

**February 14, 2003**  
**Report on preclinical studies in  $\gamma_c$ -KO mice**  
**Fabio Candotti**

Five published reports (see details below) have described the development of peripheral blood lymphocytes as well as cellular and humoral immune function in  $\gamma_c$ -KO mice transplanted with bone marrow cells transduced with retroviral vectors expressing  $\gamma_c$ . Four different strains of mice, nine different vectors, and two main transduction procedures were used in these studies. The MFG- $\gamma_c$  vector used in the French human trial of gene therapy for X-SCID was used to treat 2 mice, whereas the clinical transduction conditions were not used in any of the mouse studies. One additional general difference between the procedures used in mice and humans is the use of 5-FU unfractionated marrow in the mouse studies vs. the isolated CD34+ cells of the clinical trial. Finally, all preclinical experiments used young adult mice. Cell infusions were performed after lethal irradiation and were of  $1-4 \times 10^6$  cells/mouse ( $1 \times 10^6 / \sim 25$  gr is equivalent to  $\sim 160 \times 10^6$  cells/kg for a patient such as #4 of the French trial, assuming a weight of 4 kg at one month since this patient was reported well and free of infection at the time of treatment).

The published literature contains a total of 51 treated  $\gamma_c$ -KO mice that have been followed for 7 to 47 wks after gene therapy. No adverse events were observed. Eight  $\gamma_c$ -KOs received a secondary transplant and were followed for 23 wks without reported adverse events. Nineteen control/mock mice were followed for 6 to 36 wks post-BMT with no problems reported.  $\gamma_c$ -transduced X-SCID bone marrow cells were transplanted in 30 W/W<sup>v</sup> mice that were followed for 12-28 wks again in the absence of adverse events. Transduction efficiency of bone marrow cells is unknown in 3 out of 5 experiments and varies between  $\sim 5\%$  and  $>50\%$  in the others. The overall efficacy is somewhat less impressive than that observed in humans: reconstitution of B and NK cell numbers is incomplete, as are responses to IL-2, IL-4, IL-7 and to vaccination. Treated mice were kept in SPF.

Discussions with Drs. Warren J. Leonard, NHLBI, and Anton Berns, The Netherlands Cancer Center, and Claire Soudais, Hôpital Necker, Paris, revealed no evidence of higher incidence of spontaneous malignancies in their  $\gamma_c$ -KO colonies. In the NIH colonies, splenomegaly was commonly noted as previously described (Blood 1996; 87:956), but no increased incidence of spontaneous malignancies was documented.

Unpublished data from the Candotti's lab include additional 33 treated  $\gamma_c$ -KO animals (11 after lethal irradiation, 22 without myeloablation), 2 of which developed lymphoma at 30 and 35 wks after treatment. Molecular analysis of these malignancies is ongoing. It is worth noting that C57BL/6 mice are classified as having "high" risk for lymphohematopoietic cancer by the Jackson

Laboratories. Six mock-treated and 23 mutant  $\gamma$ c-treated animals were followed for 10-22 wks after BMT and showed no problems.

In competitive repopulation experiments part of which were published (Hum Gene Ther 2000, 11:2051), 75  $\gamma$ c-KO animals received XSCID and WT bone marrow cells and 46  $\gamma$ c-KO animals received XSCID and WT bone marrow cells in the absence of myeloablation. No malignancies were noted.

In summary, the available data refer to a limited number of  $\gamma$ c-treated mice (86) followed for less than or equal to 1 year after treatment. The models have obvious differences with the clinical trial that, in retrospect, may make them less useful than previously thought in terms of predicting toxicity outcomes. Two cases of lymphoma are being analyzed. At difference with JAK3-deficient mice, genotype-specific increased incidence of malignancy has not been recognized to date among  $\gamma$ c-KO mice.

Thanks to Anton Berns, Warren J. Leonard, Makoto Otsu and Claire Soudais for sharing information and unpublished results.

