



A Phase I Study of Gene Transfer for Patients with Fanconi Anemia Complementation Group A (FANCA)

Pamela S. Becker, MD, PhD

Hans-Peter Kiem, MD

University of Washington and Fred Hutchinson
Cancer Research Center

RAC Reviewer Comments

Fanconi Anemia: Background

- Rec inherited condition, characterized by bone marrow failure, congenital anomalies and epithelial malignancies
- Defect in DNA repair, sensitivity to DNA interstrand cross-linking (e.g.-mitomycin C)
- At least 13 complementation groups (A, B, C, D1, D2, E, F, G, I, J, L, M and N)
- Fanconi core complex associates with Bloom syndrome complex to form BRAF1
- In response to DNA damage, FANCD2 and FANCI are monoubiquitylated, and form the FANCD2 “ID” complex, which is recruited to the chromatin, and DNA repair occurs.

*Fanconi
Core
Complex*

FANCA

FANCC

FANCE

FANCF

FANCG

FANCM

FAAP24

FANCB

FANCL

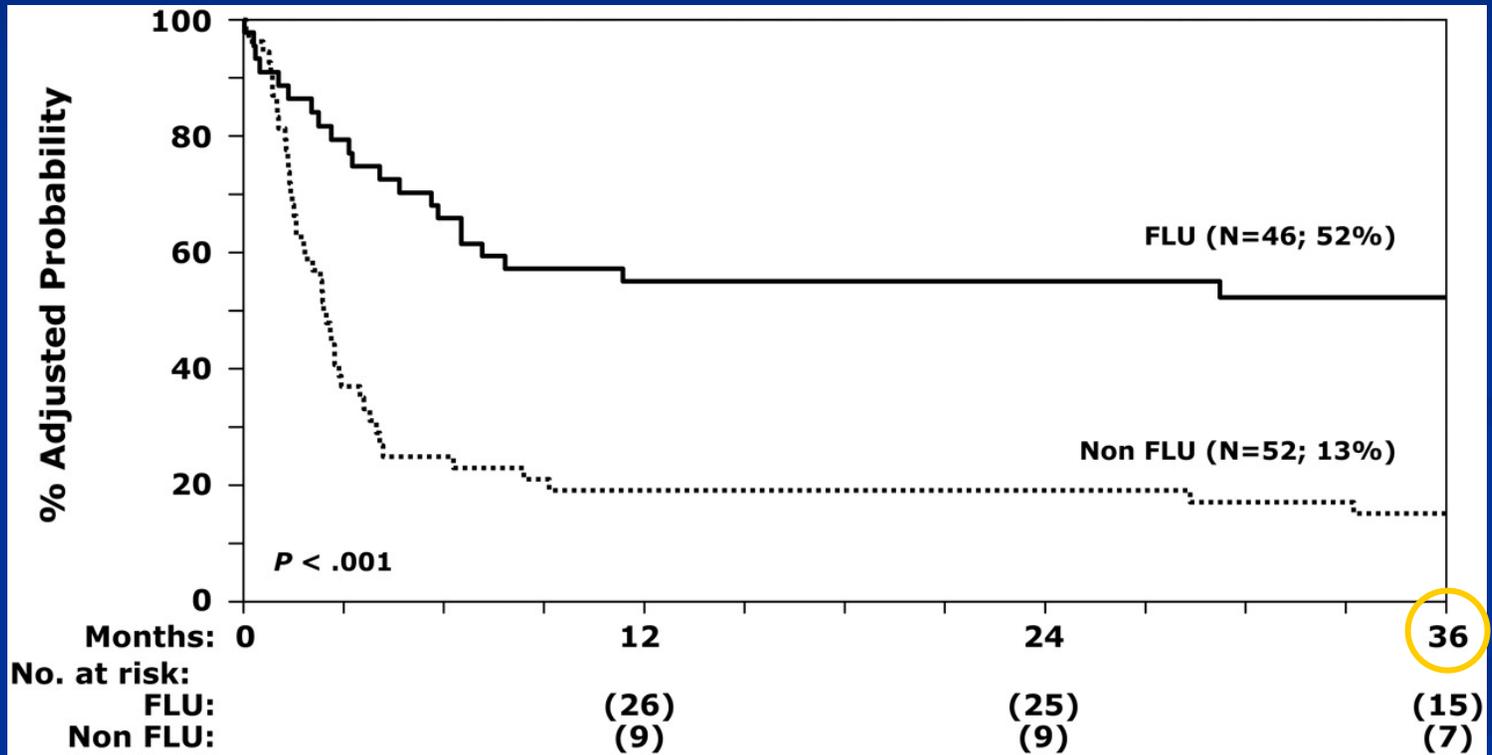
FAAP100

Current Treatment Options for FA

