A PHASE I/II, NON RANDOMIZED, MULTICENTER, OPEN-LABEL STUDY OF G1XCGD (LENTIVIRAL VECTOR TRANSDUCED CD34+ CELLS) IN PATIENTS WITH X-LINKED CHRONIC GRANULOMATOUS DISEASE

Peter Newburger U Mass Worcester

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Chronic Granulomatous Disease

First described, 1959, as “fatal” granulomatous disease

A Fatal Granulomatous Disease of Childhood

The Clinical, Pathological, and Laboratory Features of a New Syndrome

ROBERT A. BRIDGES, M.D.; HEINZ BERENDES, M.D., and ROBERT A. GOOD, M.D., Ph.D., Minneapolis

Am J Dis Child 1959; 97: 387-408

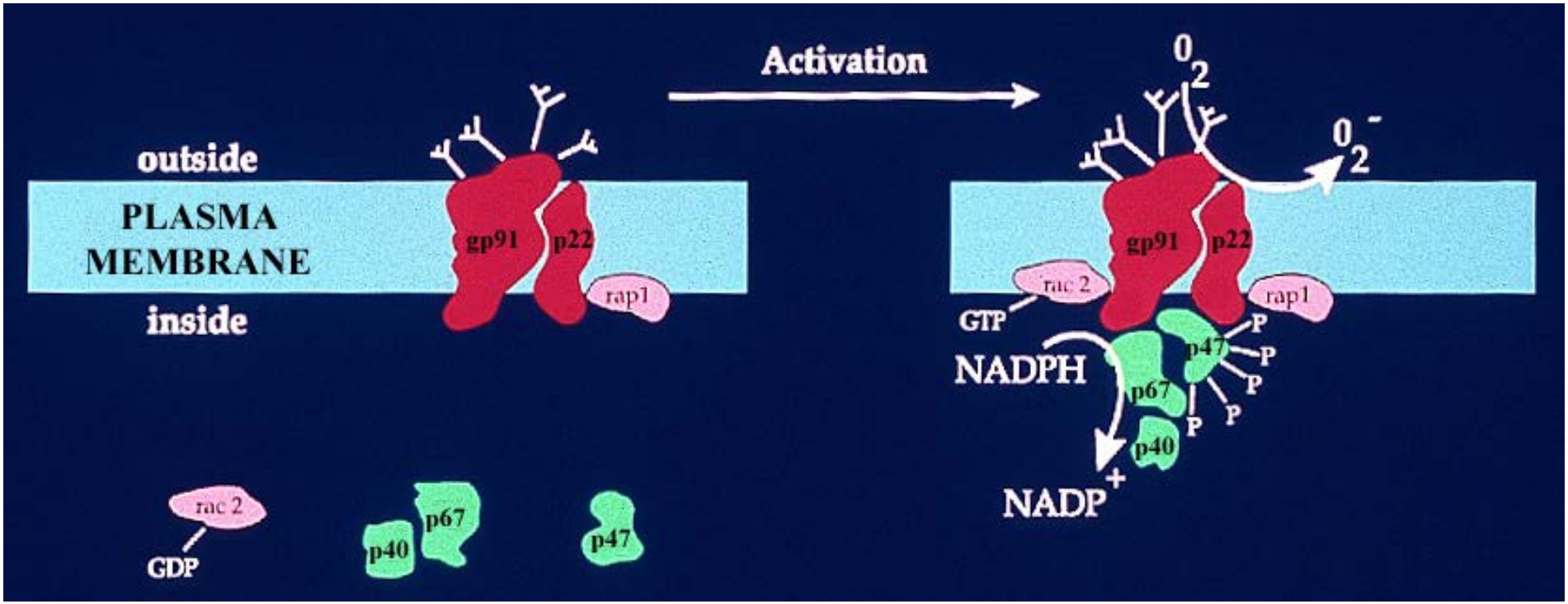
The Lancet · Saturday 4 June 1966

FATAL GRANULOMATOUS DISEASE OF CHILDHOOD

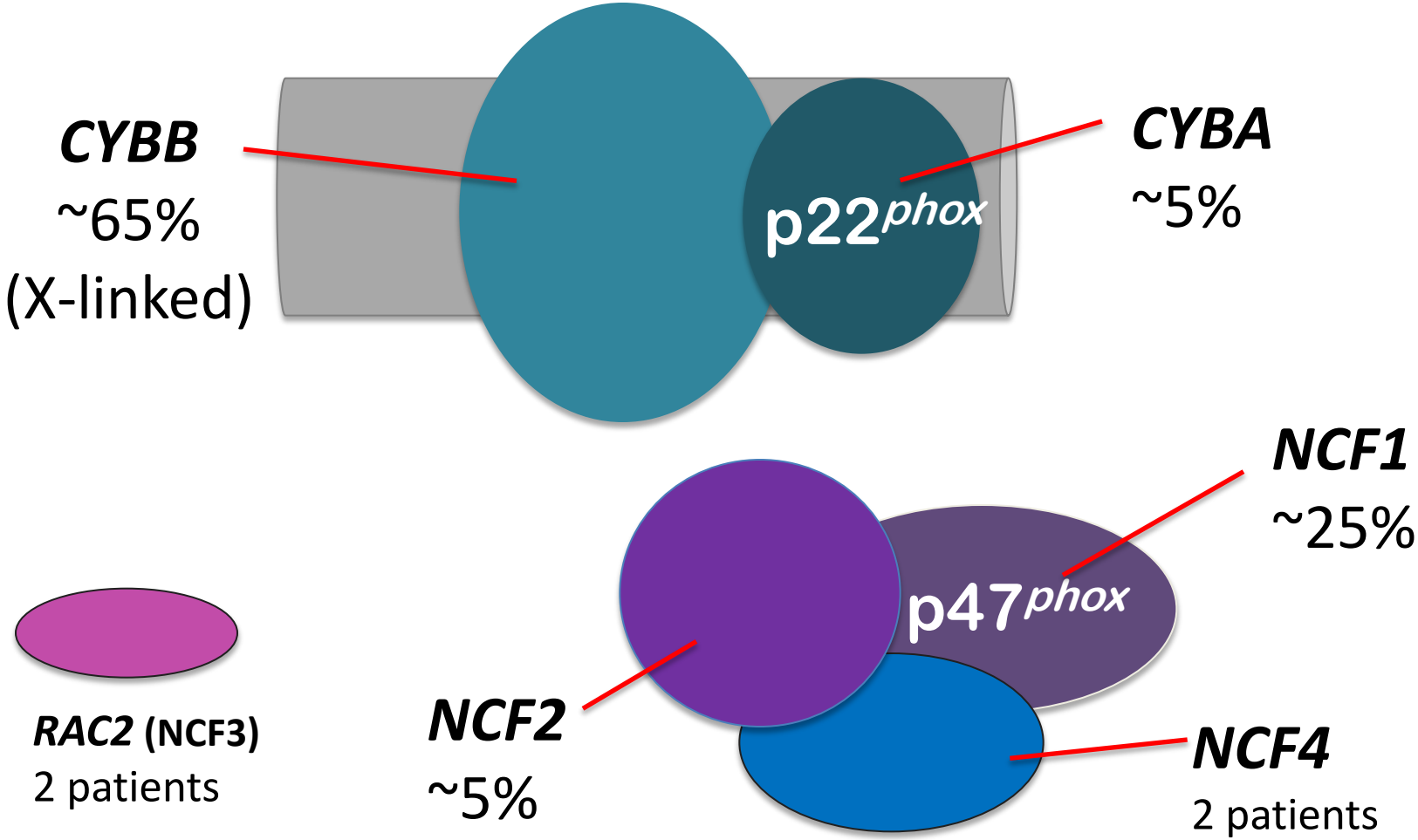
An Inborn Abnormality of Phagocytic Function

BEULAH HOLMES
PAUL G. QUIE
DOROTHY B. WINDHORST
ROBERT A. GOOD

Phagocyte NADPH Oxidase (NOX2)

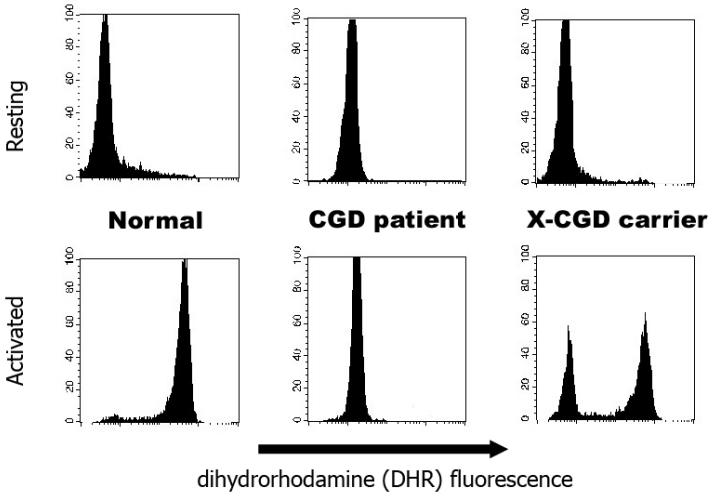


Genetic Defects in CGD



Diagnosis of CGD

- Assay of NADPH oxidase activity
  - DHR fluorescence (flow cytometry
  - Cytochrome c reduction
  - Chemiluminescence



- Genotyping

- Mean age at diagnosis:
  - X-linked 3.0 years
  - Autosomal recessive 7.8 years

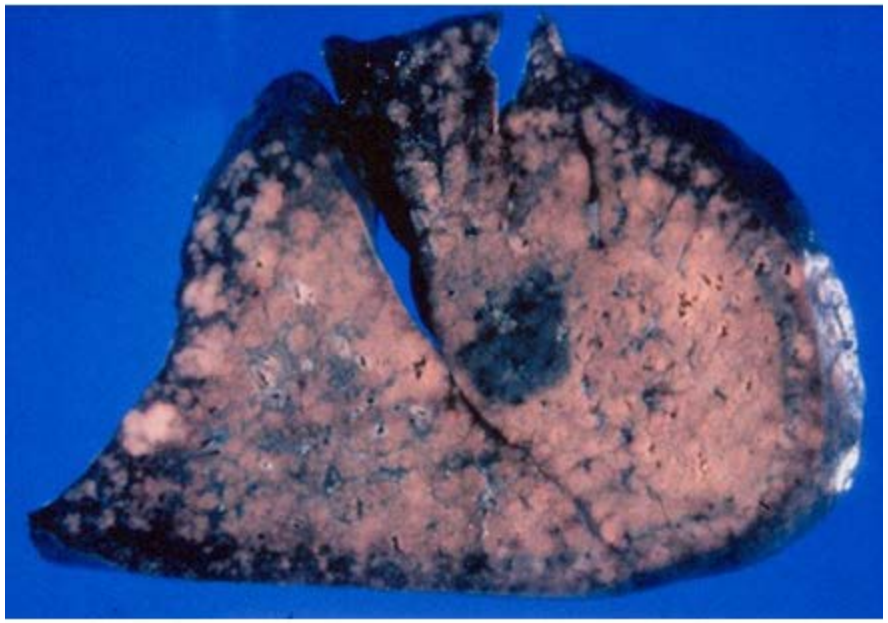
CGD National Registry data, *Medicine;* 2000

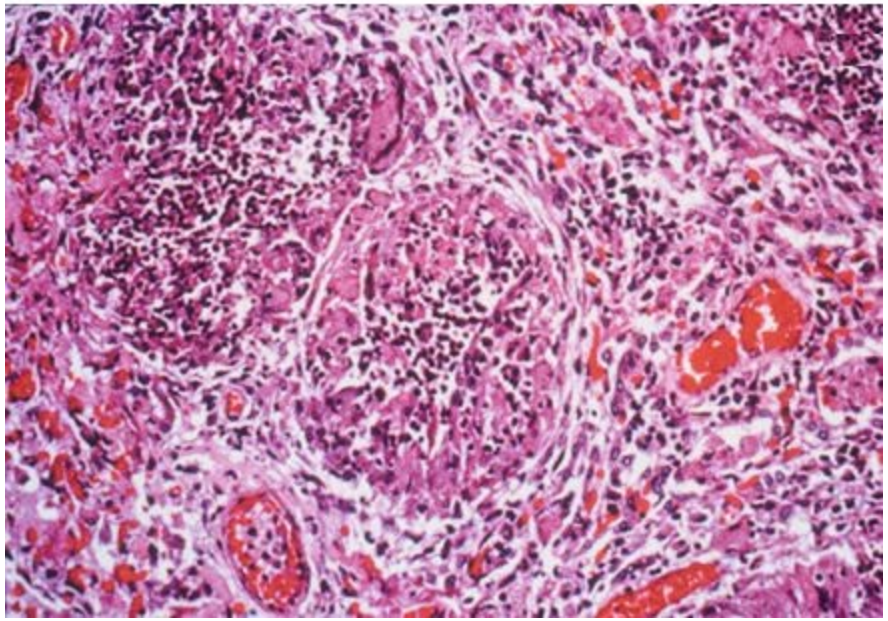
Clinical Features of CGD

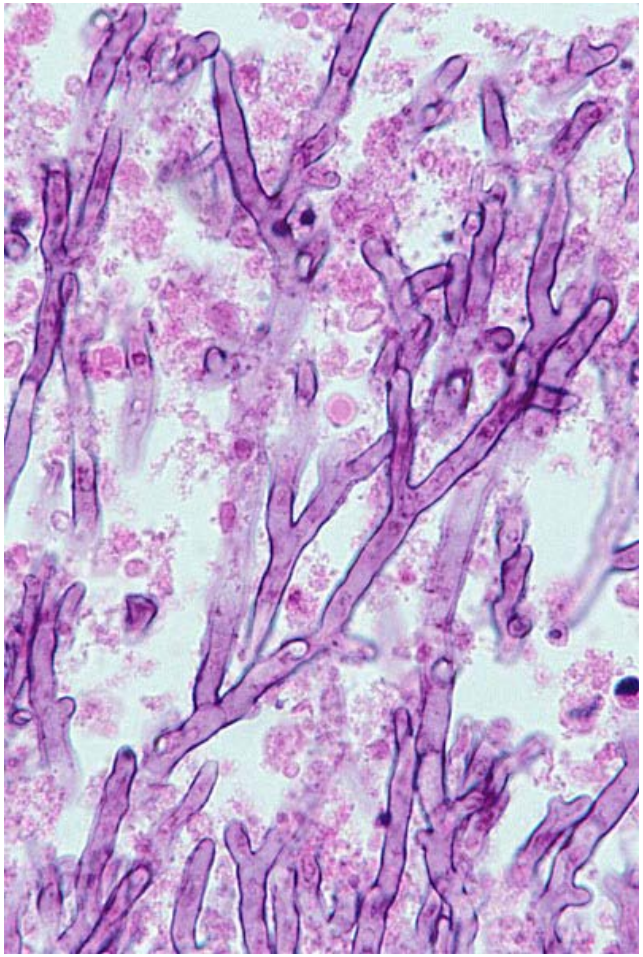
- Recurrent infections, with onset usually (not always!) early in life
- Single infections with opportunistic organisms
- Chronic inflammation:
  - Granulomatous colitis
  - Restrictive lung disease
  - Obstruction of gastric outlet, ureters
- Immune-mediated disease
  - Discoid lupus
  - Macrophage activation syndrome / hemophagocytic lymphohistiocytosis

