Θεραπευτική άκριβείας (theragnostic): Η αξία των βιοδεικτών στην Ιατρική του μέλλοντος

THE CASE OF IL-17 IN PSORIATIC DISEASE

www.autorheumatology.com

Dimitrios Bogdanos MBA, LLM, MPA, MD, PhD,
Professor of Medicine and Autoimmune Diseases

Director and Chairman,
Department of Rheumatology and Clinical Immunology

Director, Rheumatology and Clinical Immunology Clinic

Head, Laboratory of Rheumatology, Clinical Immunology & Autoimmune Diseases

UNIVERSITY OF THESSALY

Theranostics=

Thera(peutics) + (Diag)nostics

THERANostics: The roadmap to Autoprediction

No serum biomarker(s) in isolation or in combination can predict response to treatment or progression of the disease

Genetic markers (signatures) in isolation or in combination to be used in routine practise are not able to efficiently predict response to treatment

Then WHAT?

What is Autoprediction?

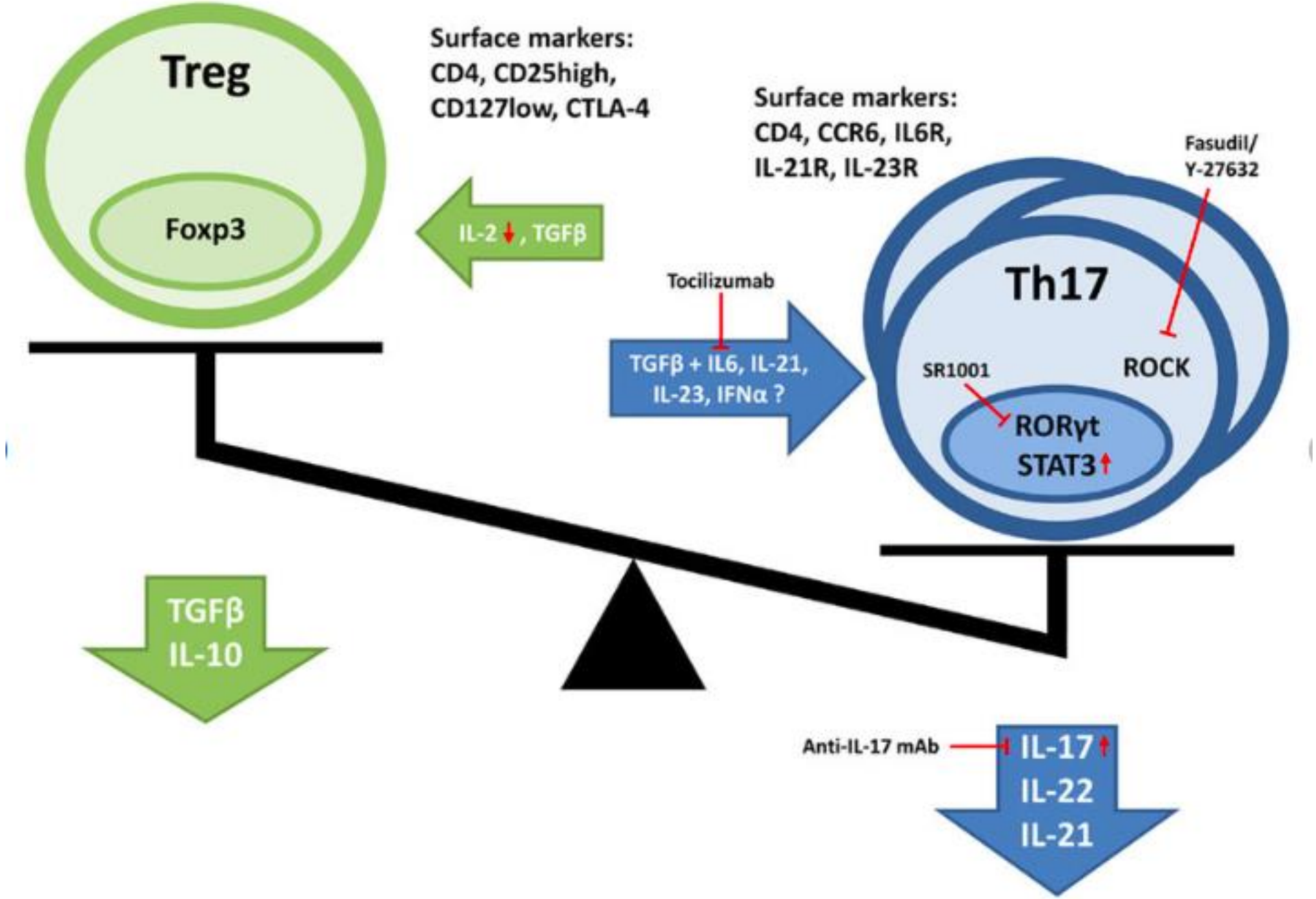
AutoPrediction©

A disease-specific, parameter-dependent algorithm, which predicts customized response

to individualized treatment

- Flexible (not all parameters needed to predict
- Semi-quantitative
- Includes demographic, clinical, laboratory and/or histological parameters
- Serological
- Immunological (autoantibody, serum cytokines, chemokines, cell subsets (Th1, Th17, Tregs, Bregs etc, surface cell subsets i.e CD24, CD27, CD39)
- Genetic parameters (SNPs), whole genome sequencing, DNA, RNA

IL-17 and anti-IL-17 as ideal markers


Treg
Foxp3
Surface markers:
CD4, CD25high,
CD127low, CTLA-4
IL-2 ↓, TGFβ
Surface markers:
CD4, CCR6, IL6R,
IL-21R, IL-23R
Fasudil/
Y-27632
Tocilizumab
TGFβ + IL6, IL-21,
IL-23, IFNα ?
Th17
SR1001
ROCK
RORγt
STAT3 ↑
TGFβ
IL-10
Anti-IL-17 mAb
IL-17 ↑
IL-22
IL-21

Aim of Theranostics

Can we predict which patients with autoimmune rheumatic disease who will make a flare of the disease and need refining of their treatment (precision medicine)?

Artificial Intelligence

Running Head: Machine-learning detection of flares

Title: Detection of flares by decrease in physical activity, collected using wearable activity trackers, in rheumatoid arthritis or axial spondyloarthritis: an application of Machine-Learning analyses in rheumatology

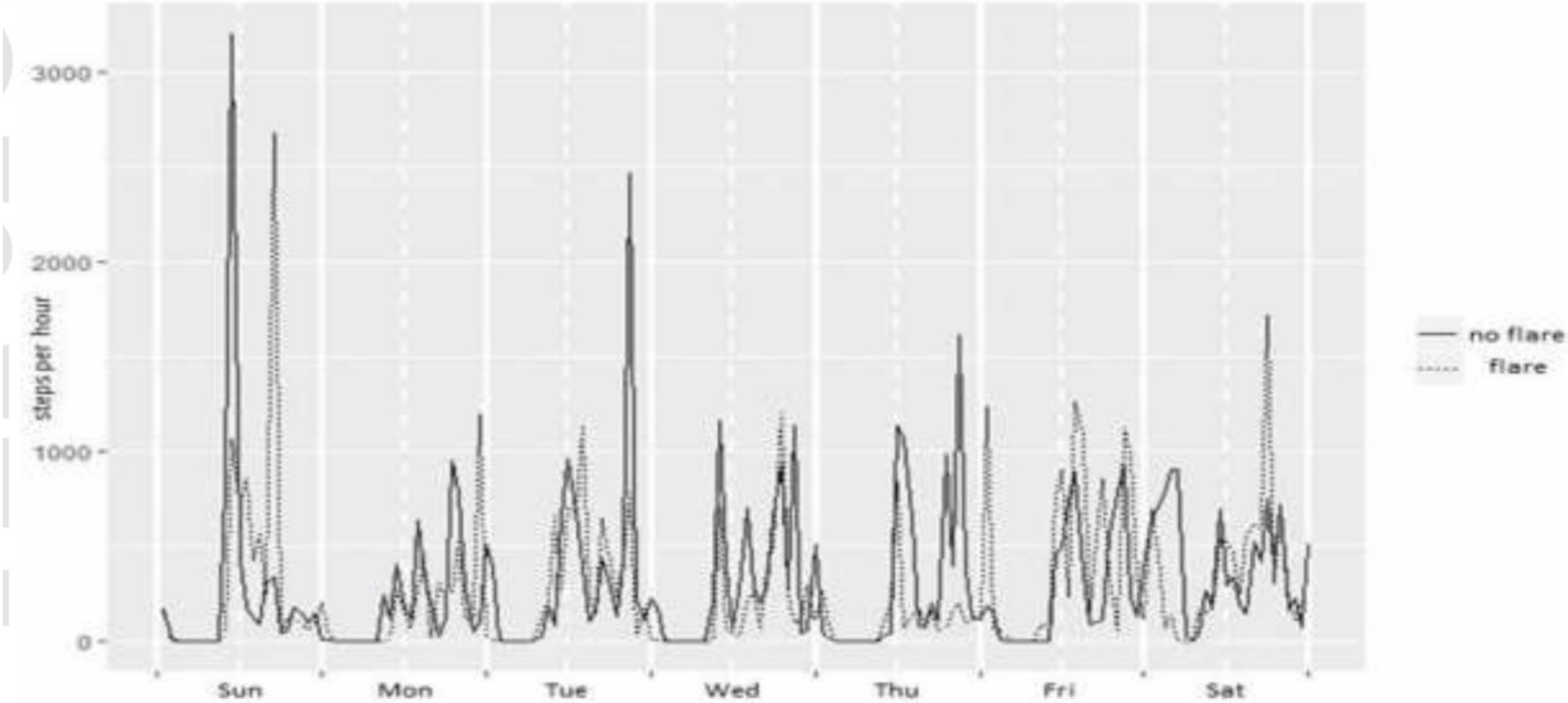
Laure Gossec MD, PhD[1], Frédéric Guyard PhD[2], Didier Leroy MSc[2], Thomas Lafargue MSc[2], Michel Seiler MSc[3], Charlotte Jacquemin, MD, MSc[1], Anna Molto MD, PhD[4,5], Jérémie Sellam MD, PhD[6], Violaine Foltz MD, MSc[1], Frédérique Gandjbakhch MD, MSc[1], Christophe Hudry MD, MSc[4], Stéphane Mitrovic, MD, MSc[1], Bruno Fautrel MD, PhD[1], Hervé Servy MSc[7]

RESULTS

Patients

Among the 170 patients included in the study, 155 (82 RA and 73 axSpA patients) were analyzed. This corresponds to 1339 weekly flare assessments and 224,952 hours physical activity assessment timeframes. Physical activity being provided at the minute, the dataset contained close to 13.5 million activity points.

