cell Dose- 20million/kg gene-modified autologous CD34+ cells
- CFU-c-From a total of 159 colonies, 43 were vector positive, ie 27.0%

Post treatment-viral screen

- Protocol-negative viral screen before treatment as eligibility criteria, and because participants are likely young adults, viral screen is repeated at regular intervals after treatment; replication competent virus screen

- Viral screen-all rely on PCR because of inability to produce antibodies

- Pre-treatment viral screen-HIV RNA PCR negative - Roche - COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

- At 6 months post treatment, HIV RNA PCR noted to be positive, 265 copies/mL.
- HIV DNA PCR (Amplicor) and p24 ELISA-negative

HIV tests

- Antibody tests: detection of HIV antibodies:
  - Enzyme immunoassay (EIA) tests
  - Western blot
- P24 Antigen tests ELISA
- HIV Polymerase Chain Reaction (PCR) tests
  - HIV DNA-detects and amplifies HIV DNA; often used to detect acute HIV infection.
  - HIV RNA-Roche COBAS/Ampliprep HIV v2

HIV RNA PCR assay: Roche - COBAS® AmpliPrep/COBAS® TaqMan® HIV-1 Test, v2.0

- Offers primers and probes to amplify the gag and LTR regions.
- Provides LTR primers with broad genotype inclusivity.
- Quantifies the clinically significant HIV-1 groups and subtypes with full subtype coverage and quantification of HIV-1 groups O and M.
- Quantitates HIV-1 RNA from 20 - 10,000,000 copies/mL.
- Limit of detection (LOD) and 100% specificity at 20 copies/mL.
- Automated, measures EDTA plasma HIV-1 RNA levels

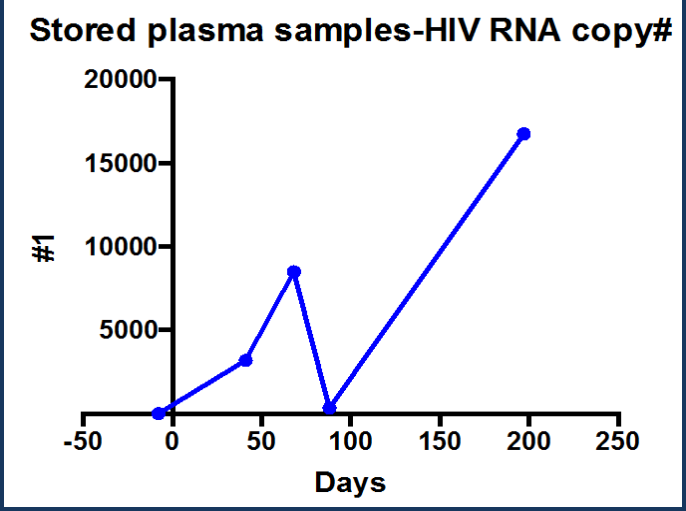
Potential causes for the positive HIV PCR RNA test:

1. Carryover from initial *in vitro* transduction of the CD34 cells.
2. HIV infection
3. Replication competent virus due to recombination
4. Detection of vector sequences from gene-modified cells

1. Carry-over from *in vitro* transduction of CD34 cells

We performed quantitative HIV RNA measurements using the Roche COBAS/Ampliprep assay on frozen plasma samples that were archived.

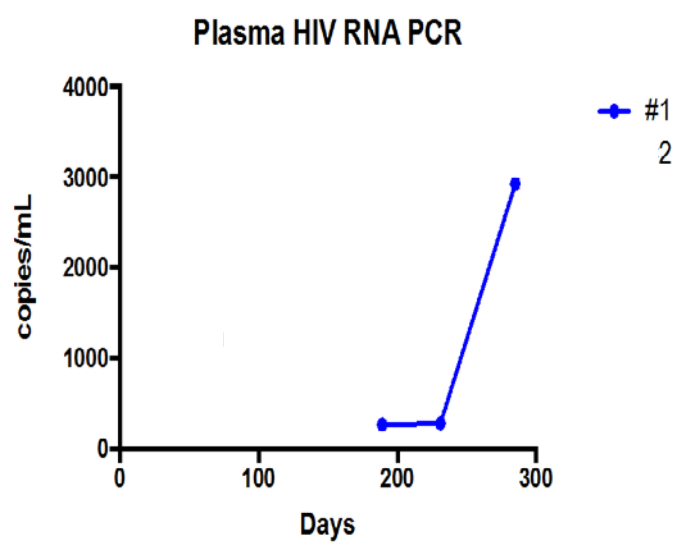
Date	HIV RNA (copies/mL)
Frozen plasma samples	
10/3/12 **(PRE-)**	Not detected
11/21/12 (7 wks)	3,194
12/19/12 (9 wks)	8,487
1/9/13 (3 mths)	342
4/30/13 (6 mths)	16,749



1. Carry-over from *ex-vivo* transduction cont.-fresh plasma samples

Serial measurement of HIV RNA by PCR (Roche COBAS Ampliprep) at each visit, from fresh blood samples:

Date	HIV RNA Copies/mL
Plasma viremia on April 30, 2013	
4/18/13 (6 mths)	265
5/30/13 (7.5 mths)	279
7/23/13 (9 mths)	2925
	HIV p24 ELISA Results
CC 4/26/13	undetected
CC 4/30/13	undetected
Plasma	undetected
Plasma	undetected



The overall rising trend of HIV RNA copies detected from archived, frozen plasma samples as well as peripheral blood fresh samples goes against the likelihood of a 'carry-over'.

