

Development of a blood-based cell-free DNA therapy selection test to predict biologic and targeted synthetic DMARDs response in rheumatoid arthritis patients (PRIMA-102)

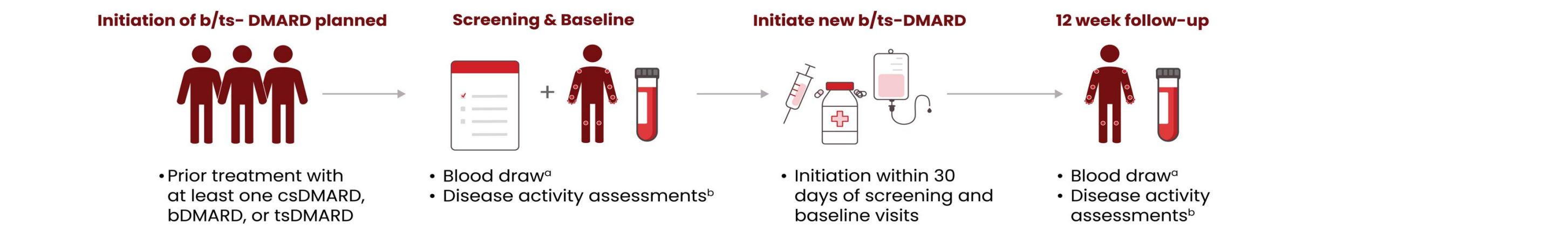
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Background

- Targeted treatments for rheumatoid arthritis (RA) have significantly improved disease management by controlling symptoms and slowing progression.
- Despite these advances, a substantial proportion of patients exhibit suboptimal responses to specific biologic and targeted synthetic DMARDs (b/ts DMARDs), often cycling through multiple treatments before achieving disease control.
- This variability in therapeutic response underscores a critical gap in treatment selection and the pressing need for a precision medicine approach.
- A predictive test to identify responders to specific b/ts DMARDs could support more informed and effective treatment decisions, improving patient outcomes and cost-effectiveness.
- This study aims to develop and validate a novel blood-based assay leveraging cell-free DNA derived from regulatory-active chromatin (cfDNA_{ac}) to predict response to bDMARDs and tsDMARDs using data from participants enrolled in the PRIMA-102 study, an ongoing prospective, multi-center, observational trial (NCT05936970).

Study Design and Enrollment Progress



- The primary endpoint is change from baseline in CDAI at 12 weeks after initiating a new b/ts DMARD.
- Response is defined according to minimally clinically important differences in CDAI¹. If high (CDAI >22) at baseline, then responder is defined as having a decrease of ≥12 points, or if moderate (CDAI > 10 and ≤22) at baseline, then responder is defined as having a decrease of ≥6 points. A non-responder is defined as not meeting one of the above responder criteria.

^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.

Platform

- 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 mRNA².

