Baseline cfDNA Active Chromatin Profiles Reflect Synovial Pathotype-Associated Biology Distinguishing Response to Targeted Treatments in Rheumatoid Arthritis

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Background

- Synovial biopsy studies have identified genes and distinct patterns of histomorphology, hypothesized to represent "pathotypes" (including lymphoid-rich, myeloid-dominant, and pauci-immune/fibroid states) associated with differential response to targeted therapies for RA.
- Because tissue is not routinely accessible, we evaluated whether circulating cell-free DNA regulatory-active chromatin ($cfDNA_{ac}$) identifies gene activity associated with response, including genes reported from synovial biopsy studies.

Methods

- Baseline plasma samples were analyzed from adult RA patients in PRIMA-102 (NCT05936970), a prospective multicenter cohort with moderate-to-high disease activity initiating a TNF inhibitor (TNFi), JAK inhibitor (JAKi), or abatacept.
- Response was defined as achievement of a minimal clinically important difference (MCID) in the Clinical Disease Activity Index (CDAI) at 12 weeks.
- $cfDNA_{ac}$ fragments derived from plasma using a novel assay were sequenced and mapped to 37,000 promoters.
- Differential response-specific promoter regulation and concordance with synovial transcriptomic signatures was assessed using multivariable linear modeling with false discovery rate (FDR) correction and unsupervised hierarchical clustering with pathway-level aggregation.

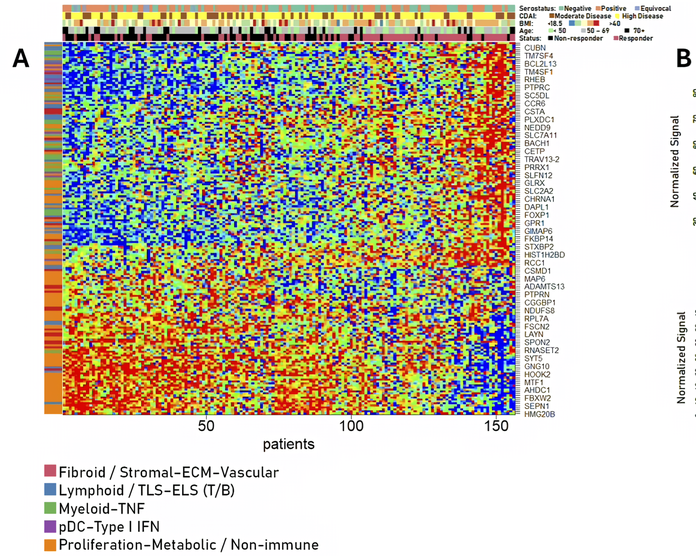


Table 1: Demographics and patient characteristics

	All	TNFi	JAKi	Abatacept
Subjects (n)	416	156	135	125
Age at Enrollment: Mean (SD)	57.6 (13.4)	56.4 (13.7)	55.6 (13.5)	61.4 (12.3)
Female: n (%)	352 (84.6%)	131 (84.0%)	115 (85.2%)	106 (84.8%)
Race: White n (%)	329 (79.1%)	121 (77.6%)	106 (78.5%)	102 (81.6%)
BMI: Mean (SD)	30.4 (7.2)	30.0 (7.3)	30.3 (6.8)	30.9 (7.4)
Duration of Disease (yr): Mean (SD)	8.4 (9.9)	7.1 (8.7)	8.4 (10.8)	10.0 (10.2)
Baseline Disease Activity Moderate: n (%)	117 (28.1%)	53 (34.0%)	36 (26.7%)	28 (22.4%)
Baseline Disease Activity High: n (%)	299 (71.9%)	103 (66.0%)	99 (73.3%)	97 (77.6%)
Baseline CDAI Score: Mean (SD)	30.9 (12.6)	30.0 (13.6)	31.4 (12.5)	31.6 (11.3)
Serostatus: Seropositive n (%)	209 (50.2%)	83 (53.2%)	69 (51.1%)	57 (45.6%)
CRP (mg/L): Mean (SD)	9.5 (18.2)	7.9 (15.0)	9.8 (19.5)	11.0 (20.1)
Concomitant Glucocorticoid Treatment: n (%)	147 (35.3%)	59 (37.8%)	44 (32.6%)	44 (35.2%)
Concomitant csDMARD Treatment: n (%)	284 (68.3%)	114 (73.1%)	75 (55.6%)	95 (76.0%)
Prior b/ts DMARD Experience 0: n (%)	98 (23.6%)	50 (32.1%)	25 (18.5%)	23 (18.4%)
Prior b/ts DMARD Experience 1: n (%)	141 (33.9%)	54 (34.6%)	50 (37.0%)	37 (29.6%)
Prior b/ts DMARD Experience 2: n (%)	92 (22.1%)	28 (17.9%)	29 (21.5%)	35 (28.0%)
Prior b/ts DMARD Experience 3: n (%)	42 (10.1%)	12 (7.7%)	17 (12.6%)	13 (10.4%)
Prior b/ts DMARD Experience 4+: n (%)	43 (10.3%)	12 (7.7%)	14 (10.4%)	17 (13.6%)
Response to On-study Treatment: n (%)	218 (52.4%)	77 (49.4%)	77 (57.0%)	64 (51.2%)

