«Τι νεότερο στη Ρευματοειδή Αρθρίτιδα»

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ΠΑΝΕΠΙΣΤΗΜΙΟ ΚΡΗΤΗΣ
ΙΑΤΡΙΚΗ ΣΧΟΛΗ

Menu

- Clinical
  - Difficult to treat RA
  - JAKis / Cardiovascular risk

- Basic Science
  - Cellular characterization
  - Biomarkers for response / prognosis

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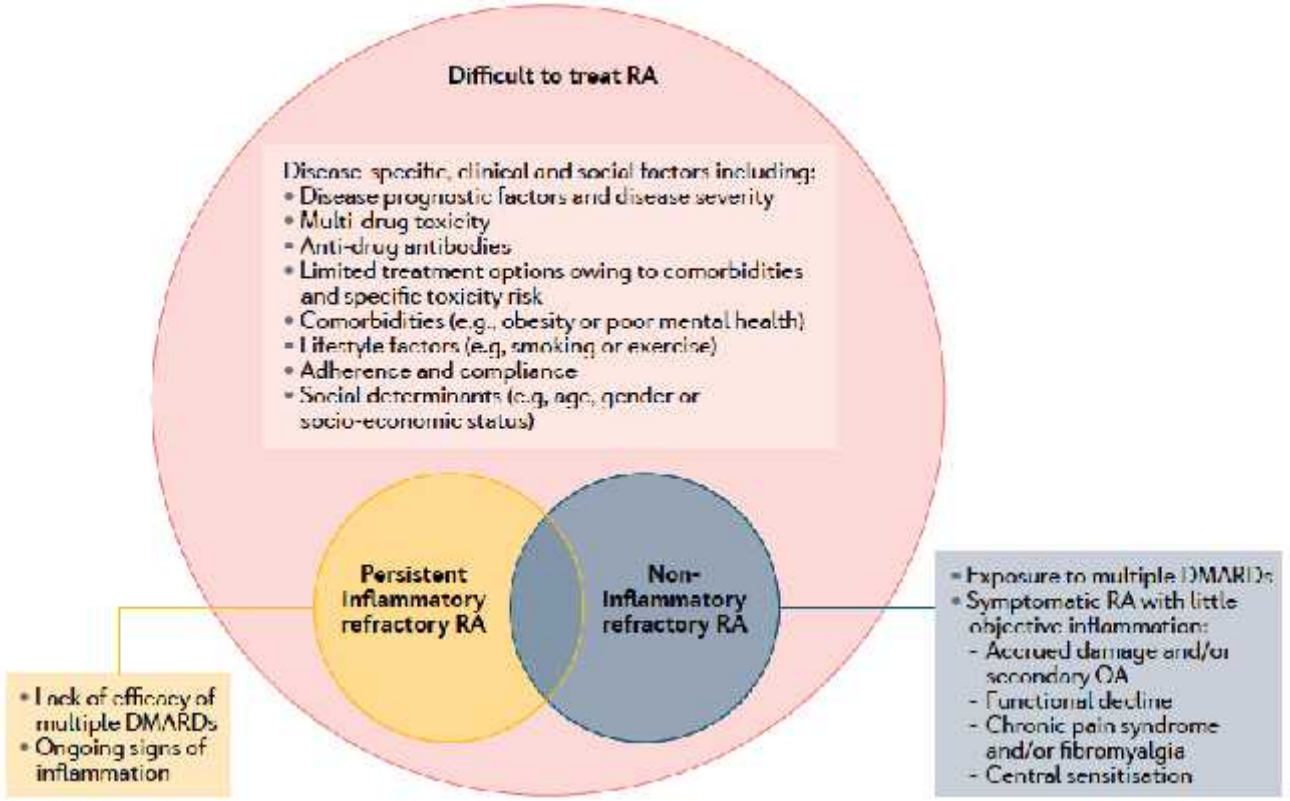
Difficult to treat RA: 7% -20% of RA on bDMARDs

Box 1 EULAR definition of difficult-to-treat RA

1. Treatment according to European League Against Rheumatism recommendation and failure of ≥2 b/tsDMARDs (with different mechanisms of action)* after failing csDMARD therapy (unless contraindicated).†
2. Signs suggestive of active/progressive disease, defined as ≥1 of:
   a. At least moderate disease activity (according to validated composite measures including joint counts, for example, DAS28-ESR>3.2 or CDAI>10).
   b. Signs (including acute phase reactants and imaging) and/or symptoms suggestive of active disease (joint related or other).
   c. Inability to taper glucocorticoid treatment (below 7.5 mg/day prednisone or equivalent).
   d. Rapid radiographic progression (with or without signs of active disease).‡
   e. Well-controlled disease according to above standards, but still having RA symptoms that are causing a reduction in quality of life.
3. The management of signs and/or symptoms is perceived as problematic by the rheumatologist and/or the patient.

All three criteria need to be present in D2T RA.



