AMERICAN SOCIETY of GENE & CELL THERAPY

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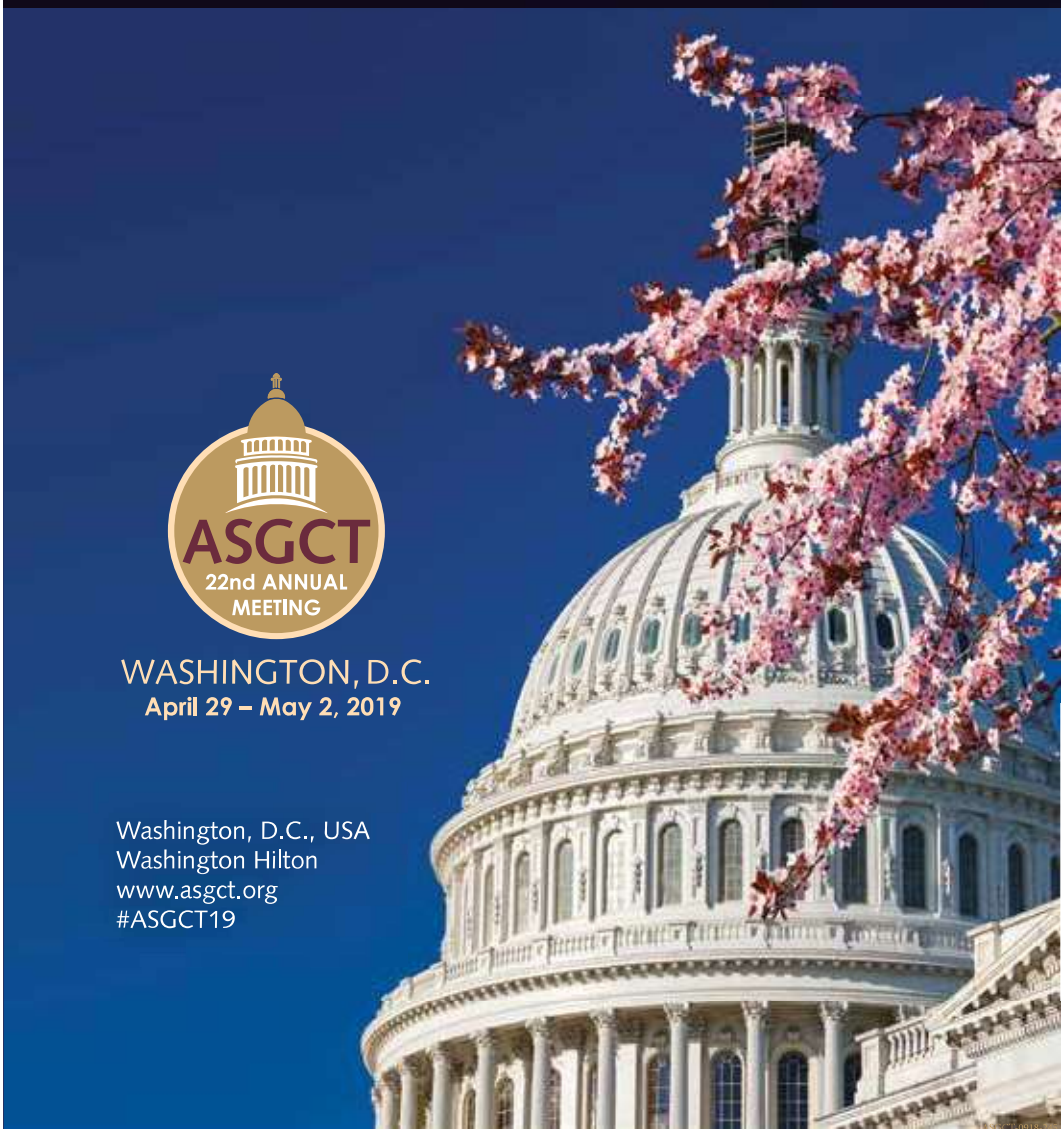
Final Program Guide



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receiving blood transfusions. Prolonged follow up analysis will provide additional information on the long-term safety and clinical efficacy of this treatment.