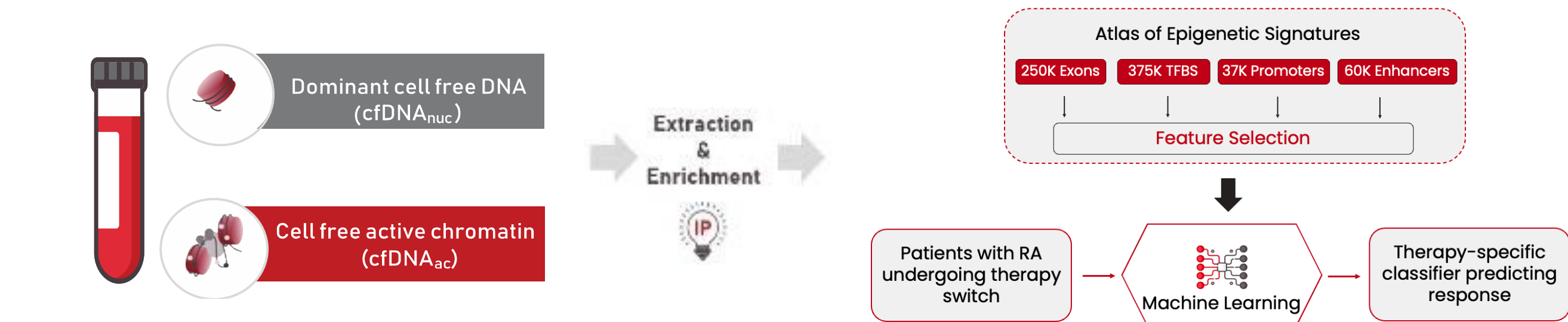
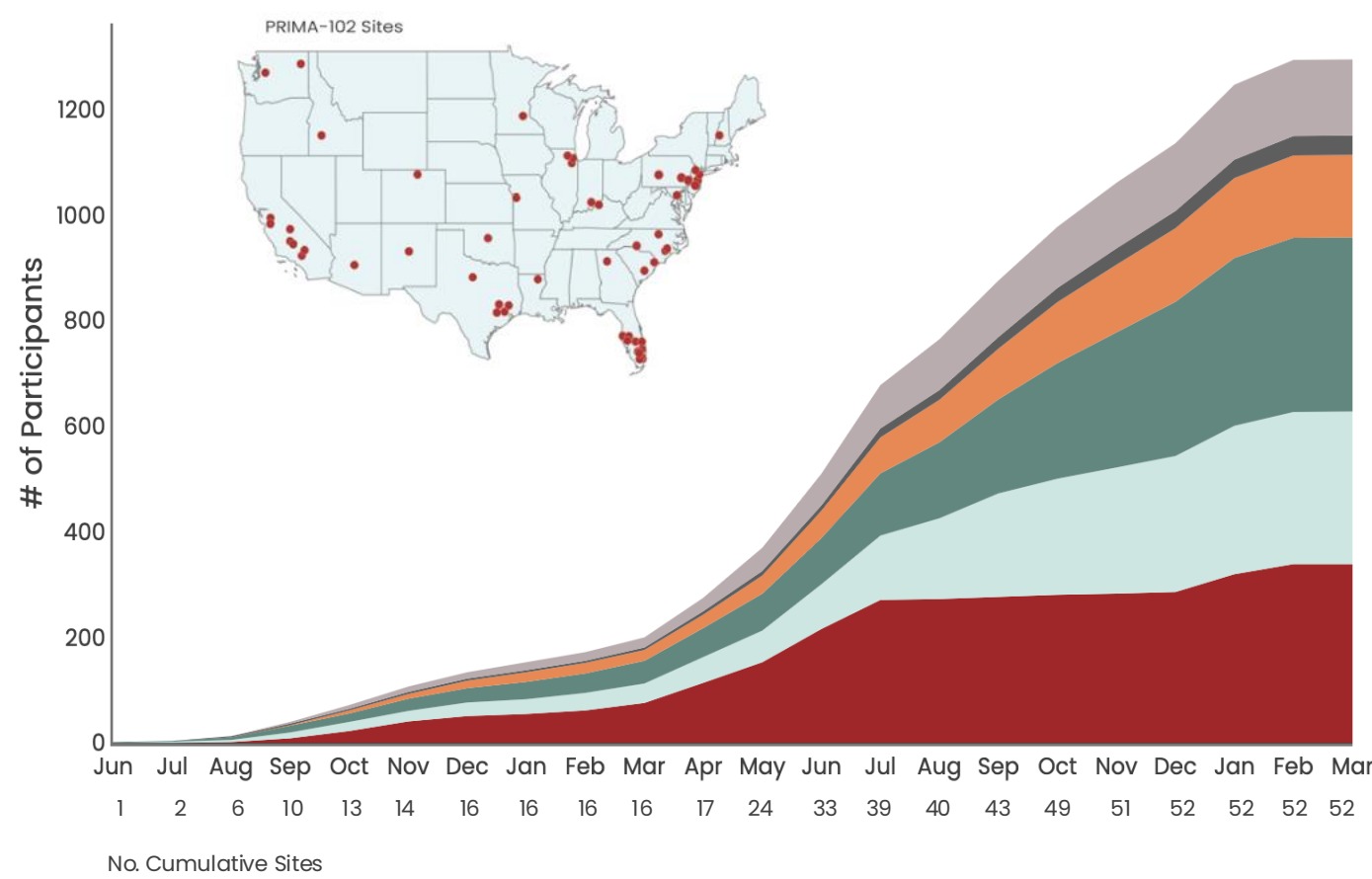


