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Assessment of CD19 exon 2 abundance as a proxy for CD19delta expression and prediction of anti-CD19 CAR therapy relapse

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1 Many B-cell lymphoma patients experience relapse after CAR T-cell therapy

B-cell lymphoma relapse has previously been linked to antigen loss[1-3], but it is currently not possible to accurately predict which patients are at risk. Splice variants may provide the answer; a splice variant is an mRNA transcript that has undergone a different post-transcriptional splicing than the one most commonly encountered. A splice variant results in the production of a protein isoform that often elicit different properties than its relative(s). These isoforms are poorly studied in context of cancer and CAR therapy, but they can have important implications. CAR T cells commonly target the exon 3 and 4 regions of the CD19 membrane protein to eliminate all B cells in a patient. However, the splice variant where exon 2 is removed might render the B cell undetectable to the CAR[1].

Here, we sat out to explore the role of splice variants in patients treated with a CD19-specific CAR. We found that the exon 2-missing CD19delta variant is more common in B-cell lymphoma patients, and it was present in all relapsing patients in the small cohort we analyzed.

2 CD19delta variant is widespread

To know if this splice variant even exists in the relevant patient group, we analyzed the TCGA and GTEx databases, counting the fraction of transcripts containing CD19 among patients in different patient groups. Interestingly, the CD19delta splice variant is more common among B-cell lymphoma patients than in other cancer types (Figure 1). With this knowledge, the next step was to link the presence of the variant to a prognostic prediction.

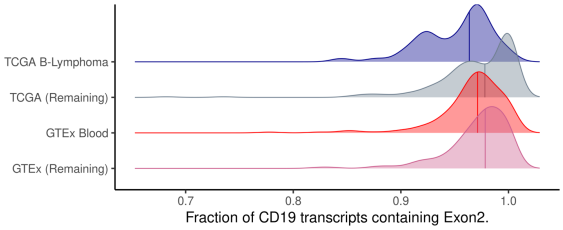

Figure 1: The distribution of CD19 transcripts containing exon 2 among patients of different cancer groups.

3 Four patients exhibited varying response to treatment

To investigate the effects of the CD19delta variant, RNA-seq data was extracted from a CD19 CAR T-cell therapy study conducted by Zhang et al.[4]. This study comprised four patients with varying tumor burden who elicited vastly differing responses to therapy (Table 1). Bone marrow samples were collected before treatment and 14 days post treatment. Our goal was to examine the correlation between response, relapse, and remission to the prevalence of the splice variant.

Patient	Responder	Relapse	TB (%) Day 0
A	Yes	1.5 months	17.92
B	No	NA	65.46
C	Yes	13 months	0.25
D	Yes	Full remission	0.5

Table 1: Overview of the four patients in the study. TB: Tumor Burden.

4 The splice variant is absent in the patient with full remission

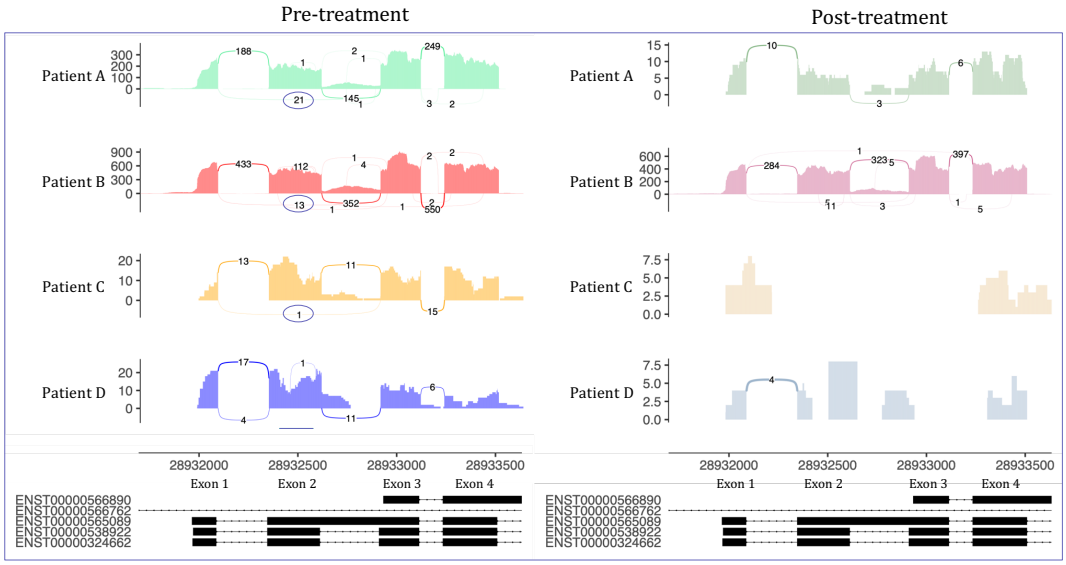

Figure 2: Sashimi plot showing the alternative splicing that occurs in CD19 in the four patients. Purple circles highlight the exon 2-excluding splice junction in each patient.

To investigate the prevalence of the splice variants in the four patients, we made a sashimi plot of the CD19 exons 1-4 and their junctions (Figure 2). From the encircled splice junctions, we noticed that the CD19delta variant was only present in the relapsing and non-responding patients (A, B, C), but absent in the patient with full recovery (D). The limited coverage in the post-treatment samples rendered it difficult to say anything significant about the presence of CD19delta variant or its prognostic capabilities.

Next steps:
We will need to redo the analysis on a larger patient cohort to draw any significance-bearing conclusions. Then, we will test a targeted qPCR panel as a diagnostic tool.

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