A Novel Blood-based Assay that Predicts Clinical Response to TNFi or JAKi in Patients with Rheumatoid Arthritis

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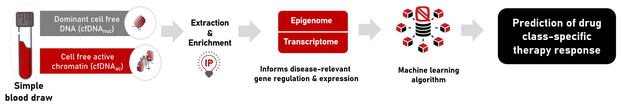
Background

- The heterogeneity of rheumatoid arthritis (RA) poses challenges in achieving optimal clinical outcomes for all patients.
- RA is treated first with conventional synthetic disease-modifying anti-rheumatic drugs (csDMARDs) followed by biologic DMARDs (e.g. tumor necrosis factor-alpha inhibitors, TNFi) or targeted synthetic DMARDs (janus kinase inhibitors, JAKi), aiming to suppress the immune system's overactive response.
- These treatments have been instrumental in controlling symptoms and slowing disease progression, however not all patients will respond to a specific therapy resulting in patients failing multiple therapies before finding an effective treatment.
- The variability in individual responses to standard therapies raises questions about the underlying factors contributing to this heterogeneity in response and underscores the need for a more targeted approach.
- In this study, we present the development of a non-invasive blood-based classifier to differentiate RA patients likely to respond to TNFi or JAKi therapy from those who will not.

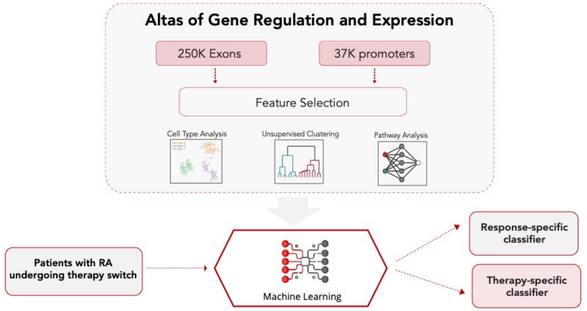
Methods

Overview of Platform

- Aqtual's novel platform provides a non-invasive, real-time assessment of organ- and disease-specific epigenomic and transcriptomic signals.
- Cell-free DNA (cfDNA) was extracted from 1 mL of plasma using a proprietary chromatin capture workflow, followed by library preparation and sequencing.



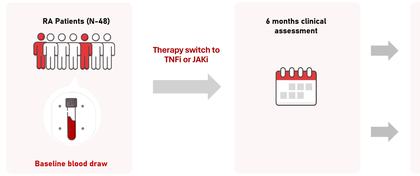
- Response- and therapy- specific signatures were identified using the following machine learning (ML) framework:



Study Design

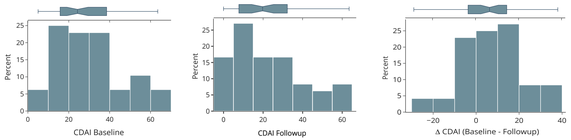
Study Objective

- The objective of this study is to evaluate the clinical feasibility of the assay to differentiate therapy response in two drug classes – TNFi and JAKi – in patients with Rheumatoid Arthritis.



Cohort Description

- A total of 48 RA participants undergoing a switch in therapy to either TNFi (n=19) or JAKi (n=29) were selected from the CorEvitas BIO-100 registry. Sample selection was based on equivalent numbers of responders and non-responder.
- The participants, aged 20-80 years (median=57), consisted of 89.5% females and 10.5% males, with 89.6% identifying as White.
- 21 patients were classified as responders and 27 as non-responders based on their Clinical Disease Activity Index (CDAI) scores collected at baseline and 6 months after therapy initiation.
- The distribution of participants by baseline and follow-up CDAI, and change in CDAI:



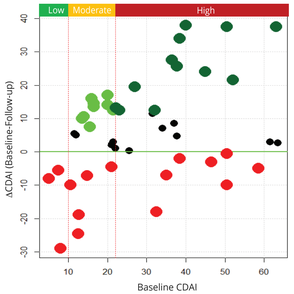
Definition of Response Classes for ML Classifier

Total Cases	TNFi	JAKi
Responders (N)	7	14
Non-responders (N)	12	15

ML Training Cases	TNFi	JAKi
Responders (N)	7	14
Non-responders (N)	6	9

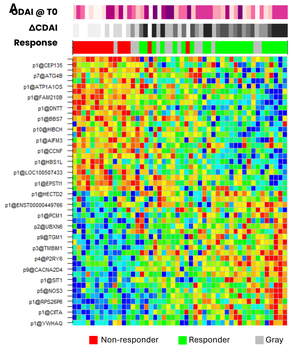
CDAI allows immediate treatment decisions to be based entirely on clinical criteria. CDAI is calculated as: CDAI = SJC + TJC +PGA + EGA

- **Responder:** Disease activity going from high to moderate with a reduction of at least 12 points on CDAI
- **Responder:** Disease activity going from moderate to low with a reduction of at least 6 points on CDAI
- **Non-Responder:** CDAI increased from baseline to follow-up
- Cases with minimal change in CDAI that were excluded in the training of the ML classier



