

11546: Identification of checkpoint inhibitor resistance mechanisms in leiomyosarcoma through enrichment of active chromatin-associated cell-free DNA.

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Background

- Leiomyosarcoma (LMS) is an aggressive sarcoma subtype with poor outcomes in the metastatic setting and limited therapeutic options. Immune checkpoint inhibitor (CPI) monotherapy has demonstrated minimal activity in LMS, highlighting the need for rational combination strategies, including PARP inhibitors and anti-VEGF agents.
- In the DAPPER trial (NCT03851614), patients with LMS were treated with durvalumab in combination with either olaparib or cediranib. We previously reported (Lopes et al. 2026) that a liquid biopsy assay enriching for active chromatin-associated cell-free DNA (cfDNA_{ac}) identified biomarkers associated with clinical benefit (CB; complete or partial response, or stable disease >6 months).
- Here, we evaluate whether cfDNA_{ac}-derived signals distinguish patients with clinical benefit (CBR) from non-responders and how these signals evolve over time (baseline to progression).

Methods

Study Workflow: Plasma Collection and cfDNA_{ac} Processing (DAPPER Trial)

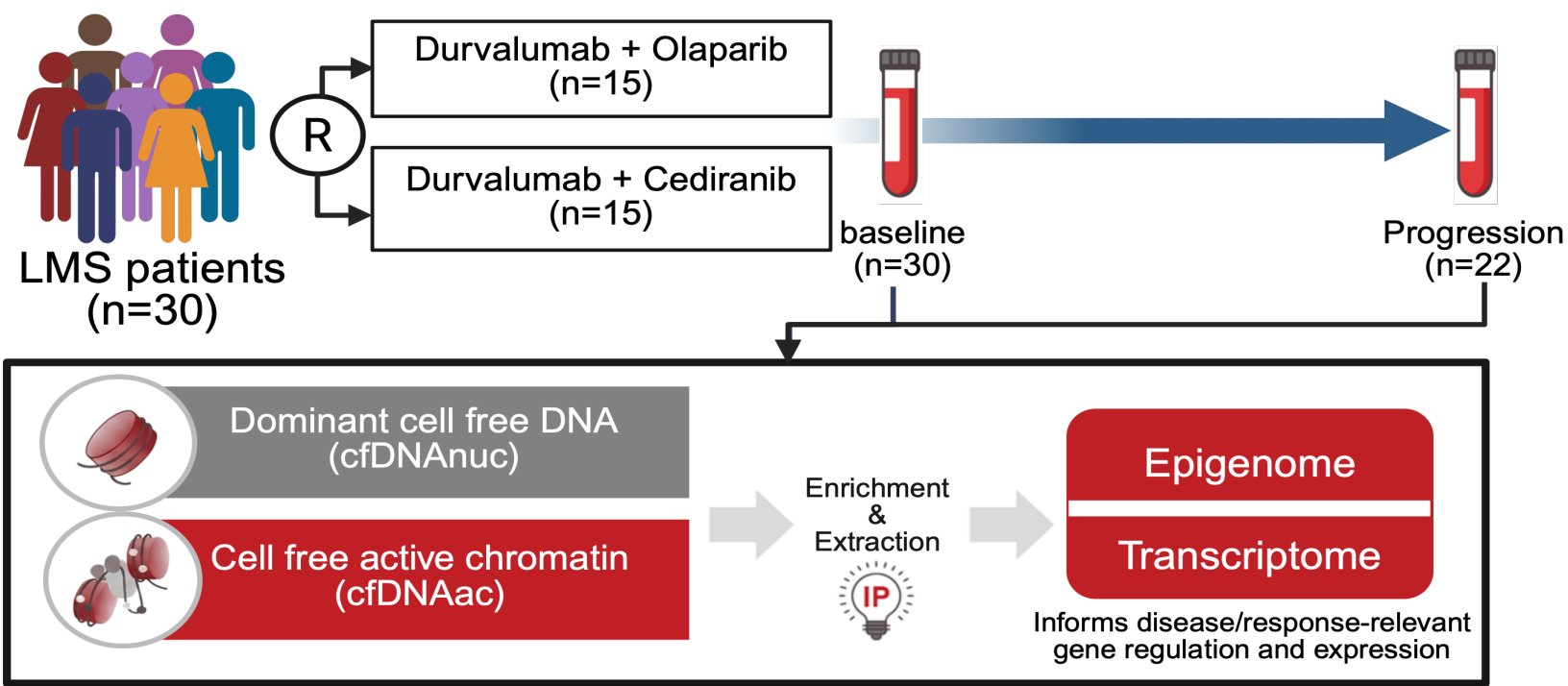


Figure 1. Overview of the DAPPER trial. Plasma samples at baseline (n=30) and disease progression (n=22) were analyzed using a novel cfDNA_{ac} capture platform. Univariate analysis and recursive feature elimination identified genomic features associated with CBR. Linear mixed-effects models assessed temporal differences in cfDNA_{ac}-derived biomarkers between CBR and non-CBR groups.