DTT represent a group with low function and quality of life population

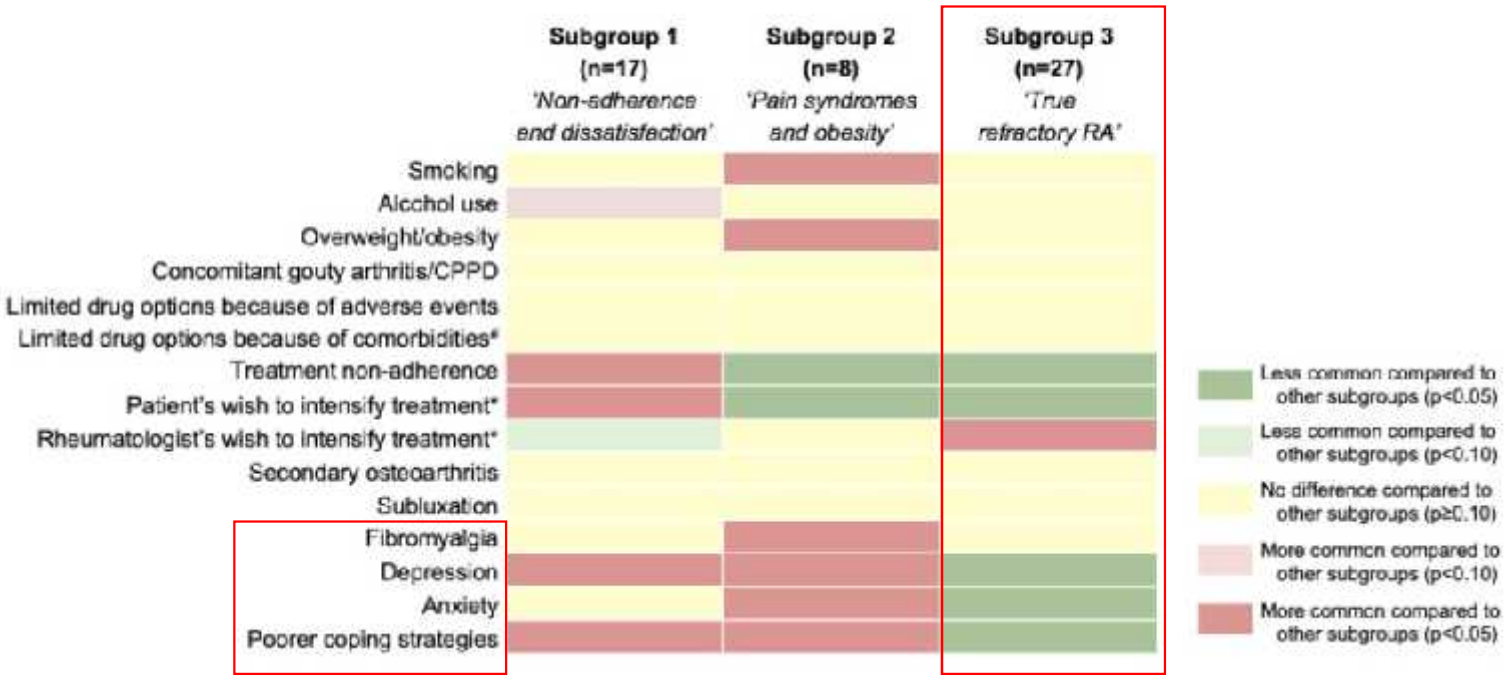
TABLE 5 Clinical burden of D2T RA

	D2T RA ($n = 52$)	Non-D2T RA ($n = 99$)	*P*-value
Physical functioning (HAQ), level	1.8 (1.4–2.1)	1.0 (0.5–1.4)	<0.001[a]
Quality of Life (EQ-5D-5L)			
Index (Dutch tariff)	0.62 (0.31–0.77)	0.81 (0.68–0.85)	<0.001[a]
VAS	51 (26–65)	75 (60–85)	<0.001[a]
Fatigue (FACIT-F), level	27 (18–34)	39 (29–45)	<0.001[a]
Pain (VAS-pain), level	58 (30–80)	23 (8–49)	<0.001[a]
Physical activity (IPAQ), %			
Low	35	13	
Moderate	23	37	<0.01[b]
High	42	50	
Total h of sitting per week	46 (28–63)	42 (28–56)	0.75[a]

Rheumatology 2020;0:1–11

Pure ongoing inflammation or comorbidities / low adherence differentiate 3 groups

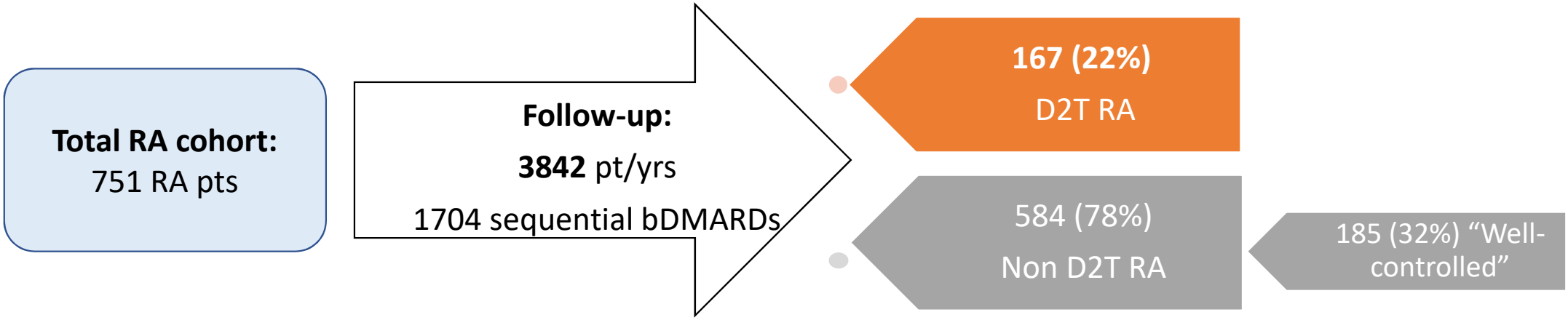
Fig. 1 Subgroups of D2T RA



Rheumatology 2020;0:1–11

Substantial number of patients are characterized as D2T:

22% of the bDMARDs cohort



EULAR 2021

Comorbidity burden at baseline predicts disease characterization as D2T

compared to “well-controlled”

	D2T versus “Well-controlled”	
	Adjusted OR*	
Sex (male vs. female)	0.44 (0.2-0.9)[a]	0.42 (0.2-0.9)[a]
Seropositivity	0.41 (0.2-0.7) [b]	0.42 (0.2-0.7) [b]
Year of therapy start	1.13 (1.05-1.2) [b]	1.13 (1.04-1.2) [b]
SDAI	1.05 (1.02-1.08) [c]	1.05 (1.02-1.07) [c]
CC ≥2 (vs. ≤1)	1.52 (0.82-2.81)	
RDCI ≥1 (vs. 0)		**2.24 (1.2-4.5) [a]**
a: p<0.05; b: p<0.01; c: p<0.001		

* Variables entered in the analyses and removed from the final models with backward selection (p≥0.10) : sex, age, RF/anti-CCP seropositivity, disease duration since diagnosis, year of therapy start, number of previous csDMARDs, type of 1st bDMARD used (TNFi vs. non-TNFi), co-therapy with methotrexate or corticosteroids (yes/no), baseline SDAI and HAQ

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Janus kinases (**JAKs**…or “Just Another Kinase”)