- Transfusion support, monitoring for development of malignancy (SCC head and neck, esophagus, vulva, cervix)
- Allogeneic stem cell transplant
 - Related donor
 - Unrelated donor
 - UCB
 - Haploidentical transplant
- ISSUES:
 - Graft versus host disease-increased risk of solid tumors (Rosenberg PS et al Blood 105: 67, 2005)
 - Significant morbidity due to graft failure, infection and GVHD



URD Transplant Overall Survival \pm Flu

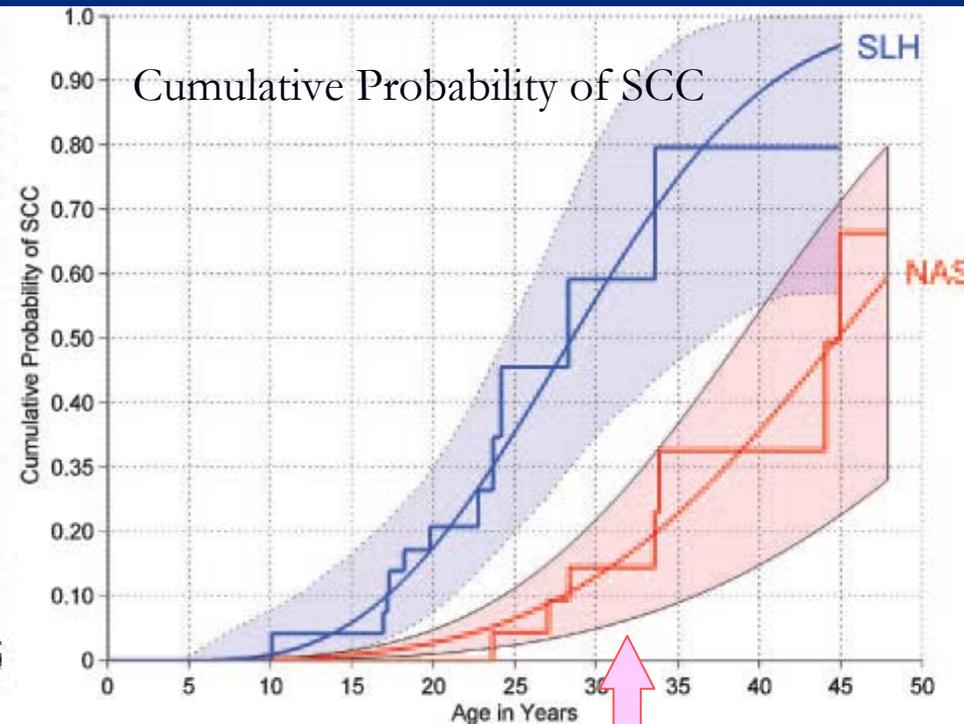
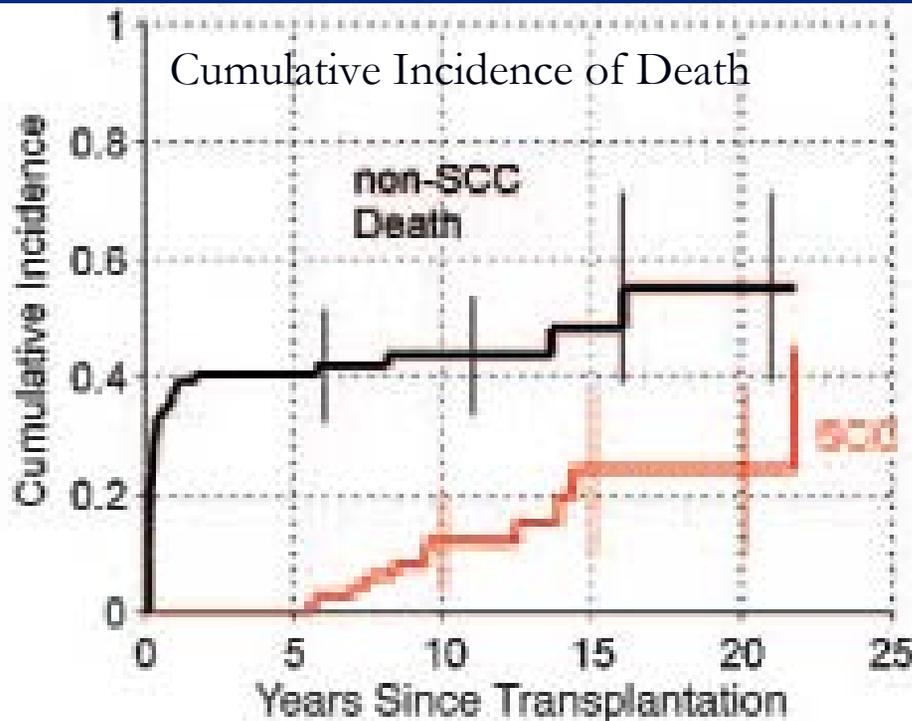