Clinical Features of X-CGD Carriers

- Discoid lupus-like cutaneous lesions and other lupus-like symptoms
- Aphthous stomatitis
- **Severe or recurrent infections if Lyon distribution is skewed to <10% active neutrophils**

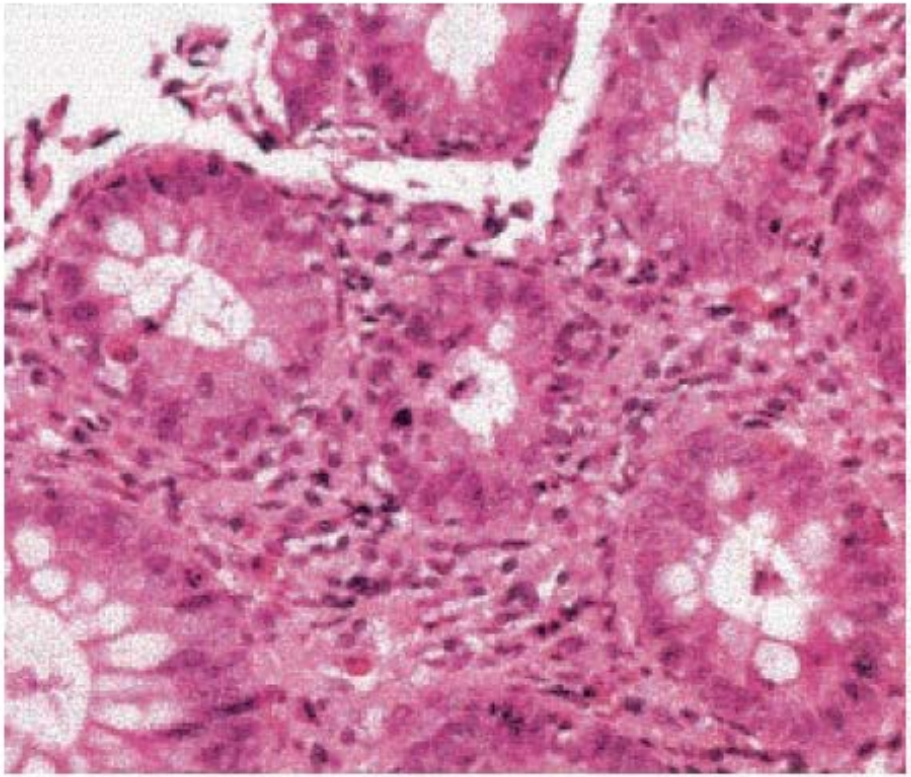




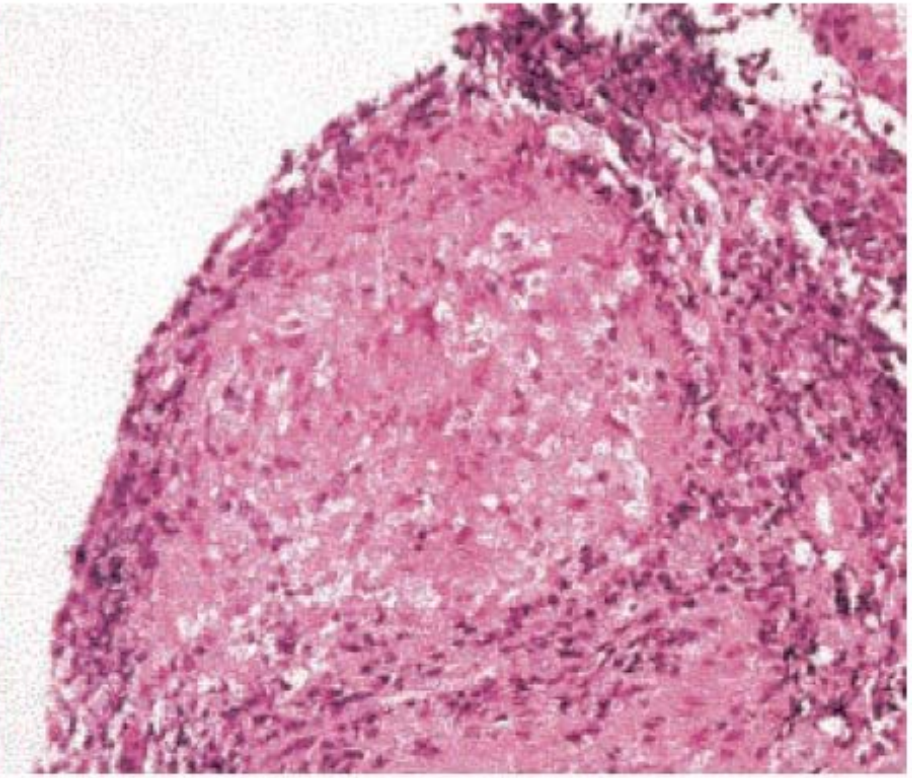


Fulminant mulch pneumonitis: an emergency presentation of chronic granulomatous disease. *Clin Infect Dis* 2007; 45:673-81

Granulomatous Colitis in CGD



Focal cryptitis

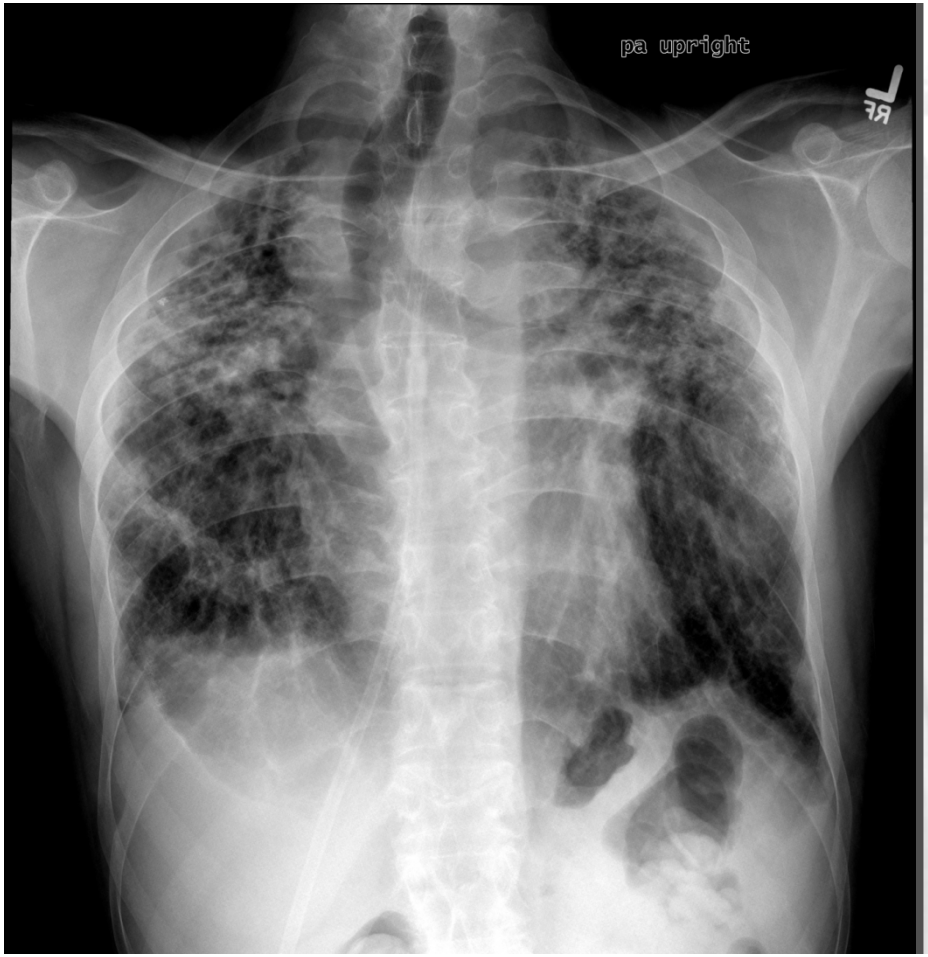


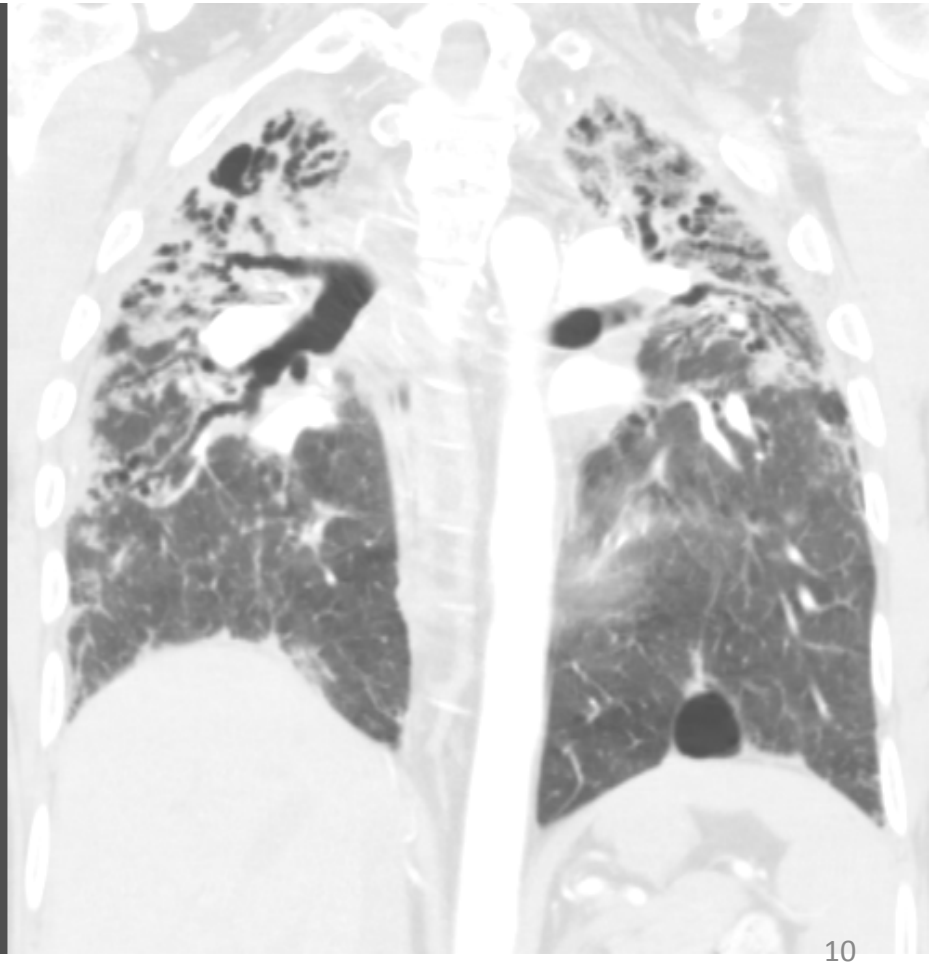
Nonnecrotizing granuloma

Gastrointestinal involvement CGD.
Pediatrics 2004; 114: 462-68

Chronic lung disease in CGD

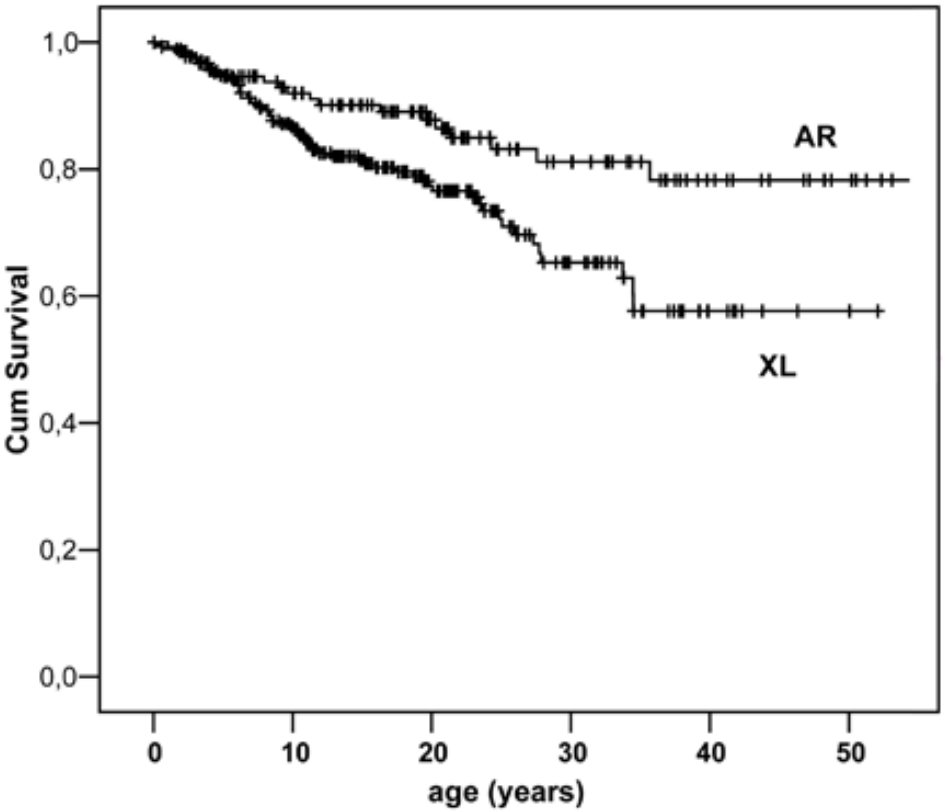
34 y.o. male with X-linked CGD, S/P multiple bacterial and Nocardial pneumonias.
Vital capacity 20% predicted; under evaluation for lung transplant (?with tandem HSCT).