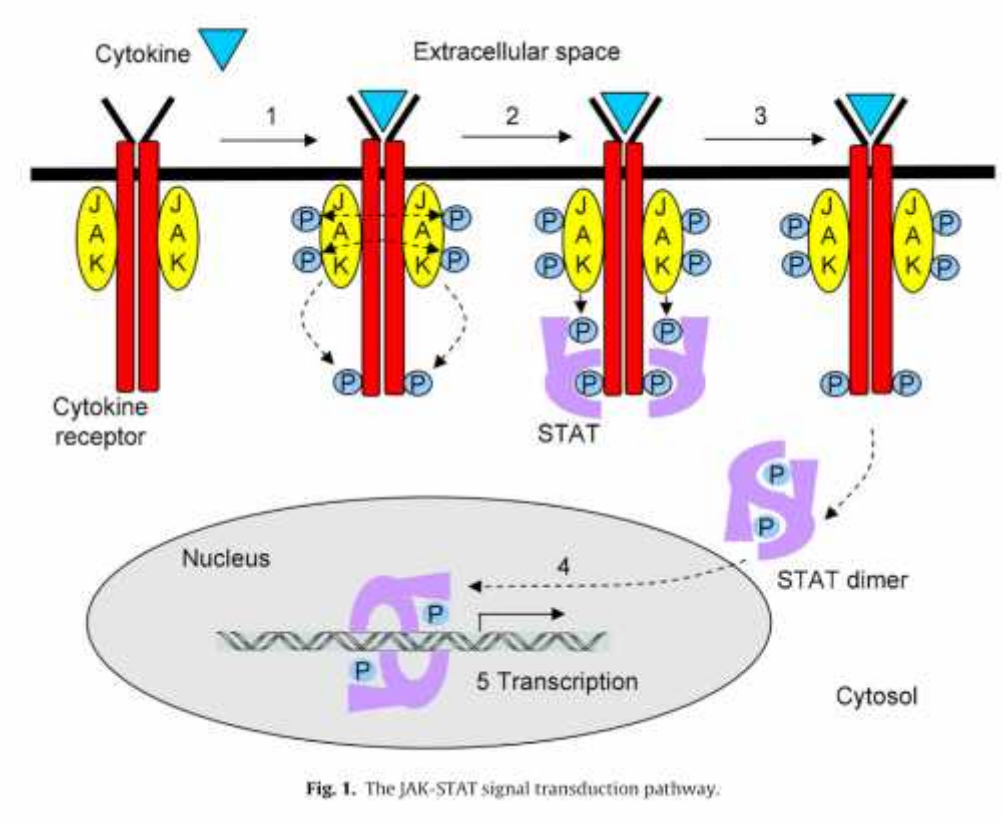
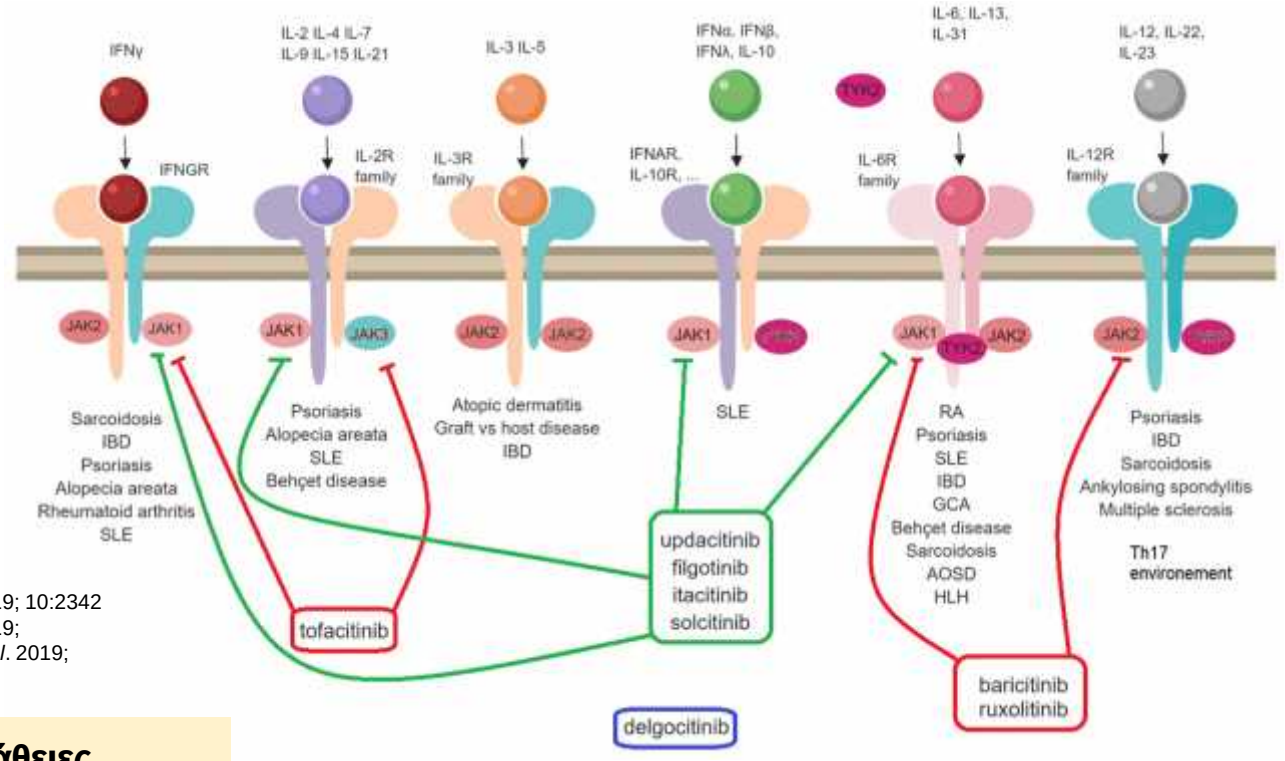


Fig. 1. The JAK-STAT signal transduction pathway.

- ✓ **Μεταγωγείς σημάτων από ~60 διαφορετικές κυτταροκίνες και αυξητικούς παράγοντες**
- ✓ **4 διαφορετικοί JAKs** (JAK1–3, TYK2)
- ✓ JAK1/2: καθολική έκφραση
- ✓ **JAK3:** αιμοποιητικά, μυελοειδή, λεμφοειδή κύτταρα
- ✓ Δρουν ως τυροσινο-κινάσες
- ✓ Σχηματίζουν **διμερή** και κατόπιν **ενεργοποιούν τις πρωτεΐνες STAT** (STAT1–4, 5A, 5B, 6)

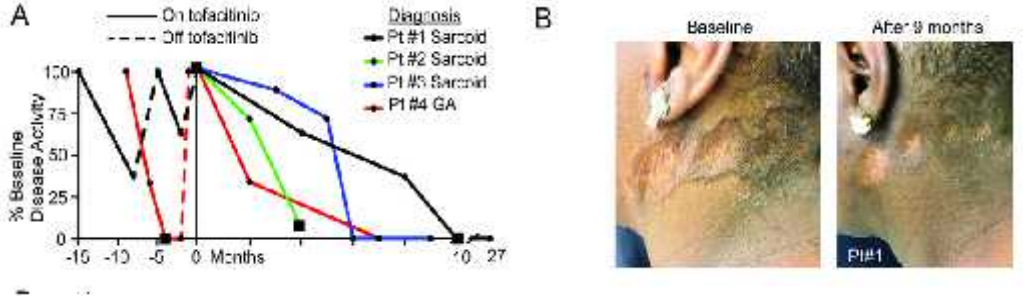
Roskoski R Jr. *Pharmacol Res*. 2016; 111:784-803; O’Shea JJ, et al. *Annu Rev Med*. 2015; 66:311-28; Gurniak CB, et al. *Blood*. 1996; 87: 3151-60

Αυξανόμενο φάσμα κλινικών εφαρμογών των αναστολέων JAK/Tyk κινασών



Howell MD, et al. *Front. Immunol*. 2019; 10:2342
Damsky W, *J Am Acad Dermatol*. 2019;
Gooderham MJ, et al. *JAMA Dermatol*. 2019;

- **Σπονδυλοαρθροπάθειες**
- **Νεανική αρθρίτιδα**
- **Γιγαντοκυτταρική αρτηρίτιδα**
- **Αυτοφλεγμονώδη νοσήματα**
- **Αυτοάνοσες δερματοπάθειες** (ατοπική δερματίτιδα, γυροειδής αλωπεκία, λεύκη, κλπ)



The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Cardiovascular and Cancer Risk with Tofacitinib in Rheumatoid Arthritis

CONCLUSIONS

In this trial comparing the combined tofacitinib doses with a TNF inhibitor in a cardiovascular risk–enriched population, risks of MACE and cancers were higher with tofacitinib and did not meet noninferiority criteria. Several adverse events were more common with tofacitinib. (Funded by Pfizer; ORAL Surveillance ClinicalTrials .gov number, NCT02092467.)

