

Development of a blood-based cell-free DNA classifier test to predict response to b/ts DMARDs in RA patients (PRIMA-102)

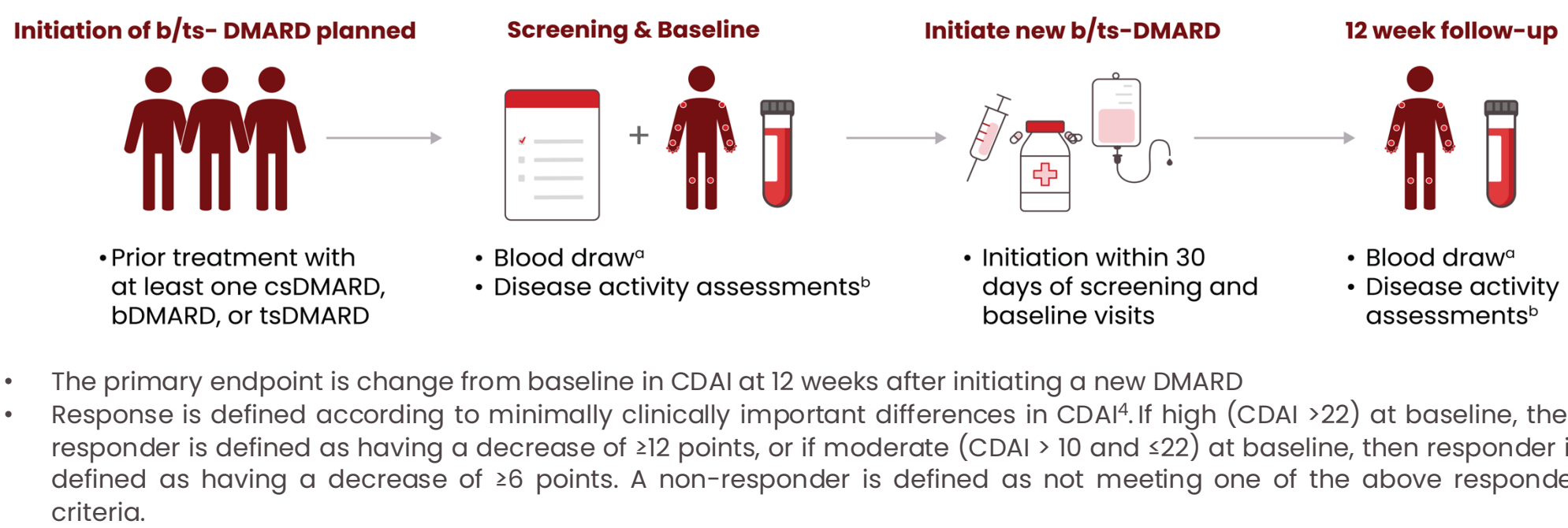
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Background

- Rheumatoid arthritis (RA) remains a significant challenge in clinical practice, particularly for patients who exhibit inadequate responses or intolerance to targeted therapies.
- RA can lead to irreversible joint damage, resulting in both short- and long-term disability, reduced quality of life, and an increased risk of cardiovascular disease, cancer, and mortality.¹
- The American College of Rheumatology (ACR)² and European League Against Rheumatism (EULAR)³ define the treatment goal as achieving low disease activity or remission using DMARDs. Current treatment approaches often involve trying multiple DMARD classes without guidance on which drug the patient is most likely to respond to, often leading to prolonged periods of medication cycling and switching.
- Here we present an interim analysis from PRIMA-102 (NCT 05936970), highlighting the development of a blood-based cell-free DNA classifier to predict therapeutic responses to biologic DMARDs (bDMARDs) and targeted synthetic DMARDs (tsDMARDs), with the aim of improving personalized treatment strategies for RA patients.

