collection rate and time from Mozobil administration. Following the completion of the apheresis procedure, the apheresis product was subjected to a ficoll density gradient separation to isolate mononuclear cells. The mononuclear cell fraction was then immunomagnetically depleted of CD11b+ cells and the CD11b- fraction was once more stained, this time with anti-CD34 immunomagnetic beads to allow positive selection of CD34+ cells. Our methods have produced 73 to 95% purity of CD34+ cells across our studies, with a cell yield between 1.34 to 53.4 x 10^6 CD34+ cells across studies. Preliminary analysis of samples collected during the apheresis procedures suggested that while total cell counts were comparable across collection conditions, there were higher concentrations of CD45+CD34+ cells in samples collected within 5-6.20hrs post-mozobil administration, than later collections (up to 7.30hrs post-mozobil administration). In addition, when collected during the earlier timeframe, a higher concentration of CD45+CD34+ cells was obtained in a sample with a collection rate of 0.5mL/min, as compared to 1mL/min. Finally, the collection color (used as an index of cells density during collection), howed a trend to greater CD45+CD34+ concentration in samples collected in the following order: dark and middle then pale red patterns. We demonstrate high degrees of purity (>70%) in isolating CD34+ cells from apheresis product of non-human primates by immunomagnetic bead separation. This method can be harnessed to produce a large variety of cells for immunotherapies, as well as to develop, test, and bring to market CAR T cells produced though allogenic methods.

183 Characterization of AAV Integrations and Rearrangements from Long and Short Reads with RAAVioli

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Recombinant Adeno Associated Viral (rAAV)-based gene therapy (GT) applications have been successfully exploited for the treatment of several disorders. rAAV mainly remains episomal in the nucleus of transduced cells, however, numerous studies demonstrated integration of fragmented or full-length AAV DNA within the transduced cell genome where double-strand DNA breaks (DSBs) or nicks have occurred. Yet, preclinical studies revealed the occurrence of hepatocellular carcinoma and clonal expansion events consequent to rAAV insertions, posing safety concerns for their clinical use. However, bioinformatics tools able to identify AAV integration sites (IS) and characterize vector rearrangements are still missing. Here, we collected data from a humanized liver mouse model, where human primary hepatocytes have been transduced ex-vivo or in-vivo with a tomato expressing AAV. PCR amplicons or DNA fragments containing AAV vector portions were sequenced by both short paired-end and long reads and then analyzed by RAAVioli (Recombinant Adeno-Associated Viral IntegratiOn analysis), to characterize vector rearrangements and IS. Python and R scripts parse the alignments to identify IS and reconstruct rearrangements using CIGAR strings. We retrieved 811 and 370 IS from short paired-end Illumina reads and long PacBio reads respectively, confirming the higher efficiency of PCR-based approach in IS retrieval. The distribution of AAV IS was sparse in the human genome similarly in both datasets, and Albumin gene was the most targeted gene as expected. Furthermore, 32 ISs were in common between the two datasets, demonstrating the reliability of RAAVioli independently from sequencing platform adopted. Both datasets showed a similar percentage (~25%) of fragments with AAV rearrangements, however more than 2 rearrangements per fragment were retrieved only in long PacBio reads. Precision and accuracy of RAAVioli pipeline was assessed through simulated datasets obtaining scores >0.95 in IS identification and rearrangement characterization. These data demonstrated that RAAVioli is a comprehensive and flexible bioinformatic tool that can efficiently map AAV IS using long and short paired ends sequencing reads. These approaches are fundamental to characterize AAV integration and recombination events in gene therapy and gene editing applications, allowing and improving the assessment of safety in AAV studies.

184 CISC: A Multi-Purpose Enrichment Tool for HDR-Edited Human T-Cells Applicable for Treg and CAR-T Therapeutic Cell Products

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Engineered T cells represent an emerging therapeutic modality. However, complex engineering strategies can present a challenge for enriching and expanding therapeutic cells at clinical scale. Additionally, lack of in vivo cytokine support can lead to poor engraftment of transferred T cells, including regulatory T cells (Treg). We have established a cell-intrinsic selection system that leverages the dependency of primary T cells on IL-2 signaling. Novel FRB-IL2RB and FKBP-IL2RG fusion proteins, referred to as chemically inducible signaling complex (CISC), were identified permitting selective expansion of transduced primary CD4+ T cells in rapamycin supplemented media when delivered as a lentivirus. LV-transduced cells strongly activated the STAT-5 signaling pathway in response to CISC heterodimerization. The CISC was subsequently incorporated into HDR donor templates designed to drive expression of the Treg master regulator FOXP3 at the endogenous *FOXP3* locus or introduce a CD19-CAR and delete the endogenous T Cell receptor at the *TRAC* locus. Following FOXP3 editing of CD4+ T cells, CISC+ engineered Treg (CISC EngTreg) were selectively expanded using rapamycin and maintained Treg activity. Significantly, CISC EngTreg cells adoptively transferred into immune deficient mice showed increased engraftment and retention, and improved therapeutic efficacy in a xenogenic graft vs host disease model when mice were treated systemically with dimerizer. *TRAC* edited CD4+ T cells displayed efficient endogenous TCR knockout in combination with CD19-CAR expression, and functional CISC CAR-T Cells were selectively enriched in the presence