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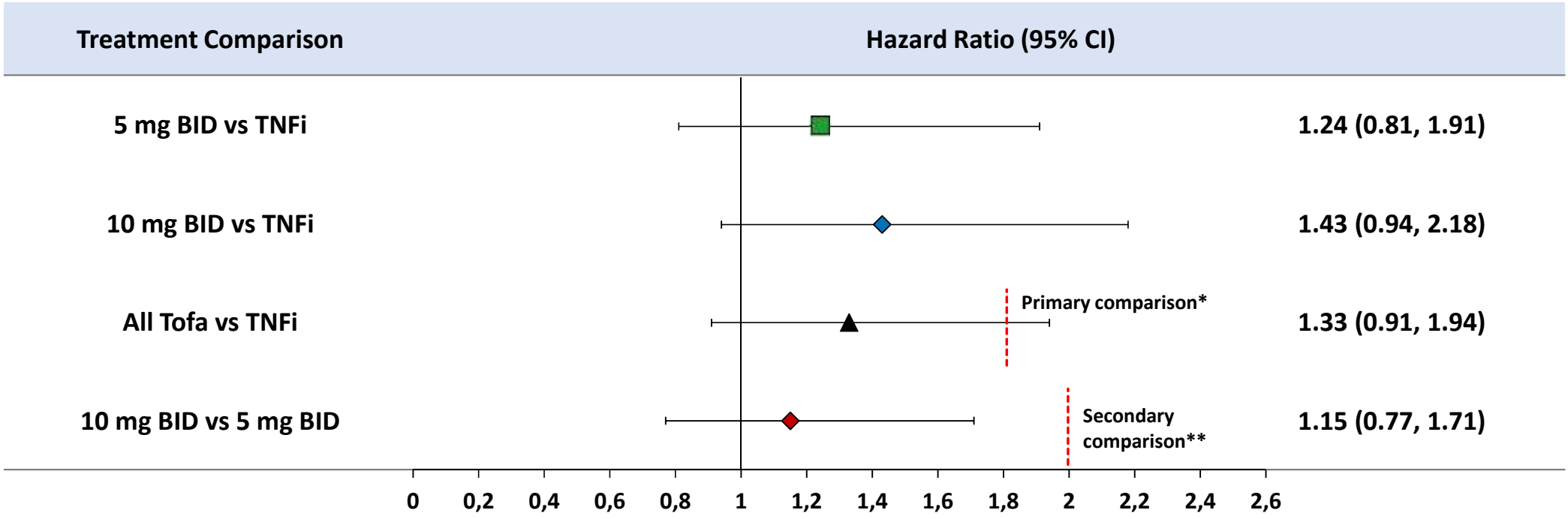
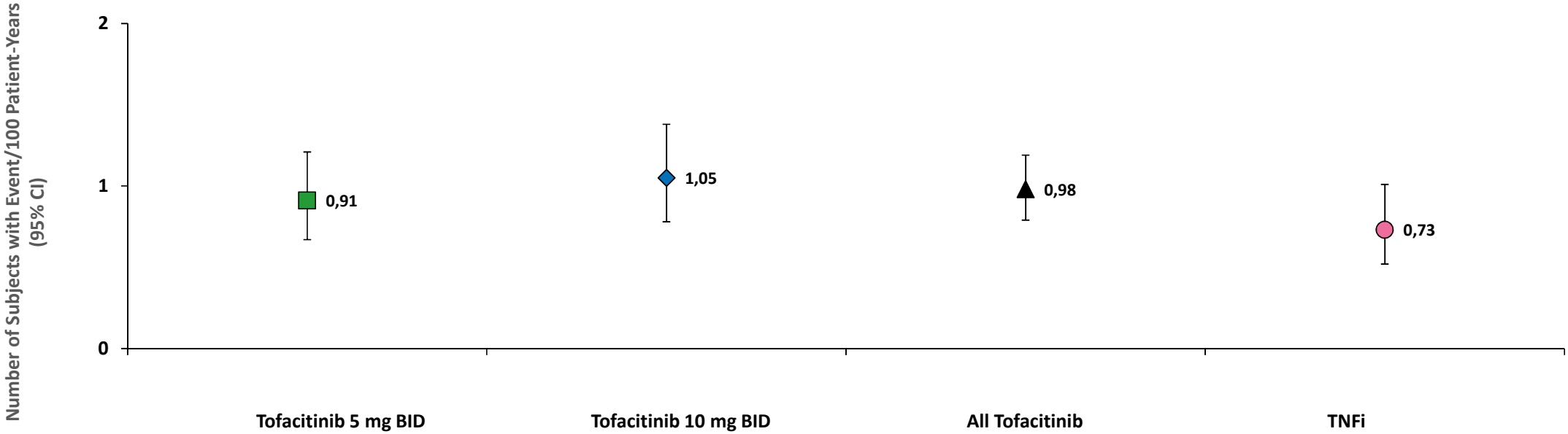
Adjudicated MACE Based on Univariate Cox Proportional Hazard Model (SAS, 60-Day On-Treatment Time[a])



a: 60-Day On-Treatment Time: the risk period is the minimum of (last contact date, or Last Study Treatment Dose date +60 days)
*For primary comparisons: Non-inferiority was met if the upper limit of the 2-sided 95% confidence interval (CI) for the hazard ratio (HR) was less than 1.8 comparing the tofacitinib doses combined vs. TNFi.
**For secondary comparisons: Non-inferiority was met if the upper limit of the 2-sided 95% CI for the HR was less than 2.0 comparing tofacitinib 10 mg BID vs. tofacitinib 5 mg BID.
Ytterberg et al. N Engl J Med 2022;386:316-26

Ytterberg et al.

Incidence Rates of MACE[a]



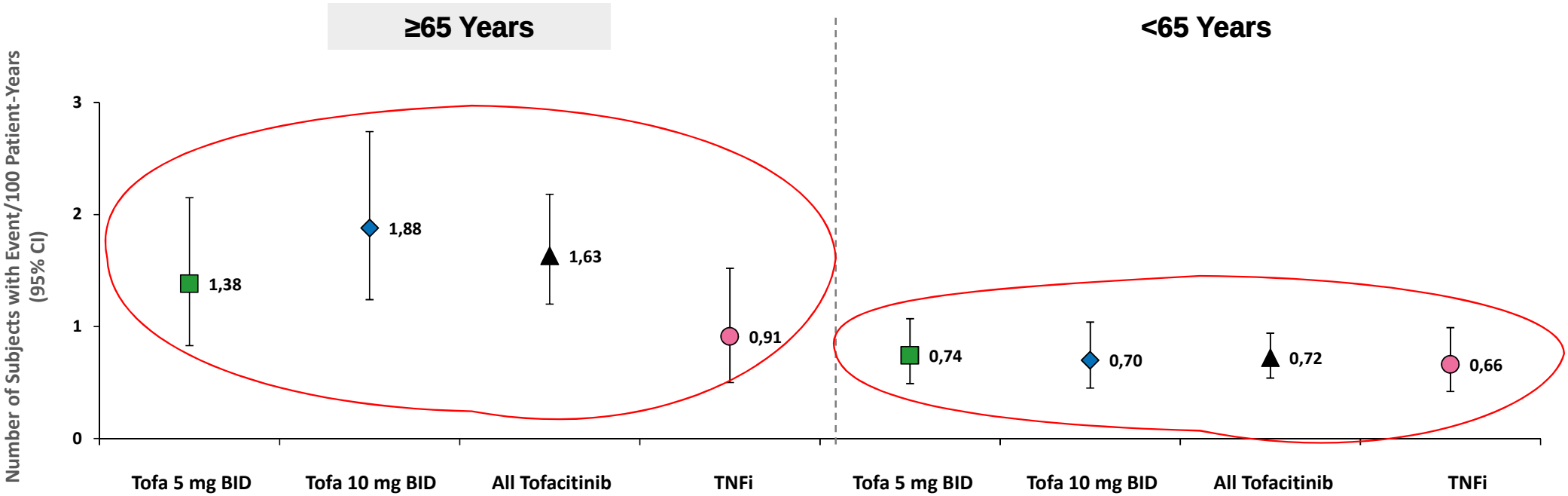
	Tofacitinib 5 mg BID	Tofacitinib 10 mg BID	All Tofacitinib	TNFi
n/N	47/1455	51/1456	98/2911	37/1451
PY	5166.32	4871.96	10038.28	5045.27
NNH (pt-yr) vs TNFi	567	319	412	-
NNH (5-yr) vs TNFi	113	64	-	-

