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**GENE & CELL  
THERAPY**

American Society of Gene & Cell Therapy

# 22nd Annual Meeting

## Final Program Guide



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cells/Kg, respectively. Patients FA-02004 and FA-02006 were infused 6 and 12 months later with 170,000 and 410,000 transduced CD34<sup>+</sup> cells/Kg. More recently, three patients were infused with lower numbers of transduced CD34<sup>+</sup> cells. Although the proportion of corrected PB cells during the first 6 months post-infusion has been consistently low (generally less than 2%), progressive increases in gene marking have been observed thereafter in the first four treated patients through the most recent follow-up period (18 to 30 months post-infusion). Patient FA-02002 currently shows the highest level of gene marking, with percentages of marked cells in BM and PB above 50% at the most recent analyses (24 and 30 months post-infusion, respectively). Insertion site analyses did not reveal patterns consistent with insertion-site mediated clonal expansion, and confirmed the engraftment of multipotent HSCs. In each of the first four treated patients evident increases in the resistance of BM progenitor cells to mitomycin-C have been observed. Moreover, after *in vitro* challenge to diepoxybutane, significant decreases in the proportion of PB T-cells with chromosomal breaks have been observed in three of the four first treated patients since the first year post-infusion. Monitoring during a 2-3 year follow-up of our first gene therapy-treated non-conditioned FA-A patients shows the absence of severe adverse events and demonstrates progressive engraftment of phenotypically corrected HSCs. Data from this clinical trial strongly suggest that gene therapy will constitute a safe therapeutic approach for the treatment of the bone marrow failure characteristic of FA.

## 52. Genome-Wide Assessment of Lentiviral Integration Sites of Gene-Corrected Lympho-Hematopoietic Cells in FA-A Patients

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Fanconi anemia (FA) is a DNA repair syndrome resulting from mutations in any of the 22 FA genes that encode for proteins participating in the FA/BRCA pathway. Three years ago a gene therapy trial utilizing autologous cells gene-modified with lentiviral-vectors (LVs) started in Spain as a potential alternative treatment for bone marrow failure in FA. Based on risks of insertional oncogenesis observed in previous clinical trials with gamma-retroviral vectors, careful insertional site studies are mandatory in clinical trials involving vectors that integrate in the genome. Analyses of transduced cells in the first four non-conditioned FA patients had been infused with gene-modified autologous CD34<sup>+</sup> cells have shown that LV-mediated hematopoietic gene therapy reproducibly confers a progressive increase of gene-modified cells in peripheral blood (PB) and bone marrow (BM). We have analyzed the LV-insertion sites

in total and enriched PB and BM cell subsets from these patients, and investigated whether the repopulation advantage of these cells was due to integration site dependent dominant clones. Insertion site analyses of hematopoietic cells from these gene therapy treated FA-A patients were conducted with LAM-PCR. A total of 2,001 unique IS were obtained. This revealed a typical lentiviral integration pattern, showing that on average 72.6% of the LV-integrations occurred within genes (80.4% in genes  $\pm$  10kb) and that no preferential LV-integrations were found in close proximity to transcription start sites. No clones harboring genes associated with hematopoietic malignancies such as LMO2, CCND2 or MN1 were detected during the observation time points (up to 30 months post gene therapy in two patients). Importantly, progressive increases in the proportion of corrected cells were not associated with the continuous expansion of one or few clones of corrected cells but rather with an increase in the number of different clones. A high proportion of IS identified in total PB were also identified in different hematopoietic cell lineages, including myeloid and lymphoid lineages expressing the CD14, CD15, CD19 and CD3 lineage markers. Taken together, our data show that the progressive hematopoietic engraftment of non-conditioned FA patients is associated with an oligoclonal pattern of reconstitution, as expected given the limited numbers of gene-modified CD34<sup>+</sup> cells (more than 1 log lower than other gene therapy clinical trials in non-malignant hematopoietic disorders). No common integration sites associated to known proto-oncogenes have been identified and no persistent clonal dominance was observed until the last observation time point (up to 30 months post gene therapy). Further ISA of these patients will allow us to confirm the safety of the LV-mediated gene therapy in FA, and will help to yield insights into clonal dynamics and repopulation kinetics of the blood forming system in this novel gene therapy trial.

## 53. A Diversity of Human Hematopoietic Differentiation Programs Identified through In Vivo Tracking of Hematopoiesis in Gene Therapy Patients

Emmanuelle Six<sup>1</sup>, Guilloux Agathe<sup>2</sup>, Adeline Denis<sup>1</sup>, Arnaud Lecoules<sup>1</sup>, Alessandra Magnani<sup>3</sup>, Romain Vilette<sup>2</sup>, Frances Male<sup>4</sup>, Nicolas Cagnard<sup>1</sup>, Marianne Delville<sup>1</sup>, Elisa Magrin<sup>3</sup>, Laure Caccavelli<sup>3</sup>, Cecile Roudaut<sup>3</sup>, Clemence Plantier<sup>3</sup>, John Gregg<sup>4</sup>, Christopher Nobles<sup>4</sup>, John K. Everett<sup>4</sup>, Salima Hacein-Bey-Abina<sup>5</sup>, Anne Galy<sup>6</sup>, Alain Fischer<sup>1</sup>, Adrian J. Thrasher<sup>7</sup>, Isabelle Andre-Schmutz<sup>1</sup>, Marina Cavazzana<sup>1</sup>, Frederic Bushman<sup>4</sup>

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