cGVHD=31%

Wagner, J. E. et al. Blood 2007;109:2256-2262

Squamous Cell Ca Post Transplant

With a transplant



Without a transplant



- **Severe Acute GVHD:** 33 fold \uparrow SCC, only 4% alive and free of SCC at 6 yrs post transplant $p=0.002$
- **Chronic GVHD:** Increased risk $p=0.03$

Preclinical Studies

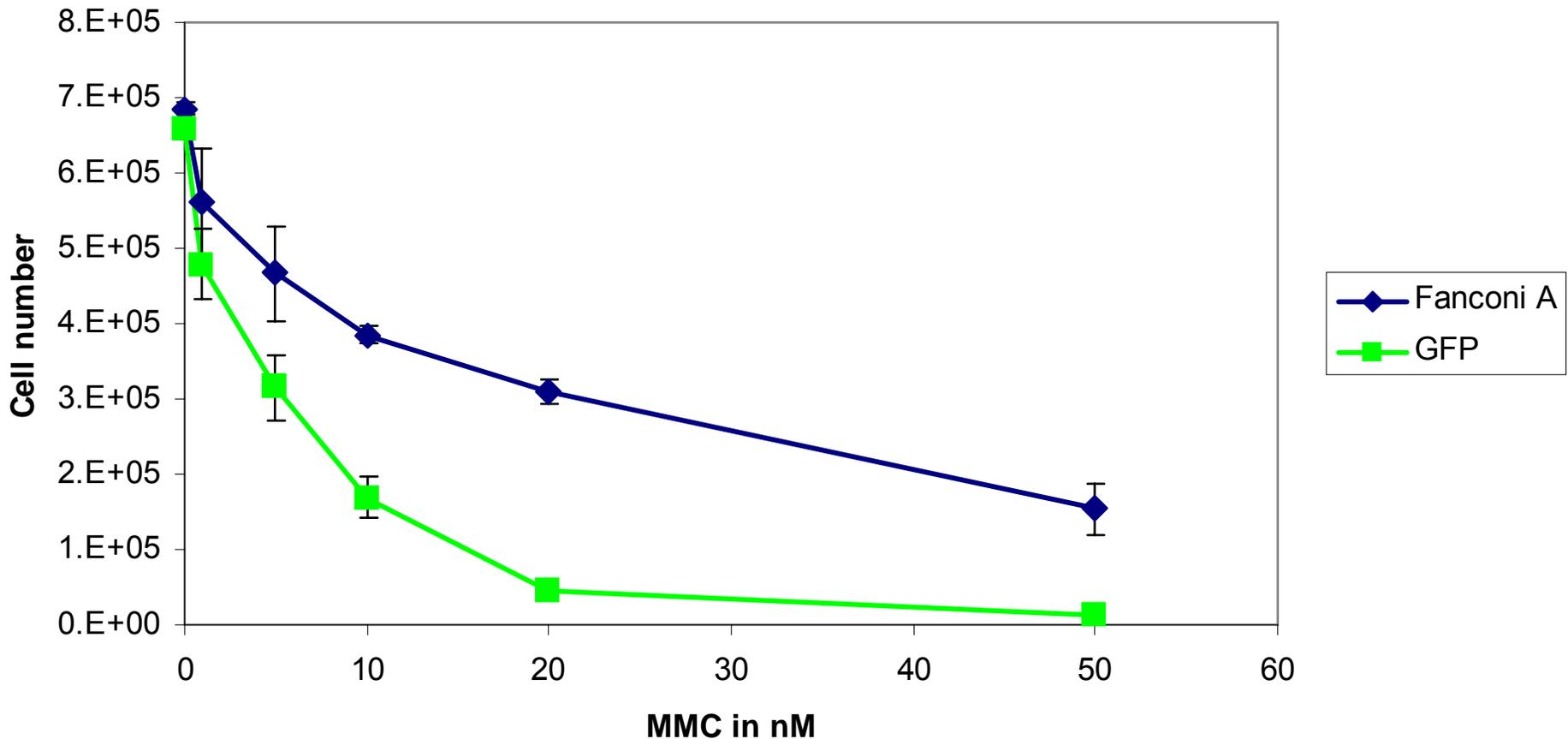
- Human Cell
 - Normal human CD34+ cells
 - FA bone marrow (FancA, unknown, FancC)
 - FA lymphoblasts (FancA)
- Murine In vivo
 - FancA-/-
 - FancC-/-

Conditions

- SIN Lentiviral vector:
 - RRLsincPPT-PGK-FA (“FancA”)
 - RRLsincPPT-PGK-GFP
- Cytokines: G-CSF, SCF, TPO, Flt3
- Retronectin coated plates
- MOI 10 (range 4-40)