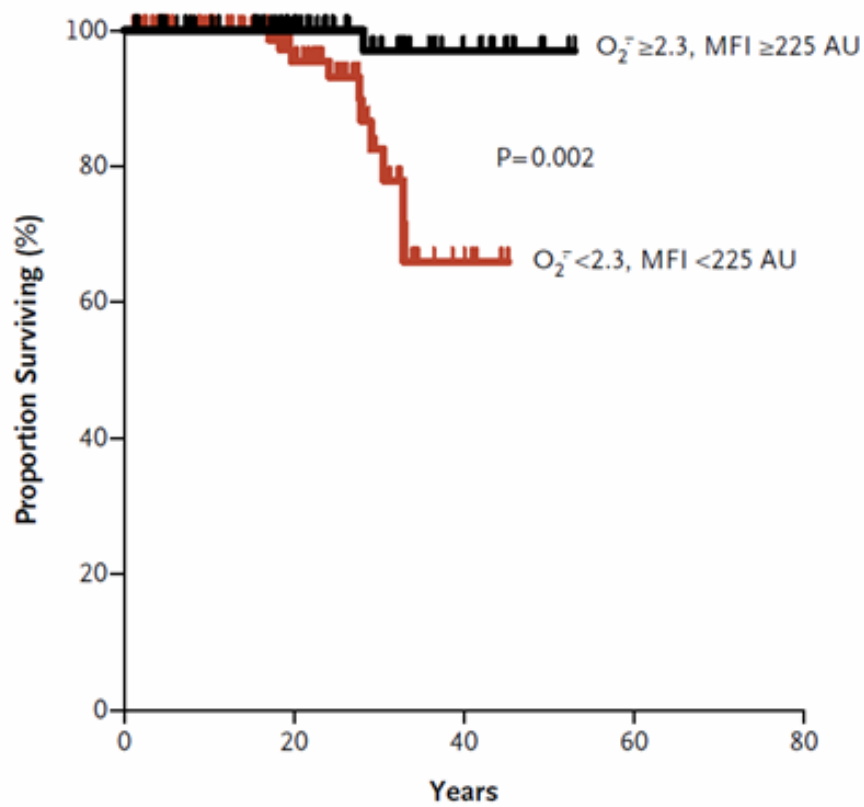
Cumulative Survival in CGD

Stratified by inheritance pattern



van den Berg et al. Chronic Granulomatous Disease: The European Experience. *PLoS ONE* 2009;4:e5234

Stratified by residual oxidase activity



Kuhns et al, Residual NADPH Oxidase and Survival in Chronic Granulomatous Disease NEJM 2010; 63:2600

Treatment of CGD

- Infection: prophylaxis
  - TMP/sulfa
  - Itraconazole
  - Interferon gamma
- Infection: therapy
  - Aggressive diagnostic approach
  - Prolonged courses of antibiotics
- Granulomatous complications:
  - Steroids (with caution!)
- Definitive:
  - HSCT
  - Gene therapy

Barriers to Transplantation

- Finding a donor
  - Matched unaffected related donor available for 10-15% of patients
  - Matched (10/10) unrelated donor available for ~60% of patients of European descent

    … much lower for some other ethnic groups
- Severe infection or chronic lung disease
- Socioeconomic and insurance issues

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Summary of Gene Therapy Trials for X-CGD

Center	Patients treated	Total Conditioning	Vector type used[#]	% Transduction efficiency°	Total dose of infused CD34+ cells per kg	Significant Engraftment > 3 months	Initial Clinical Benefit	Genotoxicity	Ref
Frankfurt	2	Liposomal busulfan 8.0 mg/kg	SF71gp91^{phox} (SFFV-LTR)	P1: 45.0% P2: 39.5%	P1: 11.3 × 10^6 P2: 9.0 × 10^6	15% gene marking in CD15$^+$ cells	Yes	Both patients developed clonal myeloproliferation and MDS with monosomy 7	Stein et al. (38) Ott et al. (40)
Zürich	2	Liposomal busulfan 7.0-8.8 mg/kg	SF71gp91^{phox} (SFFV-LTR)	P1: 25.3% P2: 32.7%	P1: 19.0 × 10^6 P2: 8.0 × 10^6	20% gene marking in CD15$^+$ cells	Yes	Development of clonal myeloproliferation	Bianchi et al. (39) and R. Seger, personal communication
London	1	Melphalan 140 mg/m^2	MFGS-gp91^{phox} (MLV-LTR)	5-20%	0.2 – 10 x 10^6	No	Yes	No	A. Thrasher personal communication
	3		SF71gp91^{phox} (SFFV-LTR)						
NIH	3	Busulfex 10 mg/kg	MFGS-gp91^{phox} (MLV-LTR)	25% - 73%	18.9 – 71.0 × 10^6	No	Yes	No	Kang et al. (37)
Seoul	2	Busulfex 6.4 mg/kg + Fludarabine 120 mg/m^2	MT-gp91^{phox} (MLV-LTR)	P1: 10.5% P2: 28.5%	P1: 5.4 × 10^6 P2: 5.8 × 10^6	No	Yes	No	Kim et al. (44)

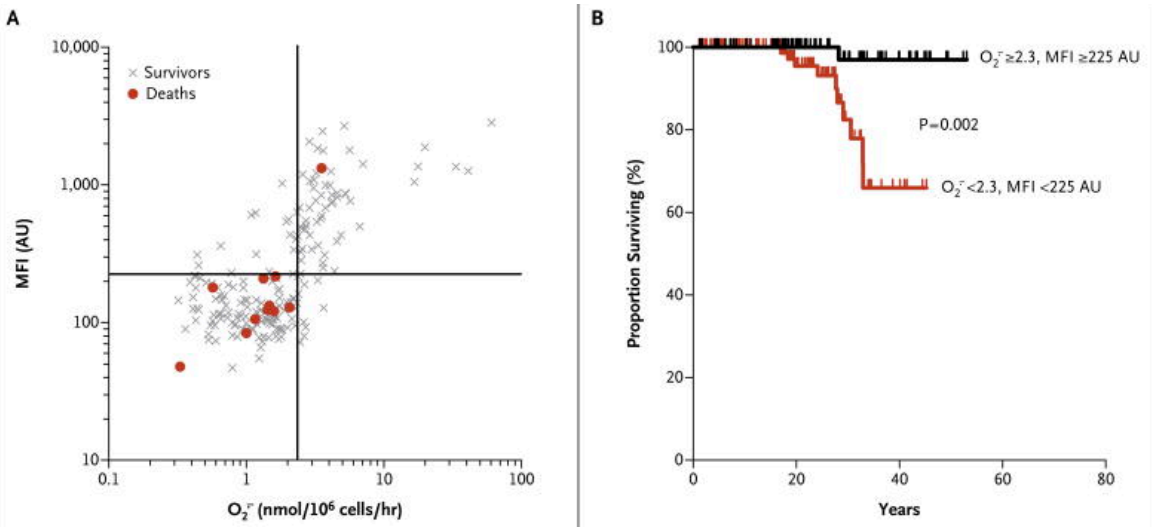
° Transduction efficiencies were measured either by the analysis of gp91phox cell surface expression using a FITC-labeled monoclonal murine anti-human antibody (7D5) and flow cytometry (Frankfurt, Zürich, London, Seoul), or by analysis of intracellular gp91phox expression using the 7D5 antibody (NIH).

[#]All vectors were pseudotyped with the GALV envelope and produced on PG13 packaging cells, except for MFGS-gp91phox vector which was produced in 293-producer cells using an amphotropic envelope.

Published in final edited form as:
N Engl J Med. 2010 December 30; 363(27): 2600–2610. doi:10.1056/NEJMoa1007097.

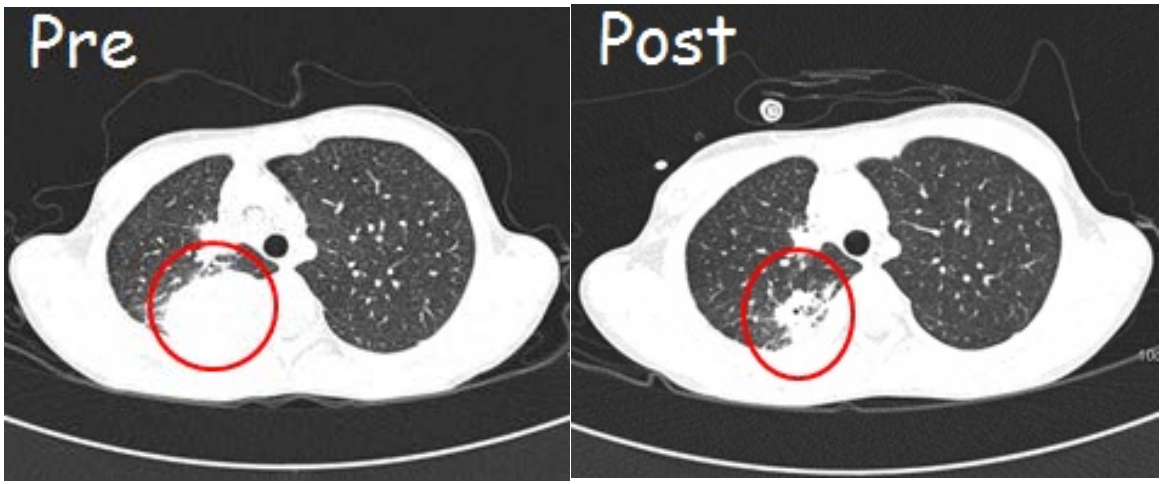
Residual NADPH Oxidase and Survival in Chronic Granulomatous Disease

Douglas B. Kuhns, Ph.D., W. Gregory Alvord, Ph.D., Theo Heller, M.B., Ch.B., Jordan J. Feld, M.D., M.P.H., Kristen M. Pike, M.S., Beatriz E. Marciano, M.D., Gulbu Uzel, M.D., Suk See DeRavin, M.D., Ph.D., Debra A. Long Priel, M.S., Benjamin P. Soule, M.D., Kol A. Zarember, Ph.D., Harry L. Malech, M.D., Steven M. Holland, M.D., and **John I. Gallin, M.D.**
Clinical Services Program (D.B.K., D.A.L.P.) and the Laboratory of Molecular Technology (K.M.P.), SAIC–Frederick, and the Biostatistical Consulting Division, Data Management Services (W.G.A.), NCI–Frederick — both in Frederick; and the Liver Diseases Branch, National Institute of Diabetes and Digestive and Kidney Diseases (T.H.), and the Laboratory of Clinical Infectious Diseases (B.E.M., G.U., S.M.H.) and the Laboratory of Host Defenses (S.S.D., B.P.S., K.A.Z., H.L.M., J.I.G.), National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda — all in Maryland; and Toronto Western Hospital Liver Centre–McLaughlin-Rotman Centre for Global Health, University of Toronto, Toronto (J.J.F.)

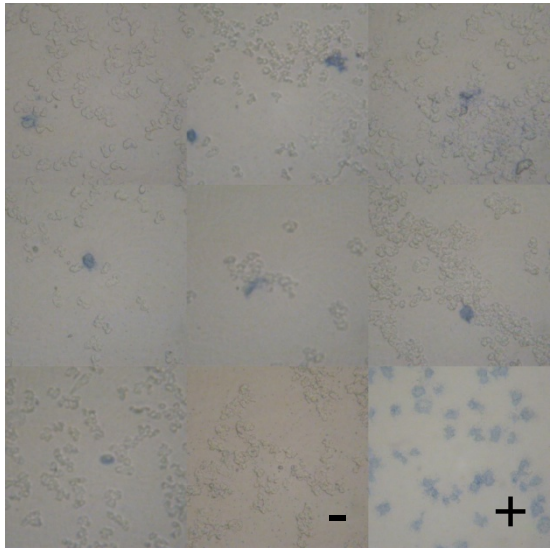


Normal subjects basal and PMA-stimulated superoxide production were 1.18±1.06 and 226.29±3.01 nmoles/10^6 cells/h.
Normal subjects basal and PMA-stimulated DHR ranges were 50-125 and 5,000-11,500.

P4:
CD34+ cell gene therapy for X-CGD incorporating melphalan conditioning (D-2)



Low frequency NBT positive PMNs



6 wks post GT

Transient therapeutic effects with mutagenesis in CGD....

