

# Safety and efficacy of autologous CD34+ hematopoietic progenitor cells transduced with retroviral vector containing a ribozyme directed against the HIV-1 tat gene, OZ1 in a multicenter, randomized, placebo-controlled Phase II trial

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## Abstract K-115



**Background:** OZ1 comprises a Moloney murine leukemia, replication-incompetent retroviral vector with a tat/tyr specific anti-HIV ribozyme. In this study, the largest randomized, double blind, placebo-controlled, phase II cell-delivered gene transfer clinical trial, we assessed the safety and efficacy of OZ1 delivered in autologous CD34+ hematopoietic progenitor cells.

**Methods:** 74 HIV-1+ adults received an infusion of CD34+ cells transduced with OZ1 or treated with medium alone. Over 100 weeks, antiretroviral therapy was interrupted two times (at weeks 24-28 and 40-100) to provide positive selective pressure for the progeny of OZ1-transduced CD34+ cells. The primary endpoint was viral load at weeks 47/48. Secondary endpoints included time-weighted area under the log HIV viral load curve (TWAUC), safety (including those specific to gene transfer studies), T-lymphocyte counts and cell marking.

**Results:** No adverse event was attributed to OZ1. The primary endpoint was not met but several other viral load parameters were significantly different in the OZ1 group including: the TWAUC, log10 copies/ml/day for weeks 40-48 and weeks 40-100, median difference log10-0.34 (p=0.024) and -0.37 (p=0.034), respectively; the number of participants with plasma viral load <4 log10 copies/ml at weeks 47/48 (15/32 vs 5/33; p=0.009), the number of participants with a TWAUC in the lowest quartile for weeks 40-100 (12 vs 5; p=0.04) and the median time to increase to 4log10 viral load copies/ml after interruption. In those participants with OZ1 expression beyond week 48, the weeks 47/48 viral load (p=0.003) and median TWAUC for both weeks 40-48 and weeks 40-100, p=0.03 and 0.005 respectively) were significantly lower than in controls. Peripheral blood lymphodepletion related to mobilization and large-volume apheresis occurred in both groups. While not statistically significant, the OZ1 group had higher CD4+ T-lymphocyte counts (eg 476 vs 437 cells/mm<sup>3</sup>) at weeks 47/48, higher CD8%, and lower CD8% over time. No evidence of resistance to OZ1 or to patients antiretroviral therapy was seen. No evidence of insertional mutagenesis was detected.

**Discussion:** This study provides the first indication that cell-delivered gene transfer is active in the setting of HIV infection. This type of therapy can reduce HIV viral load, and may preserve immune function and avoid the toxicities associated with antiretroviral therapy.

## Results

Participants were screened from July 2003 to Jan 2006 in USA and Australia. 76 eligible participants were randomized and infused with either OZ1- (38) or Placebo (36) transduced CD34+ cells manufactured in 1 of 3 local facilities. Two placebo groups did not receive an infusion. Two analysis populations were defined prior to database lock and unblinding to treatment group. Intention To Treat (ITT) was defined as all infused participants and Per Protocol (PP) as participants who complied with the protocol and entered the eight week Analytic Treatment Interruption (ATI).

Table 1. Baseline characteristics of ITT population

	OZ1 (n=38)	Placebo (n=36)
Age in years (mean ± sd)	36.8 ± 5.4	38.4 ± 4.8
Gender		
Male	92%	94%
Female	6%	6%
Race		
African American	10.5%	13.9%
Caucasian	2.9%	-
Hispanic	15.8%	5.6%
Multi/Polynesian	-	2.8%
Other	-	5.6%
Time since HIV Diagnosis (years)	7.2 ± 5.2	5.8 ± 3.9
Time since HAART commenced (years)	4.2 ± 2.8	3.9 ± 2.2
Screening CD4+ Cell Count (10 <sup>6</sup> /L)	607 ± 241	683 ± 252
Screening CD4+ Cell Count (%)	35 ± 8	35 ± 7
Screening CD8+ Cell Count (10 <sup>6</sup> /L)	825 ± 313	801 ± 327
Screening CD4+/CD8+ Cell Ratio	0.93 ± 0.40	0.97 ± 0.45
Antiretroviral treatment at screening		
NRTI only	10.5%	5.6%
NRTI + PI	34.2%	22.2%
NRTI + NNRTI	55.3%	69.4%
NRTI + NNRTI + PI	-	2.8%
Dose of CD34+ cells infused x 10 <sup>6</sup> cells/kg	9.0 ± 3.6	9.2 ± 4.2
Transduction Efficiency (%)	54.0 ± 17%	-