a. Adjudicated MACE Based on Univariate Cox Proportional Hazard Model (SAS, 60-Day On-Treatment Time). 60-Day On-Treatment Time: the risk period is the minimum of (last contact date, or Last Study Treatment Dose date +60 days). NNH was 100/IRD, which was number of PY needed to expose in a treatment to have one more event relative to control; IRD = Incidence rate difference

Ytterberg et al. N Engl J Med 2022;386:316-26

MACE IR by Age (Number of First Events/100 Patient-Years During Study Participation)

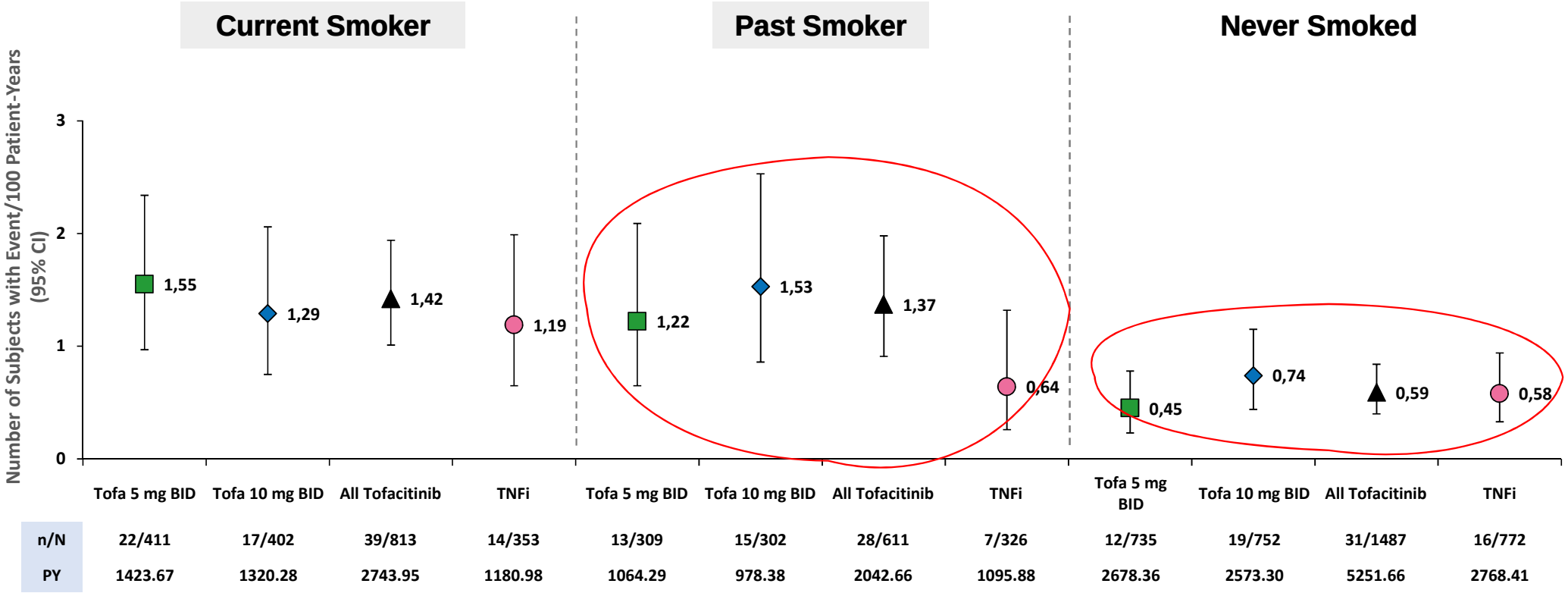
Ytterberg et al.



	Tofa 5 mg BID	Tofa 10 mg BID	All Tofacitinib	TNFi	Tofa 5 mg BID	Tofa 10 mg BID	All Tofacitinib	TNFi
n/N	19/413	27/478	46/891	14/462	28/1042	24/978	52/2020	23/989
PY	1381.65	1436.13	2817.78	1543.90	3784.67	3435.83	7220.50	3501.37

Adjudicated MACE Based on Univariate Cox Proportional Hazard Model (SAS, 60-Day On-Treatment Time). 60-Day On-Treatment Time: the risk period is the minimum of (last contact date, or Last Study Treatment Dose date +60 days).
Ytterberg et al. N Engl J Med 2022;386:316-26

MACE IR by Smoking Status (Number of First Events/100 Patient-Years During Study Participation)



	Current Smoker				Past Smoker				Never Smoked			
	Tofa 5 mg BID	Tofa 10 mg BID	All Tofacitinib	TNFi	Tofa 5 mg BID	Tofa 10 mg BID	All Tofacitinib	TNFi	Tofa 5 mg BID	Tofa 10 mg BID	All Tofacitinib	TNFi
n/N	22/411	17/402	39/813	14/353	13/309	15/302	28/611	7/326	12/735	19/752	31/1487	16/772
PY	1423.67	1320.28	2743.95	1180.98	1064.29	978.38	2042.66	1095.88	2678.36	2573.30	5251.66	2768.41

Adjudicated MACE Based on Univariate Cox Proportional Hazard Model (SAS, 60-Day On-Treatment Time). 60-Day On-Treatment Time: the risk period is the minimum of (last contact date, or Last Study Treatment Dose date +60 days).
Data on file. Pfizer Inc, New York, NY

CorEvitas (previously Corrona) US Registry PASS Study: Methodology

Corrona

Prospective, multicenter, disease-based, observational post-authorization safety study

Location	USA
Follow-up Period	5 years
Dosage	Approved dose for the US (5 mg BID or 11 mg qd)

Study Objective

To compare 5-year IRs for AEs of interest in patients starting tofacitinib vs bDMARDs using cohorts from the Corrona US RA registry

Assessment

- Clinical assessment forms were completed at enrollment and follow-up visits (requested approximately every 6 months)

Concomitant Treatment

MTX alone, MTX with other csDMARDs, or non-MTX csDMARDs

Outcomes

Incidence rates (IRs; number of first events/100 patient-years [PY]) **of:**

- **Deaths** occurring from drug initiation to January 31, 2019
- Adverse Events (AEs) occurring from drug initiation to January 31, 2019
  - Major adverse cardiovascular events (**MACE**)*
  - Serious infection events (**SIEs**)†
  - Herpes zoster (**HZ**; serious and non-serious)‡
  - **Malignancy** excluding non-melanoma skin cancer (NMSC)
  - **NMSC**
  - Venous thromboembolic event (**VTEs**)

AE=adverse event; bDMARD=biologic disease-modifying anti-rheumatic drug; BID=twice daily; Corrona=Consortium of Rheumatology Researchers of North America; HZ=herpes zoster; IBD, inflammatory bowel disorder; IR=incidence rate; MACE=major adverse cardiovascular events; PS=propensity score; qd=once daily; RA=rheumatoid arthritis; SAE=serious adverse event; SIE=serious infection event; VTE=venous thromboembolism.

