Chimeric Antigen Receptor T Cell Integration Site Analysis in Patients with B-cell Acute Lymphoblastic Leukemia

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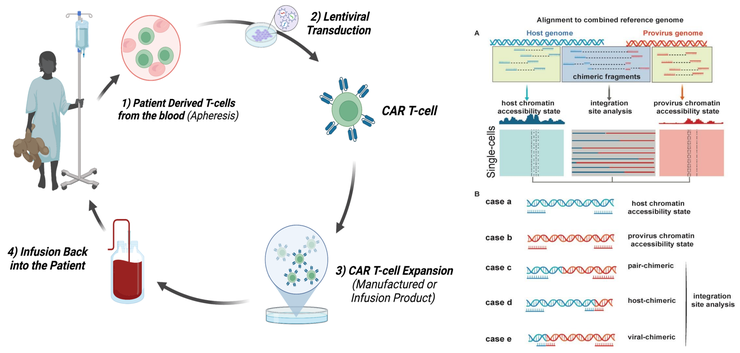
Abstract

Background:
- Minimal CAR T cell proliferation often leads to ineffective therapy.
- CAR T vector integration sites may influence therapy outcomes.
- EpiVIA analyzes ATAC-seq data to detect vector integration sites and epigenetic landscapes.

Study Design:
- Applied EpiVIA to pediatric B-cell ALL data, performing downstream analyses and computational assessments.
- CAR T cells from manufacturing (PM) and long-term follow-up (LTFU) samples.
- Explored EpiVIA's potential to identify relapse-associated factors.
- Sought to use integration sites to link LTFU cells back to PM cells

Results:
- Found no overlap in integration sites between PM and LTFU samples.
- Detected little correlation between expression-based clusters and cells with integration sites.
- EpiVIA is efficient, but sequencing depth was insufficient to detect many integration sites.

CAR T and Viral Integration Overview



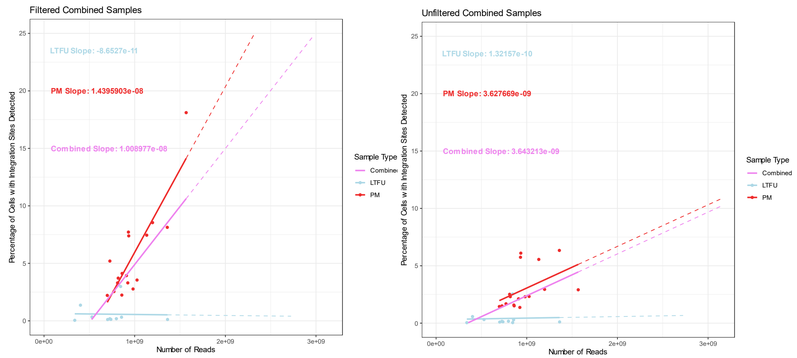
Left: How CAR T therapy works. **Right:** EpiVIA's integration site detection technique (Wang et al. 2020)

Available Data & EpiVIA Results

PM Samples			LTFU Samples				
Sample name (PM)	**Total cells (PM)**	**Percent detected (PM)**	**Sample name (LTFU)**	**Total cells (LTFU)**	**Percent detected (LTFU)**	**Percent overlap with PM**	**Time since diagnosis**
CHP959158_PM	10508	2.769318614	CHP959158_Y7_CAR	7648	0.1176778243	0	7 years
			CHP959158_Y7_G	3876	0.1805985552	0	7 years
CARTEXP01_sv40CAR	8305	2.98615292					
115_PM	7823	3.297967532	115_Y9	6587	0.04554425383	0	9 years
126_PM	3539	5.199208816	126_Y7	3874	0.3355704698	0	7 years
ET38_PM	5593	3.933488289	ET38_Y5	3961	0.1262307498	0	5 years
ET40_PM	7733	2.5475236	ET40_Y5	11588	0.112185019	0	5 years
155_PM	4974	2.211499799	155_Y8	2568	1.362928349	0	8 years
			160_Y8	5397	0.185288123		8 years
136_PM	3456	8.130787037					
164_PM	4531	8.541160892					
ET02_PM	8707	2.239577352					
ET09_PM	740	18.10810811					
100_PM	9047	3.548137504					
156_PM	6188	4.120879121					
ET12_PM	3546	7.388606881					
ET16_PM	2967	7.448601281					
ET24_PM	3239	7.718431615					
ET35_PM	7755	3.301096067					
ET14_PM	3474	3.713298791					