Figure 1: Cell-free DNA (cfDNA) was extracted from 1mL of plasma using a proprietary chromatin capture workflow, followed by library preparation and sequencing. Fragments were aligned to the human genome, deconvoluted into cfDNA_{nuc} and cfDNA_{ac} fragments, and quantified across our atlas consisting of exons, transcription factor binding sites, promoters and enhancers.

Disclosure Information: DC is the presenter and advisor for Aqtual.

Clinical Cohort Description & Results

PRIMA-102 has enrolled 1200+ participants across 22 states



Participants completed 12 weeks of TNFi, JAKi, or Abatacept therapy

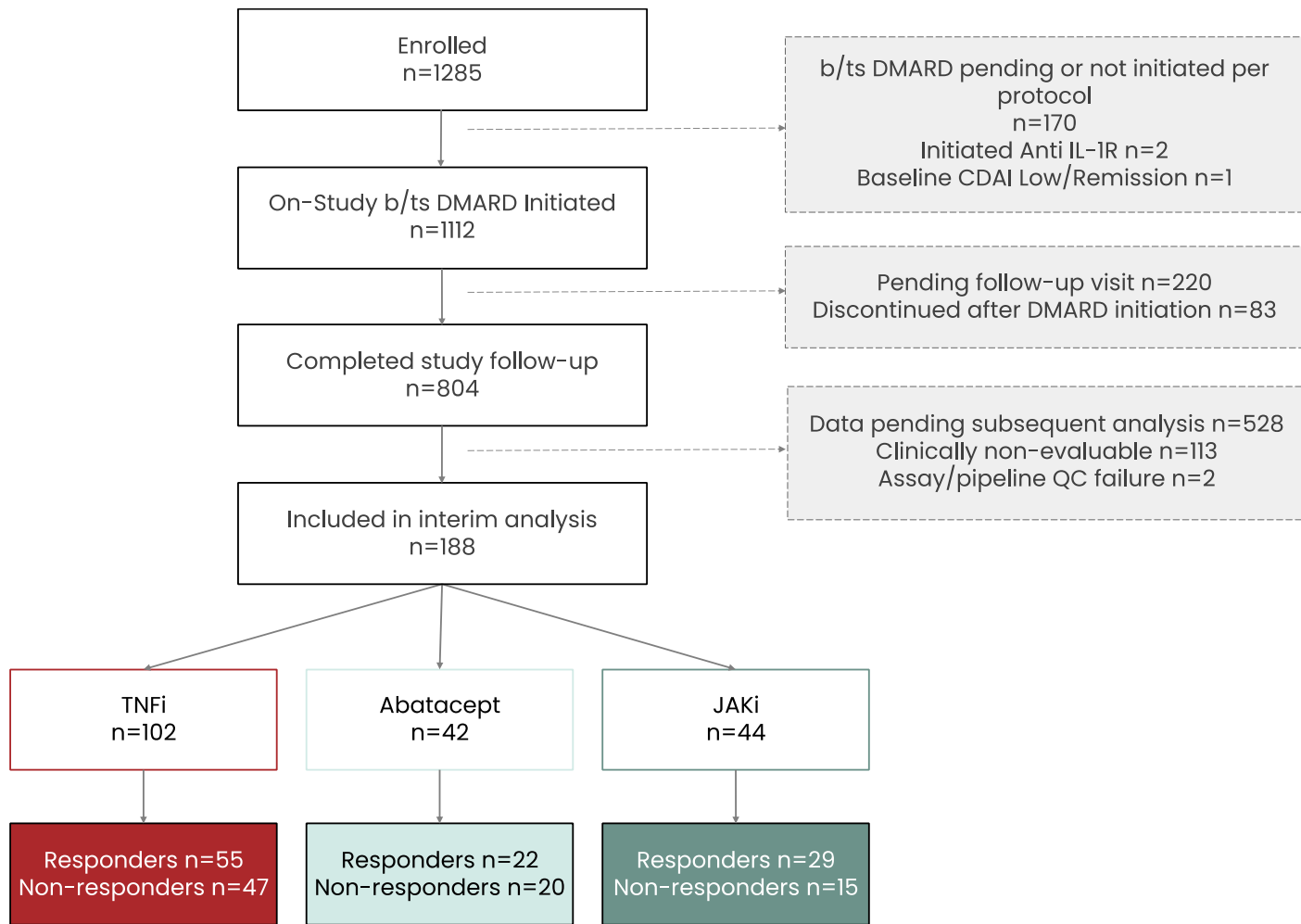


Table 1: Demographics of Participants used in Interim Analysis

	All	Training	Holdout		All	Training	Holdout
Subjects (n)	188	161	27	Subjects (n)	188	161	27
Age at Enrollment: Mean (SD)	57.8 (12.5)	57.6 (12.3)	59.2 (13.9)	Baseline CDAI Score: Mean (SD)	29.5 (13.4)	29.7 (13.6)	28.5 (12.7)
Female: n (%)	161 (85.6%)	140 (87%)	21 (77.8%)	Serostatus: n (%)			
Race: n (%)				Seropositive	98 (52.1%)	86 (53.4%)	12 (44.4%)
White	143 (76.1%)	123 (76.4%)	20 (74.1%)	Seronegative	90 (47.9%)	75 (46.6%)	15 (55.6%)
Black	16 (8.5%)	12 (7.5%)	4 (14.8%)	b/ts DMARD Initiated On Study: n (%)			
Other or Unknown	29 (15.4%)	26 (16.1%)	3 (11.1%)	TNFi	102 (54.3%)	86 (53.4%)	16 (59.3%)