Results

Feasibility of cfDNA_{ac} Detection and Discrimination of CBR vs Non-CBR

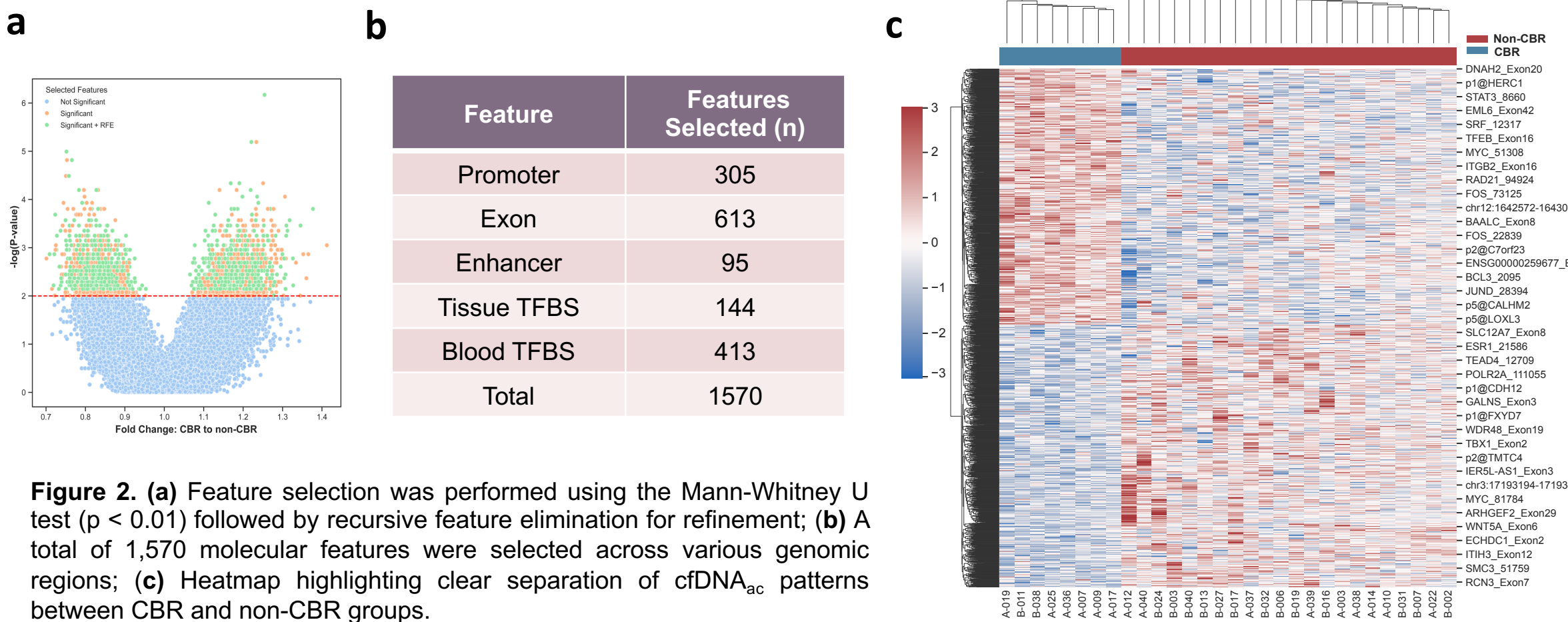


Figure 2. (a) Feature selection was performed using the Mann-Whitney U test ($p < 0.01$) followed by recursive feature elimination for refinement; **(b)** A total of 1,570 molecular features were selected across various genomic regions; **(c)** Heatmap highlighting clear separation of cfDNA_{ac} patterns between CBR and non-CBR groups.