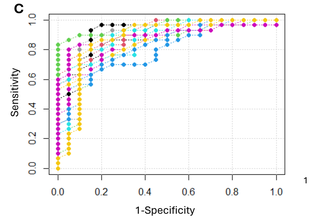
Results

Figure 1. The therapy response classifier in the combined TNFi & JAKi cohort demonstrated a sensitivity of 80% at a specificity of 85%



B

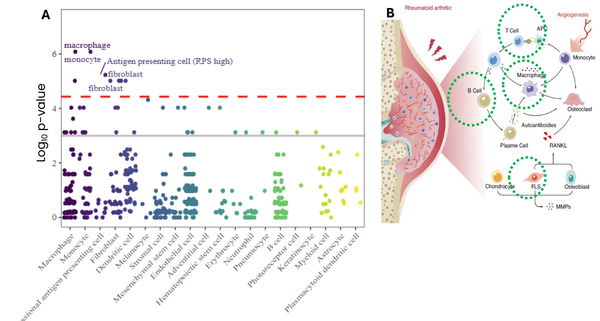
Overall	Mean	95% CI
AUC	0.91	[0.77, 0.98]
Sensitivity (%)	80.0	[63.9, 96.1]
Specificity (%)	85.0	[65.3, 99.7]



- Change in CDAI score, not baseline CDAI, was associated with treatment response

A total of 57 features with the most discriminatory power were identified, followed by false discovery rate (FDR) correction. **A.** The heatmap displays features identified through hybrid unsupervised clustering during feature selection. Upper tracks denote response status, disease activity, and change in CDAI from baseline to 24 weeks post-therapy initiation. **B.** A machine learning classifier was used to identify relevant variables to construct a predictive model using epigenetic data for patients switching therapies to TNFi and JAKi drug classes. **C.** The ROC curve shows the performance of the classifier, achieving 80% sensitivity and 85% specificity. The workflow incorporated 10,000 classification trees and a 5-repeated 5-fold cross-validation.

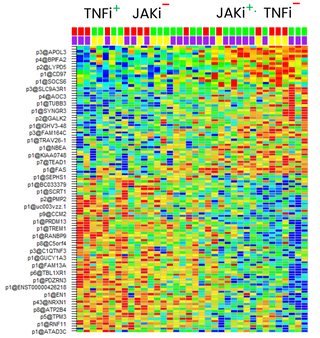
Figure 2. Classifier features predicting response are linked to RA pathobiology



- Twenty known cell types were found to be enriched in the response classifier, including cell types known to be in the joint synovium

Cell-type specific enrichment analysis was performed on feature set mapped to genes using WebCSEA (https://bioinfo.uth.edu/webcsea/). **A.** Results show enrichment across top 20 general cell types with raw and Bonferroni-corrected p-values. Bonferroni-corrected significance ($p = 3.69 \times 10^{-5}$) is indicated by a red-dashed line, and nominal significance ($p = 1 \times 10^{-3}$) by a grey-solid line. X-axis represents cell types and y-axis represents the significance level. **B.** Diagram of joint synovium with cell types identified in enrichment analysis shown.

Figure 3. A second ML classifier focused on therapy-specific response is under development



- Pathway analysis using features enriched in therapy response-classifier identified:
  - JAK/STAT signaling and cell-matrix adhesion pathways in JAKi responders
  - B-cell proliferation and MAPK pathways in TNFi responders
- These findings are consistent with known mechanisms of action for JAKi (Tanaka et al. 2022) and TNFi (Pala et al. 2018) respectively.
- The JAKi-response classifier demonstrated a sensitivity of 80% with a specificity of 83%.

Hierarchical clustering was performed on features identified in 2nd machine learning classifier, and pathways analysis was conducted with gene set enrichment analysis using coding genes as background.

Summary

- This proof-of-concept study establishes the use of a novel active chromatin capture method to develop a classifier capable of predicting responders from non-responders with a performance of 80% at a specificity of 85%.
- Cell enrichment and pathway analysis establish a link between the classifier features and tissue-specific RA pathobiology, providing insights into underlying cellular mechanisms driving treatment response variability in RA.
- A second machine learning classifier was also trained to predict outcomes within a single therapy class, JAKi. Using a 5-fold cross-validation framework, the JAKi-response classifier demonstrated a sensitivity of 80% with a specificity of 83%.
- Further efforts to expand the cohort will be necessary to develop a more robust classifier that captures the inherent heterogeneity of RA and delivers a positive prediction of drug-class specific therapy response.

Clinical Implications

Currently, there is a significant gap in our ability to guide therapy selection in RA. A non-invasive blood test that could predict therapy response could have significant implications for RA clinical practice and patient care.

Disclosure Information: SF is the presenter and an employee of Aqtual. DC is the lead investigator and a scientific advisory board member for Aqtual. JC is the senior author and a consultant for Aqtual.