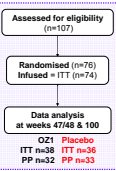


Figure 2. HIV-1 Viral Load: Intention-to-Treat Population

The mean log<sub>10</sub> viral load for the 2 treatment groups was determined throughout the study. Periods of HAART are shaded, and initial screening visit is indicated as scr. ●→●→ OZ1, ○→○→ Placebo; \* = primary endpoint difference in viral load between groups. Participants recommenced HAART at different times, so n at several time points is shown. PCR sequence data of the HIV-1 protease and reverse transcriptase genes were used to generate a virtual phenotype. Only one participant with a mutation associated with resistance to protease inhibitors failed to suppress HIV-1 replication after the recommencement of HAART.

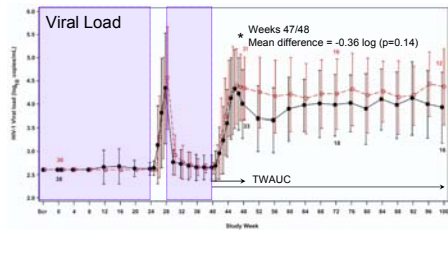


Table 2. Viral Load Primary and Secondary Efficacy Parameters.

Parameter	Analysis Population	n	Median	n	Placebo Median	Median Difference (95% CI)	p-value
Weeks 47/48 Log Viral Load (log <sub>10</sub> copies/ml)	ITT	38	4.27	36	4.63	-0.36 (-0.61, 0.08)	0.141
	PP	32	4.20	31	4.57	-0.37 (-0.63, 0.04)	0.085
Log TWAUC Viral Load Weeks 40-48 (log <sub>10</sub> copies/ml/day)	ITT	38	3.64	36	4.00	-0.37 (-0.56, 0.06)	0.123
	PP	32	3.59	33	3.93	-0.34 (-0.61, -0.07)	0.024
Log TWAUC Viral Load Weeks 40-100 (log <sub>10</sub> copies/ml/day)	ITT	38	4.20	36	4.62	-0.42 (-0.57, 0.04)	0.117
	PP	32	4.17	33	4.53	-0.37 (-0.64, -0.04)	0.034
Time to reach HIV-1 viral load in Analytic Treatment Interruption (days)*	PP	32	21 (19, 28)	31	17 (14, 21)		0.364
4 log <sub>10</sub> copies/ml	PP	31	36 (30, 43)	31	24 (19, 28)		0.041
5 log <sub>10</sub> copies/ml	PP	17	282 (50, N/A)	23	52 (39, 169)		0.084

Figure 3. Change in percentage T lymphocyte counts, from baseline, in Intention-To-Treat Population. Shown as mean percentage of CD3+ T-lymphocytes for each treatment group. Only participants who had not resumed HAART are included in the analysis at each time-point post week 40. Periods of HAART are shaded.

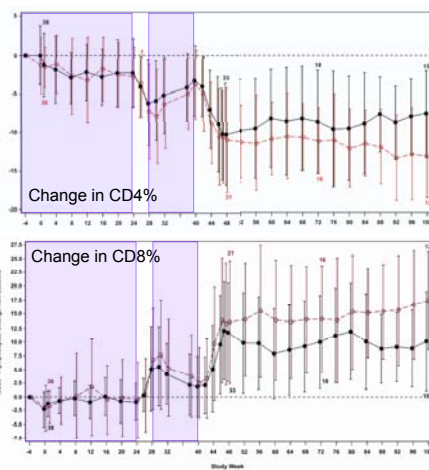


Figure 4. CD4+ T-lymphocytes as percentage of CD3+ T-lymphocytes only for participants who resumed HAART (shaded) prior to, or at week 52. ●→●→ OZ1 (10), ○→○→ Placebo (9). Baseline CD4 count is shown as a dashed line.