Fanconi A Lymphoblasts : Survival in mitomycin C

Day 4: MMC challenge of transduced Fanconi A lymphoblasts



Normal Human CD34+ cells

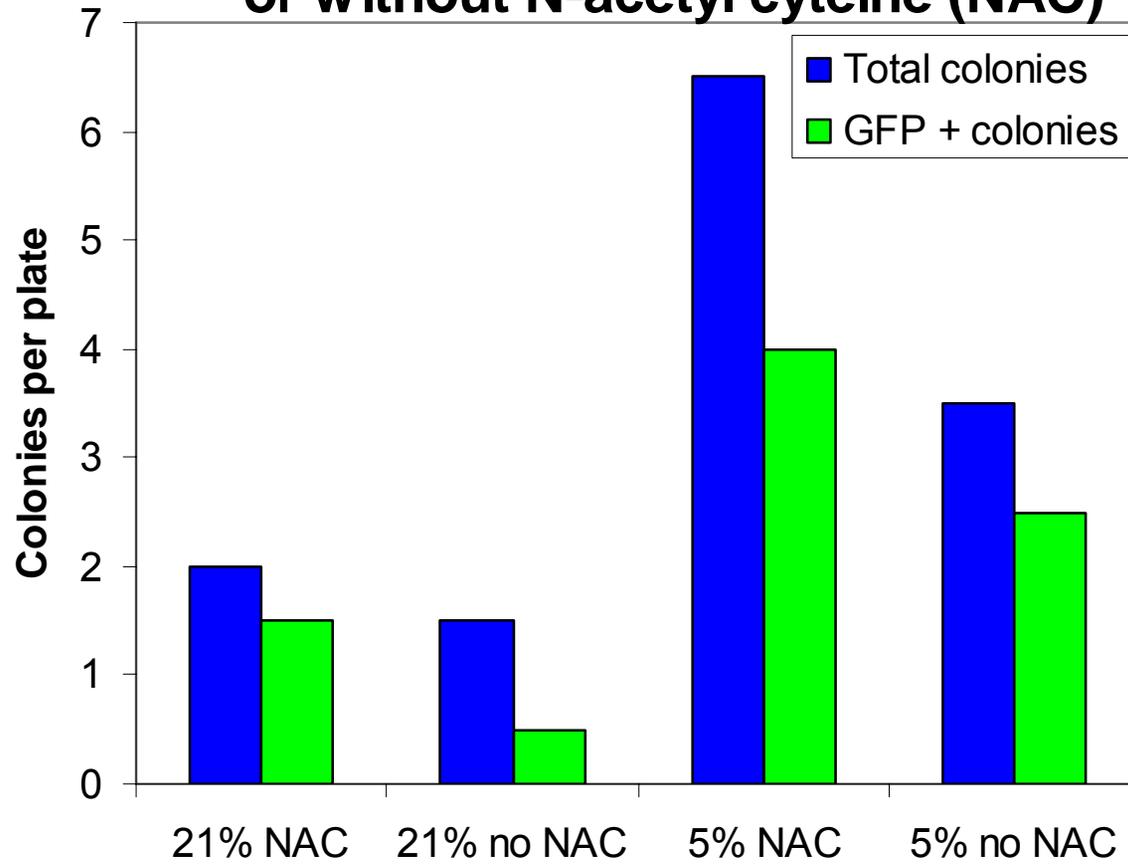
- Transduction of Normal Human CD34+ cells
- MOI 4, FancA Vector titer 2.7×10^7
- Transduction Efficiency by PCR of DNA isolated from methylcellulose colonies: 24-26%.
- LAM-PCR Insertion site analysis: 26 insertions in bulk culture of transduced cells identified in 9 sites.

Cryopreserved Human FancA BM

- Mononuclear cells isolated
- Overnight transduction, MOI 4
- Viability 55%
- Non-transduced: 0 colonies/cell clusters in 1 through 20 nM mitomycin C
- Transduced: 50 colonies/cell clusters in 1 nM mitomycin C, 9 cell clusters in 5 nM mitomycin C
- PCR + one of three plates 1 nM mitomycin C and one of 3 plates in 5 nM mitomycin C