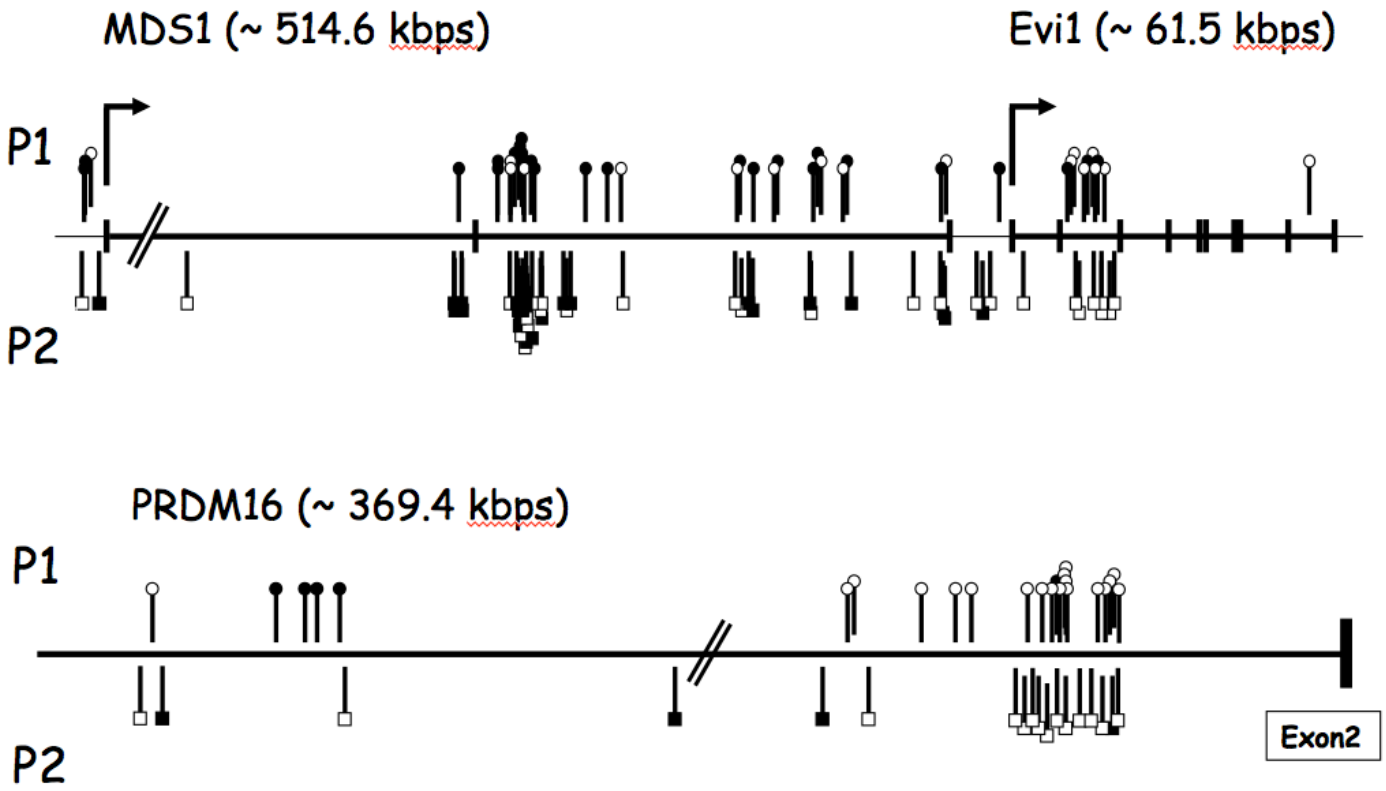
Article abstract

Nature Medicine **16**, 198 - 204 (2010)
Published online: 24 January 2010 | doi:10.1038/nm.2088

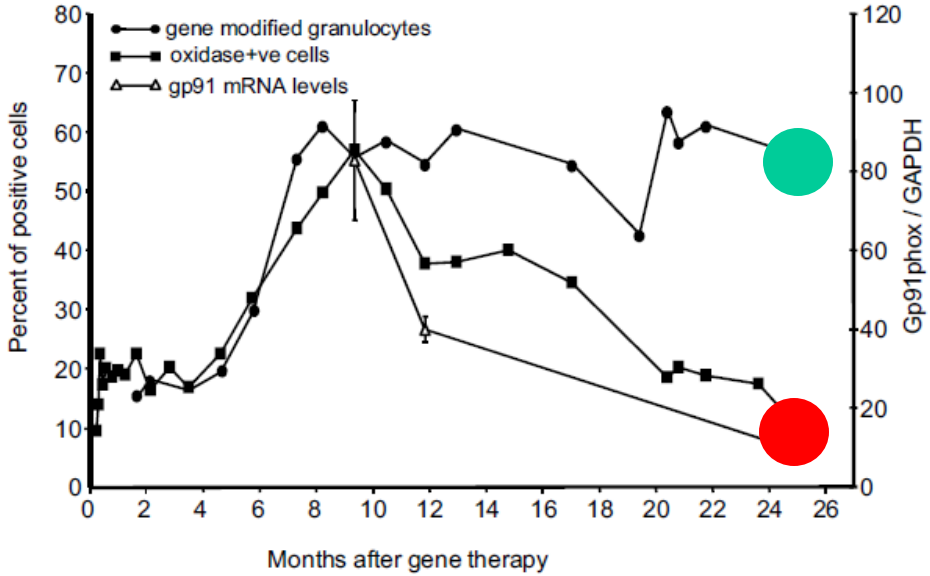
Genomic instability and myelodysplasia with monosomy 7 consequent to *EVI1* activation after gene therapy for chronic granulomatous disease

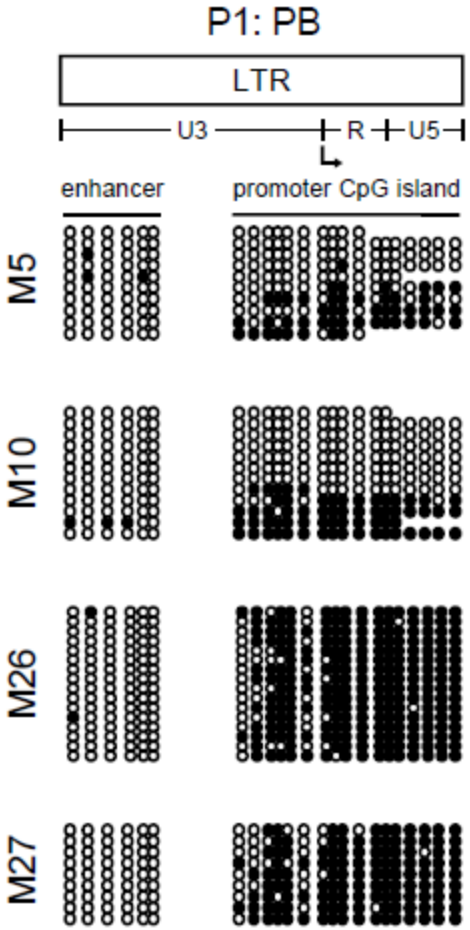
Stefan Stein[1,15], Marion G Ott[2,15], Stephan Schultze-Strasser[1], Anna Jauch[3], Barbara Burwinkel[4,5], Andrea Kinner[1], Manfred Schmidt[6], Alwin Krämer[7], Joachim Schwäble[2], Hanno Glimm[6], Ulrike Koehl[8], Carolin Preiss[1], Claudia Ball[6], Hans Martin[2], Gudrun Göhring[9], Kerstin Schwarzwaelder[6], Wolf-Karsten Hofmann[10], Kadin Karakaya[7], Sandrine Tchatchou[4,5], Rongxi Yang[4,5], Petra Reinecke[11], Klaus Kühlcke[12], Brigitte Schlegelberger[9], Adrian J Thrasher[13], Dieter Hoelzer[2,15], Reinhard Seger[14,15], Christof von Kalle[6,15] & Manuel Grez[1,15]

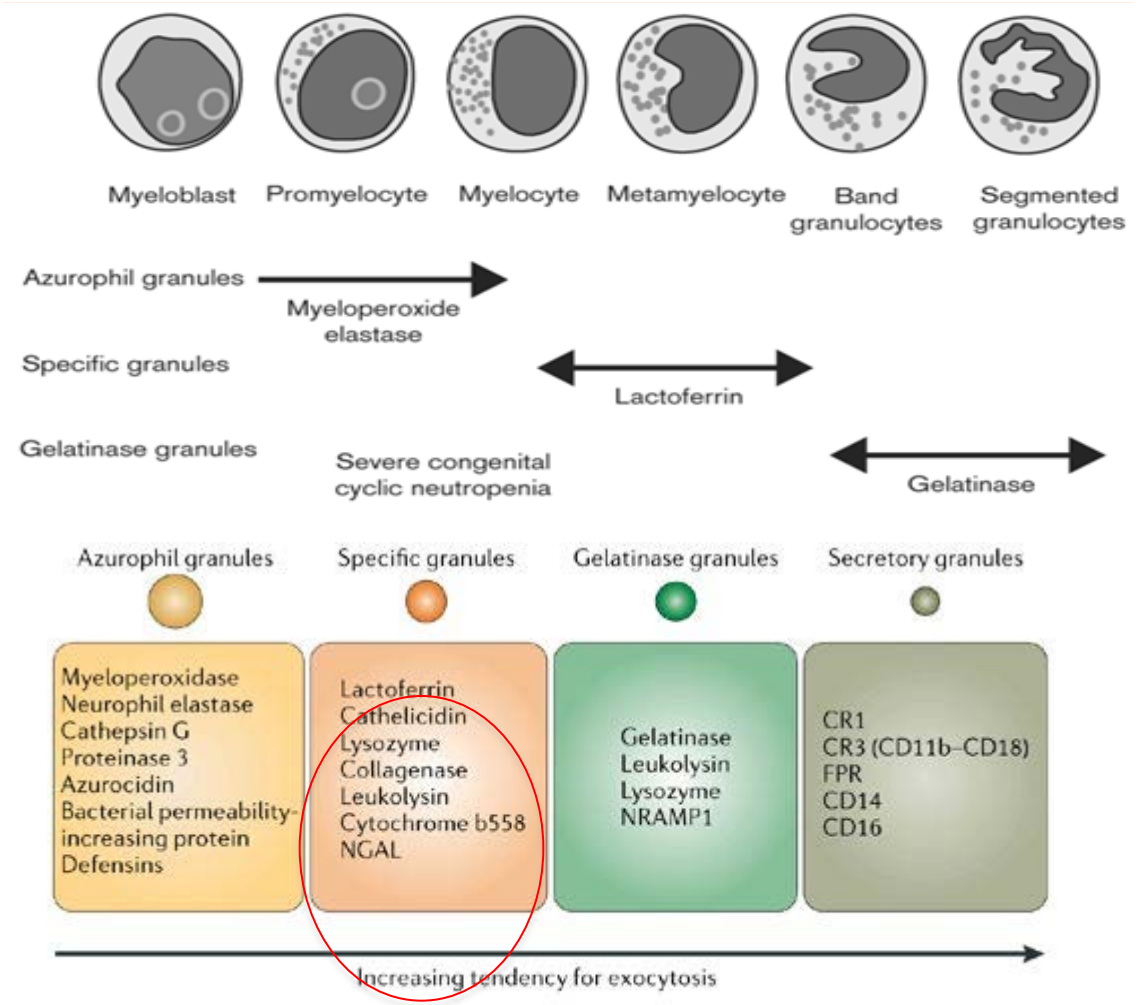
CIS within MDS/EVI1 and PRDM16…

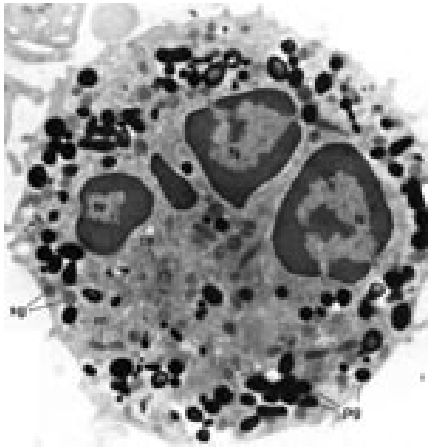


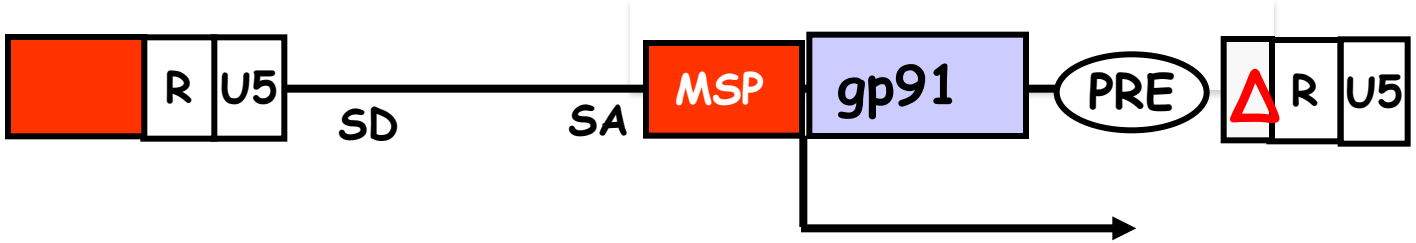
Vector silencing through LTR methylation....