Results

CBR and Non-CBR Differ at Baseline but Converge at Progression

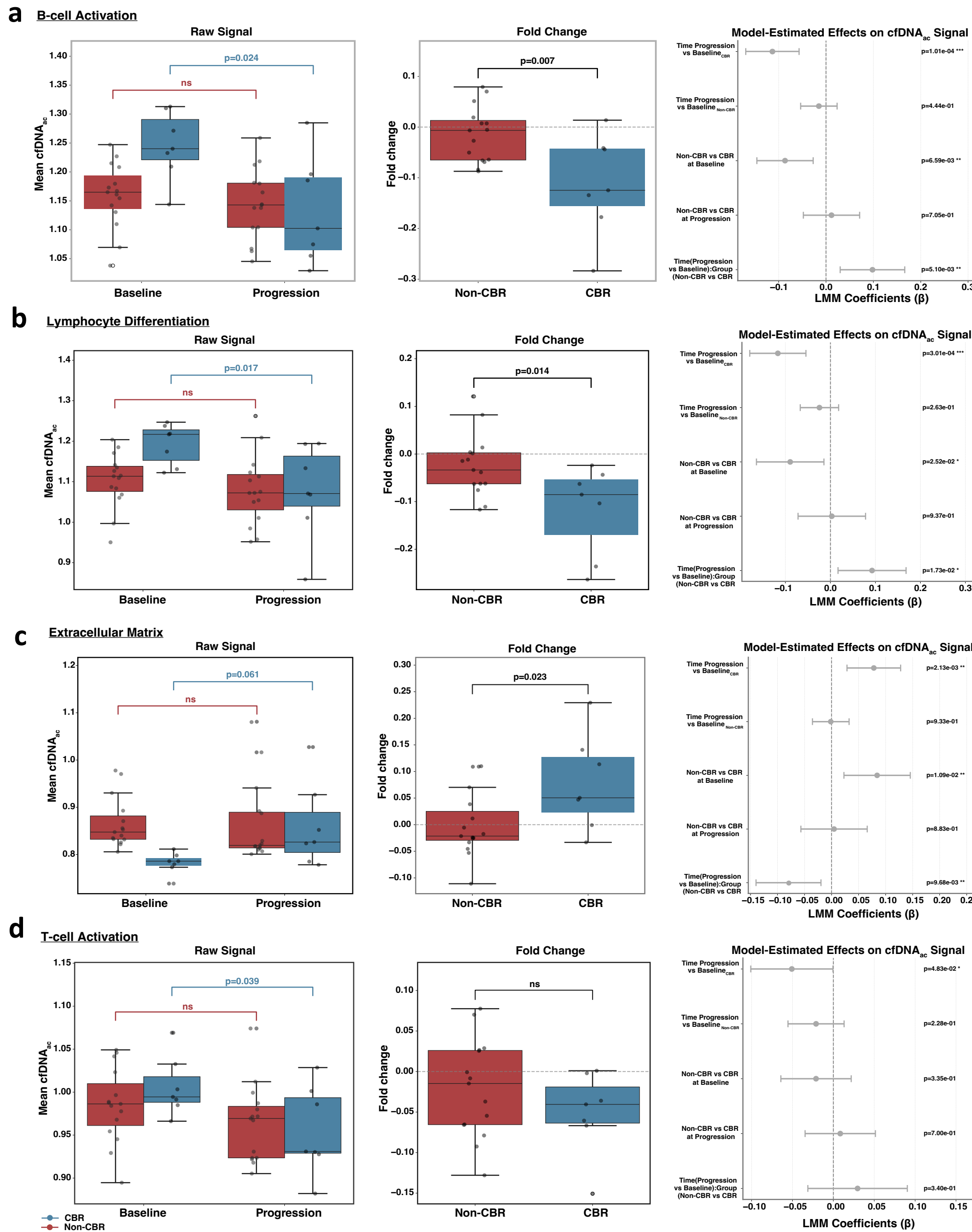


Figure 3. At baseline, CBR patients show enrichment of immune-related cfDNA_{ac} signatures, including (a) B-cell activation (b) lymphocyte differentiation and (c) T-cell activation with (d) lower extracellular matrix (ECM) organization, relative to non-CBR. At progression, CBR patients show significant decreases in B-cell activation and lymphocyte differentiation, approaching levels seen in non-CBR patients, along with a borderline increase in ECM signatures. T-cell activation decreases in raw signal but does not differ between groups by fold change. Non-CBR patients remain largely stable over time. Boxplots show raw signal (left) and within-patient fold change (middle). Forest plots (right) show linear mixed-effects model estimates ($\beta \pm 95\%$ CI) for time, group, and their interaction. Overall, B-cell activation, lymphocyte differentiation, and ECM programs show treatment-associated changes over time in CBR patients that are not observed in non-CBR patients, while T-cell activation does not differ between groups.