Study Design



^aIncludes Aqtual blood test, Rheumatoid Factor (RF), Cyclic Citrullinated Peptide (Anti-CCP), and C-Reactive Protein (CRP); ^bIncludes CDAI, DAS-28 CRP, SDAI, EULAR Response, and VAS = visual analog scale disease activity assessment.

PRIMA-102 has enrolled b/tsDMARD naïve and experienced patients with diverse treatment histories

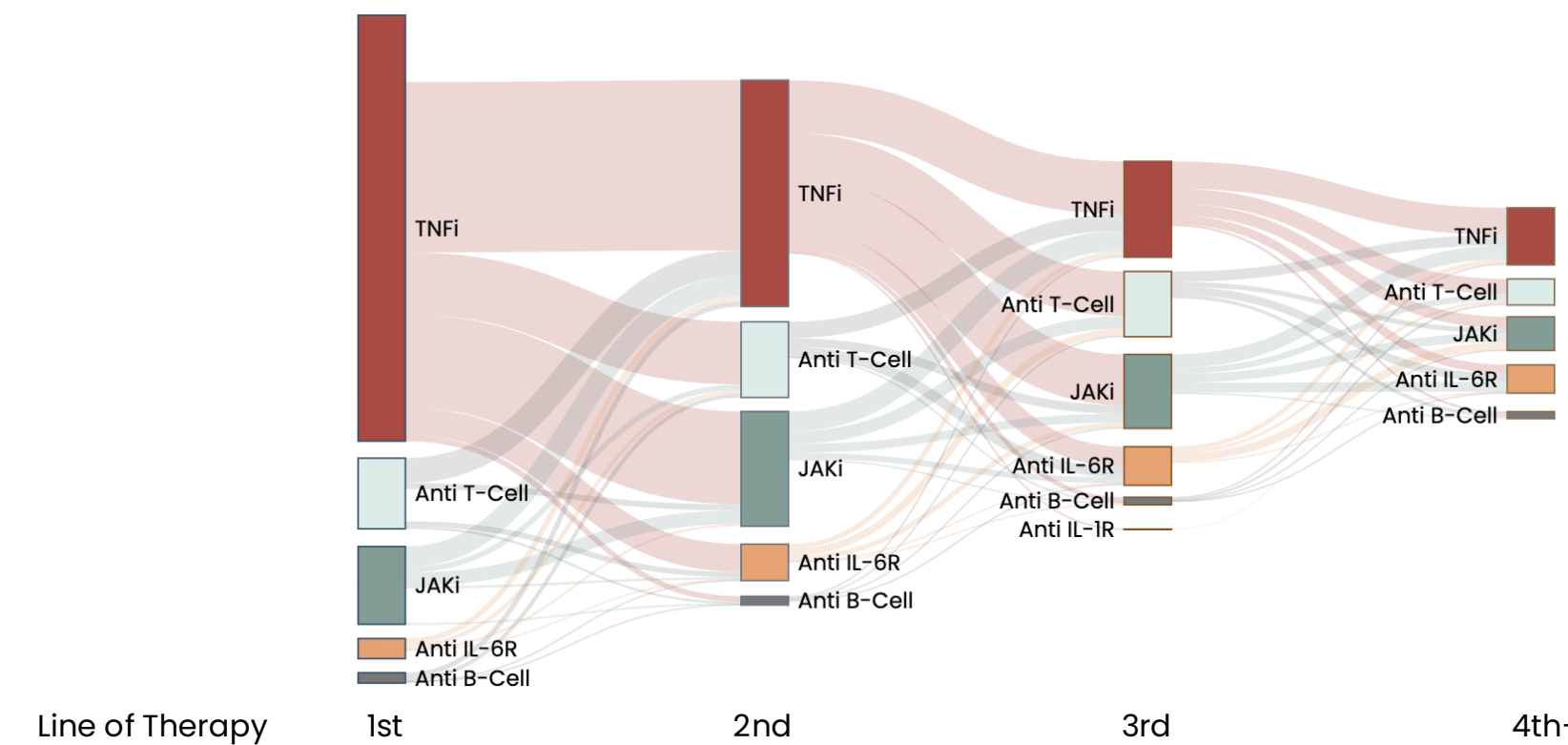
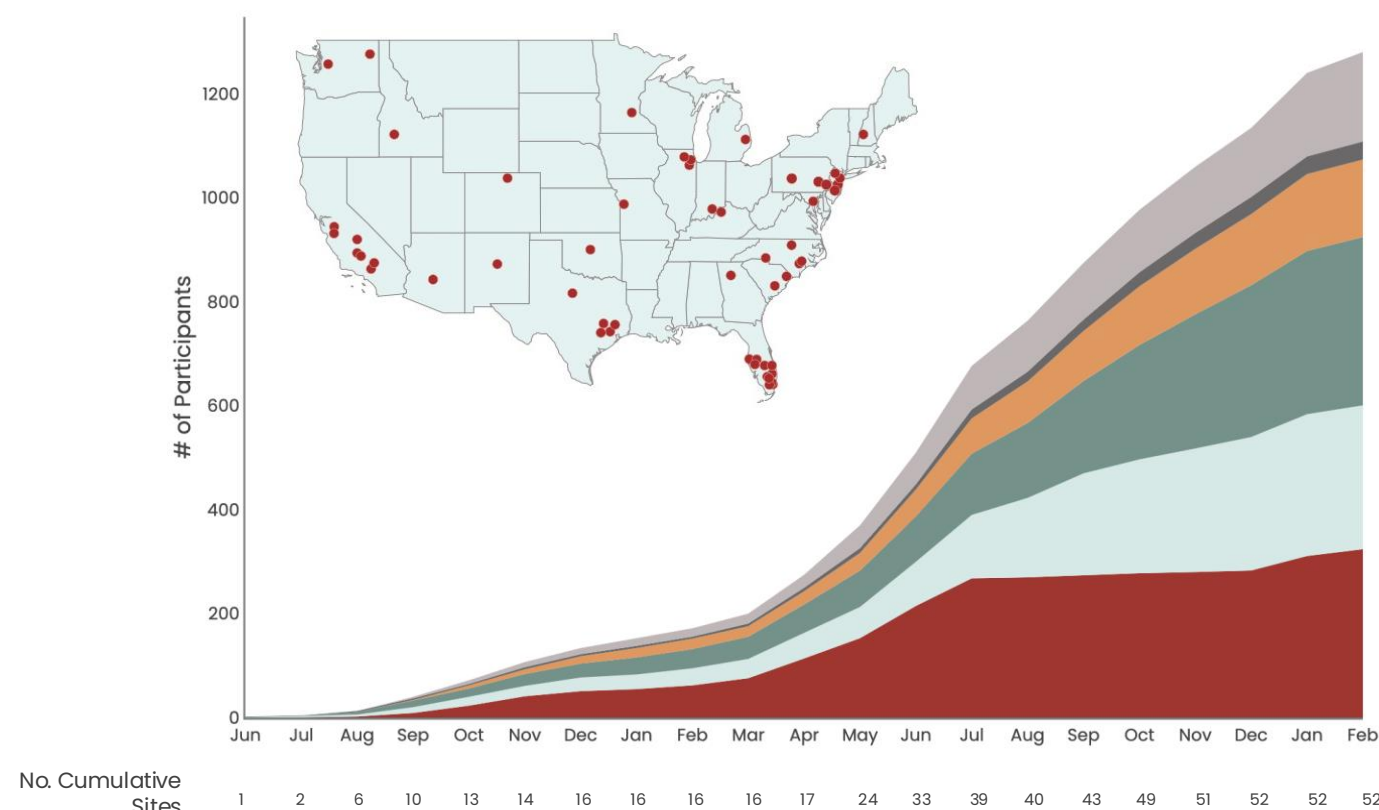