50. Gene Therapy for Sickle Cell Disease (SCD) Using RVT-1801 Lentivirus Vector and Arulite Reduced Intensity Conditioning Transplant Shows Promising Correction of the Disease Phenotype

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Myeloablative busulfan conditioning and transplant of autologous anti-sickling beta^{87}-globin gene modified hematopoietic stem cells (HSC) was shown to cure a child with SCD (NEJM 2017). The same approach was not successful in subsequent SCD adults treated similarly, until modifications to intensify busulfan dose, HSC dose and gene transfer were made (ASH, 2017, 2018). Other SCD trials (NCT03282656, NCT02247843) are also using ablative busulfan conditioning with mixed results. This conditioning comes with significant acute (cytopenia, mucositis) and chronic toxicities (infertility, secondary malignancy). We performed a Reduced Intensity Conditioning (RIC) Phase I/II Pilot Study on Gene Transfer In SCD Patients with a Modified γ-Globin Lenti-vector (RVT1801; NCT02186418) to determine its safety, feasibility and efficacy. RIC will have less toxicity, cost and implementable in many transplant centers, including resource-poor countries, where intense medical support required for myeloablative transplants is scarce, and where majority of SCD patients exist. Eligible adults with severe SCD underwent HSC collection via bone marrow (BM) harvests and/or plerixafor mobilized Peripheral Blood Stem Collection (PBSC). Patients received AruLite conditioning (IV melphalan) followed by infusion of RVT-1801-modified HSC. Patients were monitored for AEs, engraftment, vector copy number (VCN), modified fetal hemoglobin (HbF^{G16D}) expression and other laboratory and clinical parameters of SCD. As of Dec. 2018: P1 (35 yo) and P2 (25 yo), with HbS-β0 thalassemia received $1x10^6$ and $6.9x10^6$ CD34+ cells/kgbw, with VCN 0.22 and 0.46, respectively, 36 h following Arulite conditioning. Time to neutrophil recovery (> 500) was 7-9 days, and platelet recovery (> 50K) was 7-12 days. Both patients had severe disease and continued to have chronic pain requiring significant IV opiods for upto 6 mo post-transplant. 80% of the AEs were pain events; others were anticipated transient laboratory AEs associated with melphalan. Following gene-modified CD34+ cell infusion, both patients have shown a net rise in HbF^{G16D} starting from D30 post-transplant. Anti-sickling hemoglobin (HbF^{G16D}, HbF, HbA_2) levels were 32% in P1 and 22% in P2 at 15mo and 12mo, with a VCN of 0.2-0.4 in multiple lineages. Integration site analysis on both at all time points upto 6 mo shows a highly polyclonal pattern of integration. P1 had 20% HbF^{G16D} (2.1g/dl HbF^{G16D}, a rise in total Hb from 7-8.5 to 10.5-11g/dL) with stable VCN of 0.2-0.4 in multiple lineages in BM and blood at 15 mo. P2 has also shown a progressive rise in HbF^{G16D}, albeit at a lower trajectory. P1 and P2 had 48 and 20 acute sickle events, respectiely, in the 18 mo prior enrollment and chronic pain requiring chronic oral/IV opioids. Both have > 90% improvement in acute sickle episodes, and were weaned off chronic opioid use. Early results from 2 SCD patients treated with RVT1801-modified HSC using Arulite RIC showed good safety, feasibility, minimal post-transplant toxicity, rapid count recovery, and stable genetically modified cells in blood and BM. Both patients have sufficient anti-sickling globin expression to ameliorate anemia and remarkably reduce chronic pain and acute sickle events to date. These results with AruLite RIC, are promising and if replicated in future patients will provide a 'transportable' and feasible gene therapy for SCD.

51. Preliminary Conclusions Obtained in Fanconi Anemia Patients Treated by Lentiviral-Mediated Gene Therapy after 2 Years of Follow-Up

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In 2016 we started a gene therapy trial in patients with Fanconi anemia, subtype A (FA-A patients) using autologous $CD34^+$ cells. $CD34^+$ cells were mobilized to peripheral blood (PB) with G-CSF and plerixafor and transduced with the therapeutic PGK-FANCA.Wpre* lentiviral vector. Seven patients with ages between 3-6 years old have been infused either with cryopreserved or fresh $CD34^+$ cells with doses ranging from 50,000 to 400,0000 transduced $CD34^+$ cells/Kg. Patients FA-02002 and FA-02005 were treated in January and March 2016 and were infused with an estimated number of 250,000 and 230,000 transduced $CD34^+$

cells/Kg, respectively. Patients FA-02004 and FA-02006 were infused 6 and 12 months later with 170,000 and 410,000 transduced CD34+ cells/Kg. More recently, three patients were infused with lower numbers of transduced CD34+ 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 LIympho-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+ 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 ± 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+ 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

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