Figure 2. One example of activity for weeks with versus without flare, for a randomly chosen patient over all weeks of activity (mean values are presented for weeks with or without flares)



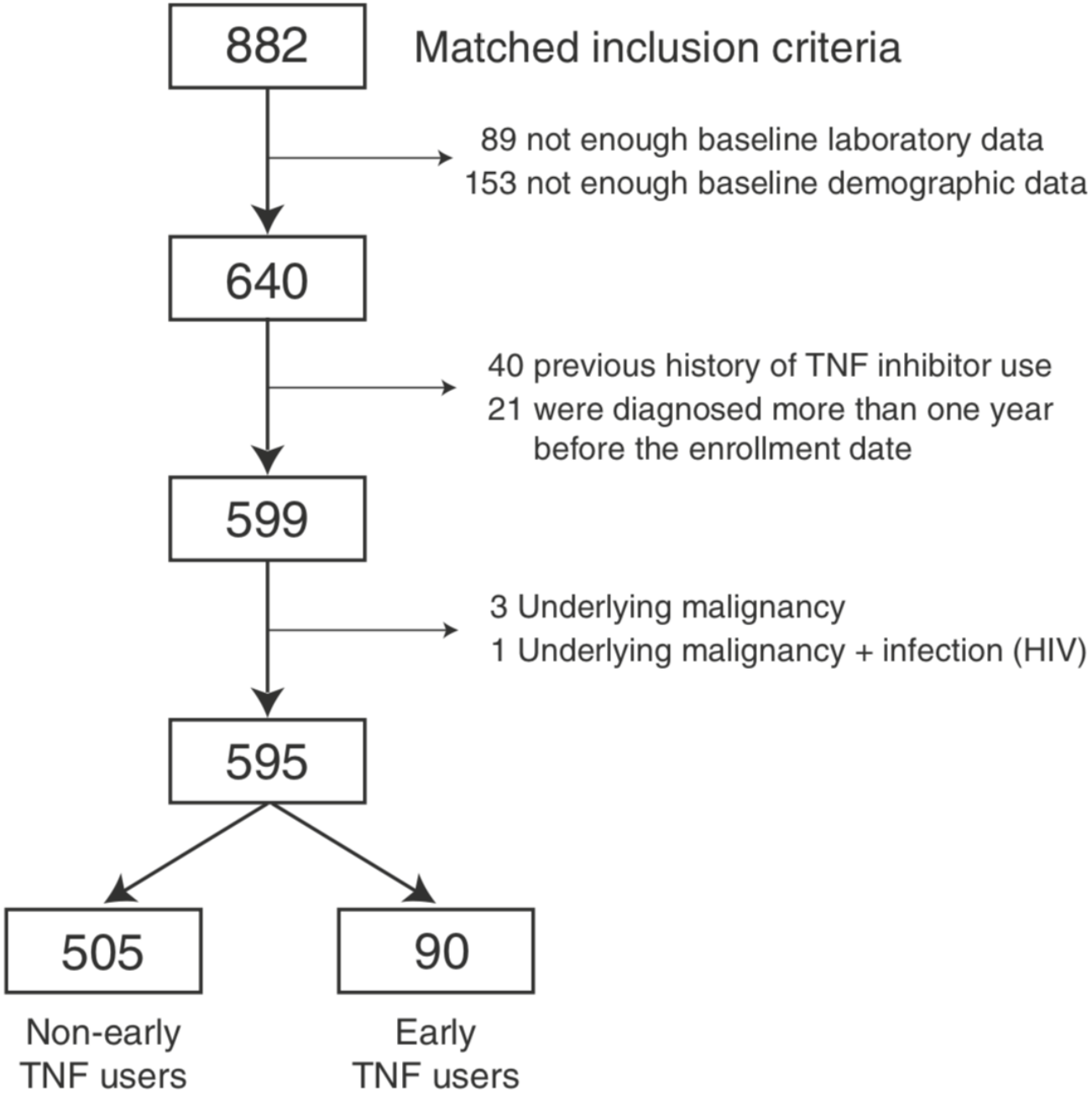
scientific reports

OPEN

Machine learning to predict early TNF inhibitor users in patients with ankylosing spondylitis

Seulkee Lee, Yeonghee Eun, Hyungjin Kim, Hoon-Suk Cha, Eun-Mi Koh & Jaejoon Lee✉

We aim to generate an artificial neural network (ANN) model to predict early TNF inhibitor users in patients with ankylosing spondylitis. The baseline demographic and laboratory data of patients who visited Samsung Medical Center rheumatology clinic from Dec. 2003 to Sep. 2018 were analyzed. Patients were divided into two groups: early-TNF and non-early-TNF users. Machine learning models were formulated to predict the early-TNF users using the baseline data. Feature importance analysis was performed to delineate significant baseline characteristics. The numbers of early-TNF and non-early-TNF users were 90 and 505, respectively. The performance of the ANN model, based on the area under curve (AUC) for a receiver operating characteristic curve (ROC) of 0.783, was superior to logistic regression, support vector machine, random forest, and XGBoost models (for an ROC curve of 0.719, 0.699, 0.761, and 0.713, respectively) in predicting early-TNF users. Feature importance analysis revealed CRP and ESR as the top significant baseline characteristics for predicting early-TNF users. Our model displayed superior performance in predicting early-TNF users compared with logistic regression and other machine learning models. Machine learning can be a vital tool in predicting treatment response in various rheumatologic diseases.


882
Matched inclusion criteria
89 not enough baseline laboratory data
153 not enough baseline demographic data
640
40 previous history of TNF inhibitor use
21 were diagnosed more than one year before the enrollment date
599
3 Underlying malignancy
1 Underlying malignancy + infection (HIV)
595
505
90
Non-early TNF users
Early TNF users

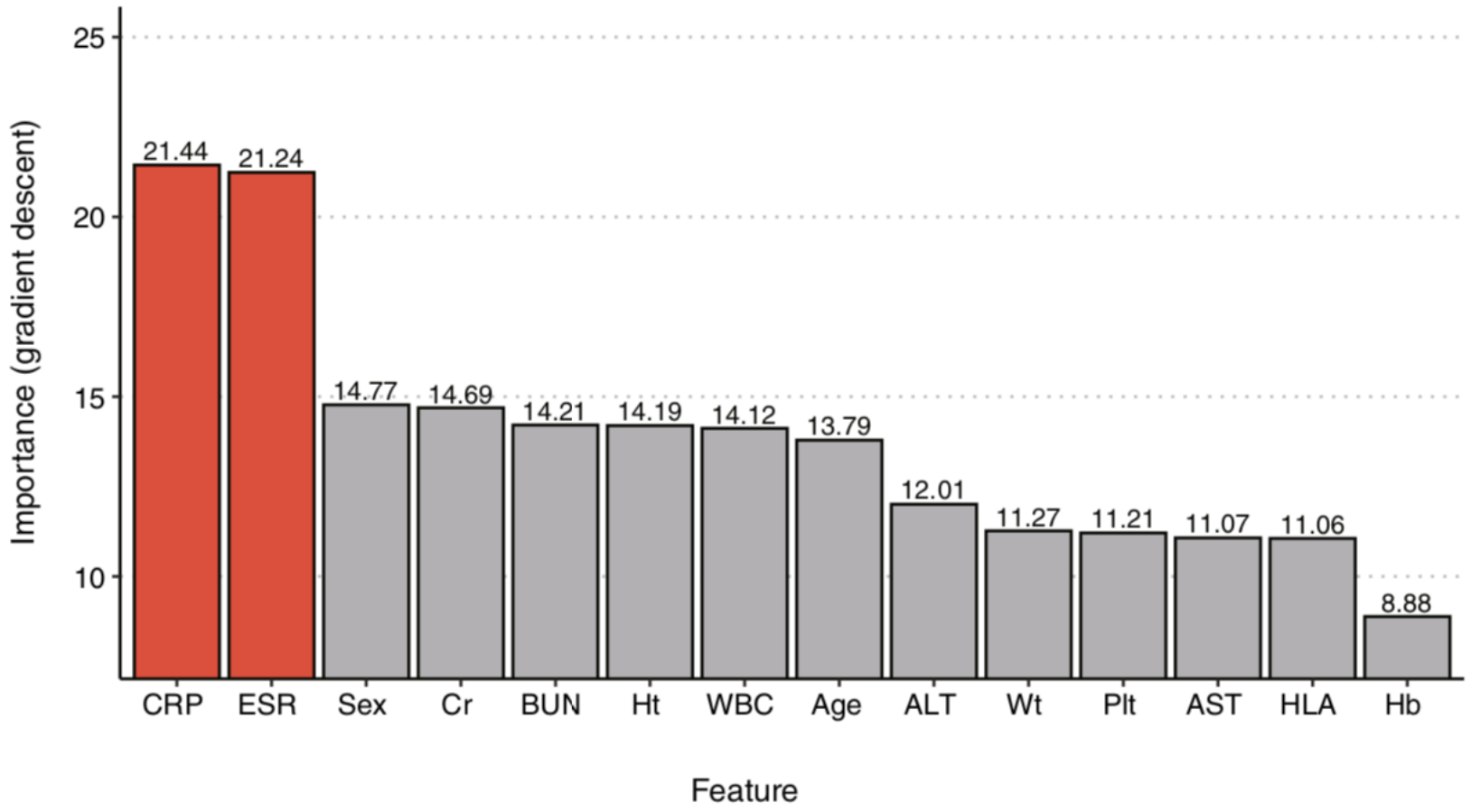


Figure 4. The result of feature importance analysis from our model trained by an ANN method. The x-axis shows the input clinical variables. The y-axis represents the feature importance score calculated by the 'risk backpropagation' method. The two most important features are highlighted in red. *Ht* height, *Plt* platelet, *HLA* HLA-B27, *Wt* weight, *Cr* creatinine, *Hb* hemoglobin.

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Journal of Medical Systems Biology and Network Science

Mary Ann Liebert, Inc. publishers

ORIGINAL RESEARCH ARTICLE Open Access

Clinical Validation of a Blood-Based Predictive Test for Stratification of Response to Tumor Necrosis Factor Inhibitor Therapies in Rheumatoid Arthritis Patients

Theodore Mellors,[1] Johanna B. Withers,[1] Asher Ameli,[1] Alex Jones,[1] Mengran Wang,[1] Lixia Zhang,[1] Helia N. Sanchez,[1] Marc Santolini,[2] Italo Do Valle,[3] Michael Sebek,[3] Feixiong Cheng,[3] Dimitrios A. Pappas,[4,5] Joel M. Kremer,[5,6] Jeffery R. Curtis,[7] Keith J. Johnson,[1] Alif Saleh,[1] Susan D. Ghiassian,[1] and Viatcheslav R. Akmaev[1,*]