Myeloblast
Promyelocyte
Myelocyte
Metamyelocyte
Band granulocytes
Segmented granulocytes
Azurophil granules
Myeloperoxide elastase
Specific granules
Lactoferrin
Gelatinase granules
Severe congenital cyclic neutropenia
Gelatinase
Azurophil granules
Specific granules
Gelatinase granules
Secretory granules
Myeloperoxidase
Neurophil elastase
Cathepsin G
Proteinase 3
Azurocidin
Bacterial permeability-increasing protein
Defensins
Lactoferrin
Cathelicidin
Lysozyme
Collagenase
Leukolysin
Cytochrome b558
NGAL
Gelatinase
Leukolysin
Lysozyme
NRAMP1
CR1
CR3 (CD11b–CD18)
FPR
CD14
CD16
Increasing tendency for exocytosis

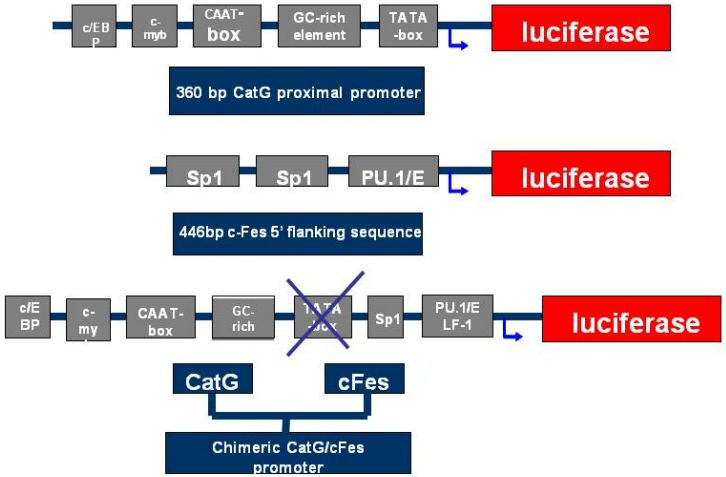


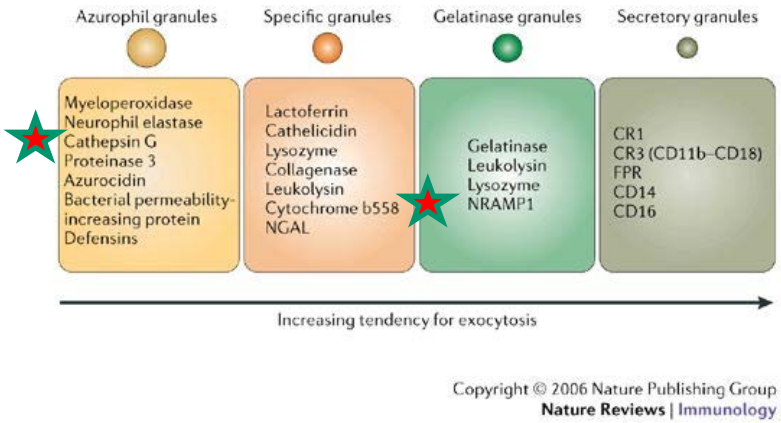

R
U5
SD
SA
MSP
gp91
PRE
Δ
R
U5

A novel chimeric promoter targets transgene expression to myeloid cells and efficiently corrects a murine model of X-CGD

Giorgia Santilli*[1], Elena Almarza*[2], Christian Brendel*[3], Chiara Beilin[1], Michael P. Blundell[1], Sneha Haria[1], Kathryn L Parsley[1], Christine Kinnon[1], Juan A Bueren[2], Manuel Grez[3] and Adrian J Thrasher[1]
•Equal contribution

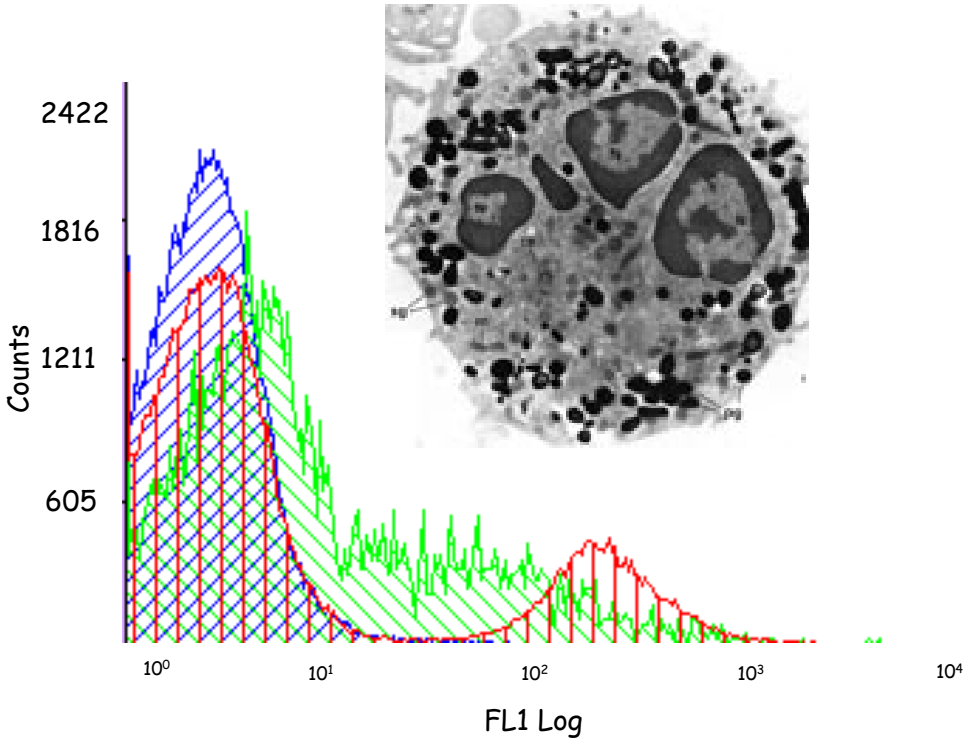
[1]Molecular Immunology Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK.
[2]Hematopoiesis and Gene Therapy Division, Centro de Investigaciones Energéticas,Medioambientales y Tecnológicas (CIEMAT), and Networking Center of Biomedical Research in Rare Diseases (CIBERER). Av. Complutense 22, 28040 Madrid, Spain.
[3]Institute for Biomedical Research, Georg-Speyer-Haus, Paul-Ehrlich-Strasse 42, 60596 Frankfurt, Germany.

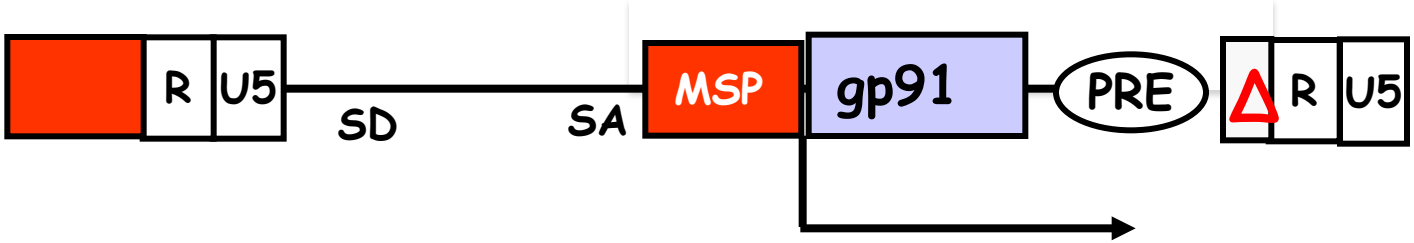




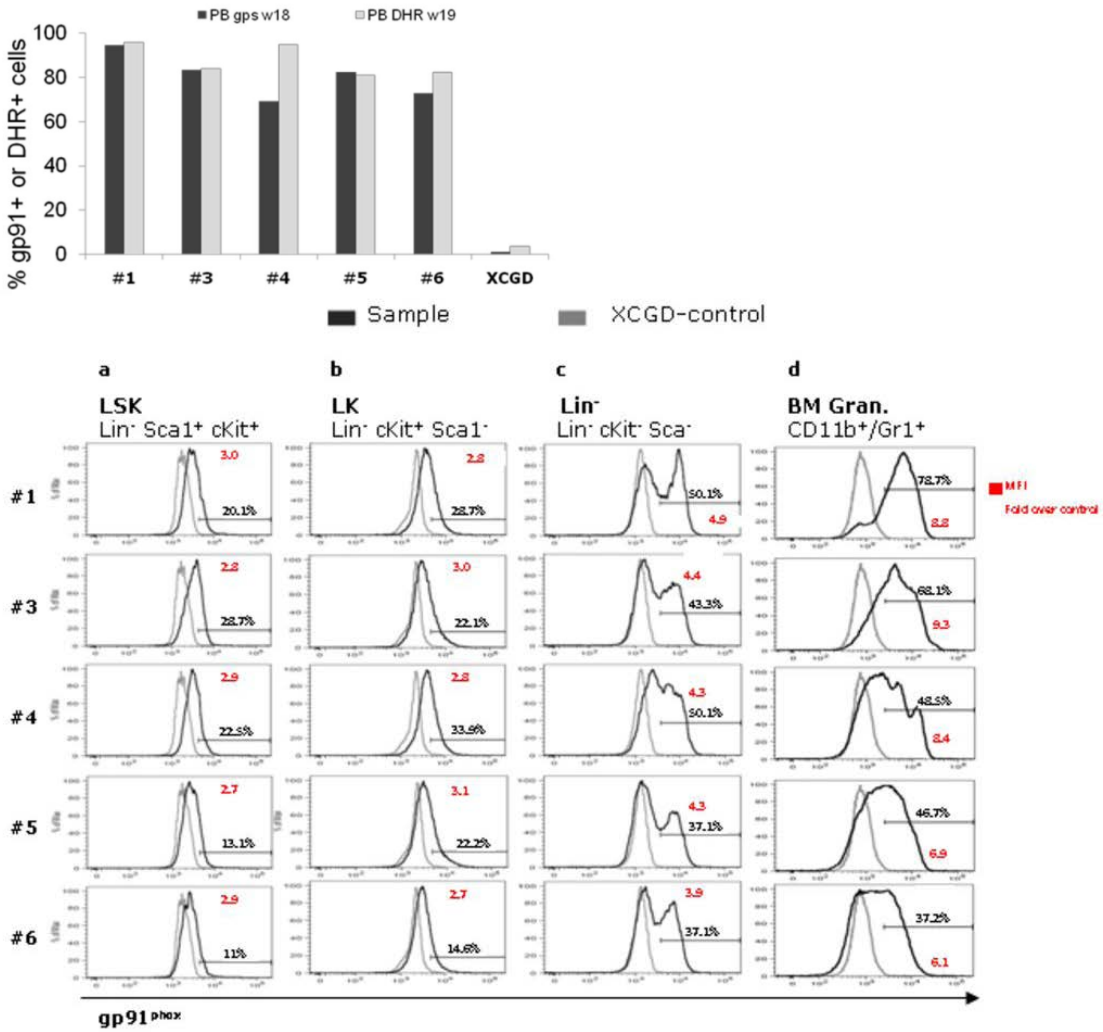
Molecular Therapy, 2011.

Expression induced on differentiation...





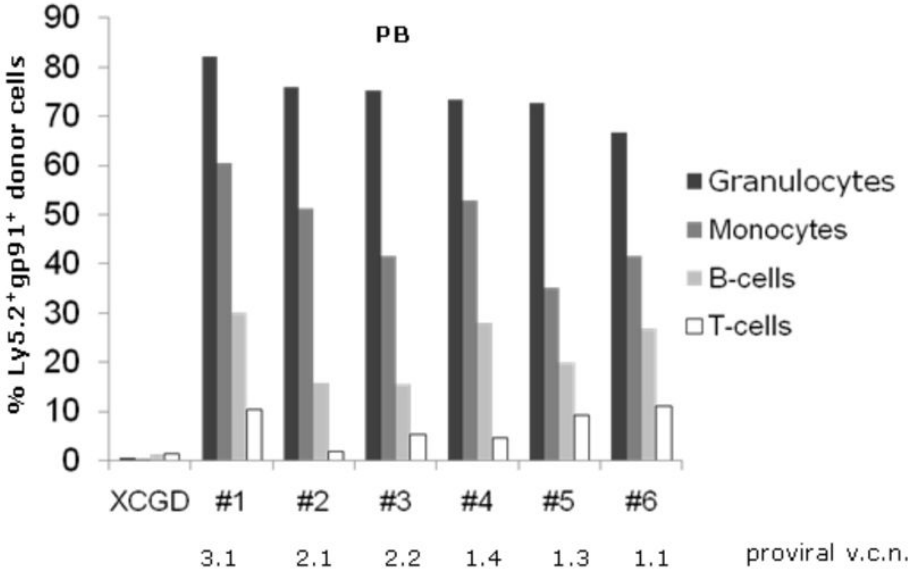
Transduced murine Lin- cells...

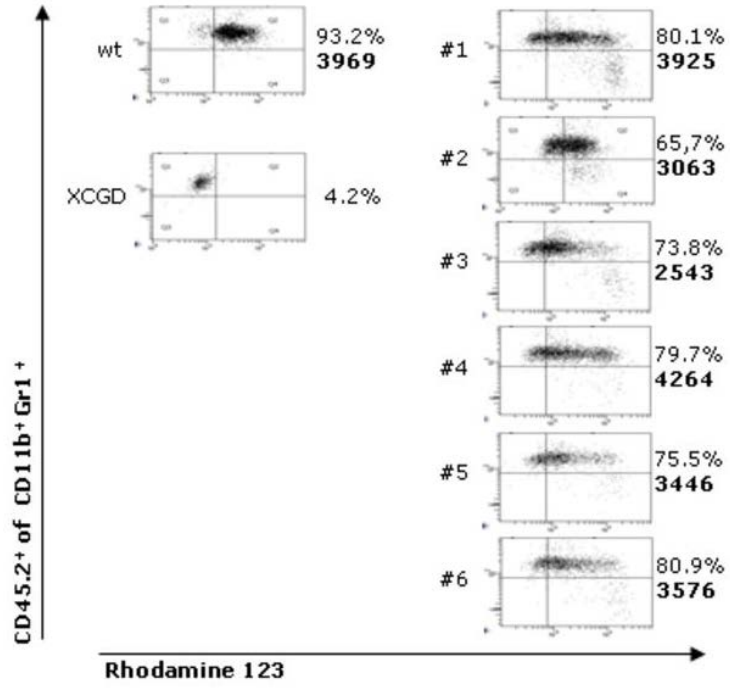


Bone marrow at >18 weeks

Transduced murine Lin- cells...