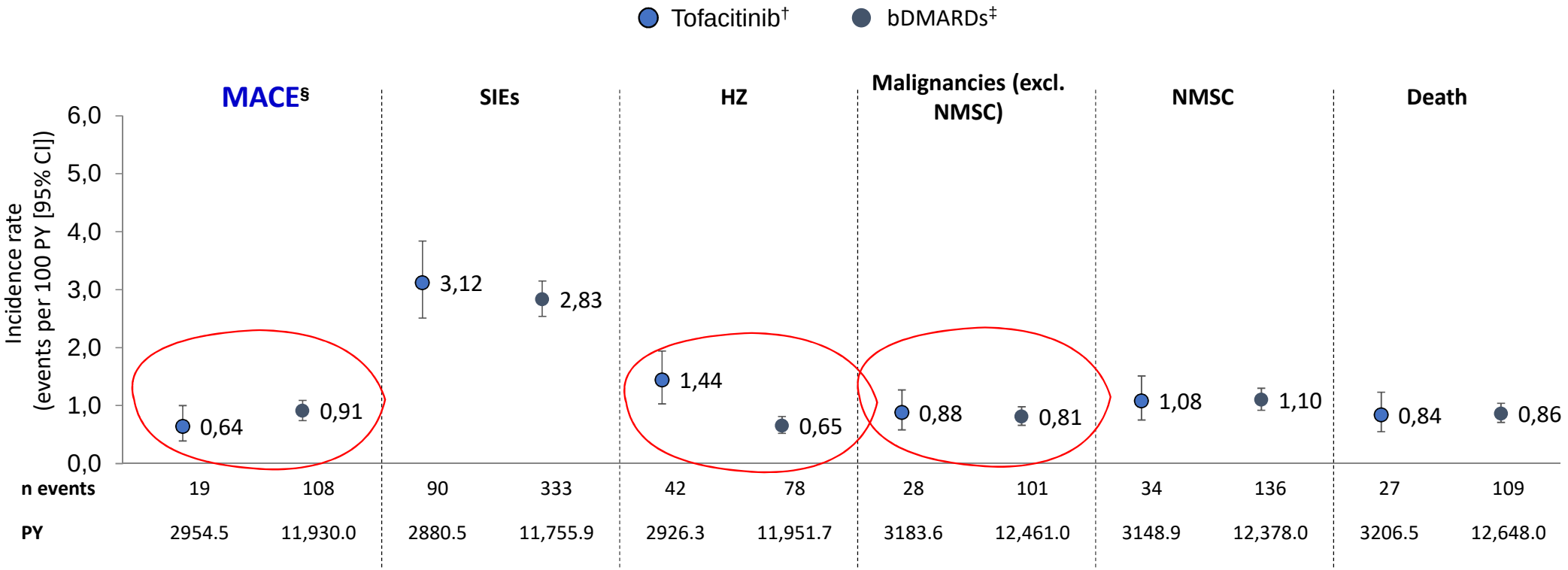
Kremer JM, et al. *ACR Open Rheumatol*. 2021;doi: 10.1002/acr2.11232.

* MACE were defined as myocardial infarction [MI], stroke/transient ischemic attack [TIA], and cardiovascular [CV] death

† SIEs were defined as infections leading to hospitalization and/or intravenous antibiotics

‡ Serious HZ was defined as any HZ infection that: led to hospitalization, disability, congenital anomaly, or death; was immediately life-threatening, medically important/serious in the opinion of the site investigator; or required treatment with parenteral therapy

Incidence Rates of Selected AEs in PS-trimmed Population



Graph adapted from Kremer JM, et al. 2021.

IR=Incidence rates are number of first events/100 PY of outcomes in the PS-trimmed population; incidence rates were based on different definitions of the risk window for outcomes with acute onset (MACE, SIEs, HZ) or latent onset (malignancies and death). Patients initiated treatment as monotherapy or in combination with a csDMARD. †XELJANZ cohort primarily received 5 mg BID.
‡bDMARD cohort included patients initiating adalimumab, certolizumab pegol, golimumab, etanercept, infliximab, abatacept, anakinra, rituximab, or tocilizumab.
§MACE defined as myocardial infarction, stroke/transient ischemic attack, and cardiovascular death.
AE=adverse event; bDMARD=biologic disease-modifying antirheumatic drug; BID=twice daily; CI=confidence interval; csDMARD=conventional synthetic disease-modifying antirheumatic drug; HZ=herpes zoster; MACE=major adverse cardiovascular events; NMSC=nonmelanoma skin cancer; PS=propensity score; PY=patient years; SIE=serious infection event.
Kremer JM, et al. ACR Open Rheumatol. 2021;doi: 10.1002/acr2.11232.

Final Set of Recommendations -2022 Update

eular

Recommendations 6-8 – 2019	
8.	If the treatment target is not achieved with the first csDMARD strategy, when poor prognostic factors are present, a bDMARD or a tsDMARD should be added. (A)

Recommendations 6-8 – 2016	
8.	If the treatment target is not achieved with the first csDMARD strategy, when poor prognostic factors are present, a bDMARD ~~or a tsDMARD~~ should be added (A); JAK-inhibitors may be considered, but pertinent risk factors* must be taken into account. (A, B)
	** The following risk factors for cardiovascular events and malignancies must be considered when intending to prescribe a JAK-inhibitor: Age over 65 years, history of current or past smoking, other cardiovascular risk factors, other risk factors for malignancy, risk factors for thromboembolic events*

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εργαλεία κατανόησης παθογένειας, ανάδειξης νέων μοριακών μηχανισμών γένεσης νόσου και βιοδεικτών πρόγνωσης

RNAseq
Single cell RNA analysis
CyTOF……..