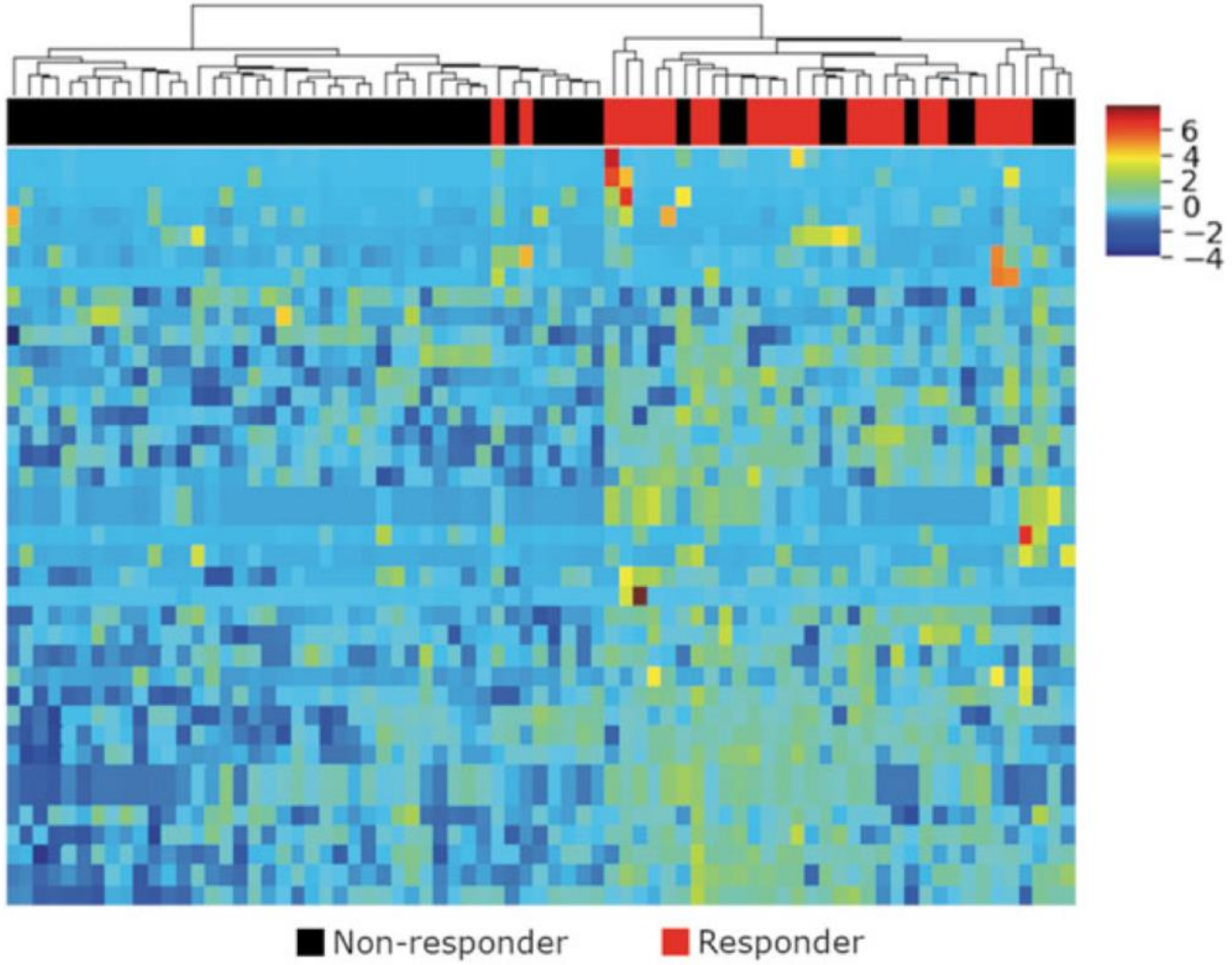
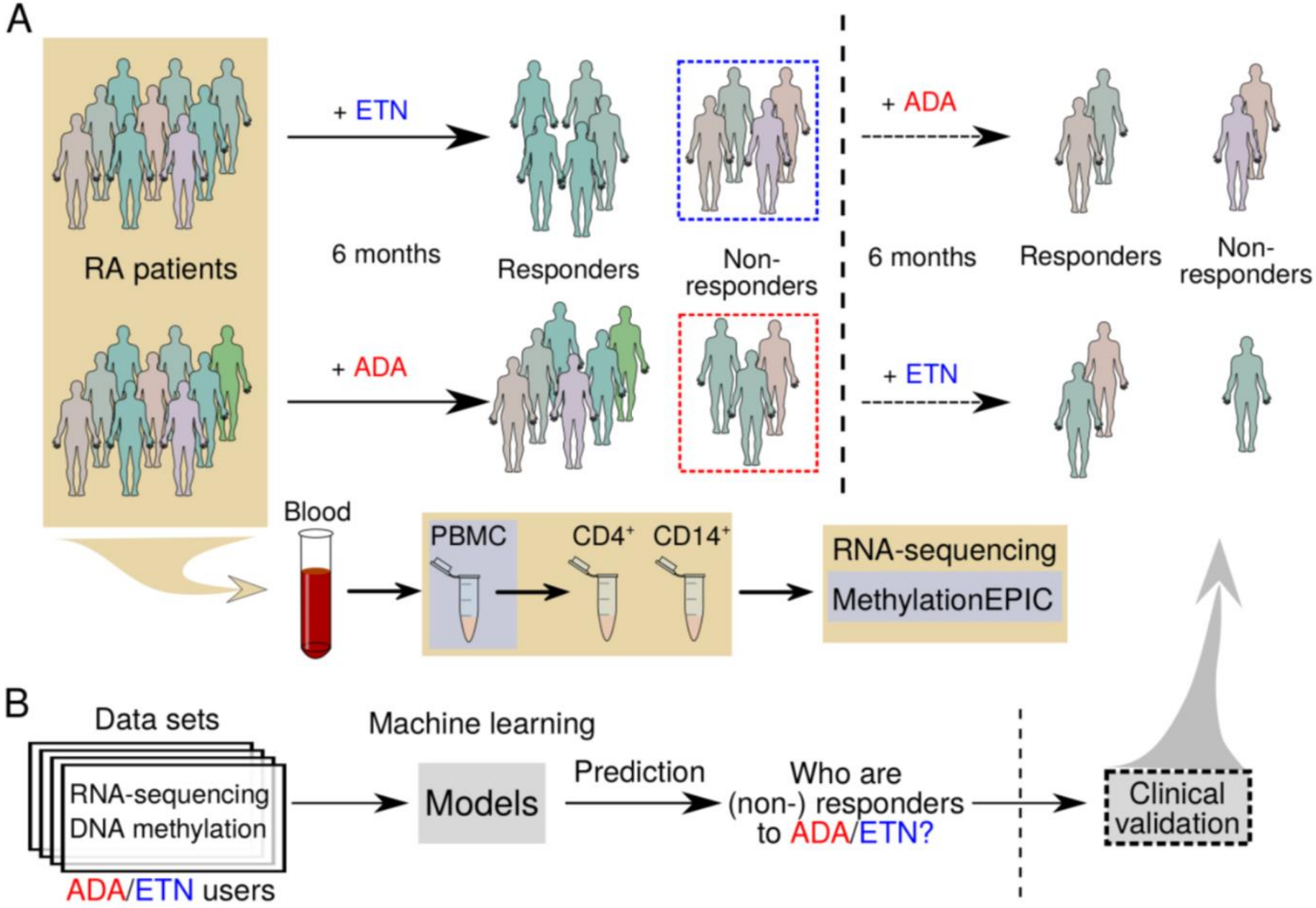
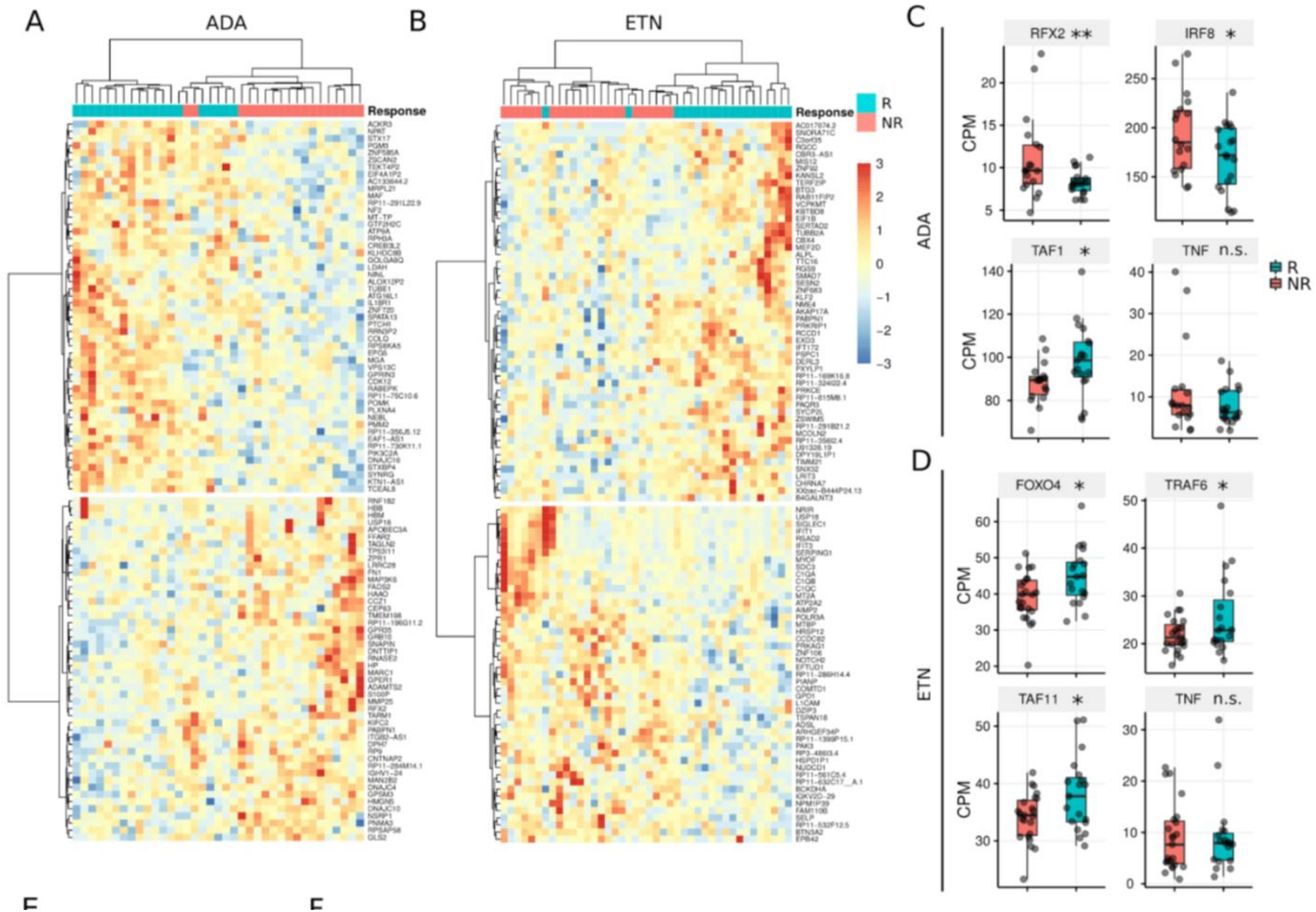
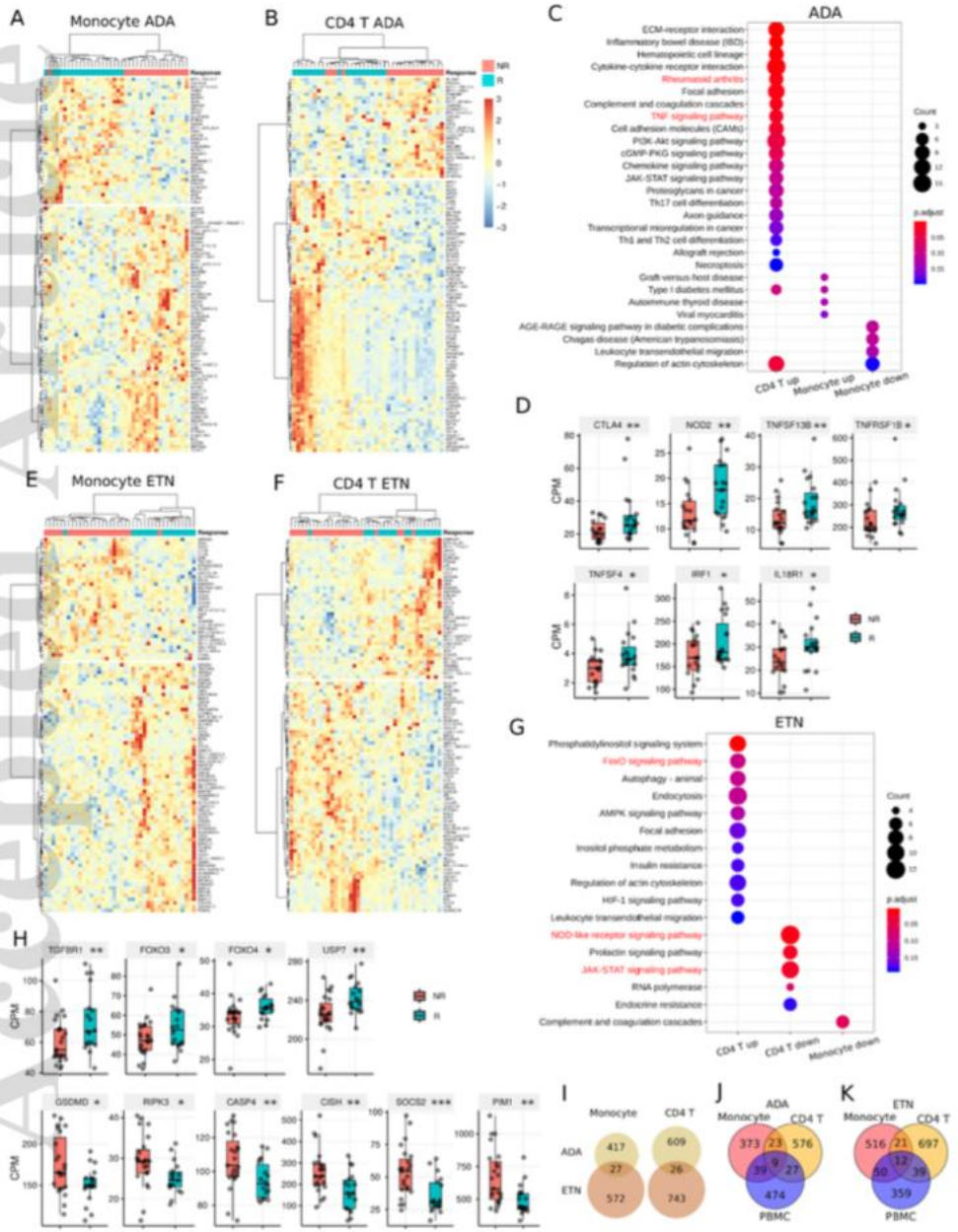