Hispanic Ethnicity: n (%)				Abatacept	42 (22.3%)	37 (23%)	5 (18.5%)
Hispanic or Latino	40 (21.3%)	34 (21.1%)	6 (22.2%)	JAKi	44 (23.4%)	38 (23.6%)	6 (22.2%)
Not Hispanic or Latino	141 (75%)	121 (75.2%)	20 (74.1%)	Any csDMARD Use: n (%)	183 (97.3%)	158 (98.1%)	25 (92.6%)
Unknown	7 (3.7%)	6 (3.7%)	1 (3.7%)	Prior b/ts DMARD Experience: n (%)			
BMi: Mean (SD)	30.4 (7)	30.6 (7.2)	29.1 (5.2)	0	34 (18.1%)	31 (19.3%)	3 (11.1%)
Duration of Disease (yr): Mean (SD)	8.1 (9.8)	8.1 (9.2)	8.2 (13)	1	73 (38.8%)	60 (37.3%)	13 (48.1%)
Baseline CDAI Activity: n (%)				2	46 (24.5%)	38 (23.6%)	8 (29.6%)
Disease Activity Moderate	67 (35.6%)	58 (36%)	9 (33.3%)	3	11 (5.9%)	10 (6.2%)	1 (3.7%)
Disease Activity High	121 (64.4%)	103 (64%)	18 (66.7%)	4+	24 (12.8%)	22 (13.7%)	2 (7.4%)

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 interim analysis demonstrates the potential of novel blood-based cfDNA_{ac} classifiers to predict therapeutic response to TNFi, JAKi, and abatacept therapies in RA patients.
- While the small training and validation cohorts to date limit the scope of these findings, recruitment is ongoing, and these findings provide a critical first step in evaluating the utility of this approach.
- The larger cohort in the ongoing PRIMA-102 study will be pivotal in validating these classifiers for future clinical use and advancing personalized treatment strategies for RA.