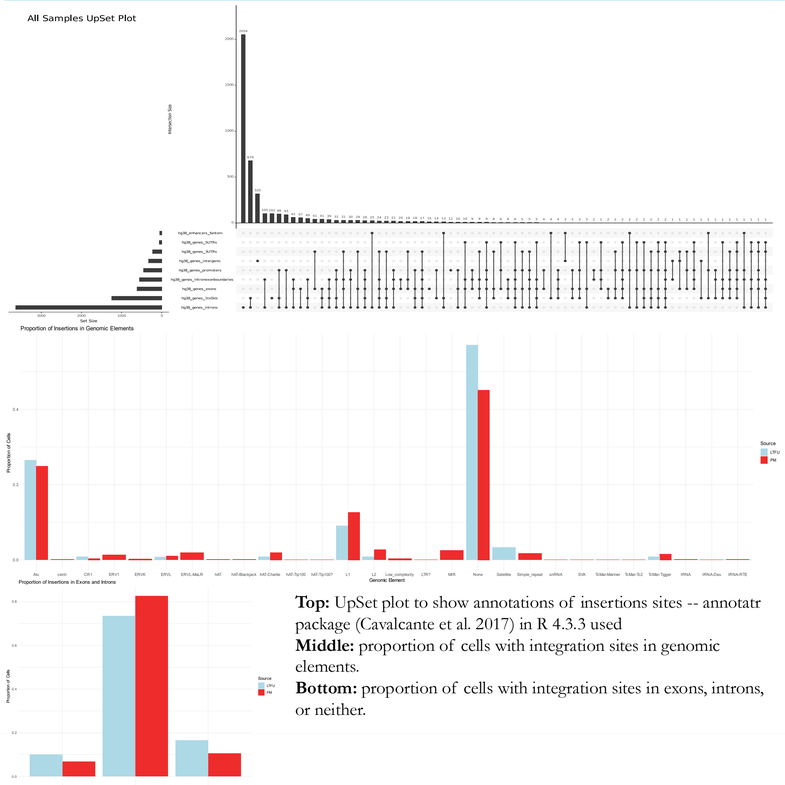
Sample table using the filtered Seurat object. Samples were filtered based on cell quality.
Total cells: Number of cells in the Seurat object
Percent detected: Percentage of total cells that EpiVIA detected to have integration sites
Percent overlap with PM: percent of integration sites that appeared in both the PM and LTFU sample
Time since diagnosis: number of days or years from when CAR T therapy was originally administered

EpiVIA detects more integration sites in more deeply sequenced samples



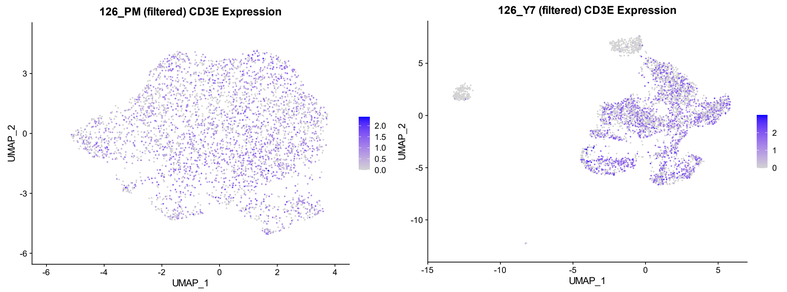
Percentage of cells detected to have insertions PM, LTFU, and combined filtered **(left)** or unfiltered **(right)** samples plotted against number of reads in various BAM files

Integration sites mostly occurred in *Alu* repeats, L1 transposons, introns



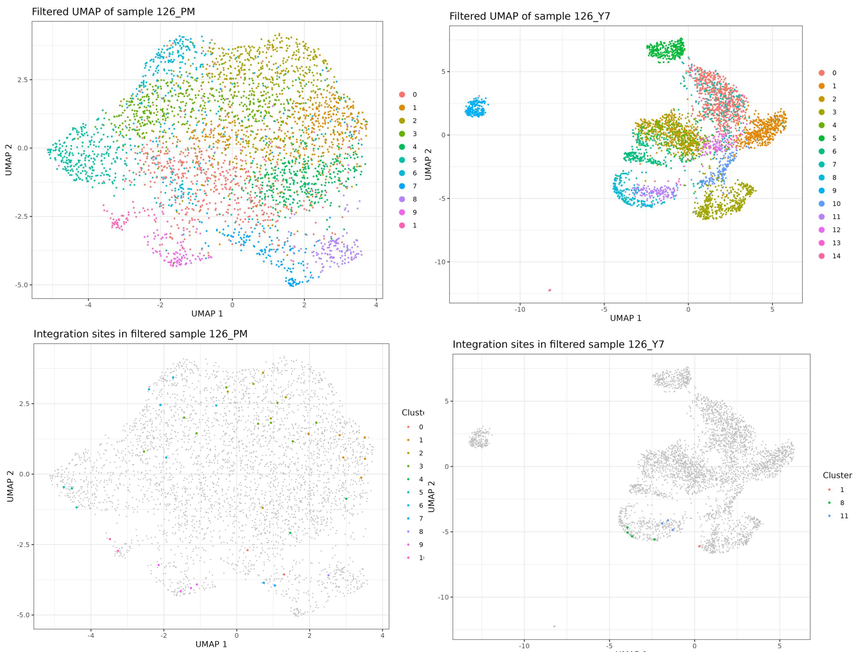
Top: UpSet plot to show annotations of insertions sites -- annotatr package (Cavalcante et al. 2017) in R 4.3.3 used
Middle: proportion of cells with integration sites in genomic elements.
Bottom: proportion of cells with integration sites in exons, introns, or neither.

CD3E analysis shows T cell presence



UMAP plot of CD3E expression in a PM sample **(left)** and the corresponding LTFU sample **(right)**

No relationship between clusters and cells with integration sites



UMAP plot of a PM sample **(left)** or the corresponding LTFU sample **(right)** colored by gene expression cluster **(top)** or coloring just cells with detected integration sites **(bottom)**

Future Directions

Based on our analysis, and because our samples have not been sequenced at 100% coverage, EpiVIA has the potential to detect more integration sites. By resequencing our samples at greater depth, we may be able to better determine EpiVIA's efficacy.

Bibliography: Wang et al. (2020). "Joint profiling of chromatin accessibility and CAR-T integration site analysis at population and single-cell levels." PNAS **117**(10): 5442-5452.

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