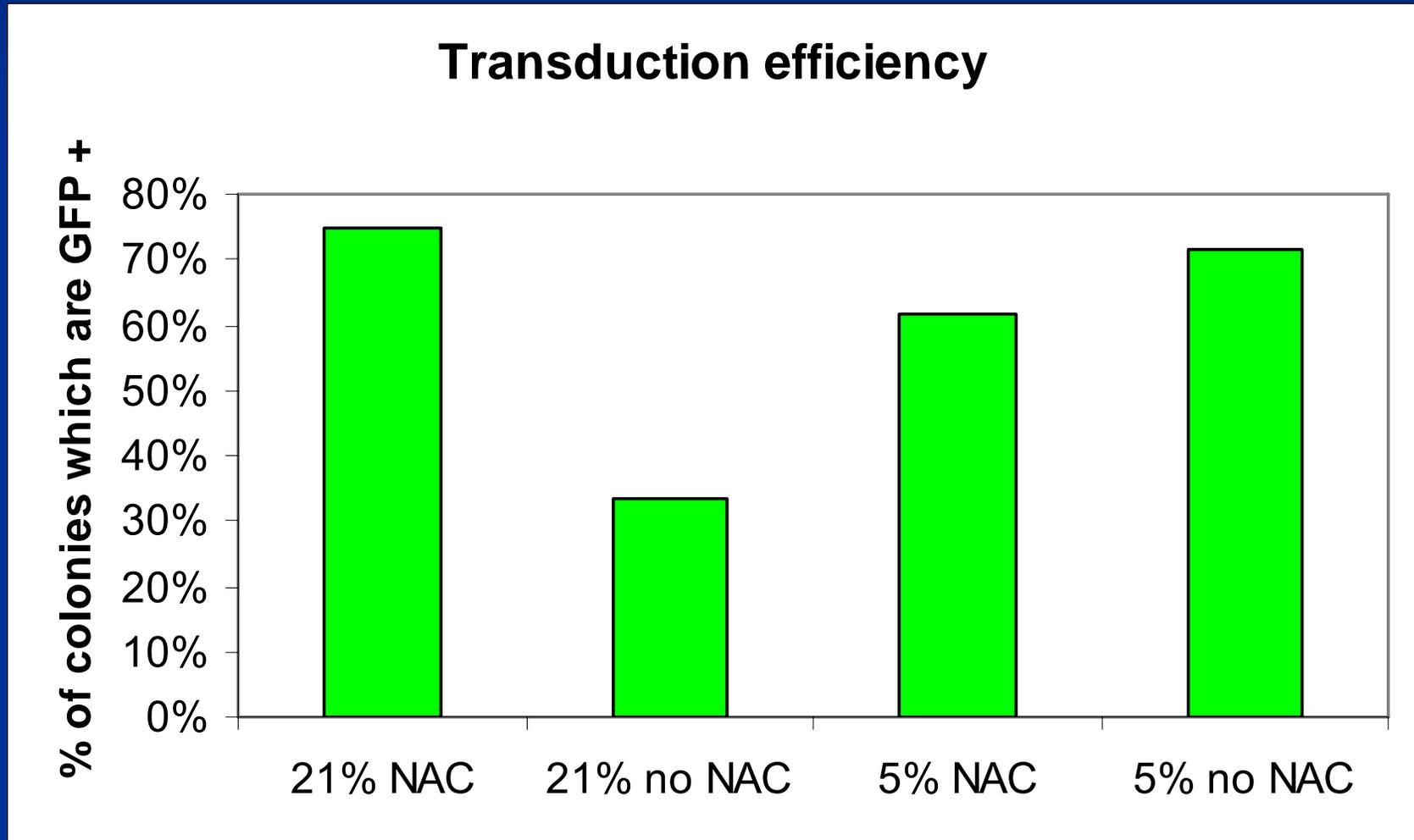
Lenti-GFP transduced FA bone marrow

Lenti-GFP transduced Fanc BM in different oxidative conditions, 5% or 21% Oxygen, with or without N-acetyl cyteine (NAC)



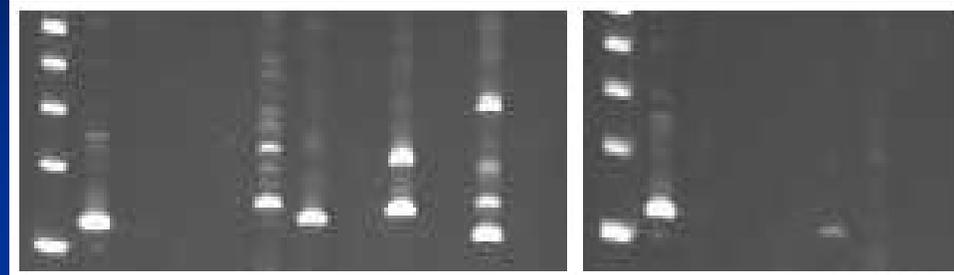
Transduction Efficiency of Colony forming cells from FA bone marrow