(Ly5.2 CGD into Ly5.1 WT)



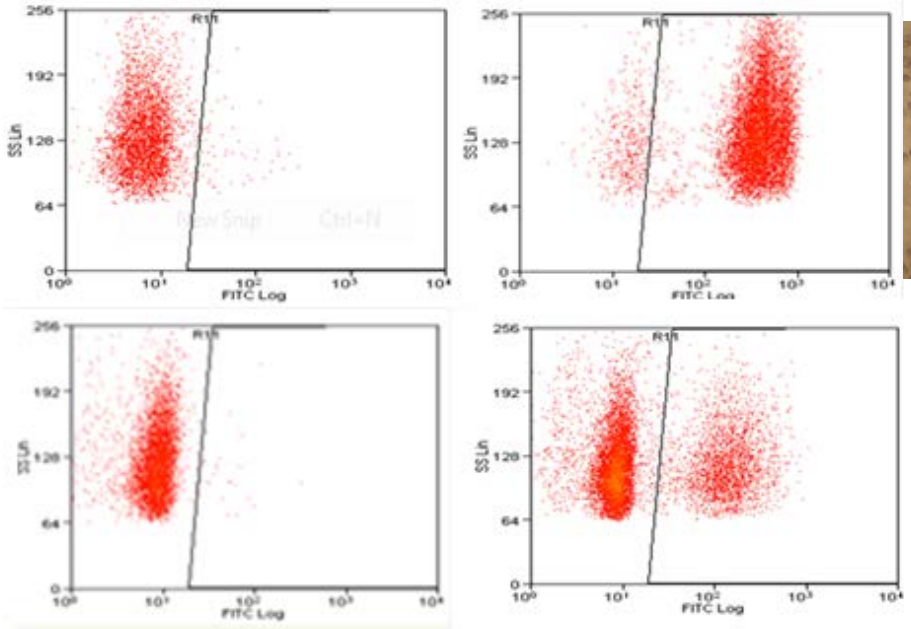


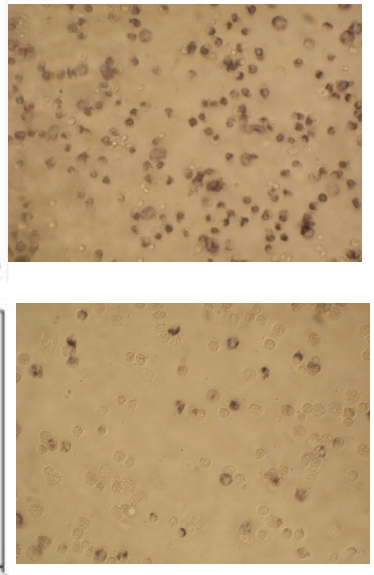
Peripheral blood at 9 weeks

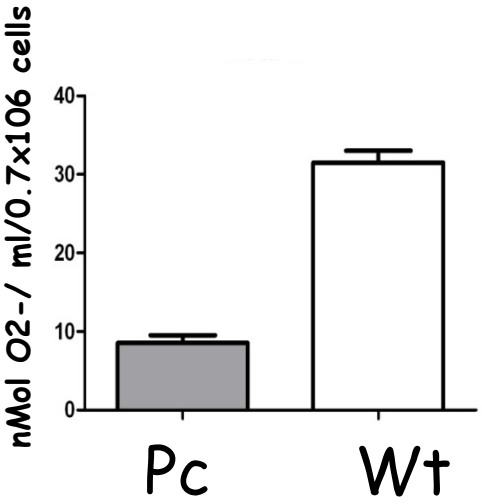
P1: Biochemical correction 14-21 days post gene therapy (low intensity conditioning)……

Wt

Pc

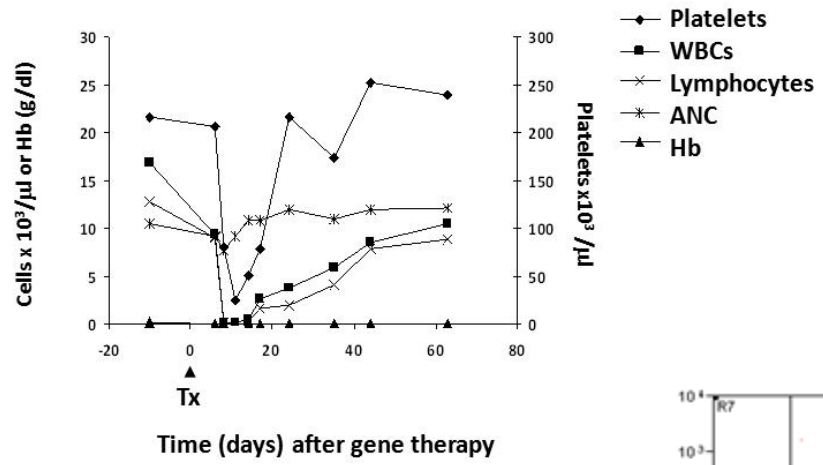


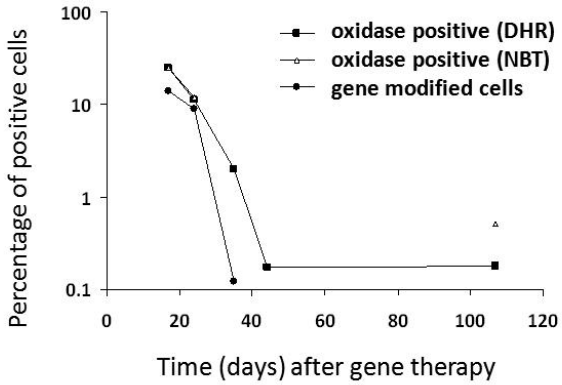


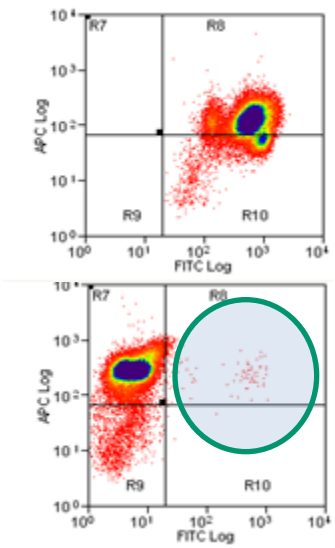


Polyclonal integration profiles d21 and d33

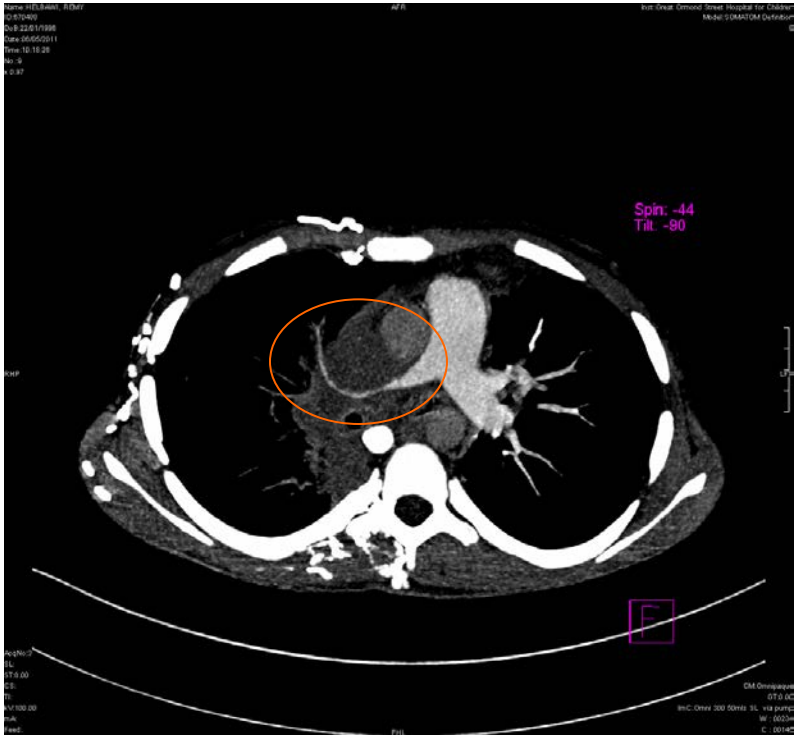
Biochemical correction 3mo post gene therapy……

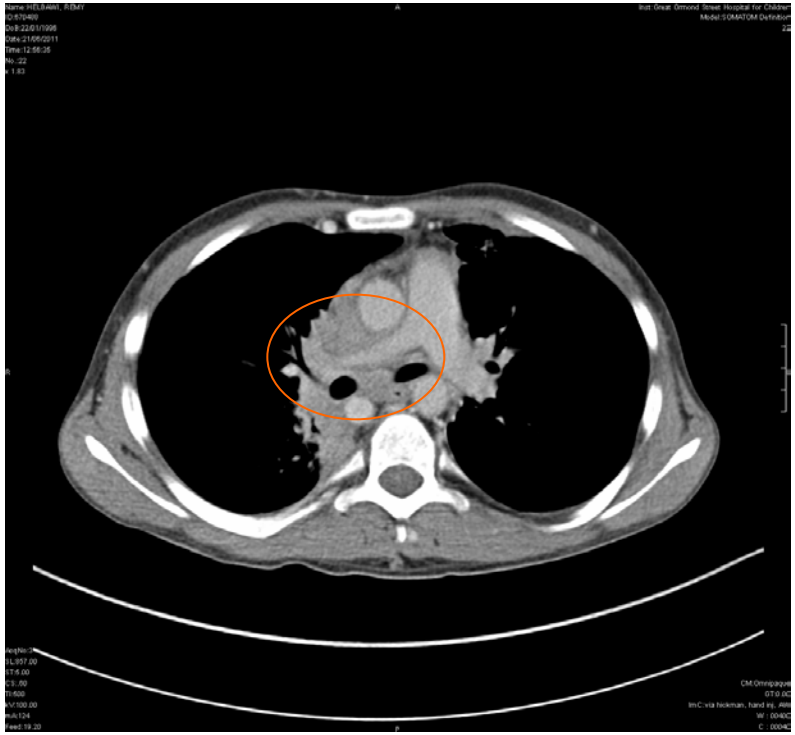




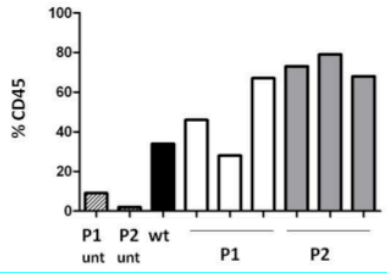


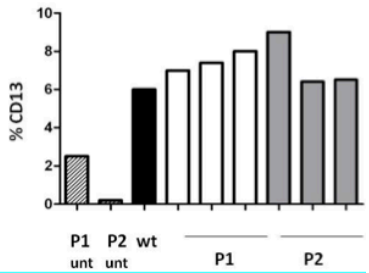
Clinical improvement……

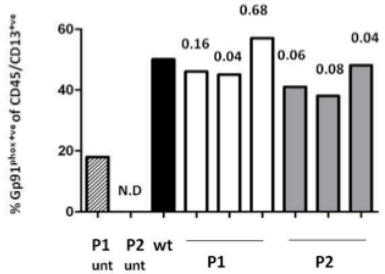


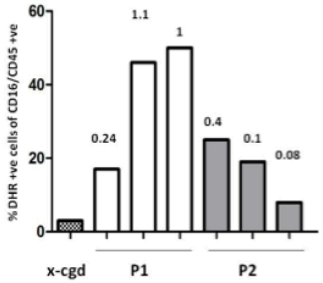


NSG engraftment (4mo post engraftment)……..