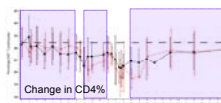
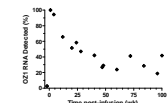


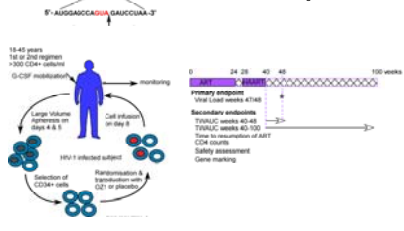
Figure 5. Expression of OZ1 in PBMC from participants who had not resumed HAART: ITT population.



## Introduction and Study Design

OZ1, an anti-HIV *pro-tat* ribozyme, was shown to be safe in two phase I clinical trials. In this large, multicenter, placebo-controlled study of safety and efficacy, participants on stable HAART were randomized to treatment or placebo, underwent peripheral blood stem cell collection and received a single infusion of autologous CD34+ cells on day 0. The study design included two treatment interruptions. The impact of OZ1 on plasma HIV-1 viral load was assessed at the end of the second, eight-week, or analytic treatment interruption (the primary endpoint). Difference in Log time weighted area under the curve (TWAUC) between the treatment groups was determined for weeks 40-48 and 40-100. Additional secondary endpoints were evaluated.

Figure 1. Sequence of HIV *pro-tat* target in HIV-1, gene modified cell manufacturing schema and outline of Clinical Trial design.



## Statistical Methods

Formal group comparisons of continuous primary and secondary parameters were performed using the Wilcoxon Rank Sum test with 95% confidence intervals provided for the medians and the difference in the medians. Binary endpoints were analyzed using Fisher's Exact test, with 95% confidence intervals. Participants who recommenced HAART prior to week 48 had an imputed value, the last value recorded, assigned and carried forward to week 48. In all analyses, statistical significance was taken at the two-sided 5% level, with no adjustment for multiple secondary parameters and analyses.



## Compliance

This study was sponsored by Johnson & Johnson Research Pty Limited in compliance with ICH GCP guidelines. Approval for the study was obtained from the appropriate Institutional Review Boards, Institutional Biosafety Committees, the US Food and Drug Administration (Centre for Biologics Evaluation Review), National Institutes of Health (Recombinant DNA Advisory Committee) and the Therapeutic Goods Administration and the Office of Gene Technology Regulator in Australia. The study was listed on a public clinical trial registry at www.clinicaltrials.gov; NCT0074997.

## Safety Evaluations

There was no death or clinically severe cardiovascular, renal or hepatic adverse event reported for randomized participants. Most experienced G-CSF-related adverse events including elevated liver enzymes or thrombocytopenia. Community-acquired infections were reported in both groups, at CD4+ counts from 342-467 cells/ml and 19-36% of T lymphocytes. All 3 participants who experienced a serious adverse event were in the Placebo group. None of these serious adverse events was assessed as being related to G-CSF, leukapheresis, cell infusion or other study procedures. No predominant integration site or replication competent retrovirus was detected at any time point. No hematopoietic cell clonal expansion or other event suggestive of insertional mutagenesis was observed. There was no evidence for the evolution of ribozyme resistant virus.

## Conclusions

This is the largest cell-delivered gene therapy trial conducted to date, and the only randomized controlled phase II study of a potential cell-delivered gene therapeutic. Although the primary efficacy endpoint was not reached, HIV-1 viral loads were consistently lower in the OZ1 group for all analyses. The impact of OZ1 on HIV-1 viral load is further supported by a correlation with OZ1 expression beyond week 48 and a correlation with CD34+ cell dose. The CD4+ and CD8+ T-lymphocyte count data were consistent with the HIV-1 viral load differences between the treatment groups. No safety concerns associated with OZ1 gene transfer were identified. All serious adverse events were unrelated to study procedures or study drug infusion. Importantly, no death, AIDS-defining event, severe infection, clinically significant cardiovascular, renal or hepatic event was reported in randomized participants. This study supports the concept that OZ1 cell-delivered gene therapy is safe, and exhibits modest efficacy and that such approaches can be developed according to the same regulatory standards as conventional products.

## In Press

The detailed description of this study and results has been accepted for publication by Nature Medicine.

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