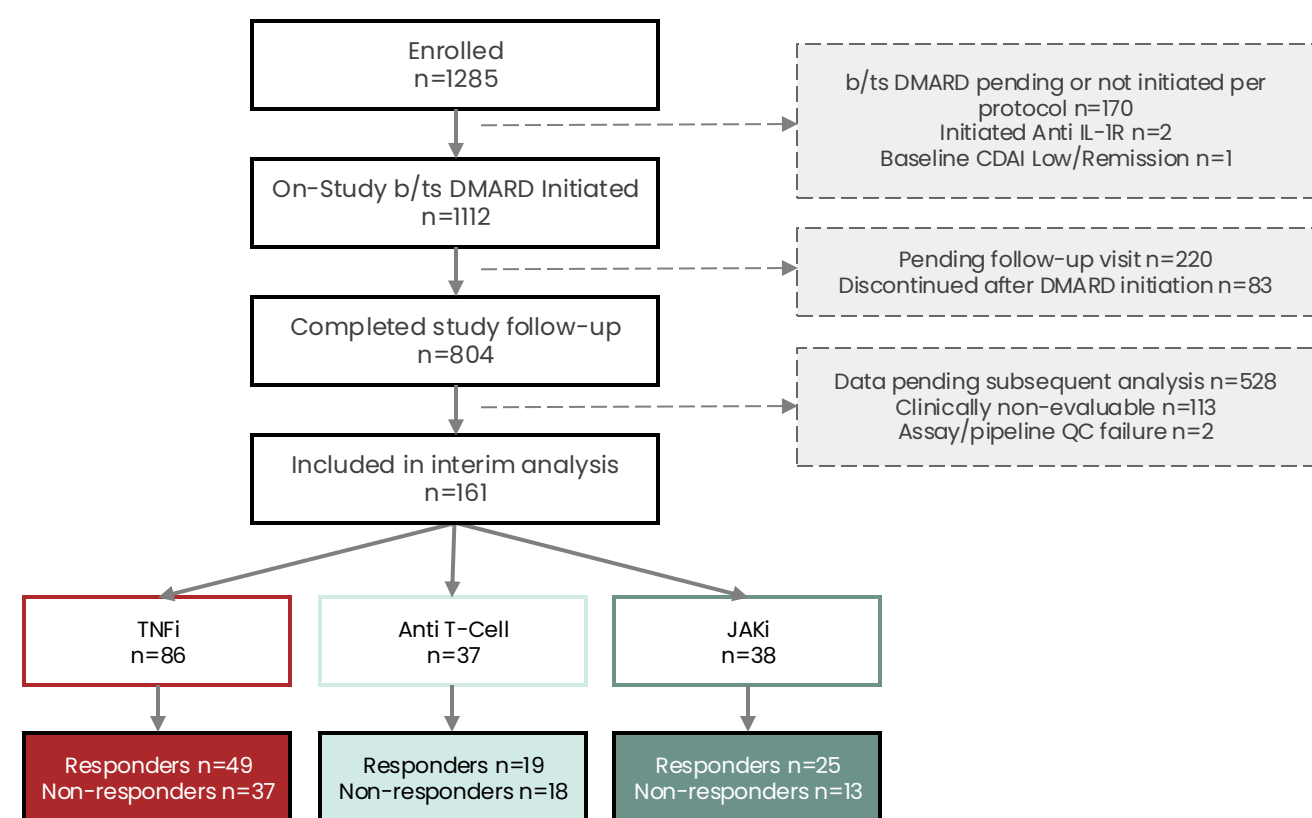
Figure 1: The Sankey plot illustrates prior and current on-study lines of therapy for PRIMA-102 participants who initiated a new b/tsDMARD (n=1112). Node sizes represent the number of participants per b/tsDMARD class, while connecting lines show cycling and switching patterns. For the 1st line of therapy, participant counts are: TNFi (n=783), Anti T-Cell (n=130), JAKi (n=143), Anti IL-6R (n=37), and Anti B-Cell (n=19). Participants naïve to b/tsDMARDs lack transition lines. Those starting their 2nd line at enrollment have transitions linking their 1st and 2nd lines of therapy, while those starting their 3rd line show transitions linking 1st, 2nd, and 3rd lines of therapy. Participants starting 4th line or greater show transitions linking all prior lines of therapy.