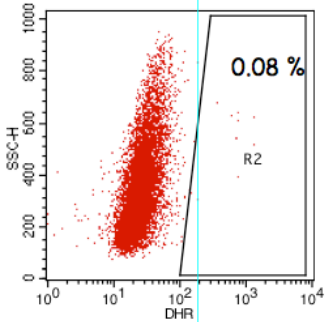








X-CGD Naïve



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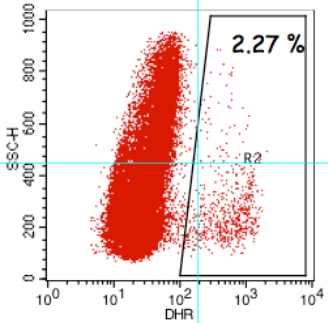
Normal



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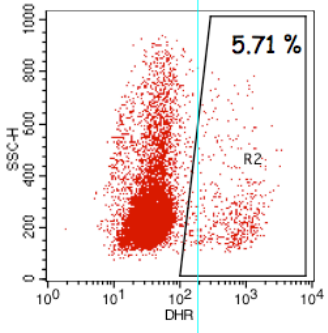
X-CGD
pCCL-Chimeric-gp91

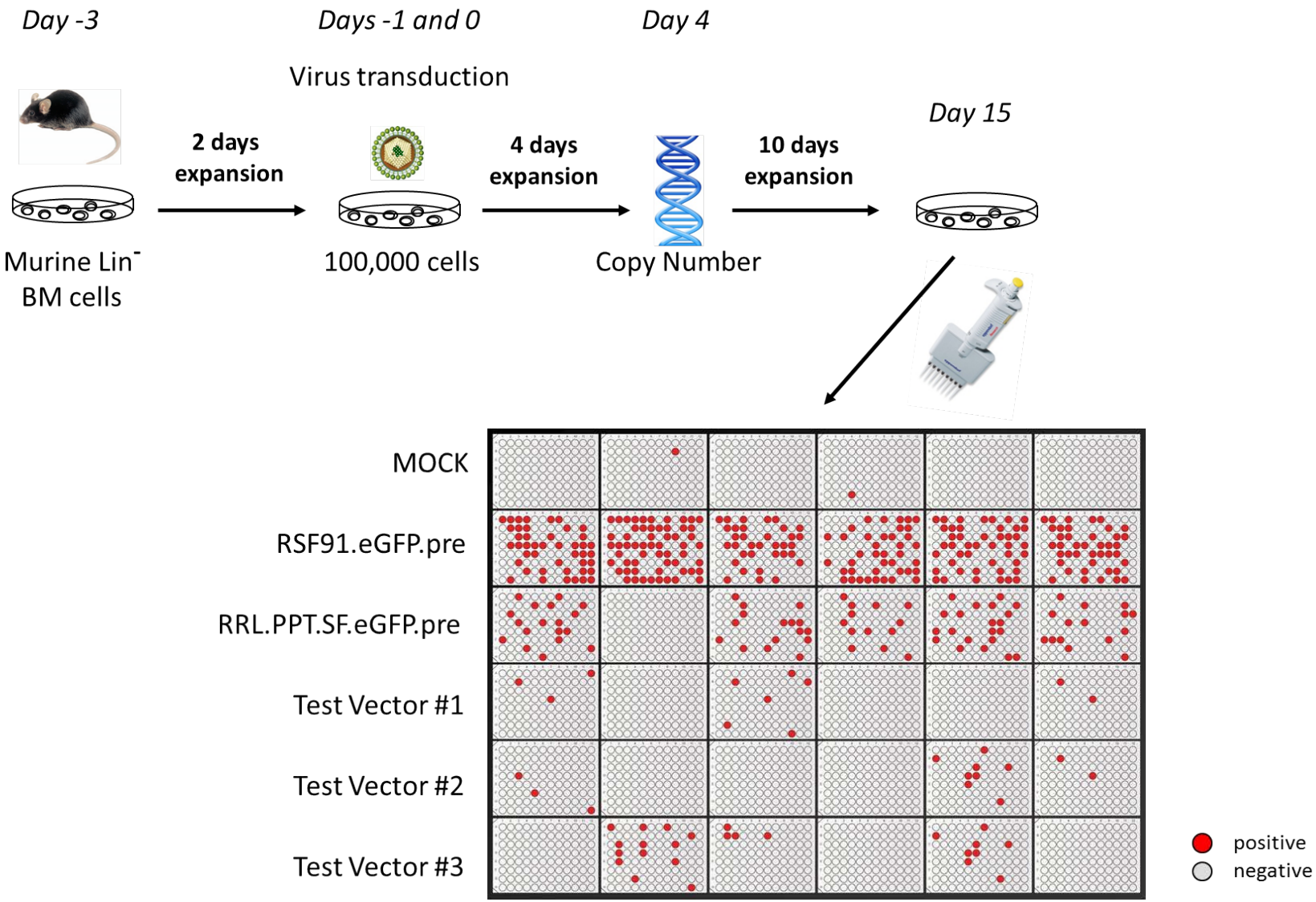


751

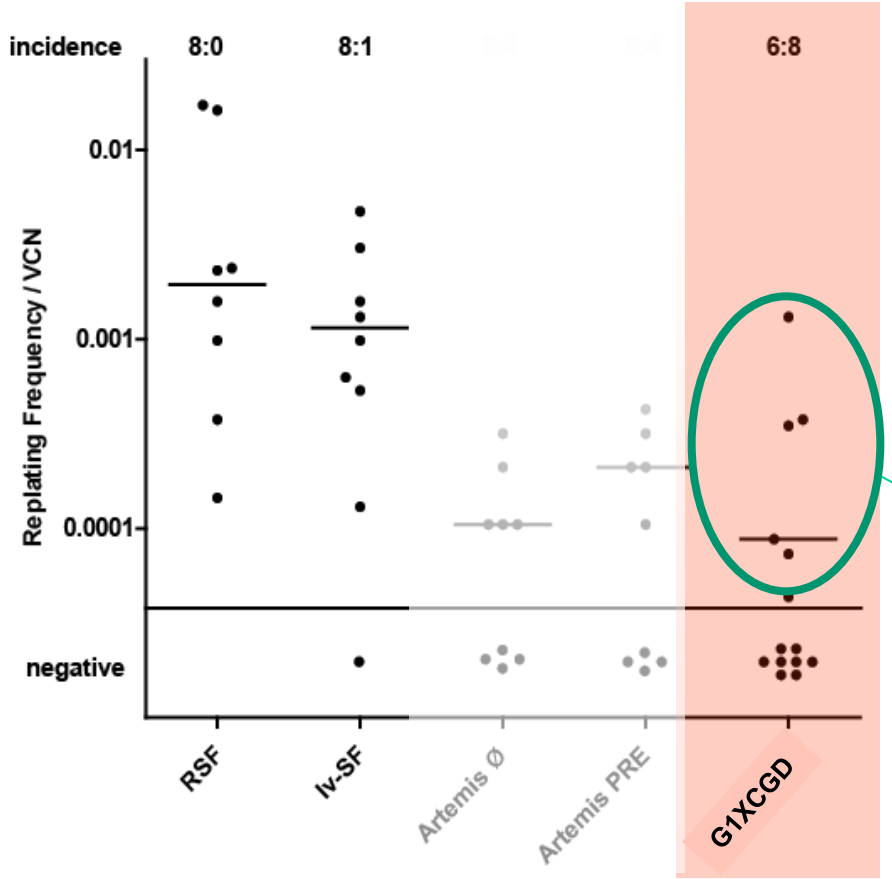
Preclinical genotoxicity assessment of vector G1XCGD in mice

Manuel Grez / Michael Rothe

IVIM assay workflow and analysis…



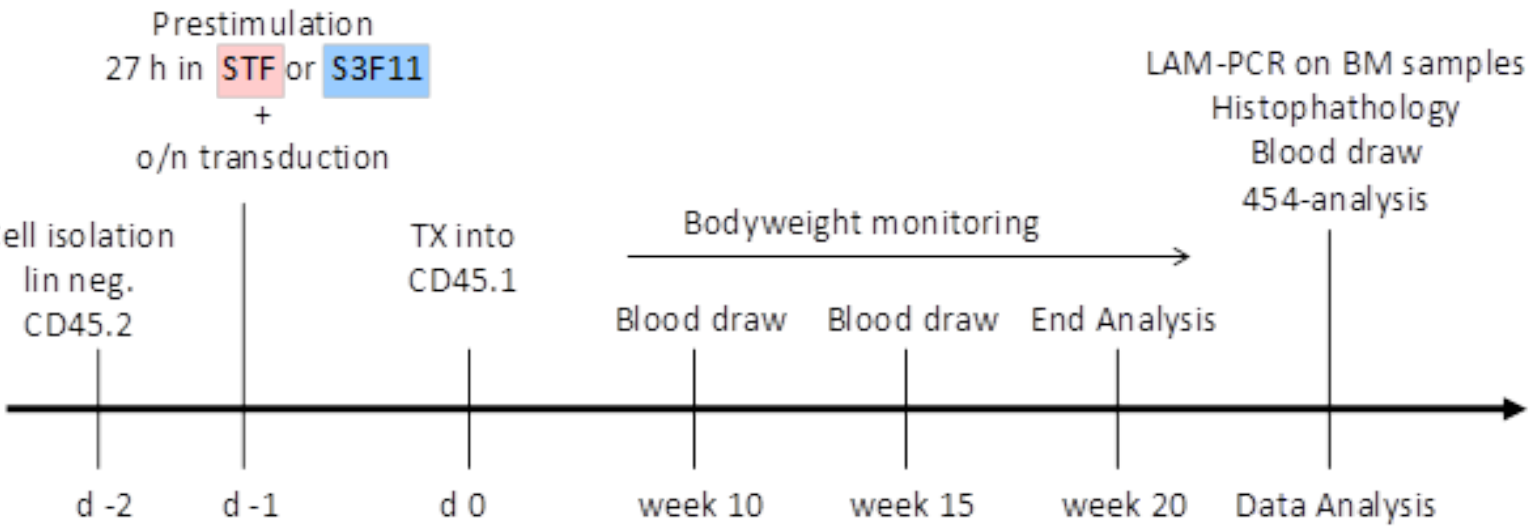
Reduced insertional mutagenesis…



Insertional Site Analysis
Mapable sequences: 3
Ccdc147 , *Scube1 and Zmat3*
No *Mds1* or Evi1 integrations

Low copy, low fitness

Outline for G1XCGD *in vivo* genotox study



Groups (R, L, G) of 7 animals for the **Test Vectors**:

eGFP marker

R-A: RV-SF in STF VCN 1

R-B: RV-SF in S3F11 VCN 1

eGFP marker

L-1: LV-SF in STF VCN 1

L-2: LV-SF in STF VCN 3-5

G-1: G1XCGD in STF VCN 1

G-2: G1XCGD in STF VCN 3-5

Controls:

M: Mock cells 5 animals

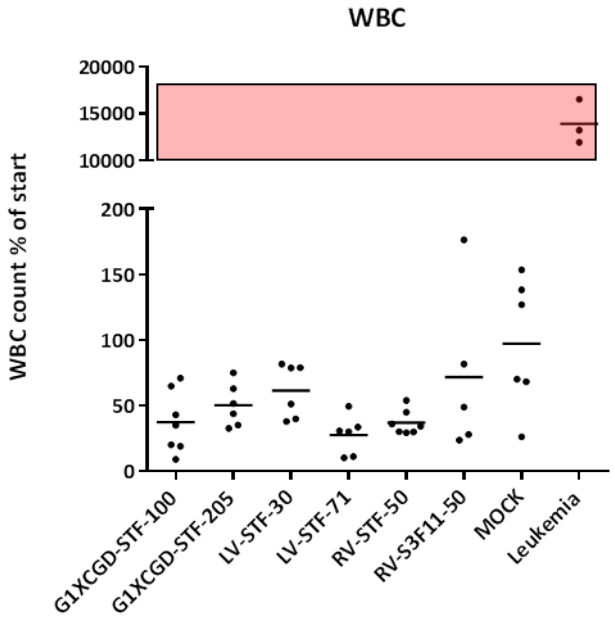
B: B-cell leukemia 3 animals
RV-SF induced

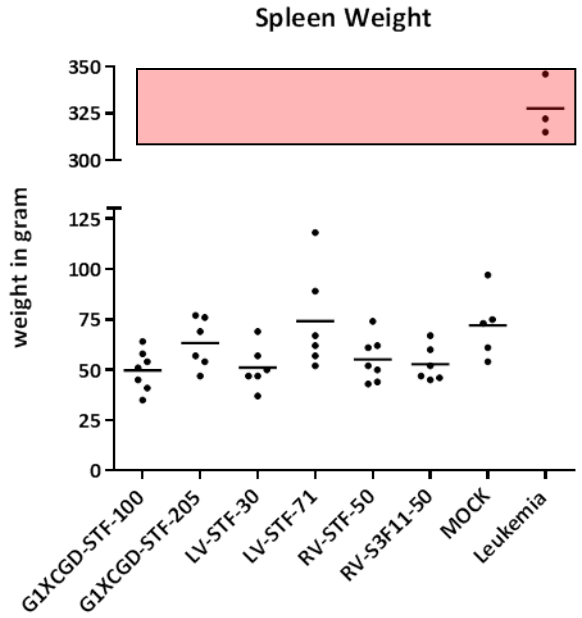
Prestimulation Conditions:

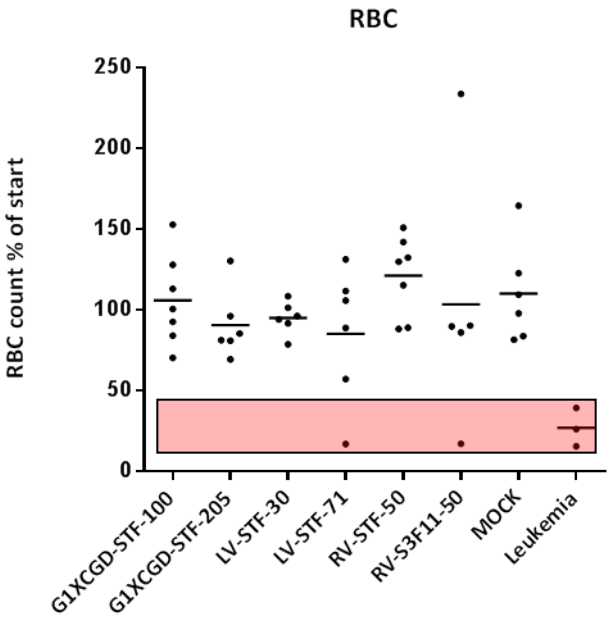
STF : 100 ng/ ml mSCF, hTPO and hFlt-3L

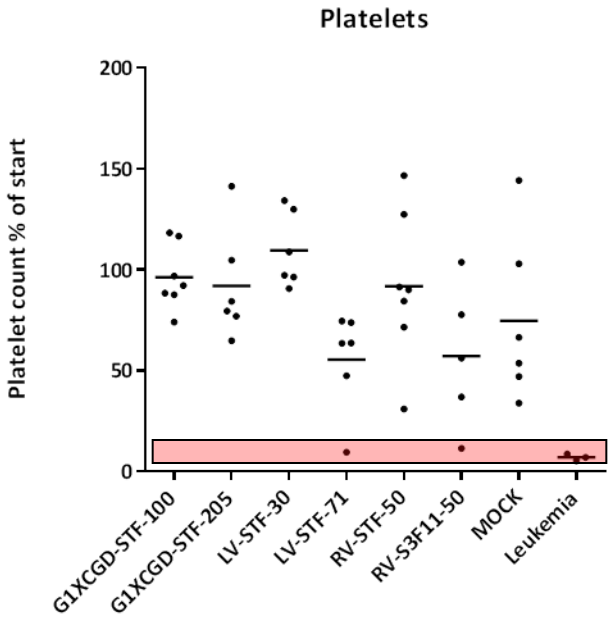
S3F11: 50 ng/ml mSCF; 100 ng/ ml hFtl3-L and IL-11; 20 ng/ml IL3