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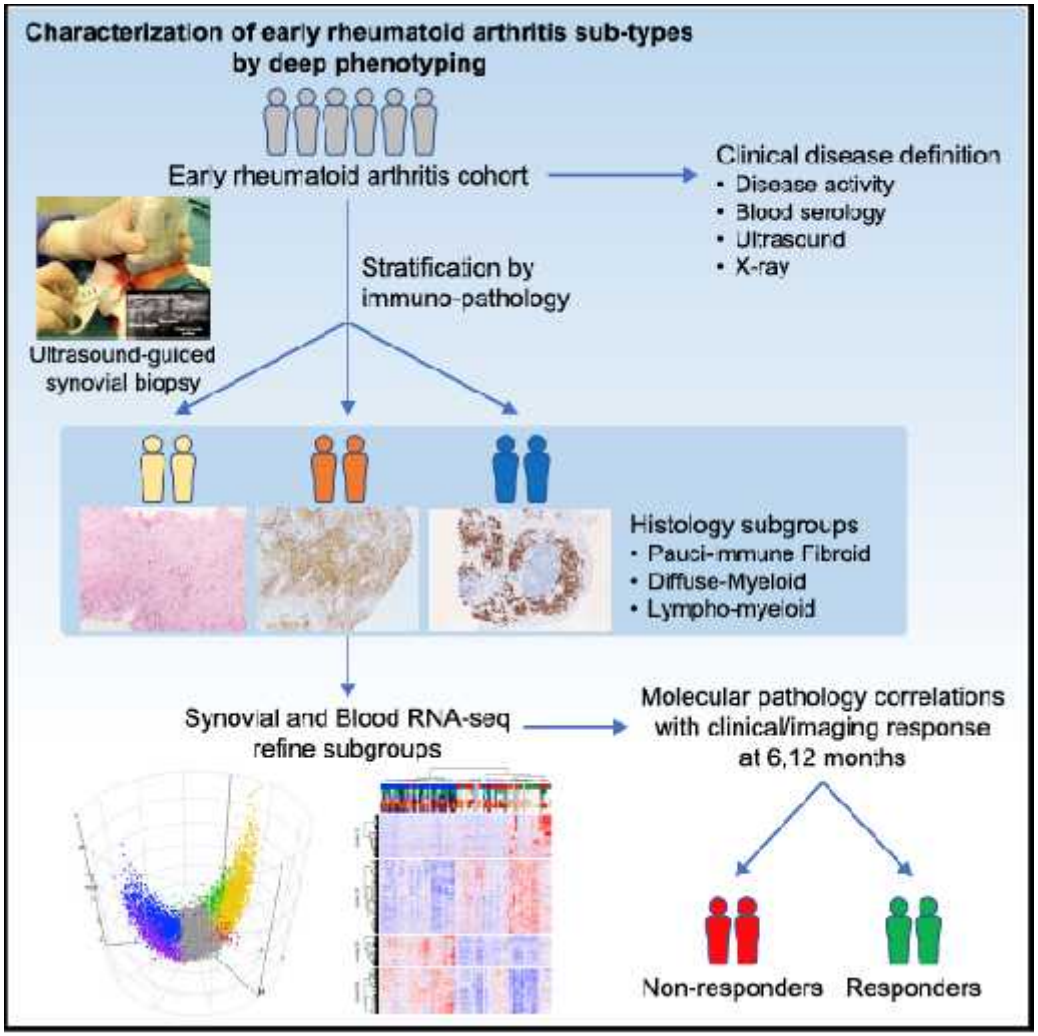

PEAC cohort:
Early RA- treatment naive

Lewis et al., 2019, Cell Reports 28, 2455

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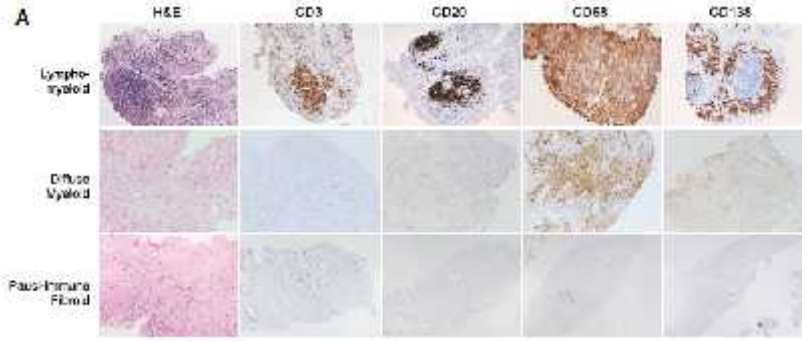
Immunohistochemistry of synovial biopsies:

- ✓ lympho-myeloid (50%)
- ✓ diffuse myeloid (20%)
- ✓ pauci-immune fibroid (20%)



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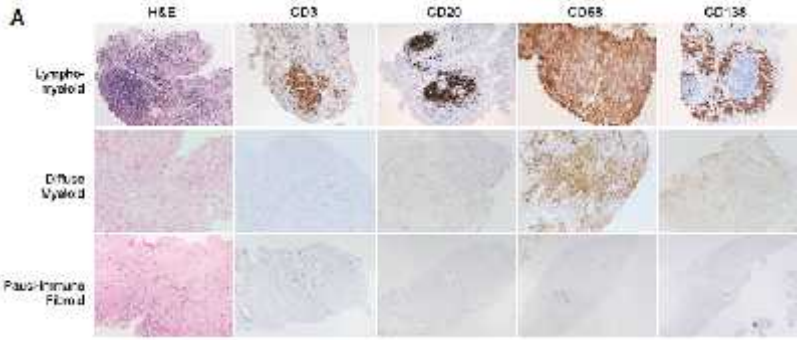
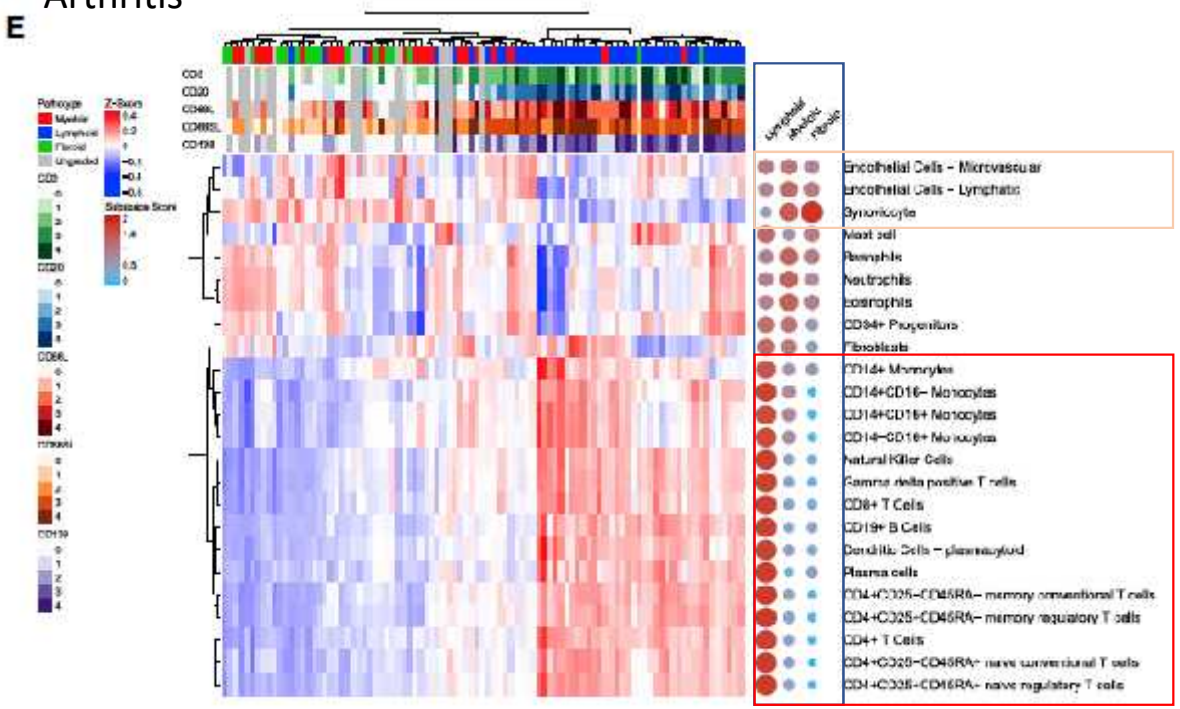