Chromosome 15-Specific CNV Dynamics Distinguish CBR and Non-CBR Across Baseline and Progression

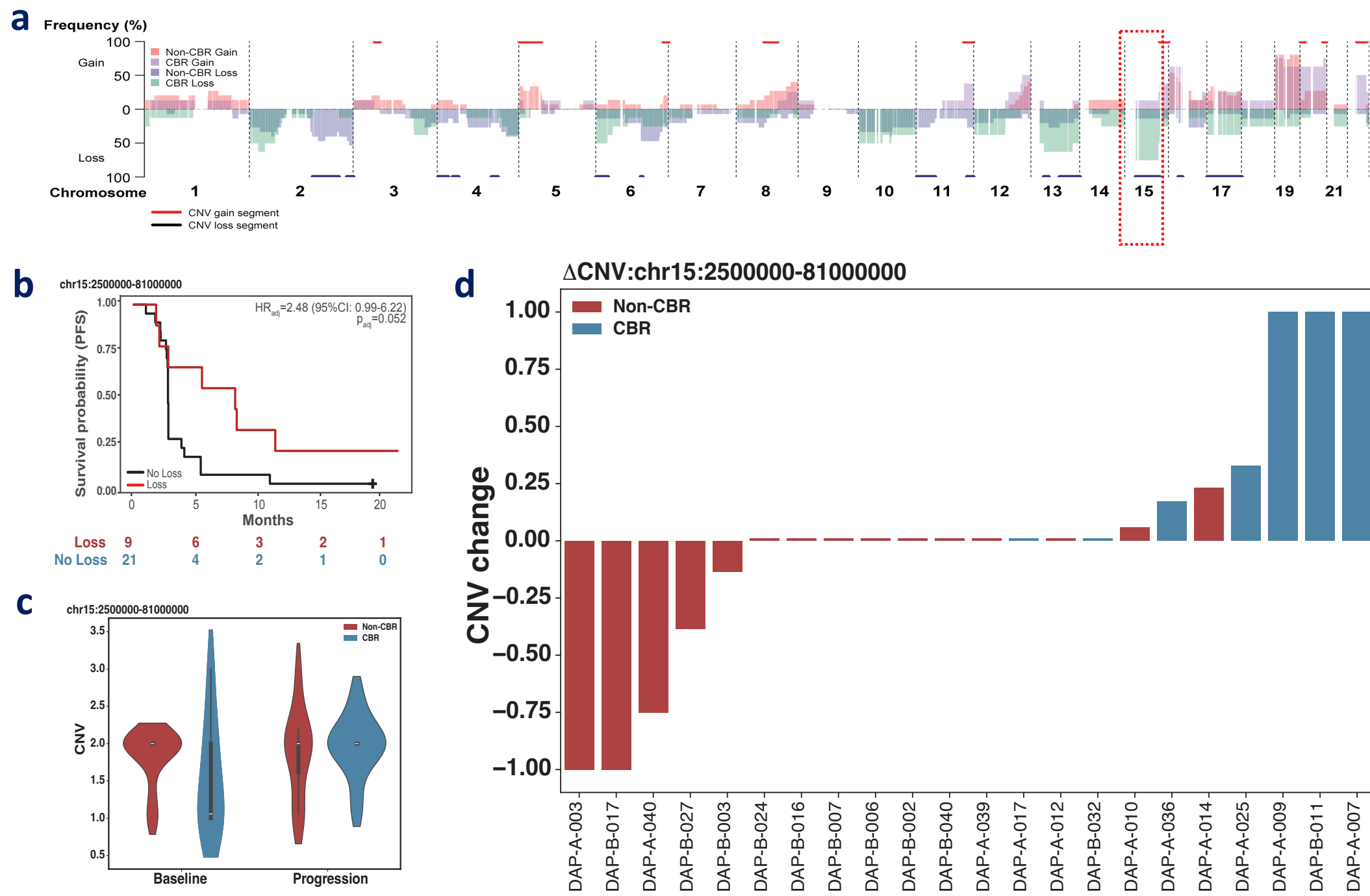


Figure 4. (a) Genome-wide segmentation of CNV gains (top) and losses (bottom) comparing CBR and non-CBR groups, highlighting regions of differential loss on chromosome 15. (b) Kaplan-Meier analysis indicating that chromosome 15 CNV loss is associated with improved progression-free survival (HR = 2.48, 95% CI: 0.99-6.22, $p = 0.052$). (c) Violin plots showing chromosome 15 CNV distributions at baseline and progression for CBR and non-CBR groups, illustrating temporal and group-specific differences. (d) Waterfall plot of per-patient CNV change (progression minus baseline) across chr15:25–81 Mb, stratified by response group. Each bar represents an individual patient; CBR patients exhibit greater CNV shifts consistent with dynamic remodeling, whereas non-CBR patients show relatively stable profiles ($p = 0.0073$, two-sample t-test).

Conclusions

cfDNA_{ac} profiling provides a non-invasive, longitudinal view of tumor microenvironment dynamics in LMS patients treated with CPI-based combinations. At baseline, CBR patients show enrichment of immune-related programs that diminish and converge to similar profiles as non-CBR patients at time of progression. By capturing both functional and genomic changes linked to response and emerging resistance, cfDNA_{ac} supports broader application across sarcoma subtypes.

References

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