Clinical Cohort Description

PRIMA-102 has enrolled 1200+ participants across 22 states



Participants completed 12 weeks of TNFi, JAKi, or T-cell therapy



Platform Technology and Methods

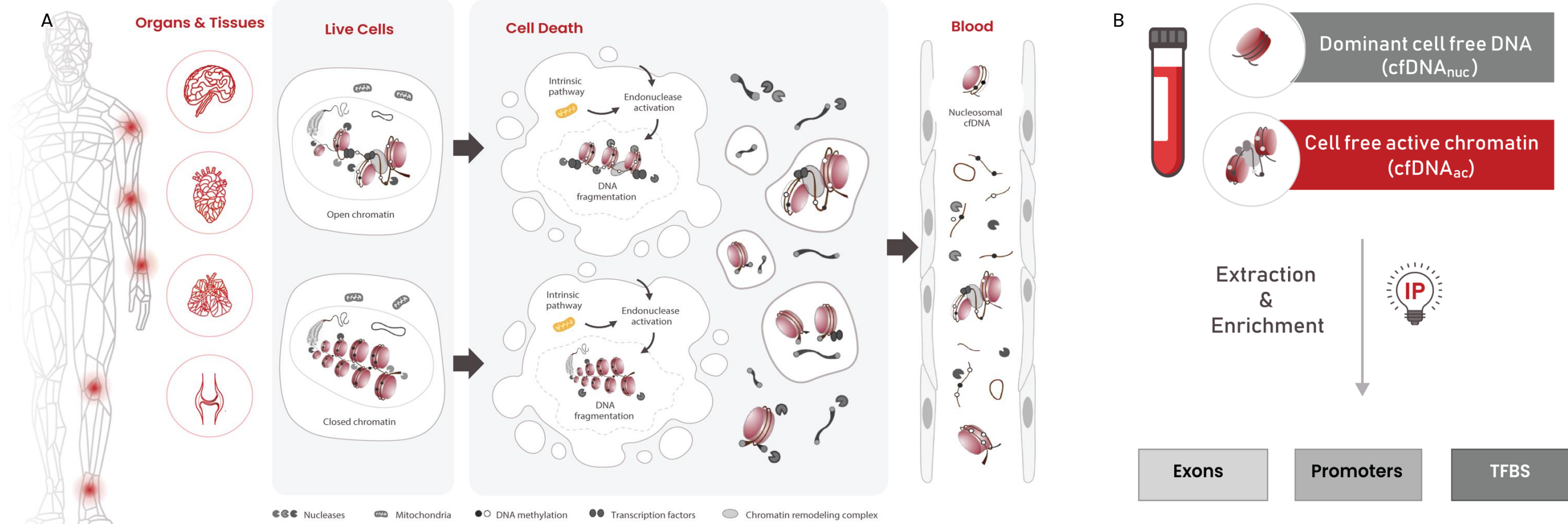


Figure 2: A. Unlike conventional tissue biopsies, cfDNA released into the bloodstream upon cell death provides a non-invasive and dynamic source of molecular information. Distinct cfDNA populations arise from cell death: 1. nucleosomal fragments (cfDNA_{nuc}) and 2. active chromatin fragments (cfDNA_{ac}). cfDNA_{ac} fragments in the blood enable the assessment of gene regulation and expression in the absence of mRNA. **B.** Cell-free DNA (cfDNA) was extracted from lml of plasma using a proprietary chromatin capture workflow, followed by library preparation and sequencing. Fragments were aligned to the human genome, deconvoluted into cfDNA_{nuc} and cfDNA_{ac} fragments, and quantified across our atlas of ~895,000 features consisting of exons (~285K), promoter (~37K) and transcription factor binding sites (~610K).

