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# Progress toward Effective Gene Therapy for Chronic Granulomatous Disease

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**SUMMARY**: Previous clinical studies of ex vivo gene therapy for chronic granulomatous disease (CGD) without marrow conditioning have resulting in transient correction of the oxidase defect in over 0.1% of circulation neutrophils. Use of improved RD114 envelope pseudotyped vectors capable of transducing >95% of $CD34^+$ stem cells ex vivo, together with non-ablative marrow conditioning will be incorporated into the next generation of clinical trials of ex vivo gene therapy for CGD. These maneuvers might result in clinical benefit to CGD patients from gene therapy.

Patients with chronic granulomatous disease (CGD) have defective phagocyte oxidase and recurrent life-threatening infections (1). We have conducted clinical trials of ex vivo gene therapy treating five patients with autosomal recessive p47phox-deficient and five patients with X-linked gp91phox-deficient CGD (2-5). We transduced autologous mobilized $CD34^+$ peripheral blood hematopoietic stem cells (PBSC) ex vivo in serum-free medium in gas permeable bags with amphotropic envelope pseudotyped MFGS retrovirus encoding normal p47phox (20% transduction rates) or gp91phox (70% transduction rates using Retronectin®), respectively (4). In eight of ten patients, peak levels of 0.004 to 0.13% oxidase normal corrected peripheral blood neutrophils and monocytes were observed at 3 - 6 weeks with the effect lasting several months per cycle of gene therapy. Repeated cycles prolonged correction >1 year, but was not permanent. We conclude that clinically beneficial gene therapy for CGD will require improved transduction of primitive stem cells, use of non-ablative marrow conditioning and possibly also co-expression of an in vivo selectable gene. We used feline endogenous virus RD114 envelope pseudotyped MFGS-gp91phox vector to achieve >95% ex vivo transduction. In a NOD/SCID mouse xenograft model engrafted with human X-linked CGD patient PBSC transduced with amphotropic MFGS-gp91phox versus RD114 MFGS-gp91phox vector, in vivo levels of gene corrected human neutrophils were 2.2% versus 22%, respectively (6). CGD vectors also containing selectable P140K mutant methyguanine methyltransferase gene allowed use of $O^6$-benzyl guanine/temozolamide in vivo selection to further enhance human neutrophil correction several fold in the NOD/SCID mouse xenograft model (7). A new clinical trial of gene therapy for X-linked will be started in about a year using in vivo selectable RD114-MFGS-gp91phox-IRES-MGMT vector and incorporating non-ablative marrow conditioning; an approach that may achieve clinical benefit for CGD patients.

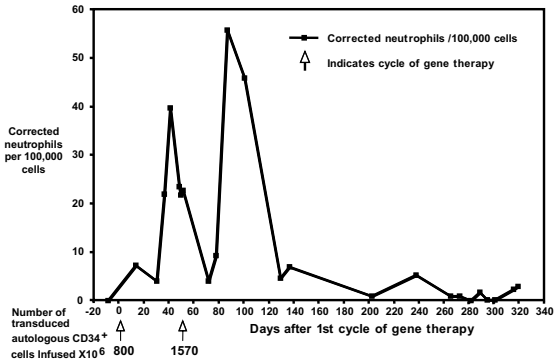


Fig. 1. Number of oxidase normal neutrophils per 100,000 in the peripheral blood of a patient with X-linked CGD treated with ex vivo gene therapy. The dihydrorhodamine assay using PMA stimulation of neutrophils was used where oxidase positive cells showed a 3 log increase in fluorescence compared to uncorrected oxidase negative cells. The gating is set so that background is zero cells. This patient received two cycles of gene therapy about 50 days apart where ex vivo transduction was >60% in both treatments. Marking at a very low level persisted for almost a year. Not shown is that no marking was seen after about one year in this patient.

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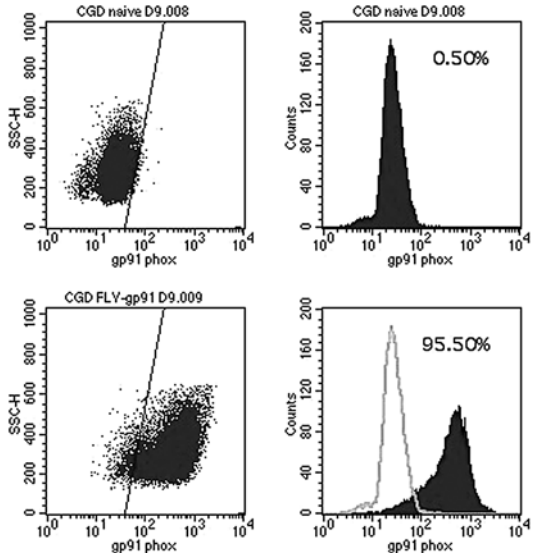


Fig. 2. Enhanced ex vivo transduction of $CD34^+$ PBSC from patient with X-linked CGD using an RD114 envelope pseudotyped MFGS-gp91phox vector assessed by flow cytometry with anti-gp91phox antibody. Transductions were performed with ultracentrifuge concentrated virus at a titer of about $2 \times 10^7$ infectious particles per ml at an MOI of 20. Transductions were performed daily overnight x 3 on days 2 to 5 of culture. Shown is the analysis at day 7 of non-transduced CGD PBSC (upper panels) and transduced CGD PBSC (lower panels). The same data is plotted as side scatter (SSC) versus anti-gp91phox fluorescence in dot plot format (left panels), and as cell counts/channel versus anti-gp91phox fluorescence in histogram format (right panels). The open histogram in the bottom right panel reproduces the pattern from the panel above showing the non-transduced control cells. This is a typical outcome with the RD114 vector where transductions ex vivo are greater than 95% of human CD34+ PBSC.

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