2. Wildtype/Natural HIV Infection

- To assess for natural HIV infection-used several sets of primers that broadly amplify HIV subtypes A,B,C,D,F, and G-all samples were negative.

  HIV Drug Resistance Program, NCI-Mary Kearney, Steven Hughes, John Coffin and Xiaolin Wu at LMT, SAIC.

- Ruled out natural HIV infection at this point

3. Replication competent virus detection: National Gene Vector Lab/Indiana U

- RCL screen-per FDA recommendations at 3, 6, 9, 12 mths, yearly
- Extended co-culture system- test sample inoculated into C8166-45 (a cell line derived from human cord blood lymphocytes, permissive for infection and growth of HIV-1), then cultured for ≥21 days to allow amplification of RCL, thereby increasing assay sensitivity.
- Cell-free supernatant from the cell culture is used to infect naive C8166-45 cells and the cells passaged for an additional 7 days. The supernatant is then collected for p24 ELISA to detect HIV-1 capsid protein and Product-Enhanced Reverse Transcriptase (PERT) Assay, respectively. Presence of RCL in the test sample is indicated by detection of Reverse Transcriptase and p24 antigen in the cell supernatants.
- Conclusion: No RCL was detected as indicated by the absence of detectable quantities of p24 protein and reverse transcriptase.

3. Replication competent virus detection: LIR, NIAID

- Quantitative coculture assay
  - Purify CD4+ T cells, place in culture in limiting dilution approach, then maximally stimulated for viral production (PHA, anti-CD3, IL2)
  - Culture maintained for 14-21 days
  - Replication competent cells identified by HIV p24 ELISA
  - the assay is used to detect infectious HIV originating from cells that carry either integrated or unintegrated proviral DNA, or HIV reservoir in HIV-infected individuals
- Result-no p24 was detected from the patient's quantitative cell coculture assay.

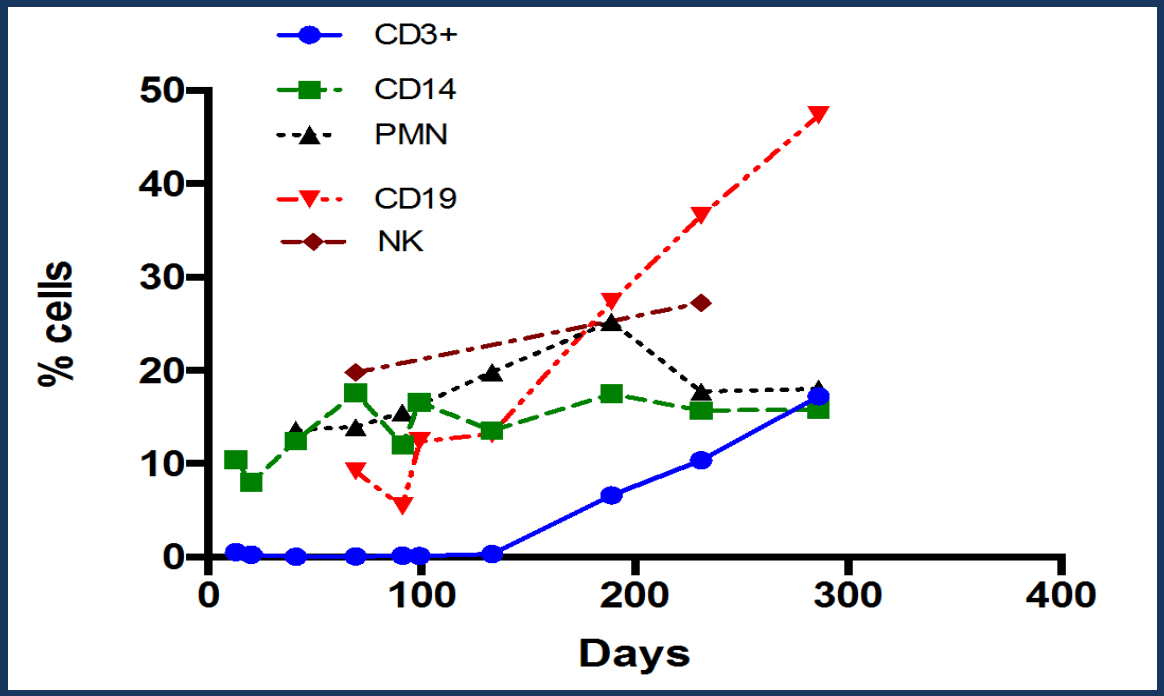
(Tae-Wook Chun Current Opin in HIV and AIDS 2013; 8(2); 111-116.)