References: 1. Curtis et al., *Arthritis Care Res.* 2015; 2. Lai et al., *Commun. Biol.* 2024

- The classifiers, comprising signatures of cfDNA_{ac}, demonstrated strong discriminatory performance across all drug classes, achieving an overall area under the curve (AUC) of ≥ 0.82.
- Sensitivity at 80% specificity ranged from 70.6% to 76.4%.
- The lack of significant overlap shows that the features identified are specific to each drug class.

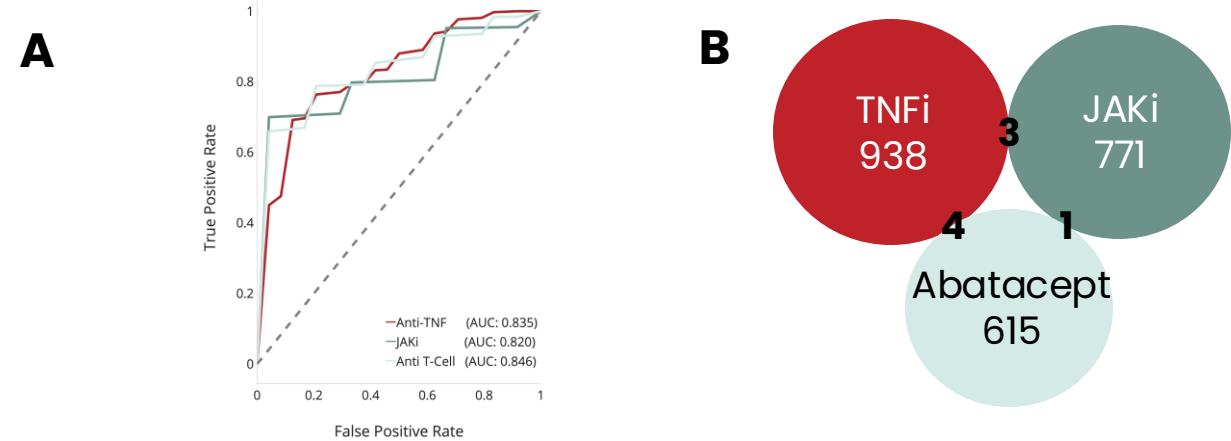


Figure 2: **A.** Receiver Operating Characteristic (ROC) curve shows the performance of each classifier achieving at least 70% sensitivity at 80% specificity in all drug classes. **B.** Venn Diagram shows overlap of selected features (i.e., different cfDNA_{ac} fragments) between each drug class.

Table 2: Features were identified through our ML framework that significantly differentiated responders and non-responders based on MCID.

Drug Class	No. of Features	Mean AUC	95% CI	Sensitivity @ 80% Specificity
TNFi	945	0.835	(0.816, 0.853)	75.10%
JAKi	775	0.820	(0.793, 0.847)	70.60%
Abatacept	620	0.846	(0.816, 0.876)	76.40%

Of 161 samples, 70 were used to develop classifiers for each mechanism of action by identifying features discriminating responders from non-responders. Machine-learning (ML) models were trained with 25 bootstrap iterations and tested on the remaining 91 samples. The performance of the three classifiers was further validated using an independent, blinded hold-out cohort of 27 randomly selected participants, balanced across b/ts DMARD classes and response categories (Table 3).

Table 3. Performance of the 3 classifiers on an independent blinded

Drug Class	No. of Participants	No. of Responders	No. of Non-Responders	Sensitivity	Specificity
TNFi	16	10	6	80%	100%
JAKi	6	3	3	100%	100%
Abatacept	5	3	2	66.70%	100%

- TNFi: 8/10 responders were classified correctly. All 6 non-responders were classified correctly.
- JAKi: All 6 responders and non-responders were classified correctly.
- Abatacept: 2/3 responders were classified correctly, and all non-responders were classified correctly.