Results

TNFi CTAP Cluster

Cell Type	CTAP Cluster Annotation ⁵	Gene
T cell	CD4+ CD146+ memory	LGALS3
T cell	CD4+ GNLy+	HOPX
B cell	B-0: CD24+CD27+CD11b+ switched memory	MS4A1
Myeloid	M-0: MERTK+ SELENOP+ LYVE1+	IFI27
Myeloid	M-6: STAT1+ CXCL10+	MARCO
Myeloid	M-7: IL1B+ FCN1+	IL1B
Myeloid	M-7: IL1B+ FCN1+	PID1
Myeloid	M-8: PLCG2+	VCAN
Myeloid	M-13: pDC	TCF4
Myeloid	M-0: MERTK+ SELENOP+ LYVE1+	LYVE1
Myeloid	M-0: MERTK+ SELENOP+ LYVE1+	SLC40A1
Stromal	F-0: PRG4+ CLIC5+ lining	DIRC3
Stromal	F-4: DKK3+ sublining	MATN2
Stromal	F-7: NOTCH3+ sublining	DIO2
Stromal	F-5: CD74-high HLA-high sublining	CHI3L2
Endothelial	E-2: ICAM1+ venous	SELP
Endothelial	E-3: NOTCH4+ Arterial	IGFBP3

Anti T-cell CTAP Cluster

Cell Type	CTAP Cluster Annotation ⁵	Gene
T cell	CD4+ CD146+ memory	LGALS3
T cell	CD8+ GZMK/B+ memory	GZMB
T cell	CD8+ GZMB+/TEMRA	ZEB2
T cell	MT-high (low quality)	PLCG2
T cell	CD4+ GZMK+ memory	DTHD1
T cell	CD4+ OX40+NR3C1+	CORO1B
B cell	B-7: HLA-DR+IgG+ plasmablast	XBP1
NK	NK-7: CD56bright CD16- GNLy+CD69+	DUSP2
NK	NK-8: CD56bright CD16- IFN response	MX1
Myeloid	M-4: SPP1+	LPL
Myeloid	M-7: IL1B+ FCN1+	IL1B
Myeloid	M-7: IL1B+ FCN1+	PID1
Myeloid	M-8: PLCG2+	PLCG2
Stromal	F-4: DKK3+ sublining	MATN2
Stromal	F-8: RSPO3-hi lining	CLU
Endothelial	E-1: LIFR+ venous	SELP
Endothelial	E-3: NOTCH4+ Arterial	VEGFC

JAKi CTAP Cluster

Cell type	CTAP Cluster Annotation ⁵	Gene
B cell	B-0: CD24+CD27+CD11b+ switched memory	MS4A1
B cell	B-4: AID+BCL6+ GC-like	CCR6
B cell	B-0: CD24+CD27+CD11b+ switched memory	SCIMP
B cell	B-0: CD24+CD27+CD11b+ switched memory	SHISA8
B cell	B-1: CD24++CD27+IgM+ unswitched memory	CD22
Myeloid	M-4: SPP1+	LPL
Myeloid	M-8: PLCG2+	VCAN

- Gene-specific features distinguishing responses to TNFi, anti T-cell, and JAKi therapies were mapped onto a published study⁹ that profiled the inflamed synovium of RA patients using single-cell transcriptomics and histology and stratified patients into distinct synovial cell-type abundance phenotypes (CTAPs).
- Our analysis revealed a biologically-relevant distribution of CTAPs across all drug classes, with significant enrichment of synovial-specific features in our signal (p-value < 10⁻⁷).