Viability for O/N transduction fresh BM cells with cytokines, hypoxic: 90%



Transduction of *Fanca*^{-/-} murine Lin⁻ BM: Insertions by LAM-PCR, Copy Number by Quantitative Real-Time PCR

L + 1 2 3 4 5 6 7 8 9 10 L + 11 12 13 14 15 16



L: 100bp Ladder
+: Lentivirus B-cell
line with 1 provirus

Estimated Provirus Copy Number: 1 - - - 2 1 - 2 - 3 - 1 - - - - 1 -

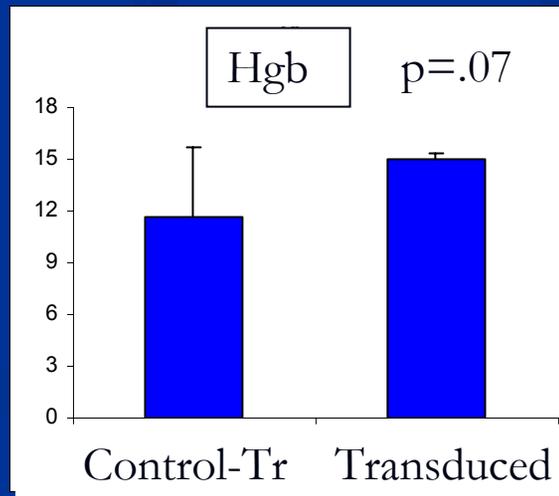
Estimated Transduction Efficiency of CFC: $5/16 = 31\%$

Integrations/cell = 2 with one, 2 with two, 1 with three. Average = 1.8

Separate Expt: Quant real time PCR: 1.6 copies/cell

TRANSPLANT

- Transduced cells infused in *Fanca*^{-/-} mice
- Cyclophosphamide at day 7
- CBC at 3 weeks post cyclophosphamide



Primary Objective

- To determine the safety of lentiviral gene transfer for patients with Fanconi anemia complementation group A.

Secondary Objectives

1. To determine the feasibility of collection of the number of CD34+ cells from Fanconi anemia complementation group A patients that would be expected to have potential for therapeutic benefit after transduction and infusion.
2. To determine the transduction efficiency for human CD34+ cells transduced with a clinical grade lentiviral vector encoding the gene for Fanconi anemia complementation group A.
3. To determine if the clinical grade transduction will result in phenotypic correction of gene modified cells by in vitro assays.
4. To determine if infusion of FANCA gene-modified cells will result in engraftment and improvement in blood counts in FA patients.

Inclusion Criteria

1. FA diagnosis by chromosome fragility
2. FA complementation group A
3. Normal BM cytogenetics and negative FISH for MDS panel
4. Age ≥ 6 years, weight > 10 kg
5. Informed consent; **Proposed: New Assent form**
6. Platelet count $> 20,000$, able to achieve a platelet count of $> 50,000$ with transfusion
7. Modified Lansky Play-Performance Score or Karnovsky score of $\geq 70\%$.
8. Suitable haploidentical donor or unrelated donor
(**patients will be eligible for allo tx to follow**)

Exclusion Criteria

1. Malignancy other than minor cancer
2. Myelodysplastic syndrome, beyond RA
3. Pregnancy or lactation.
4. Investigational drug
5. Physical or emotional status that would prevent informed consent, protocol compliance, or adequate follow-up
6. HLA matched sibling donor for transplant
7. Significant associated diseases including HIV, uncontrolled HTN, unstable angina, CHF, poorly controlled DM, coronary angioplasty within 6 months, MI within the last 6 months, uncontrolled cardiac arrhythmia.
8. Active ongoing viral, bacterial, or fungal infection.