FIG. 2. Discriminatory genes indicative of nonresponse to anti-TNF therapy in RA patient blood samples. Hierarchical cluster analysis[42] of RNA expression data for 37 genes illustrates two main groupings, one predominantly nonresponders and the other responders, thereby substantiating the discriminatory nature of these genes for anti-TNF response prediction. The heatmap represents the relative RNA expression level in arbitrary units.

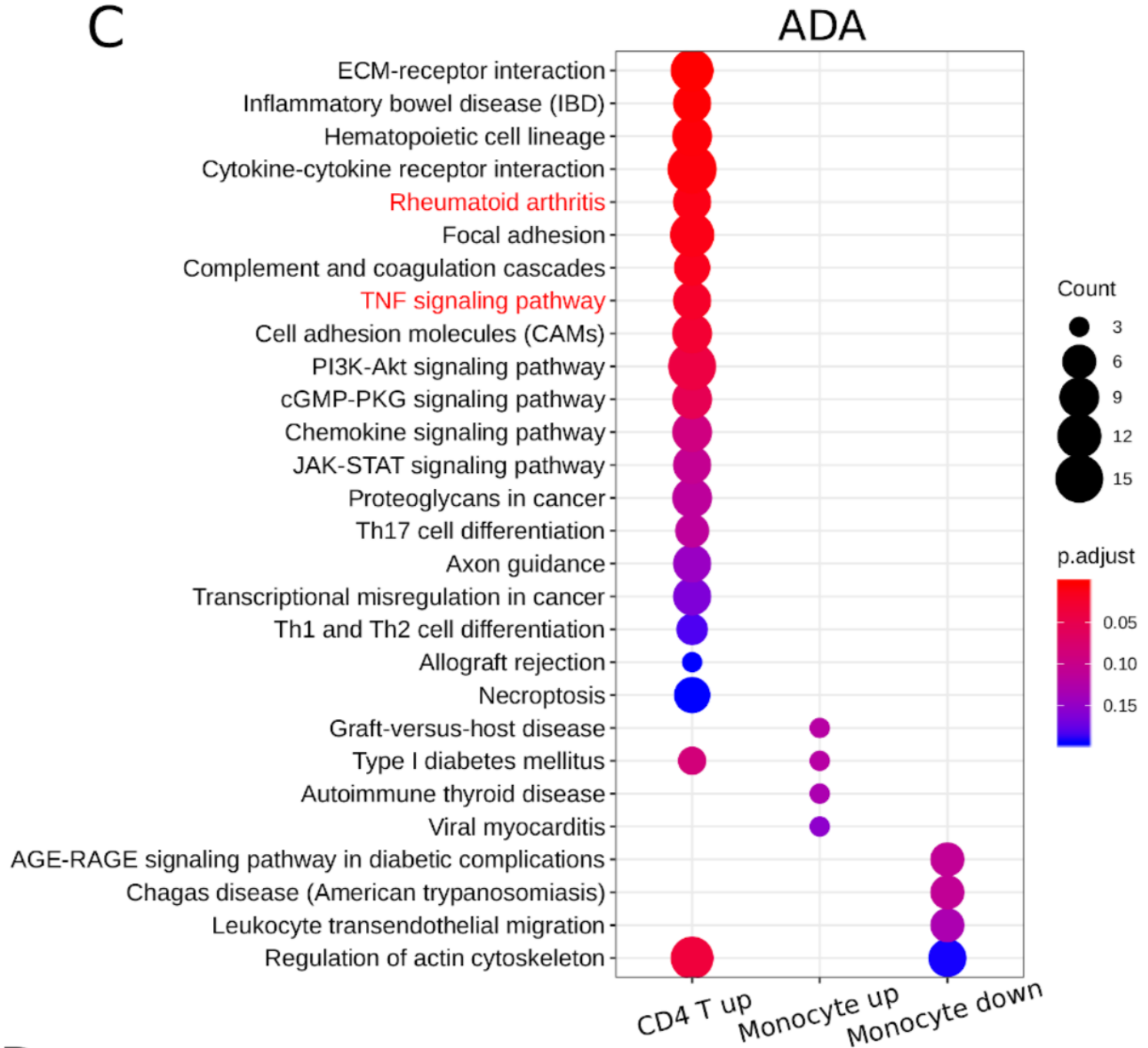
1. **Isolation of peripheral blood mononuclear cells (PBMCs)** from these patients prior to ADA or ETN treatments.
2. Then **genome-wide gene expression** and **DNA methylation** profiling were performed on these PBMCs, ***using RNA-sequencing and Infinium MethylationEPIC BeadChip***, respectively.
3. RNA-sequencing on CD14+ monocytes and CD4+ T cells isolated from the PBMCs were performed to gain more insights into different anti- TNF response.
4. Using the gene expression and DNA methylation signatures, they then built and internally validated machine learning models to predict response to ADA and ETN

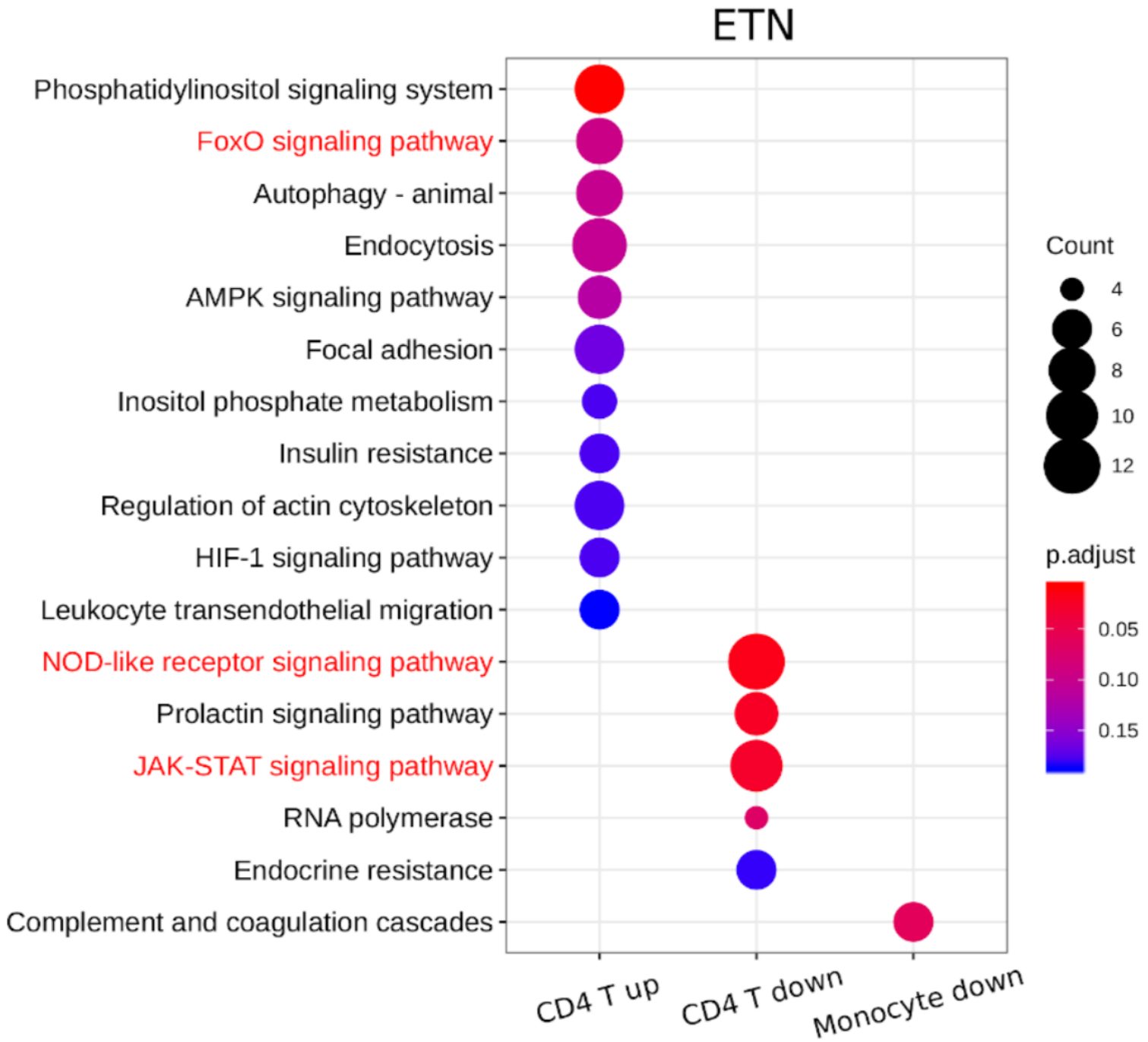

A
RA patients
+ ETN
6 months
Responders
Non-responders
+ ADA
6 months
Responders
Non-responders
+ ADA
+ ETN
Blood
PBMC
CD4+
CD14+
RNA-sequencing
MethylationEPIC
B
Data sets
RNA-sequencing
DNA methylation
ADA/ETN users
Machine learning
Models
Prediction
Who are
(non-) responders
to ADA/ETN?
Clinical
validation


