## 36. Genome-Wide Insight Into the Transcriptional Modulations Triggered By Lentiviral Transduction in Human Hematopoietic Stem Cells

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Recent studies suggest that hematopoietic stem cells (HSC) can sense foreign nucleic acids and pathogen-associated molecular patterns (PAMPs). Exposure to lentiviral vectors (LV) upon gene transfer may thus trigger acute host responses in HSC that could potentially impact on their biological properties, although no comprehensive studies are available to date. We have performed a high throughput RNA-Seq analysis on human cord-blood (CB)-derived CD34+ hematopoietic stem and progenitor cells (HSPC) exposed to research- or clinical-grade VSV-g pseudotyped (SIN) LV at a high multiplicity of infection, matching current clinical vector dose requirements. As controls, cells were exposed to non-transducing Env-less, genome-less or heat inactivated control vectors or kept in culture untreated. RNA was extracted at different times early after transduction, processed and ran in Illumina HiSeq2000. Analysis of Differential Expression in Time Course was performed using LIMMA R/BioConductor library. Key pathways were assessed by Term Enrichment Analysis considering KEGG pathways and Gene Ontology Biological processes. Transduction with both research- and clinical-grade LV significantly triggered DNA damage and apoptosis-related responses. In particular, p53 signaling was among the most significantly altered pathways ($p<3.47x10^{-14}$) and induction of several key players, including a 8-fold increase in p21 mRNA, was further confirmed by Taqman. This signaling occurred also in bone-marrow-derived CD34+ cells and was integration-independent as Integrase-Defective LV (IDLV) induced p21 to a similar extent as LV. Furthermore, equal induction was observed in all CD34+ subpopulations, including in the most primitive CD38-CD133+ fraction. Finally, LV/IDLV exposure lead to a slight but significant increase in the percentage of apoptotic HSPC in culture ($p<0.001$) as compared to control vector exposed cells and untreated controls. Experiments are ongoing to further investigate the potential short and long-term consequences of this signaling on the biological properties of HSPC *in vitro* and *in vivo*. Overall, our results suggest for the first time that LV transduction triggers transcriptional changes in HSPC involving pathways pivotal for their biology. Better understanding of the potential functional consequences this may have will be important for the development of improved gene therapy protocols.

# Clinical Gene and Cell Therapy

## C-2. Eteplirsen, a Phosphorodiamidate Morpholino Oligomer (PMO) for the Treatment of Duchenne Muscular Dystrophy (DMD): 168 Week Update on Six-Minute Walk Test (6MWT), Pulmonary Function Testing (PFT), and Safety

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**Background/Objective**:

DMD is a rare, degenerative, X-linked recessive genetic disease that results in progressive muscle loss and premature death. DMD is caused by mutations in the dystrophin gene that lead to a reading frame shift and premature translation termination. Exon skipping, a promising disease-modifying approach for DMD, can be induced by eteplirsen, a charge neutral PMO that selectively binds to exon 51 of dystrophin pre-mRNA, restoring the open reading frame and enabling production of an internally truncated yet functional dystrophin protein as found in the less severe dystrophinopathy, Becker muscular dystrophy (BMD).

**Methods**:

Twelve eligible boys, aged 7-13 years were randomized 1:1:1 to eteplirsen 30 or 50 mg/kg/wk, or placebo for 24 weeks. All patients transitioned into the ongoing open-label extension trial at Week 25 taking eteplirsen 30 or 50 mg/kg. Initial placebo-treated were denoted "placebo-delayed" after starting the PMO. Efficacy endpoints included 6MWT, PFT, and %-dystrophin positive fibers. Safety assessments included AE recording, ECG, ECHO, and safety laboratory testing.

**Results**:

After more than 3 years of treatment, all patients previously evaluable on 6MWT (mITT; n=10) showed continued ambulation. The boys on continuous eteplirsen from Week 1 (n=6) declined 76.7 meters in walking ability through week 168. This was 65 meters better than placebo-delayed (n= 4; p£0.017), starting eteplirsen at Week 25. Of particular interest, the placebo-delayed declined 68 meters through Week 36 and then showed a course almost identical to the eteplirsen cohort (placebo-delayed declined 73 meters; continuous eteplirsen declined 76 meters). This is most likely related to the time needed for the production of meaningful levels of dystrophin (~12 weeks post-treatment). All patients, even those age 12-15, continue to demonstrate an ability to climb stairs, to rise from supine, and to raise a hand to the mouth at a rate higher than what was observed in a natural history study of glucocorticoid-naïve or steroid-treated boys with DMD (Henricson et al., 2013). All 12 patients, including the two non-ambulatory patients, demonstrated PFT stability from baseline through Week 168, including MIP (+11.1%, p=NS), MEP (+13.5%, p=NS), and MIP/MEP %-predicted (-2.4%/-6.3%, p=NS).

No deaths, discontinuations, treatment-related SAEs, immune activation including infusion reactions, or clinically significant abnormal laboratory, ECG, or ECHO findings were reported.

**Conclusion**:

Eteplirsen treated patients demonstrated a significant clinical benefit on the 6MWT over 168 weeks. The observed decline in walking distance contrasts with the decline observed in DMD natural history of comparative age. Eteplirsen was well tolerated, with no clinically significant treatment related adverse events.