Antigen Escape via CD19 Splice Variants in B-Cell Acute Lymphoblastic Leukemia Post CAR T-Cell Therapy

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Introduction

•CD19 CAR-T therapy induces 70–90% remission in relapsed/refractory B-ALL.
•Despite this, 30–60% of patients experience disease relapse after treatment.
•CD19-negative relapse has emerged as a major form of therapeutic failure.
•Antigen escape via CD19 splice variants is a key driver of immune evasion.
•Splice variants often result in isoforms that escape CAR recognition but retain partial function.
•The most common mechanism: exon 2 skipping removes FMC63 epitope.
•Understanding molecular escape mechanisms is critical for improving CAR-T durability.

Objectives

•Investigate the role of CD19 splice variants in mediating antigen escape after CAR-T therapy.
•Determine prevalence of CD19-negative relapse across studies and cohorts.
•Characterize specific molecular events—exon skipping, mutations, or intron retention.
•Evaluate functional consequences of variant CD19 isoforms.
•Examine timing of escape clone emergence (pre-existing vs post-treatment).
•Assess implications for CAR design and treatment planning.
•Identify strategies for early detection and therapeutic targeting.

Methods

Retrospective literature review (2015–2024) including >500 B-ALL patients.
Focused on clinical trials and case series of CD19 CAR-T therapy with antigen monitoring.
Collected data on relapse types (CD19+ vs CD19–), variant prevalence, sequencing findings.
Assessed molecular events: exon 2–5 disruptions, biallelic loss, splicing alterations.
Integrated single-cell transcriptomics where available.
Evaluated pre-treatment samples to detect escape-prone subclones.
Correlated escape variants with clinical outcomes and remission durability.

Figure 1: CD19-Negative Relapse Rate

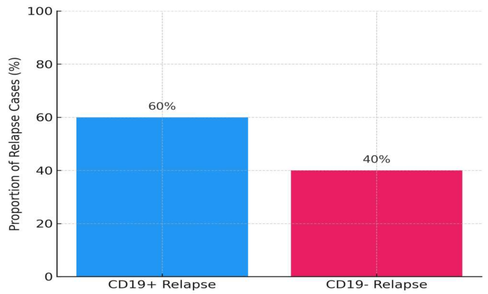


Figure 2: CD19 Exon 2 Skipping and Loss of FMC63 Epitope

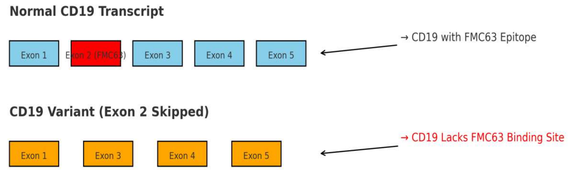
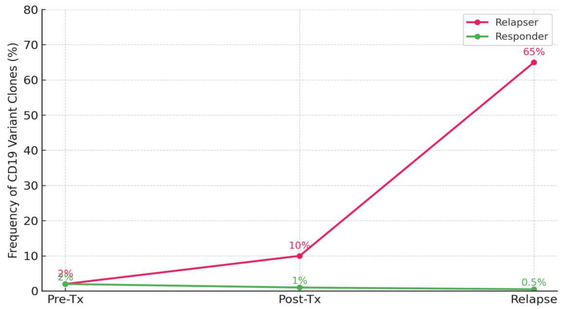


Figure 3: Evolution of CD19 Variant-Expressing Clones Over Time



Results

•CD19-negative relapse occurred in ~30–50% of all relapse cases across cohorts.
•Exon 2 skipping was the most common splicing abnormality, leading to CAR resistance.
•One study found 100% (12/12) relapsed cases had exon 2–5 mutations, often biallelic.
•Spliced isoforms retained partial signaling function, aiding leukemic survival.
•Intron retention and loss-of-heterozygosity were other less common escape mechanisms.
•Single-cell studies showed that escape clones were detectable before infusion (<3%).
•These clones expand under selective pressure from CAR-T exposure.

Conclusion

•CD19 splice variants enable immune escape by disrupting CAR recognition sites.
•These variants retain signaling capacity, promoting survival and relapse.
•Pre-existing escape clones may drive relapse under treatment pressure.
•Dual-target CARs (e.g., CD19/CD22) or alternative epitope targeting may overcome escape.
•Screening for splice variants pre-infusion may guide personalized CAR design.
•Early molecular detection could enable proactive relapse-prevention strategies.
•Clinical protocols should consider integrating single-cell analysis for risk stratification.

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SOHO ANNUAL MEETING 2025

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