A
ADA
Response
B
ETN
Response
R
NR
3
2
1
0
−1
−2
−3
C
ADA
RFX2 **
IRF8 *
TAF1 *
TNF n.s.
CPM
R
NR
D
ETN
FOXO4 *
TRAF6 *
TAF11 *
TNF n.s.
CPM



C


ADA
ECM-receptor interaction
Inflammatory bowel disease (IBD)
Hematopoietic cell lineage
Cytokine-cytokine receptor interaction
Rheumatoid arthritis
Focal adhesion
Complement and coagulation cascades
TNF signaling pathway
Cell adhesion molecules (CAMs)
PI3K-Akt signaling pathway
cGMP-PKG signaling pathway
Chemokine signaling pathway
JAK-STAT signaling pathway
Proteoglycans in cancer
Th17 cell differentiation
Axon guidance
Transcriptional misregulation in cancer
Th1 and Th2 cell differentiation
Allograft rejection
Necroptosis
Graft-versus-host disease
Type I diabetes mellitus
Autoimmune thyroid disease
Viral myocarditis
AGE-RAGE signaling pathway in diabetic complications
Chagas disease (American trypanosomiasis)
Leukocyte transendothelial migration
Regulation of actin cytoskeleton
CD4 T up
Monocyte up
Monocyte down
Count
3
6
9
12
15
p.adjust
0.05
0.10
0.15


ETN
Phosphatidylinositol signaling system
FoxO signaling pathway
Autophagy - animal
Endocytosis
AMPK signaling pathway
Focal adhesion
Inositol phosphate metabolism
Insulin resistance
Regulation of actin cytoskeleton
HIF-1 signaling pathway
Leukocyte transendothelial migration
NOD-like receptor signaling pathway
Prolactin signaling pathway
JAK-STAT signaling pathway
RNA polymerase
Endocrine resistance
Complement and coagulation cascades
CD4 T up
CD4 T down
Monocyte down
Count
4
6
8
10
12
p.adjust
0.05
0.10
0.15

Flow cytometry Data: cellular markers (immune)

Salomon *et al. Arthritis Research & Therapy* (2017) 19:33
DOI 10.1186/s13075-017-1244-x

Arthritis Research & Therapy

RESEARCH ARTICLE Open Access

Th17 and CD24^{hi}CD27^{+} regulatory B lymphocytes are biomarkers of response to biologics in rheumatoid arthritis

Sarah Salomon[1†], Caroline Guignant[2†], Pierre Morel[3], Gauthier Flahaut[2], Clément Brault[2], Clément Gourguechon[2], Patrice Fardellone[1], Jean-Pierre Marolleau[3], Brigitte Gubler[2] and Vincent Goëb[1*]

Analysis of surface & intracellular markers

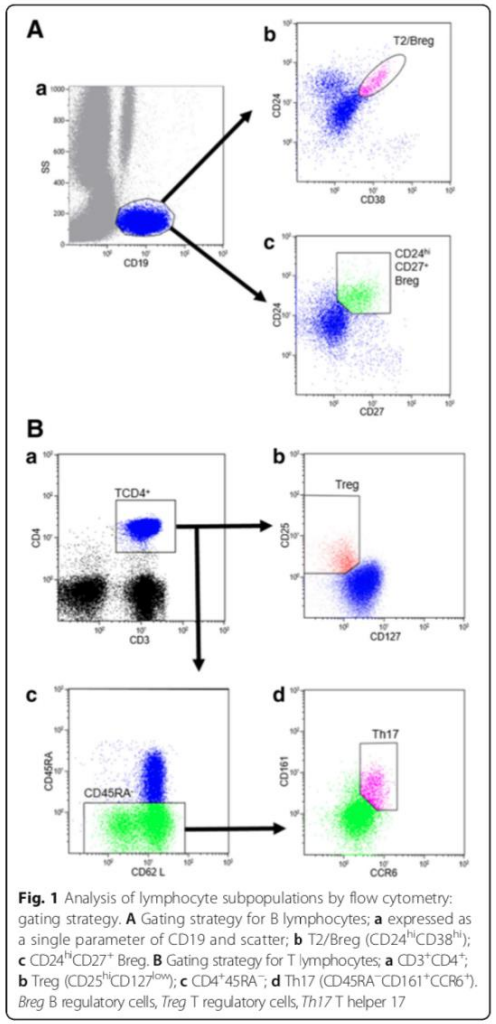


Fig. 1 Analysis of lymphocyte subpopulations by flow cytometry: gating strategy. **A** Gating strategy for B lymphocytes; **a** expressed as a single parameter of CD19 and scatter; **b** T2/Breg ($CD24^{hi}CD38^{hi}$); **c** $CD24^{hi}CD27^{+}$ Breg. **B** Gating strategy for T lymphocytes; **a** $CD3^{+}CD4^{+}$; **b** Treg ($CD25^{hi}CD127^{low}$); **c** $CD4^{+}45RA^{-}$; **d** Th17 ($CD45RA^{-}CD161^{+}CCR6^{+}$). *Breg* B regulatory cells, *Treg* T regulatory cells, *Th17* T helper 17

Response to treatment is strongly associated with fluctuation of cell subsets

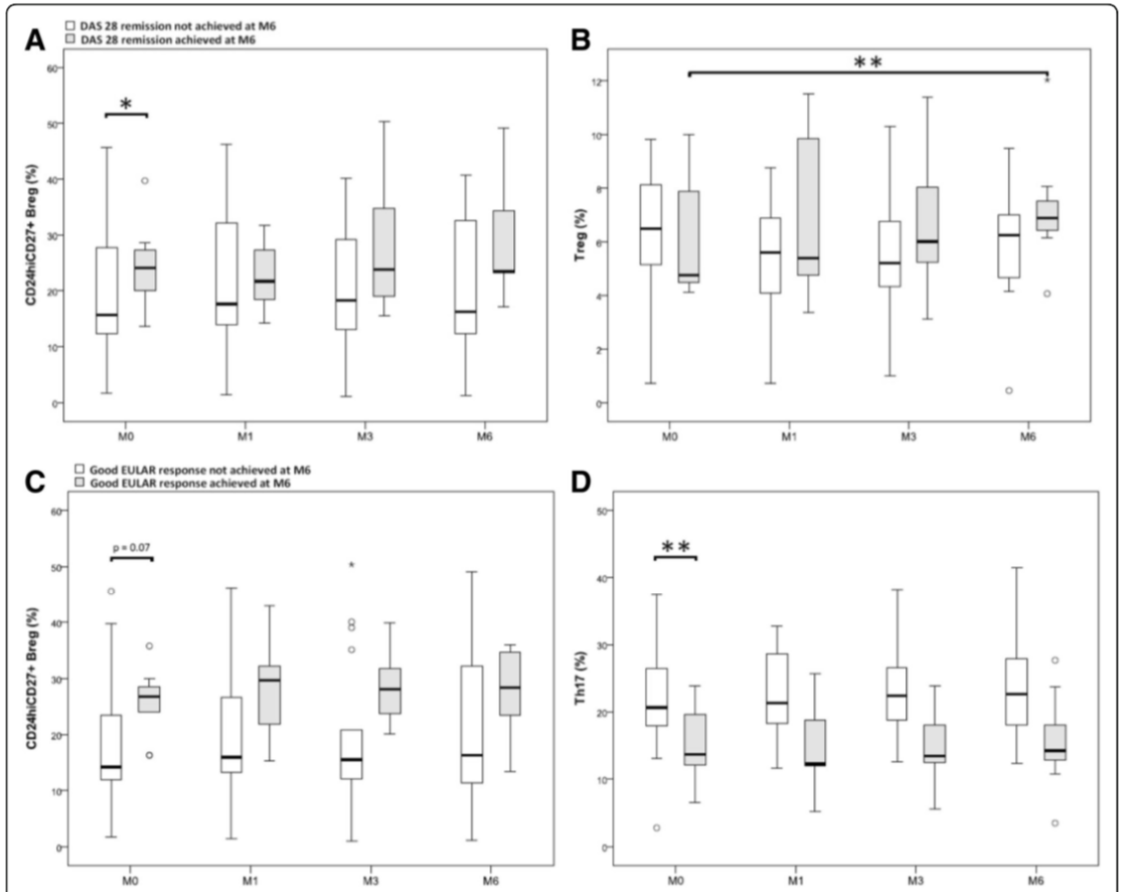


Fig. 2 Baseline levels and evolution of lymphocyte subpopulations in patients with rheumatoid arthritis according to Disease Activity Score in 28 joints (*DAS28*) remission or European League Against Rheumatism (*EULAR*) response at month (M)6. **a** Baseline levels and evolution of $CD24^{hi}CD27^{+}$ B regulatory cells (*Breg*) in percentage between M0 and M6 in the patients who achieved DAS 28 remission (n = 7) or not (n = 19) at M6. **b** Initial levels and evolution of T regulatory cells (*Treg*) in percentage between M0 and M6 in the patients who achieved DAS28 remission (n = 7) or not (n = 19) at M6. **c** Initial levels and evolution of $CD24^{hi}CD27^{+}$ Breg in percentage between M0 and M6 in patients who achieved good EULAR response at M6 (n = 9) or not (n = 17). **d** Baseline levels and evolution of T helper 17 (Th17) cells in percentage between M0 and M6 in patients who achieved good EULAR response at M6 (n = 9) or not (n = 17). Results in box plots are median and IQR; difference observed between the two groups was assessed by the Mann–Whitney test at M0 and the general linear model (GLM) procedure and MIXED SAS for repetitive analyses. *$p < 0.05$, **$p < 0.01$