4. Vector sequences from gene-modified cells (cont.):

- *In vitro* test:
  - Healthy donor CD34 cells thawed, exposed to same clinical vector cl20-i4-EF1a hgcOPT x2, cells washed extensively, then spiked in to healthy donor plasma, Roche COBAS HIV RNA Ampliprep-millions of HIV RNA copies detected .
- Patient serum (John Gray, Robert Throm- SJCRH Vector Facility):
  - Patient serum-DNA removed, +/-reverse transcriptase to make cDNA copies of RNA in serum, then qRT-PCR using primers specific for codon-optimized gag sequences present in lentivector production system, OR sequences in therapeutic vector genome responsible for packaging ('psi')
  - QRT-PCR for the codon optimized gag was negative, but HIV psi sequences from vector genome were detected, though not amplified by an RT step, ie likely cellular genomic DNA from the genetically modified cells.
- Conclusion: RCL not detected in patient sample (sensitivity of PCR about 1.5×10^4 RCL tu/mL)
- Recommends repeat testing of patient's samples

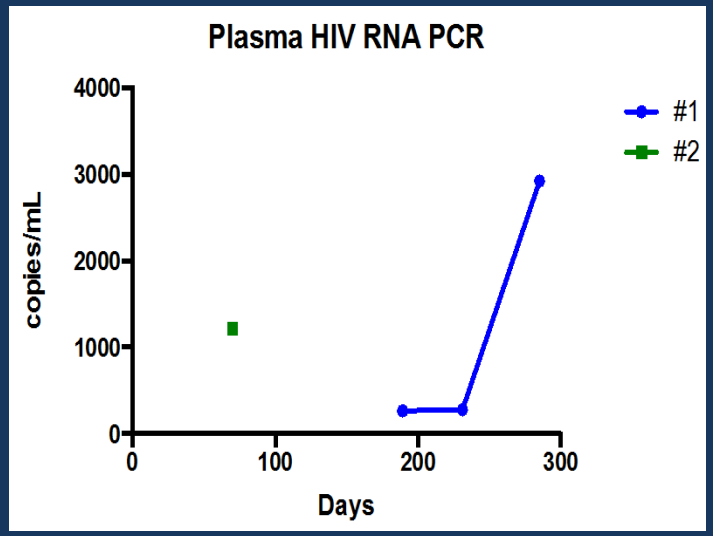
4.Vector sequences from gene-modified cells (Subject 1)

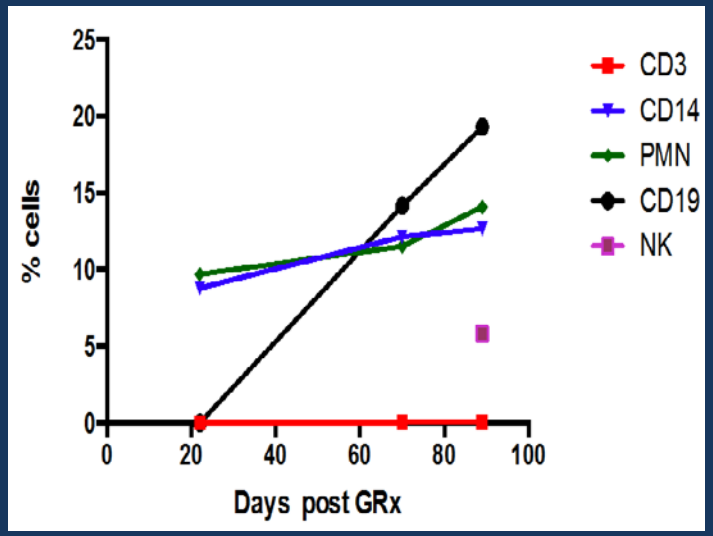
- Consistent with the possibility that the Roche Ampliprep HIV RNA assay is detecting vector sequences from gene-modified cells, Subject 1 is clearly having stable or increasing circulating levels of marking in multiple lineages-in CD14, PMN, B and T respectively.



Subject #2:

- Very similar to Subject 1- a 24 yo Caucasian male, previous haplo-identical transplant, similar indications for treatment
- HIV RNA PCR at 2 months post infusion was 1,211 copies/mL
- Gene marking in multiple lineages in peripheral blood





Conclusion and Implications

- Conclusion- HIV RNA PCR assay detects vector-related sequence in provirus containing cells
- Issues raised- false positive HIV tests-social, economic considerations, potential impact on health insurance
- Steps to address the false positive HIV RNA PCR tests: protocol amendment, consent/assent document, letter

Acknowledgements:

HIV Drug Resistance Program, NCI

- Mary Kearney
- Xiaolin Wu
- Stephen Hughes
- John Coffin

Laboratory of Immune Regulation, NIAID

- Tae Wook Chun
- Susan Moir

National Gene Vector Laboratory, Indiana University

- Ken Cornetta

St Jude Children's Research Hospital

- Robert Throm
- John Gray
- Brian Sorrentino