Figure 3

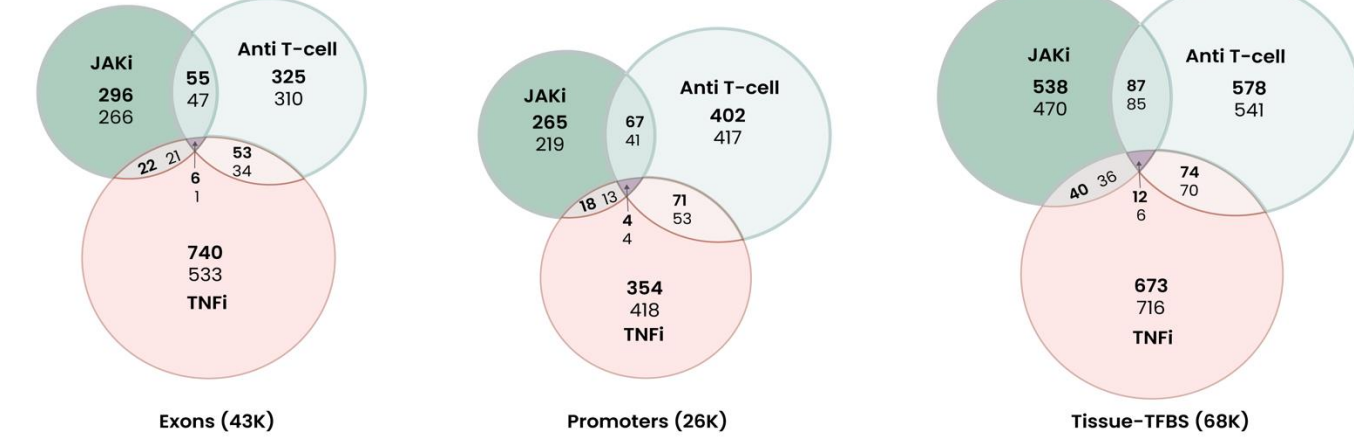


Figure 3: Empirical Bayes moderated t-statistics and corresponding p-values were used to identify features exhibiting differential signals associated with therapy responses across various drug classes. Venn diagrams show overlaps of top ranking differentially regulated active chromatin features— exon, promoter, tissue-specific TFBS— associated with TNFi, JAKi, and anti T-cell response.

Figure 4: A linear mixed-effects model was used to assess the contributions of general responder status and drug class-specific effects to the overall response variability. These are representative epigenetic features out of 1.6K features that were identified to have significant contextual or therapy-specific response effects in the linear model (adj p-value < 0.01). **A.** JAKi-specific, **B.** Anti T-cell-specific, **C.** JAKi- and TNFi- specific, or **D.** across all drug classes. Reported adjusted p-values were corrected for multiple comparisons using the Benjamini-Hochberg procedure.

Discussion and Conclusion

- PRIMA-102 is an ongoing, prospective, multi-center, observational study (NCT05936970) with over 1200 participants enrolled across the U.S., representing a diverse range of treatment histories.
- This study explores the use of cell-free DNA from active chromatin, cfDNA_{ac} as a novel approach to predict therapy response in RA patients.
- Our analysis revealed a biologically-relevant distribution of cell-type abundance phenotypes across all drug classes, with significant enrichment of synovial-specific features, supporting the relevance of our epigenetic markers to RA drug-specific responses and pathology.
- This interim analysis demonstrates the potential of leveraging cfDNA_{ac} to predict drug class-specific therapy responses, offering a promising avenue for personalized treatment strategies for RA patients experiencing inadequate response or intolerance to current therapies.

References: 1. www.cdc.gov/arthritis/basics/rheumatoid-arthritis.html; 2. Fraenkel, et al., Arthritis Care Res. 2021; 3. Smolen, et al., Ann Rheum Dis. 2020; 4. Curtis et al., Arthritis Care Res. 2015; 5. Zhang et al., Nature 2023

Disclosure Information: SF is the presenter and an employee of Aqtual. DC is the senior author and advisor for Aqtual.