Blood counts…

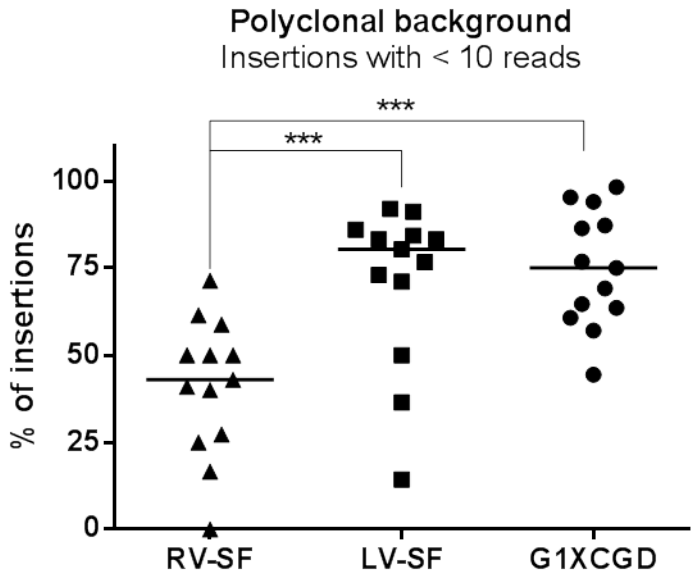


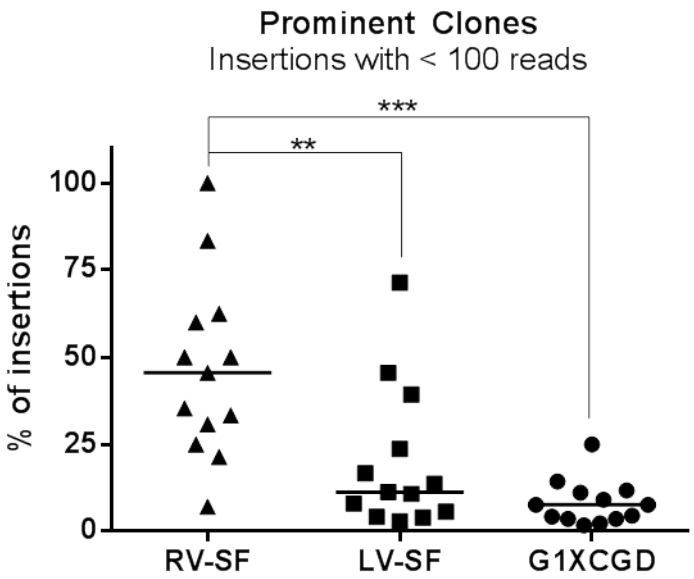






Polyclonal engraftment background....





Conclusions....

- Likely clinical benefit in terms of long term survival from low levels of sustained correction

- IVIM reveals significantly reduced immortalisation rate compared with RV or LV vectors incorporating SFFV LTR and **no** recovery of Evi1/MDS1 insertions.

- Engraftment was more polyclonal *in vivo* than with RV-SF vectors

- Mice treated with G1XCGD exhibited **no** unexpected pathology up to 26 weeks

GENETHON
CURE THROUGH INNOVATION
PRIX GALIEN 2012

Clinical Study & Regulatory Status in Europe

- Phase I/II clinical study sponsored by Genethon and supported by the EC FP7
- Non randomized
- Open-label
- Multicenter
  - 4 countries: **UNITED KINGDOM, GERMANY, SWITZERLAND, FRANCE**
  - 1 site per country
- Patient Number: Approximately 5 patients per site
- Follow-up : 2 years post administration
- Primary objectives:
  - Evaluation of safety
  - Evaluation of efficacy by biochemical and functional reconstitution in progeny of engrafted cells and stability at 12 months

Approved Clinical Trial in United Kingdom, site is opened (March 2013),
(1 patient enrolled)

A PHASE I/II, NON RANDOMIZED, MULTICENTER, OPEN-LABEL STUDY OF G1XCGD (LENTIVIRAL VECTOR TRANSDUCED CD34+ CELLS) IN PATIENTS WITH X-LINKED CHRONIC GRANULOMATOUS DISEASE

Peter Newburger U Mass Worcester

-- Chronic Granulomatous Disease - Pathogenesis and Treatments

Adrian Thrasher University College London

-- Chronic Granulomatous Disease - Prior gene therapy and new lentiviral vector

David Williams Harvard Medical School

-- Chronic Granulomatous Disease - Proposed trial and responses to RAC reviews

A PHASE I/II, NON RANDOMIZED, MULTICENTER, OPEN-LABEL STUDY OF G1XCGD (LENTIVIRAL VECTOR TRANSDUCED CD34+ CELLS) IN PATIENTS WITH X-LINKED CHRONIC GRANULOMATOUS DISEASE

IND Sponsor and Coordinating Investigator of the Leading Center:
Donald B. Kohn, M.D.
University of California, Los Angeles (UCLA)

Primary Institution:
Mattel Children's Hospital, UCLA
Site PI: Donald B. Kohn, M.D.

Collaborating Institution:
Children's Hospital Boston
Site PI: David A. Williams, MD

Clinical Collaborators
Peter Newburger - U Mass, Worcester
Thomas Coates - Children's Los Angeles
Joseph Church - Children's Los Angeles

Vector Production /Scientific Coordination
GENETHON
91000 EVRY - FRANCE

Objectives

Primary objectives:

Evaluation of safety.

Evaluation of efficacy of biochemical and functional reconstitution in progeny of engrafted cells and stability at 12 months.

Secondary objectives:

Clinical efficacy and longitudinal evaluation of clinical effect in terms of augmented immunity against bacterial and fungal infection.

Transduction of CD34+ hematopoietic cells from X-CGD patients by *ex vivo* lentivirus-mediated gene transfer.

Evaluation of engraftment kinetics and stability.

Diagnosis and Inclusion Criteria

N=10. Male X-CGD patients >23 months of age.

Molecular diagnosis confirmed by DNA sequencing and supported by laboratory evidence for absent or reduction > 70% of the biochemical activity of the NAHPD-oxidase.

At least one ongoing or refractory severe infection and/or inflammatory complications requiring hospitalization despite conventional therapy.

No HLA-matched donor available after 3 months search.

No co-infection with Human Immunodeficiency Virus (HIV) or hepatitis B virus (HBsAg positive) or hepatitis C virus (HCV RNA positive).

Written informed consent for adult patient.

Parental/guardian and where appropriate child's signed consent/assent.

Exclusion Criteria

10/10 HLA identical family or unrelated or cord blood donor unless there is deemed to be an unacceptable risk associated with an allogeneic procedure.

Contraindication for leukapheresis.

Contraindication for administration of conditioning medication.

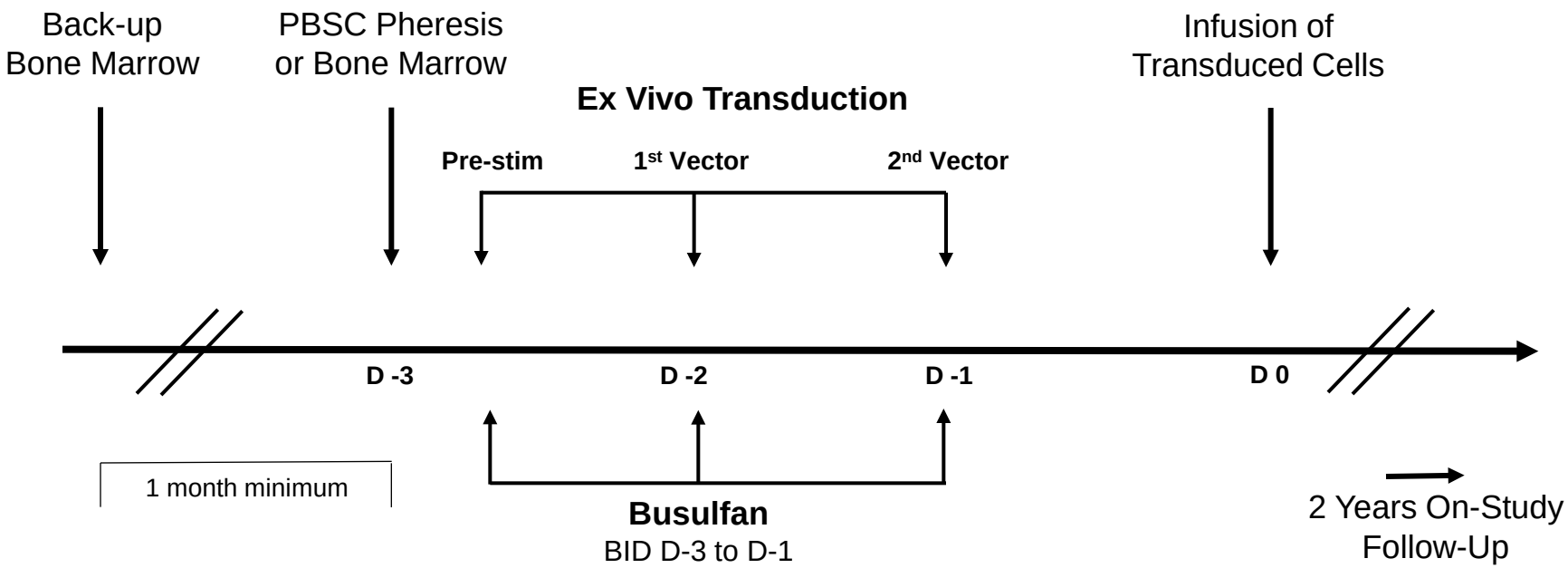
Administration of gamma-interferon within 30 days.

Participation in another experimental therapeutic protocol within 6 months.

Any other condition that, in the opinion of the Investigator, may compromise the safety or compliance of the patient or would preclude the patient from successful completion of the study.

Patient/Parent/Guardian unable or unwilling to comply with the protocol requirements.

Procedure Schema



Primary endpoints:

Safety of the procedure as measured by the incidence of adverse events.

Restoration and stability of granulocyte NADPH oxidase activity, measured as >20% normal activity in >5% of cells.

Secondary endpoints:

Normalization of nutritional status, growth, development, severe infection and/or inflammatory complication .

Percentage of transduced CD34+ hematopoietic cells infused and blood cells over time (at month 1, 2, 3, 6, 9, 12, 18 and 24 months).

Immunological reconstitution as measured by evidence of restored neutrophil oxidase function and immunity against bacterial and fungal infections over time.

RAC Reviewer Questions and Responses

1. Longevity of expression in pre-clinical studies:
What is the longest follow up of expression in vivo in human CGD-affected and non-affected cells? Also what was the follow up in NSG mice, i.e. number of mice with persistent expression and were any secondary NSG mouse studies done with human cells.

Response: In the X-CGD murine bone marrow transplants, primary mice were analyzed after 6 months following transplant and the secondaries were studied 19-21 weeks later (total ~10 months). Expression was seen in secondaries as follows: 9-59% gp91phox (+) with 0.2-0.7 VCN.

G-CSF mobilized PBSC from XCGD subject in 3 NSG mice. After 8 weeks, human cells in BM showed 20% expressing gp91phox with ave. VCN= 0.35.

2. Inclusion of Minor Subjects in FIH study:

Response:

Age range is >23 months ("Male X-CGD patients >23 months of age"), including adults, as in EU protocol.

Prior trials have included pediatric subjects (Malech, Ott).

Based on positive effects in prior trials, there is clear prospect of direct benefit for eligible pediatric subjects, which fulfills the 21 CFR 50.52 requirement for inclusion of children in phase I studies.

May be the only possible therapy for subjects with "ongoing or resistant severe infections" who do not have a fully-matched donor for allogeneic HSCT.

3. For cord blood transplants especially double cord blood transplants 10/10 is usually not required for a standard transplant. Would they still be excluded?

Response: While cord blood transplants may be done with less than 10/10 HLA-matching, especially using two partially matching units, these transplant do have increased risks for non-engraftment and GVHD. It is unlikely that transplant using two incompletely matched cord blood units would be done for CGD as primary therapy, and this too would be experimental in nature.

4. Other donor issues: The consent form states that transplant from a suitable donor is one option if the subject's immune system fails to recover. If later discovery of a suitable donor is a reasonable possibility, should the eligibility criteria be changed to require a longer search for a suitable donor? If later location of a suitable donor is not a reasonable possibility, then the consent form should make it clear that transplant is an unlikely option.

Response: Low likelihood of a new fully matched donor becoming available in the months after transplant if none was found during the initial search. So, "rescue transplant" if prolonged aplasia would likely be from partially matched donor. We clarified this in the consent.

5. What are criteria of harvesting marrow vs. peripheral blood and what is the timing of back-up and harvest of CD34 cells for transduction?
What is the CD34 cell dose required for the back-up marrow or PBSCs?

Response: PBSC for adults and adolescents, BM for subjects under ~10-13 years, or under 20 kg, with clinician discretion.

For BM, there will be at least one month between 2 collections.

For PBSC, should get enough for both back-up and CD34+ cells for transduction from single pheresis or 2 successive days.

Back-up will be at least 1x10e6 CD34+ cells/kg.