Bregs predict response to Biologics

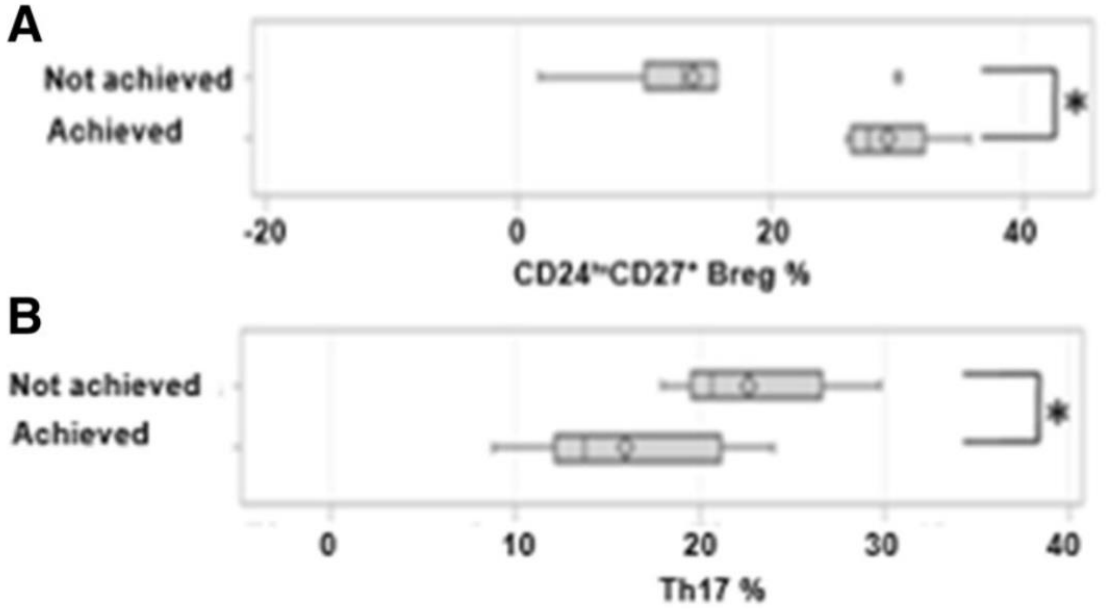


Fig. 3 Baseline lymphocyte subpopulation levels according to the European League Against Rheumatism (*EULAR*) response at month 6 (*M6*) by type of biologic drug. **a** Comparison of baseline levels of $CD24^{hi}CD27^{+}$ B regulatory cells (*Breg*) in percentage, between the patients who achieved a good EULAR response at M6 (n = 4) or not (n = 6) under abatacept. **b** Comparison of T helper 17 cell (*Th17*) levels in percentage at M0 between patients who achieved good EULAR response at M6 (n = 5) or not (n = 11) under anti-cytokine treatment; box-plots show median and IQR; difference observed between the two groups using the *t* test. $*p < 0.05$

ARTHRITIS & RHEUMATOLOGY
Vol. 68, No. 2, February 2016, pp 494–504
DOI 10.1002/art.39437

Breg Cells Are Numerically Decreased and Functionally Impaired in Patients With Systemic Sclerosis

Athanasios Mavropoulos, Theodora Simopoulou, Areti Varna, Christos Liaskos, Christina G. Katsiari, Dimitrios P. Bogdanos, and Lazaros I. Sakkas

Clinical Immunology 184 (2017) 26–32

Contents lists available at ScienceDirect

Clinical Immunology

journal homepage: www.elsevier.com/locate/yclim

IL-10-producing regulatory B cells (B10 cells), IL-17 + T cells and autoantibodies in systemic sclerosis

Athanasios Mavropoulos, Christos Liaskos, Theodora Simopoulou, Dimitrios P. Bogdanos, Lazaros I. Sakkas *

Department of Rheumatology and Clinical Immunology, Faculty of Medicine, School of Health Sciences, University of Thessaly, Larissa 40500, Greece

Clinical Immunology 184 (2017) 33–41

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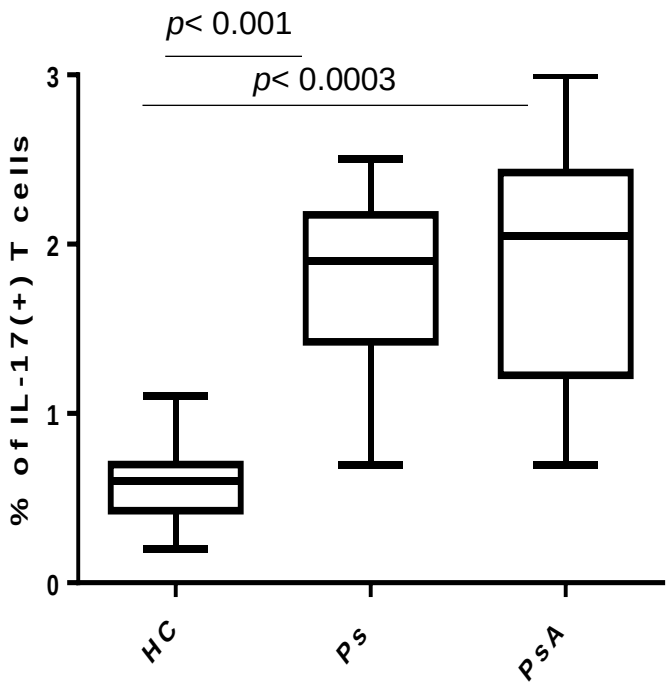
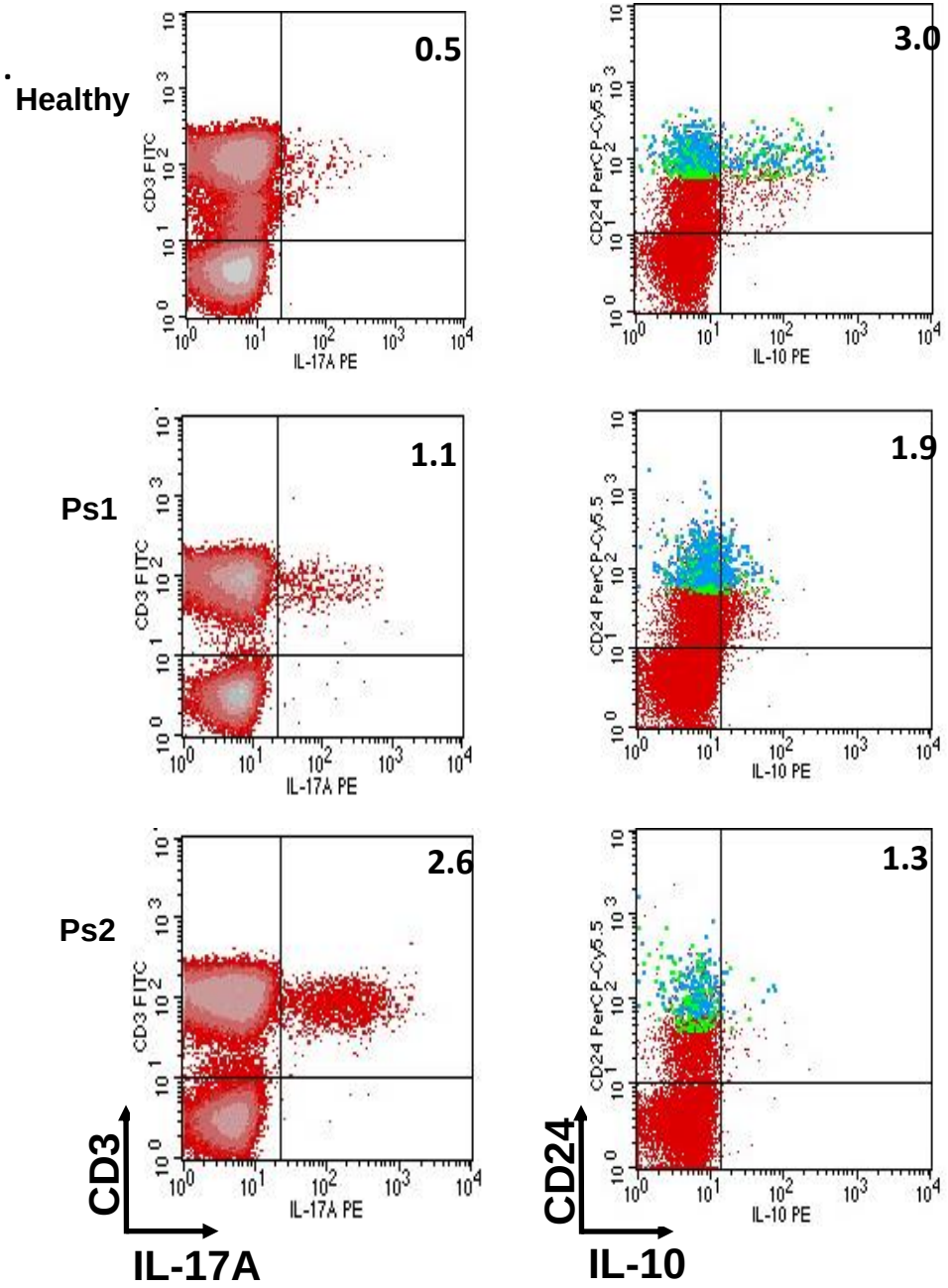
IL-10 producing Bregs are impaired in psoriatic arthritis and psoriasis and inversely correlate with IL-17- and IFNγ-producing T cells☆

Athanasios Mavropoulos [a], Areti Varna [a], Efterpi Zafiriou [b], Christos Liaskos [a], Ioannis Alexiou [a], Aggeliki Roussaki-Schulze [b], Marianna Vlychou [c], Christina Katsiari [a], Dimitrios P. Bogdanos [a], Lazaros I. Sakkas [a,*]

A.



B.



Mavropoulos Clin Immun 2017

RHEUMATOLOGY

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doi:10.1093/rheumatology/kez204
Advance Access publication 17 June 2019

Original article

Apremilast increases IL-10-producing regulatory B cells and decreases proinflammatory T cells and innate cells in psoriatic arthritis and psoriasis

Athanasios Mavropoulos[1], Efterpi Zafiriou[2], Theodora Simopoulou[1], Alexandros G. Brotis[3], Christos Liaskos[1], Aggeliki Roussaki-Schulze[2], Christina G. Katsiari[1], Dimitrios P. Bogdanos[1] and Lazaros I. Sakkas[1]

Our patient-tailored approach

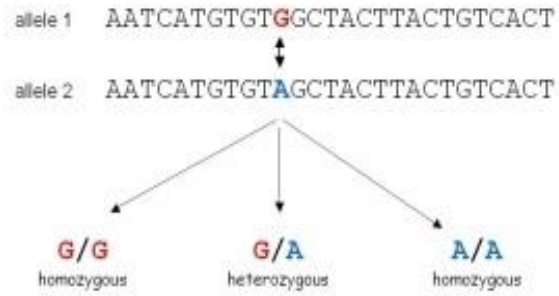
Patients at baseline or before new treatment are:

