the purification of various LV pseudotypes that are biologically-active, stable and with sufficient recovery in the perspective of preclinical studies and clinical applications.

535.   Increasing Accuracy and Precision of Vector Integration Site Identification of Sequencing Reads With a New Bioinformatics Framework

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In hematopoietic stem cell (HSC) gene therapy (GT) applications patients are transplanted with autologuos HSCs that have been ex-vivo genetically modified with integration competent vectors to express a therapeutic transgene. Specific PCR techniques coupled to next generation sequencing and bioinformatics analysis allow the high throughput retrieval, sequencing and mapping of proviral/genomic DNA junctions present in the blood and bone marrow derived cell populations sampled at different time points after therapy. The increase in sequences available for IS mapping is accompanied by an increase in false positives derived by sequencing errors or sequencing read parsing and mapping on the reference genome. In particular, by analyzing IS datasets form vector marked human and mouse tumor cells, clones with defined integration sites and GT patients, we observed that when multiple sequences arising from the same IS are aligned on the reference genome >10% mapped near (+/- 4 bases) the true insertion site. Without correction, these misaligned sequences not only result in an overestimation of the overall number of IS but in some cases also in the generation of false common insertion sites, worrisome hallmarks of insertional mutagenesis. To mitigate this issue we and others, based on empirical observations, merge sequencing reads mapping within +/- 3 bp into a single IS. Although this adjustment reduces the impact of the "wobbling" around the true ISs, a dedicated method and model is still missing.

To further increase the accuracy of genomic positioning of sequencing reads we developed a new bioinformatics framework as post-processing plugin for pipelines that correctly partitions sequencing reads in a given genomic position by considering the relative abundance and distribution of each sequence cluster using local modes and Gaussian scores through an adaptive approach that varies the parameters of the Gaussian curve and proposes different solutions. To chose the best solution, the algorithm first evaluates each solution by exploiting 100 simulations of the input reads and then selects the resulting best solution using the Kolmogorov-Smirnov test. The simulation step is designed to test the mappability of the IS genomic interval and to quantify the impact of the observed nucleotide variations of the reads with respect to the reference genome (PCR artifacts or real genomic differences) that may lead to different mapping results that justify a larger span of the mapped reads surrounding the putative IS. The algorithm returns the list of IS and relative number of reads with the p-value of the best solution.

We performed 3 ad-hoc in vitro experiments on a cell clone with 6 known IS in which we measured the precision of IS placement obtaining an average of 100% with our new method whereas <30% using our previous method based on a rigid sliding window approach of 4 bp. We applied our new approach to our clinical trial datasets obtaining improvements in IS genomic placement and overestimation with a reduction of potential false IS of 3% without changing the biological results.

536.   Dual-Vector Prodrug Activator Gene Therapy Using Retroviral Replicating Vectors Derived from Amphotropic Murine Leukemia Virus and Gibbon Ape Leukemia Virus

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Retroviral replicating vectors (RRVs) have been shown to achieve efficient tumor transduction and enhanced therapeutic benefit in a wide variety of cancer models. An amphotropic murine leukemia virus (AMLV)-based RRV encoding the yeast cytosine deaminase (CD) prodrug activator gene, designated Toca 511 (*vocimagene amiretrorepvec*), is currently being investigated in combination with Toca FC (extended-release 5-FC) in multi-center clinical trials in the United States for patients with recurrent high-grade glioma (http://www. clinicaltrials.gov NCT01156584, NCT01985256, NCT01470794). This treatment has been safe and well tolerated and median overall survival exceed historical benchmarks. RRV-mediated prodrug activator gene therapy represents the ultimate form of 'intracellular' chemotherapy, generated selectively and directly from within the infected cancer cells themselves, without incurring systemic toxicity. Moreover, preclinical data support subsequent activation of the immune system selectively against the cancer. However, some patients may not respond to this treatment, therefore, combination with additional therapeutic agents may be desirable to optimize treatment outcomes. Here we evaluated two different RRVs derived from AMLV and gibbon ape leukemia virus (GALV), encoding two different prodrug activator genes, CD and herpes simplex virus thymidine kinase (TK) in Hep3B human hepatocellular carcinoma cells.

Both RRVs expressing the green fluorescent protein gene (AMLV-GFP and GALV-GFP) efficiently replicated in Hep3B cells and spread in culture. Additionally, AMLV-GFP can spread in GALV-mCherry pretransduced Hep3B cells but not in AMLV-mCherry pretransduced cells. Similarly, GALV-GFP can spread in AMLV-mCherry pretransduced cells but not in GALV-mCherry pretransduced cells. This mutually exclusive infection pattern is likely due to receptor interference resulting in superinfection resistance when the same viral strain is used. Notably, however, replication and spread of either RRV in culture was not affected by pretransduction with the counterpart RRV encoated with the other envelope.

In order to investigate the effect of combined prodrug-dependent cell killing vs. multiple vector copy transduction *in vitro*, Hep3B cells were transduced with the AMLV-CD (Ca), GALV-CD (Cg), AMLV-TK (Ta), GALV-TK (Tg), respectively or in combination (Ca/Cg, Ta/Tg, Ca/Tg, Cg/Ta). The resultant cells were used to evaluate the cytotoxic effect of RRV-mediated prodrug activator gene therapy with CD and TK in the presence of their respective prodrugs, 5-fluorocytosine (5-FC) and Ganciclovir (GCV). *In vitro* cytocidal effects obtained by combining different prodrug activator genes (Ca/Tg and Cg/Ta) were significantly greater than when the same prodrug activator genes (Ca/Cg and Ta/Tg) were delivered with two different vectors. These data indicate the potential utility of dual-vector prodrug activator gene therapy using two different RRVs carrying different prodrug activator genes.