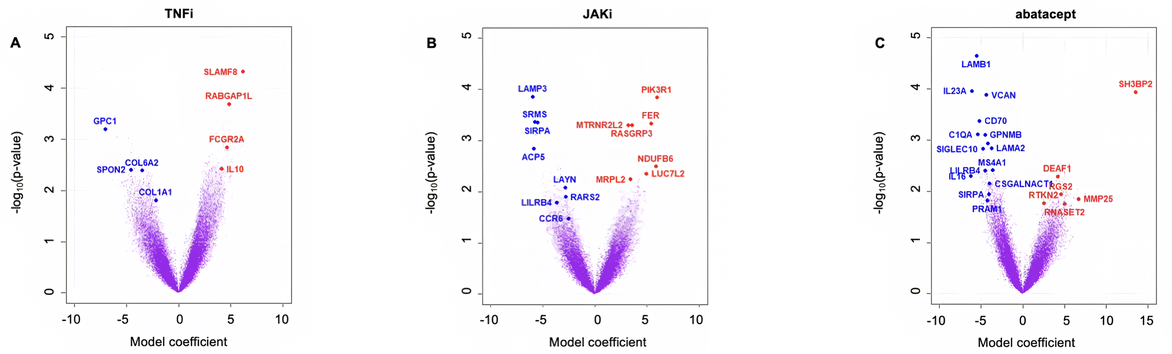
Results

Figure 1: Baseline cfDNA promoter signatures associated with TNFi response



(A) Heatmap of baseline cfDNA promoter signals differing between responders and non-responders (FDR < 0.05). Rows denote promoters and columns denote patients; both were clustered by unsupervised hierarchical clustering. (B) Boxplots of selected cfDNA promoter biomarkers stratified by TNFi response status.

Figure 2: Baseline cfDNA promoter signatures associated with response



Volcano plots of differential baseline cfDNA promoter signals associated with response (right) and non-response (left) to TNFi (2A), JAKi (2B), and abatacept (2C) treatment. Each point represents a gene; the x-axis shows effect size and the y-axis shows –log10(P). Genes corresponding to pathotype-associated programs are labeled. Vertical distance corresponds to strength of evidence. Horizontal position corresponds to the direction of the effect (response or non-response).

- Of the **416 participants** in the analysis, 156 initiated TNFi, 135 JAKi, and 125 abatacept, with **218 (52.4%)** meeting response criteria (Table 1). Follow-up visits occurred a mean of 92.2 days (range, 60–193 days) after treatment initiation.
- **TNFi** responders showed higher $cfDNA_{ac}$ signal for immune-associated genes while non-responders demonstrated higher signal at stromal and extracellular matrix–associated loci, consistent with fibroid synovial biomarkers (Figures 1 and 2A), overlapping directionally with immune-rich versus pauci-immune synovial pathotypes.
- **JAKi** responders showed higher $cfDNA_{ac}$ signal at cytokine signaling integration, transcriptional regulation, and mitochondrial regulatory loci, whereas nonresponders showed higher signal at antigen-presenting cell and inhibitory myeloid loci, partially overlapping with inflammatory macrophage and dendritic cell synovial signatures (Figure 2B).
- **Abatacept** responders demonstrated relative enrichment of adaptive immune regulatory loci, consistent with less chronically activated immune states, whereas nonresponse was associated with higher signal at myeloid and fibroblast-macrophage interaction loci (Figure 2C).
- $cfDNA_{ac}$ signatures showed consistent directionality with established synovial pathotypes (χ^2 $p < 10^{-18}$ for each drug class) with large effect sizes (**Cramér's V ≈ 0.85–0.88**), indicating strong alignment with known synovial biomarkers.
- Cross-drug-class comparisons demonstrated limited overlap between response-associated promoter signatures (**Jaccard indices < 0.10** for pairwise comparisons).

Conclusion

Baseline $cfDNA_{ac}$ profiling captures biologically meaningful biomarkers associated with response concordant with published synovial tissue biomarker profiles, and largely distinct by drug class. These findings support the potential utility of $cfDNA_{ac}$ for reflecting tissue-relevant biology and informing disease subtypes for patient stratification and treatment selection in RA. Findings are exploratory; validation in larger prospective cohorts is needed.

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Abbreviations: CDAI, Clinical Disease Activity Index; $cfDNA_{ac}$, cell-free DNA regulatory-active chromatin; FDR, false discovery rate; JAKi, JAK inhibitor; MCID, minimal clinically important difference; RA, rheumatoid arthritis; TNFi, TNF inhibitor.

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