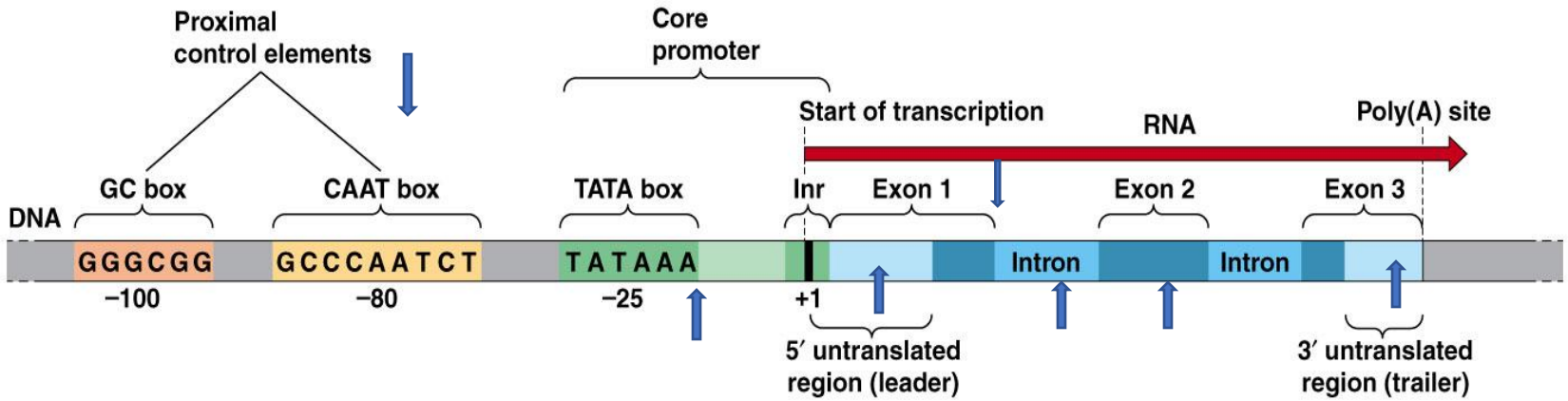
1) Tested for antibodies (profiling or multiple reflexing depending on the disease) i.e true ACPA pos versus anti-CCP neg
2) **Tested for activation markers by flowcytometry of peripheral blood mononuclear cells**
3) **PBMCs are stored and re-analyzed when decision making regarding biologics treatment must be made**
4) **Tested using multi flow cytometry for Th1, Th2, Th17, Tregs, Bregs etc**
5) Tested for cytokine expression if anti-cytokine biologics are applied

(ie cell-subset specific TNF producing cells if an anti-TNF is to be used

Genetic variation- SNPs (750,000 SNPS)

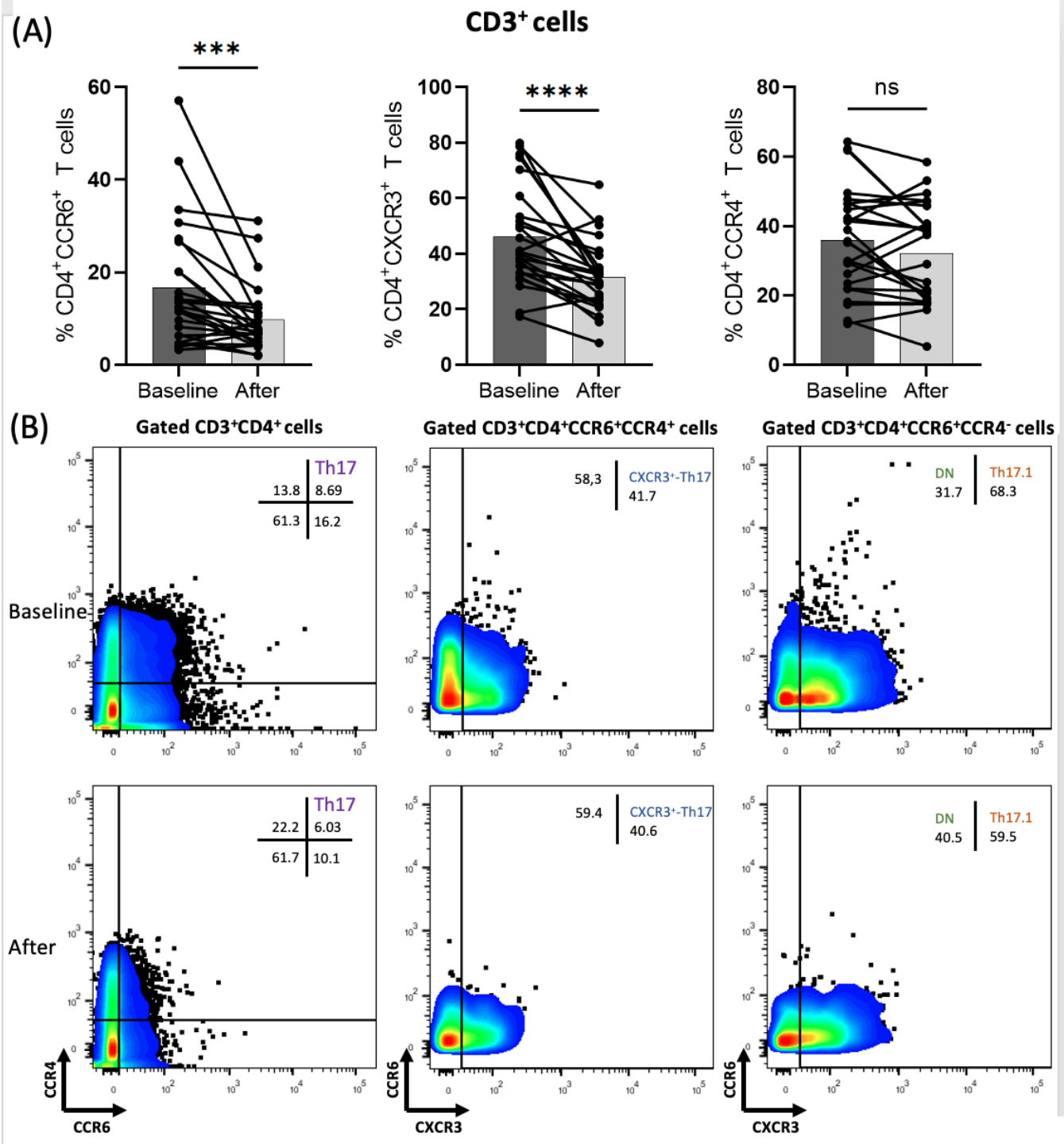
- **Single Nucleotide Polymorphism** : the most common form of genetic variation
- Change in **one** nucleotide
- Usually one common (major) and one rare (minor) allele
- **Millions** of SNPs across the genome
- Different effects based on their location