Procedures for Cell Collection

- G-CSF 16 $\mu\text{g}/\text{kg}$ sc bid
- Proposed: AMD 3100 4th day, \pm 5th day
- If CD34+ $\geq 5/\text{mcl}$, then apheresis days 4,5
- If CD34+ $< 5/\text{mcl}$, then BM harvest
- If apheresis collection < 2 million CD34+/ kg , then BM harvest

Laboratory procedures in GMP facility

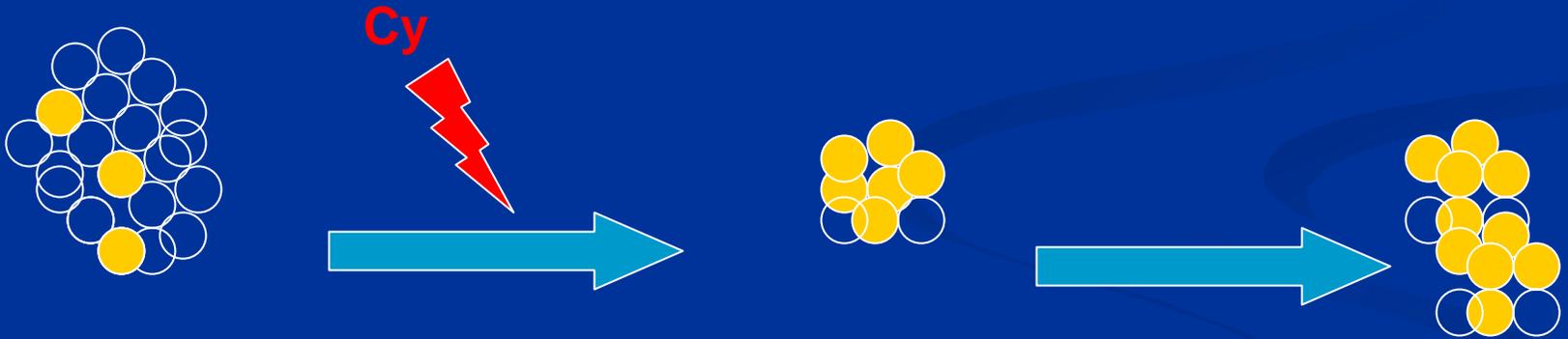
- Progenitor cell enrichment (CD34 selection or lineage depletion)
- Overnight transduction in cytokines on recombinant fibronectin peptide CH-296
- Microbiological testing, endotoxin assay, RCL testing to follow

Gene modified cell infusion

- ~~Preconditioning d-1: Cy 20 mg/kg IV~~
 - Proposed Amendment: No preconditioning for initial 3 pts
- Gene modified cell infusion target at least 1 million CD34+ cells/kg

Post infusion cyclophosphamide → ? Expansion of transduced cells

- 3 months post infusion, if <5% transduced cells, but detectable, then cyclophosphamide 10 mg/kg IV



Cyclophosphamide

- Use in Fanc mouse models
- Create “space”
- Wide use in FA patients as part of transplant preparative regimen
- Question whether this increases risk for MDS

Post Infusion Monitoring

- Blood counts
- Peripheral blood cells for quant PCR
- LAM-PCR for insertion site analysis
- Monitoring for clonal dominance
- Bone marrows-colony growth in mitomycin C, colony PCR, quant PCR
- Chemistry panel
- Replication competent lentivirus (RCL)
- Toxicity

Issues

- Low stem cell numbers in FA patients
- Difficulty harvesting stem cells by apheresis or marrow harvest
- Need for brief period of ex vivo culture to maximize stem cell survival and reduce chance of heme malignancy
- ? Utility of low oxygen conditions, reducing agents
- Post transplant risk of development of cancer

Rationale for Lentivirus for Fanconi anemia gene transfer

- No need for cytokine prestimulation
- Integration in non-cycling cells (quiescent stem cells)
- Reduced cell culture time (fragile FA cells)
- Improved engraftment (possibly)
- Improved location of insertions relative to transcription start sites
-Intro to Dr Kiem



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