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Details of the reviewed experiments:

**Warren J. Leonard's laboratory, NHLBI, NIH  
(Blood 1999, 94:3027)**

*Mice*

$\gamma$ c KO (Immunity 1995, 2:223), C57BL/6 background.

*Transduction procedure*

Vector: MPSV LTR +  $\eta$  $\gamma$ c; Phoenix ecotropic envelope.

5-FU marrow + IL-3, IL-6, SCF x 2 days.

Co-culture x 2 days at 32 °C + 24 hrs on preloaded plates.

$1 \times 10^6$  cells/mouse into 6-8 wk-old recipient, 800 rads.

8  $\gamma$ c KO treated followed for 9 wks – no adverse events.

4  $\gamma$ c KO mock-treated, followed for 6-10 wks – no adverse events.

No problems in the colony.

Transduction efficiency: not reported.

Efficacy: ~ WT-BMT, but low NK cells, lower responses to IL-2.

**James P. Di Santo's laboratory, Paris  
(Blood 1999, 94:3027)**

### *Mice*

$\gamma_c$ /RAG2 KO (JI 1999, 162:2761), C57BL/6 background.

### *Transduction procedure*

Vector: MoMLV LTR + myc; BOSC 23 ecotropic envelope.

5-FU marrow + IL-6, SCF, Flt3-L, fibronectin, polybrene, 1:1 vector x 3 days at 37°C.

$2 \times 10^6$  cells/mouse into 4-12 wk-old recipients, 300 rads.

7?  $\gamma_c$ /RAG2 KO treated followed for 7-47 (1 at 40, 1 at 47) wks, no adverse events.

8  $\gamma_c$ /RAG2 KO secondary transplants followed up to 23 wks, no adverse events.

7?  $\gamma_c$ /RAG2 KO mock-treated followed for an unspecified number of wks.

Transduction efficiency: not reported.

Efficacy: ~ WT-BMT, but low  $\gamma\delta$  T cells and NK cells, lower responses to IL-2 and IL-7.

### Unpublished

3?  $\gamma_c$ /RAG2 KO mice failed reconstitution possibly due to low retroviral titer.

2?  $\gamma_c$ /RAG2 KO treated with MFG- $\gamma_c$  clinical grade vector and followed for almost one year. Peripheral reconstitution was delayed but at 47 weeks post-graft there were: 10-14% of T cells and 38-42% of B cells. The human transgene was shown to be present in spleen cells by PCR.

No unexpected death was observed in the animal entered in the study.

### **Fabio Candotti's laboratory, NIH (Mol Ther 2000, 1:145)**

### *Mice*

$\gamma_c$  KO (JEM 1999, 190:1059), C57BL/6 background.

### *Transduction procedure*

Vector: MND LTR + myc; GP+E-86 ecotropic envelope.

5-FU marrow + IL-3, IL-6, SCF, polybrene x 2 days.

Co-culture x 2 days at 37 °C.

$1-1.5 \times 10^6$  cells/mouse into 8-16 wk-old recipient, 900 rads.

8  $\gamma_c$  KO treated followed for 24-32 wks – no adverse events (1 found dead).

2  $\gamma$  KO received EGFP-BMT followed for 24-36 wks (1<sup>st</sup> euthanized because infected).

Transduction efficiency: 5-25% CFUs, ~25% bone marrow cells, 14-38% in periphery at 3 months.

EGFP transduction efficiency: ~64% injected cells.

Efficacy: low PBLs, low B and NK cells, low response to IL-2 and vaccination.