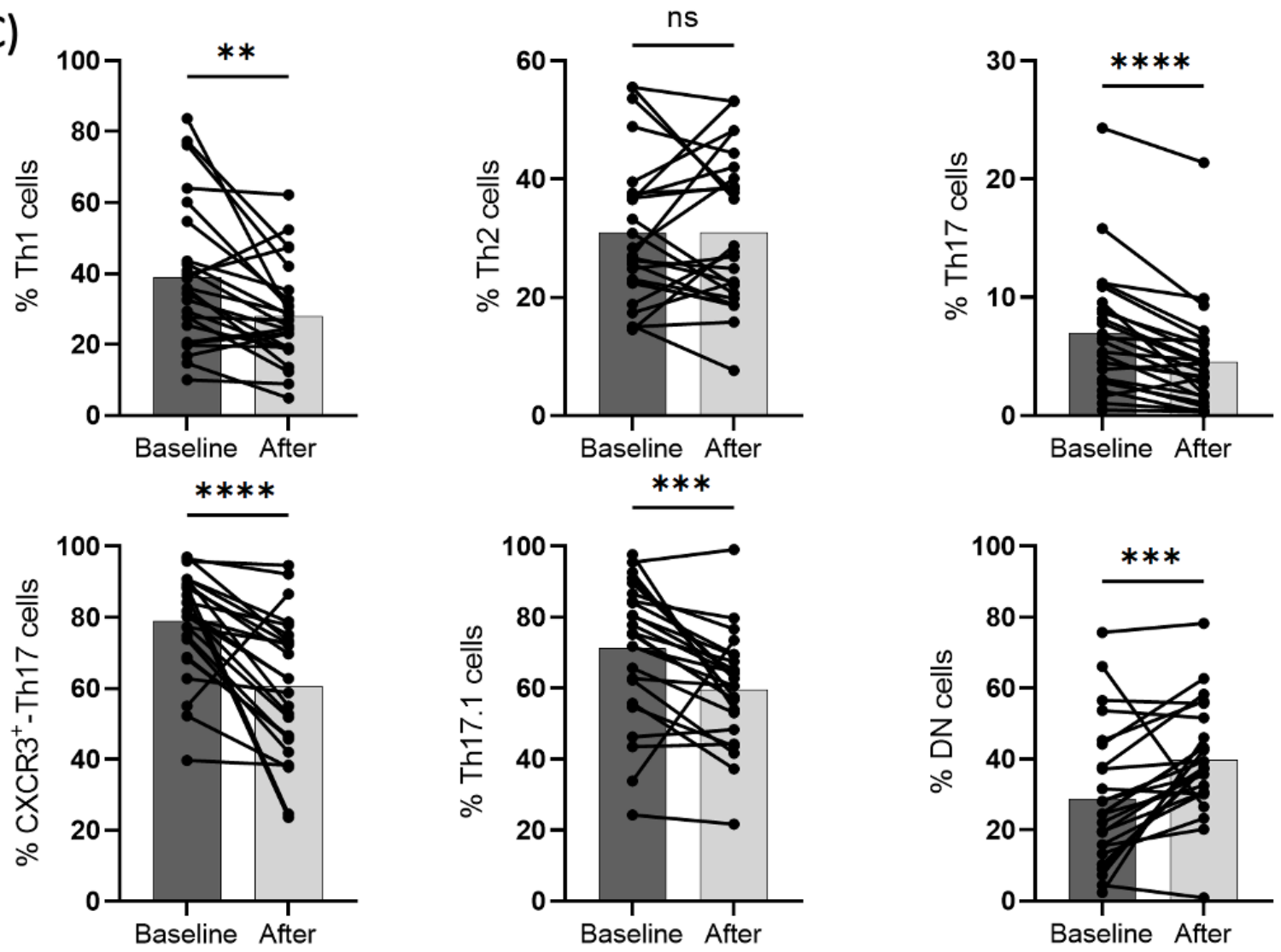


Aim

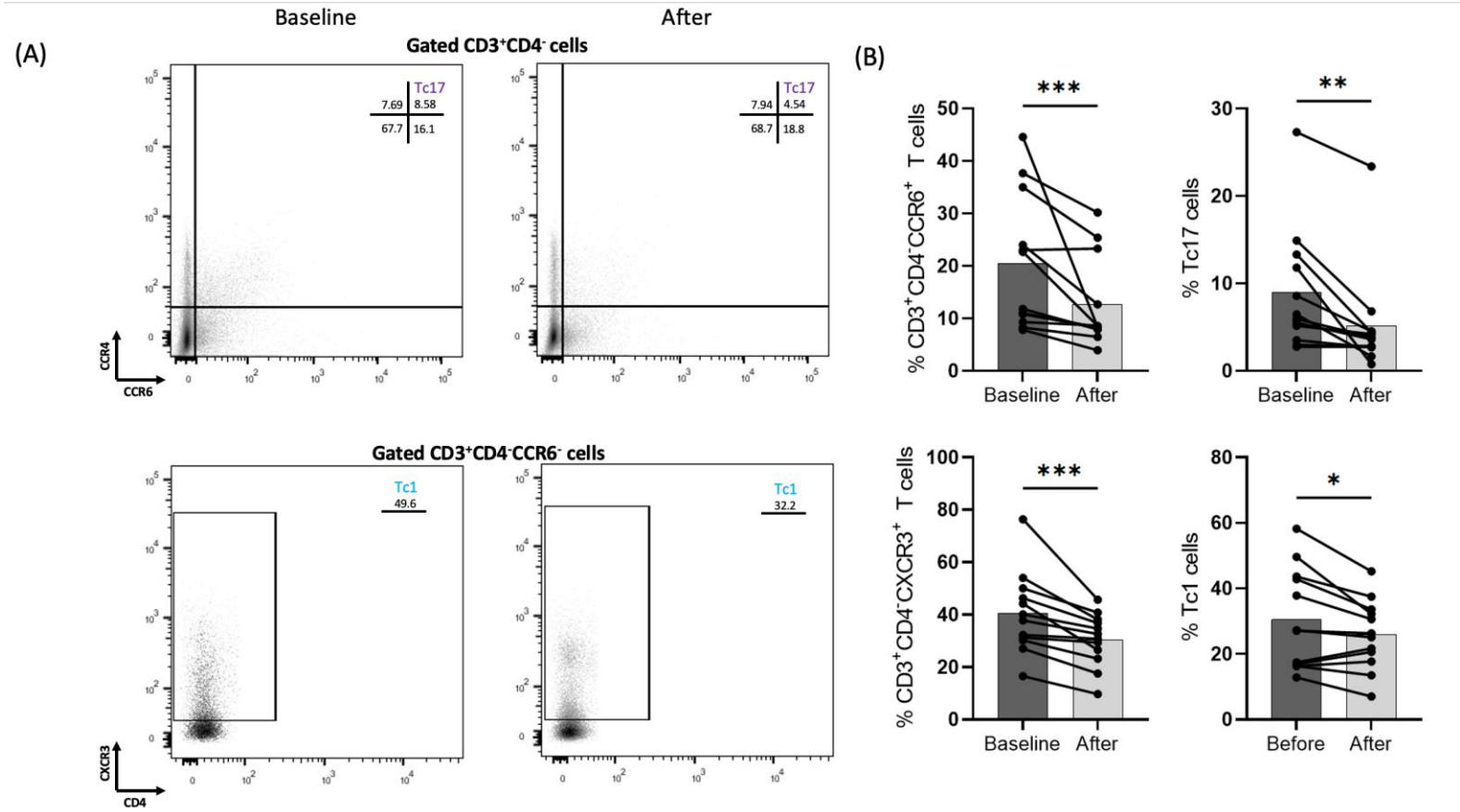
- **To test whether we can predict if people with psoriatic arthritis (PsA) will respond to certain biologic drugs using blood tests.**


(A)
CD3+ cells
***
****
ns
% CD4+CCR6+ T cells
% CD4+CXCR3+ T cells
% CD4+CCR4+ T cells
Baseline
After
(B)
Gated CD3+CD4+ cells
Gated CD3+CD4+CCR6+CCR4+ cells
Gated CD3+CD4+CCR6+CCR4- cells
Baseline
After
Th17
13.8
8.69
61.3
16.2
22.2
6.03
61.7
10.1
58,3
CXCR3+-Th17
41.7
59.4
40.6
DN
31.7
Th17.1
68.3
40.5
59.5
CCR4
CCR6
CXCR3

(C)


**
% Th1 cells
100
80
60
40
20
0
Baseline
After
ns
% Th2 cells
60
40
20
0
Baseline
After
****
% Th17 cells
30
20
10
0
Baseline
After
****
% CXCR3+-Th17 cells
100
80
60
40
20
0
Baseline
After
***
% Th17.1 cells
100
80
60
40
20
0
Baseline
After
***
% DN cells
100
80
60
40
20
0
Baseline
After

IL-17 Therapeutic Blockade Decreased $CD3^+CD4^-CCR6^+$, $CD3^+CD4^-CXCR3^+$, Tc1 and Tc17 cell Populations in Psoriasis Patients



> Clin Exp Immunol. 2022 Oct 21;210(1):79-89. doi: 10.1093/cei/uxac069.

A sharp decrease of Th17, CXCR3+−Th17, and Th17.1 in peripheral blood is associated with an early anti-IL-17-mediated clinical remission in psoriasis

Sotirios G Tsiogkas [1], Athanasios Mavropoulos [1], Efthimios Dardiotis [2], Efterpi Zafiriou [3], Dimitrios P Bogdanos [1]

frontiers

Anti-IL-17 Biologics Diminish Circulating T Follicular Helper Cell Sub-Populations in Patients with Psoriasis

Sotirios G. Tsiogkas[1], ATHANASIOS MAVROPOULOS[1], Efthimios Dardiotis[2], DIMITRIOS P. BOGDANOS[1*], Efterpi Zafiriou[3]

[1]Department of Rheumatology and Clinical Immunology, Faculty of Medicine, University of Thessaly, Larissa, Greece, Greece, [2]Department of Neurology, Faculty of Medicine, University of Thessaly, Larissa, Greece, Greece, [3]Department of Dermatology, Faculty of Medicine, University of Thessaly, Larissa, Greece, Greece

What is Autoprediction?

AutoPrediction©

A disease-specific, parameter-dependent algorithm, which predicts customized response

to individualized treatment

- Flexible (not all parameters needed to predict
- Semi-quantitative
- Includes demographic, clinical, laboratory and/or histological parameters
- Immunological (autoantibody, serum cytokines, chemokines, cell subsets (Th1, Th17, Tregs, Bregs etc, surface cell subsets i.e CD24, CD27, CD39)

Systemic Sclerosis

87 patients with Systemic Sclerosis, who responded to treament

92 patients with Systemic Sclerosis, who did not respond to treatment

Serum/plasma
Blood
DNA/RNA
PBMC
Saliva
Urine
Stool Sample

534 analytes

(17 autoab specificities,
45 cytokines,
19 angiogenic factors,
17 fibrogenic factors
27 cell-surface markers,
19 chemokines, …)

Systemic Sclerosis

87 patients with Systemic Sclerosis, who responded to treament

92 patients with Systemic Sclerosis, who did not respond to treatment

Type of Treatment

IVIG
MMF
CYC
Cyclosporin
MTX
AZT
Rituximab
Anti-TNF
Infliximab
….
Specific treatment for Pulmonary arterial hypertension, digital ulcers, etc

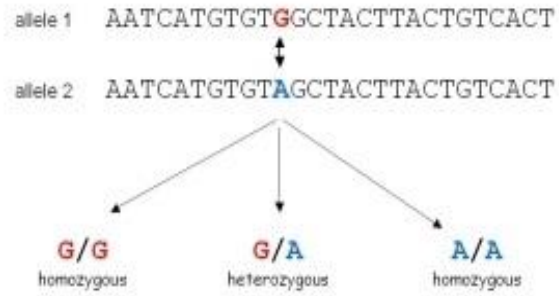
Immunogenetic, Genetic, Pharmacogenetics,

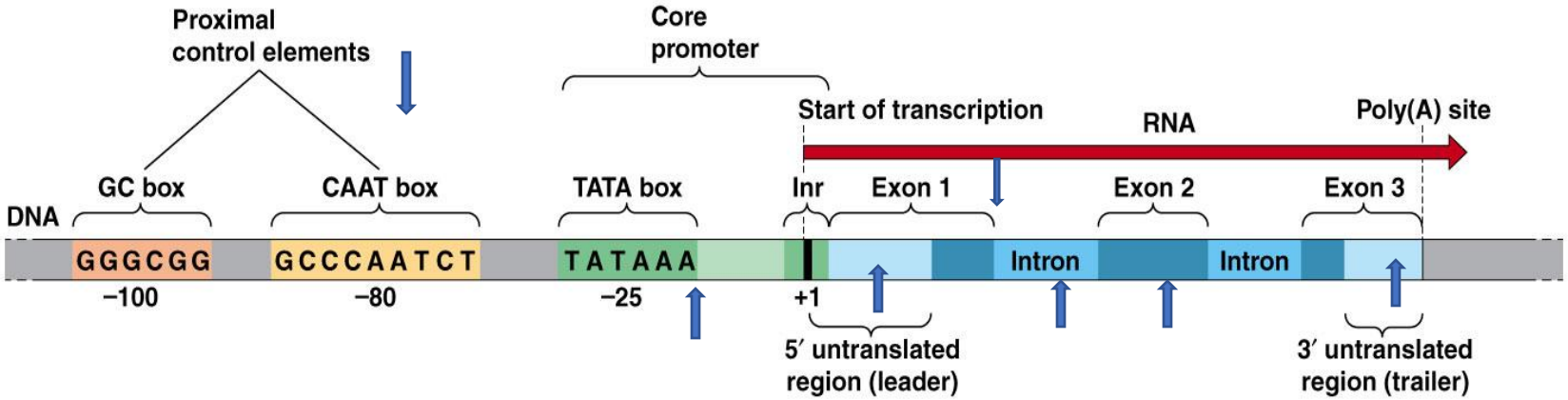
- **594,322 SNPs (single nucleotide polymorphisms)**

Infinium Global Screening Array-24 v2.0 BeadChip, Illumina

Genetic variation- SNPs

- **Single Nucleotide Polymorphism** : the most common form of genetic variation
- Change in **one** nucleotide
- Usually one common (major) and one rare (minor) allele
- **Millions** of SNPs across the genome
- Different effects based on their location





AutoPrediction -examples

- Prediction algorithm for response of SSc patients to individual treatment-
- **infliximab** (27parameters 14 with positive predictive value and 13 with negative predictive value
- **IVIG** (17 parameters, 12 with positive and 5 with negative predictive value

General Comments

- **Less than 30%** of the predictive score can be attributed to autoantibodies (mono or poly-specificity or combination
- **More than 40%** of the total predictive score is to be attributed to cell-subset phenotyping
- **Less than 20%** of the predictive score can be based on serum cytokines
- **Less than 10%** of the scoring is due to genotyping

Example A

Algorithm % prediction (0-92%)= ZxPre-Predict algoritm
(3.2autoab1)+(9.1autoab2)-(6.7autoab3)-
(1.2 autoab levels/cut off autoab4)+ ……..
(3.1cytokine1)+ (1.7cytokine2)+…….. +
(3.9 xCD4+CD27+) (+2.2CD4-CD39+)+ ……
+(9.2 B10)+ (1.3CD4C+D25+)…….

Prediction algorithm for response of SSc to infliximab
(27 parameters 14 with positive predictive value &
13 with negative predictive value

Example A continued

Pre-Predict algorithm (sex, previous treatment, age,
histology, pulmonary fibrosis (Y/N),
pulmonary portal hypertensions (Y/N)…
previous serious infection),
pneumococcus vaccine immunity (Y/N)…….
Increased CRP (Y/N)….

The roadmap to Autoprediction?

DRCI (Department of Rheumatology and Clinical Immunology, University of Thessaly)

www.autorheumatology.com

MINISTRY OF DEVELOPMENT
GENERAL SECRETARIAT FOR RESEARCH AND TECHNOLOGY
The way forward

Thank you for your attention

THANK YOU!

BIOZ
Laboratory of Genetics, Comparative and Evolutionary Biology