#### Unpublished

1. 5  $\gamma$  KO treated followed for 9-30 wks (one missing at 30 wks), one w/ lymphoma.  
3  $\gamma$  KO mock-treated followed 13 wks (one missing at 30 wks), no problems.  
Transduction efficiency: not studied.  
Efficacy: 5/5 mice showed good lymphocyte development at 9 wks. Similar findings in 4/5 mice at 30 wks  
Lymphoma mouse was leukopenic at 9 wks (WBC: 2944), but seemed to have responded to therapy as per CD8+ T cell and B cell development.  
Moderate epatosplenomegaly noted at the time of scheduled euthanasia.
2. 6  $\gamma$  KO treated followed for 35 wks (2 dead at 19 wks), one with lymphoma.  
5  $\gamma$  KO mutant  $\gamma$ -treated followed for 19-22 wks (1 missing at 19 wks)  
3  $\gamma$  KO mock-treated followed 10 wks, 1 found dead, no other problems.  
Transduction efficiency: not studied.  
Efficacy: 4/6 mice showed good lymphocyte development at 19 wks.  
Lymphoma mouse had normal leukocyte numbers at 19 wks (WBC: ~9900) and seemed to have responded to therapy as per CD8+ T cells and B cell development. Euthanized at ~35 wks because sick, slight splenomegaly.
3. 6  $\gamma$  KO mutant  $\gamma$ -treated followed for 8-17 wks, no problems  
Transduction efficiency: not studied.  
No expected efficacy.
4. 12  $\gamma$  KO mutant  $\gamma$ -treated followed for 7-14 wks, no problems  
Transduction efficiency: not studied.  
No expected efficacy.

**Fabio Candotti's laboratory, NIH  
(Blood 2001, 97:1618)**

*Mice*

$\gamma$ c KO (Blood 1996; 87:956), C57BL/6 background.

*Transduction procedure*

Vector: MND LTR +  $\eta$  $\gamma$ c; GP+E-86 ecotropic envelope.

5-FU marrow + IL-3, IL-6, SCF, polybrene x 2 days.

Co-culture x 2 days at 37 °C.

$4 \times 10^6$  cells/mouse into 8 wk-old recipient, 900 rads.

8  $\gamma$ c KO treated followed for 18-22 wks (2 missing at last follow-up), no adverse events.

6  $\gamma$ c KO mock-treated followed for 8-12 wks (3 found dead), no adverse events.

Transduction efficiency at 22 wks: 6.2% BM, ~35% spleen, >50% thymus.

Efficacy: low B and NK cells, low response to IL-2, IL-4, IL-7.

Unpublished data

1. 22 not myeloablated mice received  $\eta$  $\gamma$ c, F/U < 12 wks, no adverse events.
2. Competition studies (**Hum Gene Ther 2000, 11:2051** and unpublished).  
75 lethally irradiated mice received XSCID and WT BM cells, F/U 16-28 wks, no adverse events.  
46 not myeloablated XSCID mice received XSCID and WT BM cells, F/U 3 to <12 wks, no adverse events.
- 3.?  $\gamma$ c gene transfer into WT bone marrow cells.  
17 mice treated, lethal irradiation, ~10%  $\gamma$ c gene transfer into CFUs, F/U 10-52 wks, no adverse events.

**David Bodine's laboratory, NIH  
(Mol Ther 2001; 3:565)**

*Mice*

$\gamma$ c KO (JEM 1999, 190:1059), C57BL/6 background.

*Transduction procedure*

Vectors: MLV, MPSV, MND, and HaSV LTRs +  $\eta$  $\gamma$ c; GP+E-86 ecotropic envelope.

5-FU marrow + IL-3, IL-6, SCF, polybrene x 2 days.

Co-culture x 2 days at 37 °C.

$2 \times 10^6$  cells/mouse into 8 wk-old recipient, 900 rads.

20  $\gamma_c$  KO treated followed for 16-28 wks, no adverse events.

30  $W/W^v$  mice received  $h\gamma_c$ -transduced X-SCID cells and were followed for 12-28 wks, no adverse events.

Transduction efficiency at 16-28 wks: 100% peripheral T cells.

Efficacy: 85% mice showed lymphoid development.