Synovium RNA Sequencing (cell specific gene signatures)
Correlates with Histological Pathotype in Early Rheumatoid Arthritis



Lewis et al., 2019, Cell Reports 28, 2455

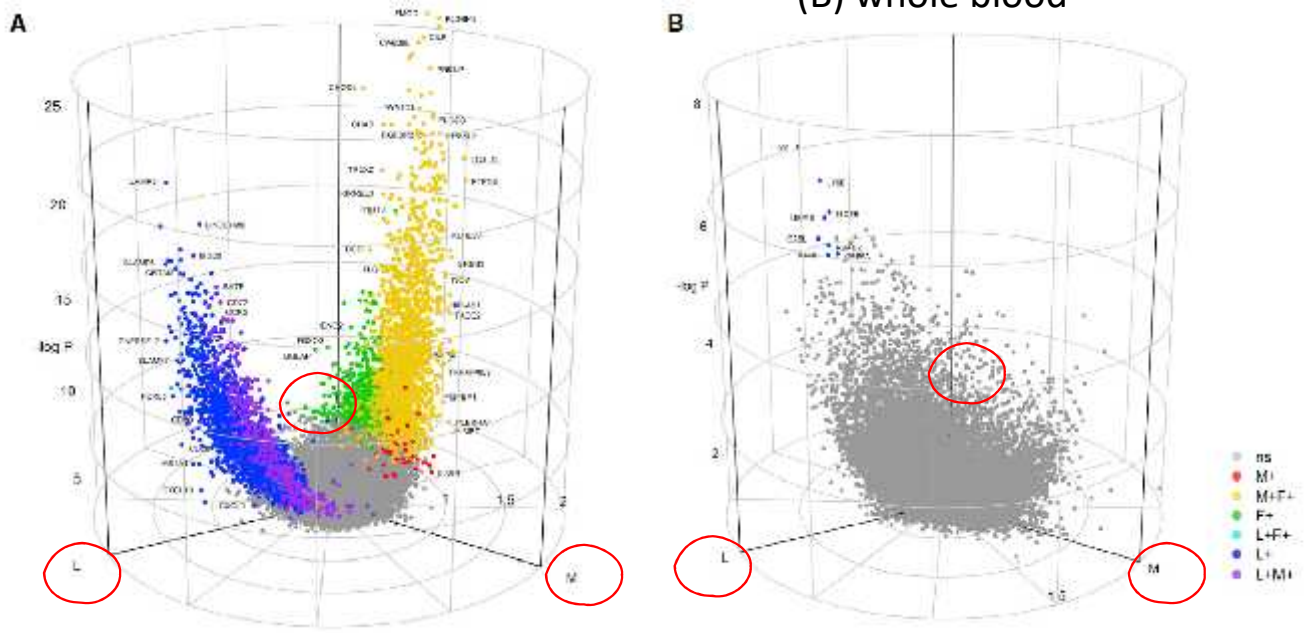
Gene expression analysis (clustering and PCA) and patients' classification:

the synovium gives clean delineation of the different histological subtypes while the blood transcriptome shows significantly less differentiation

Differentially expressed genes comparing RNA sequencing of (A) synovial tissue and (B) whole blood

(A) synovial tissue

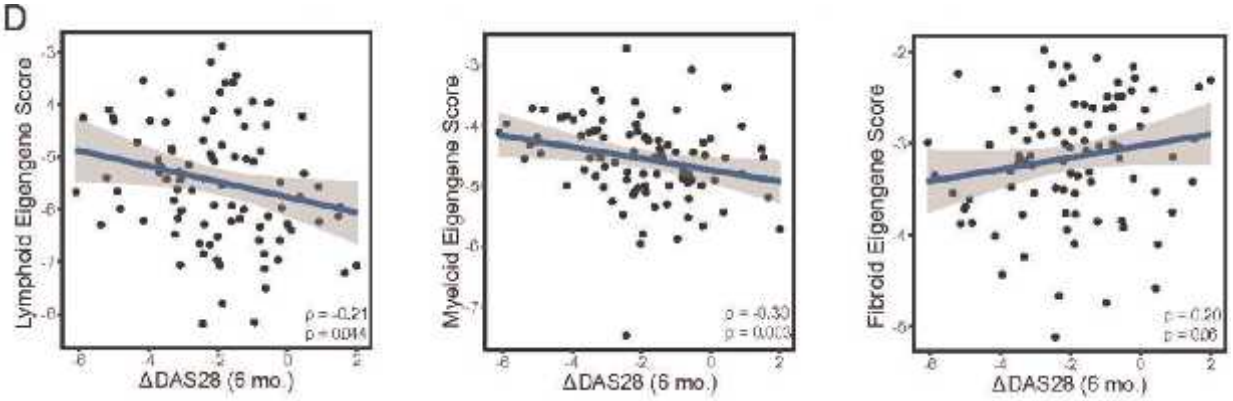
(B) whole blood



Lewis et al., 2019, Cell Reports 28, 2455

Baseline Synovium gene expression analysis (RNAseq) is more informative in predicting clinical responses and radiographic progression

Although histology did NOT predicted responses,
Higher myeloid and lymphoid eigengene expression was associated with **larger decreases in DAS28**



Correlation of pretreatment lymphoid, myeloid and fibroid eigengene scores with change in DAS28-ESR after 6 months of DMARD treatment

Humby F, et al. Ann Rheum Dis 2019;78:761

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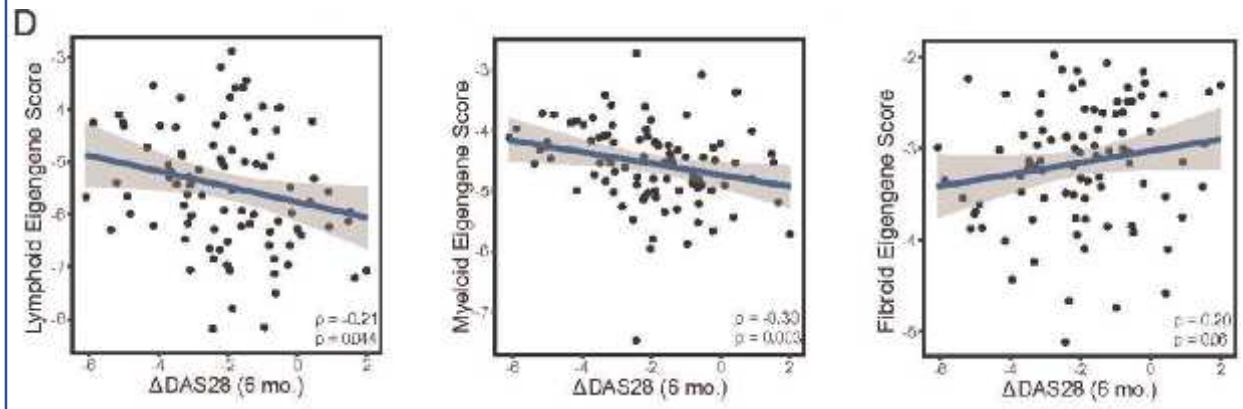

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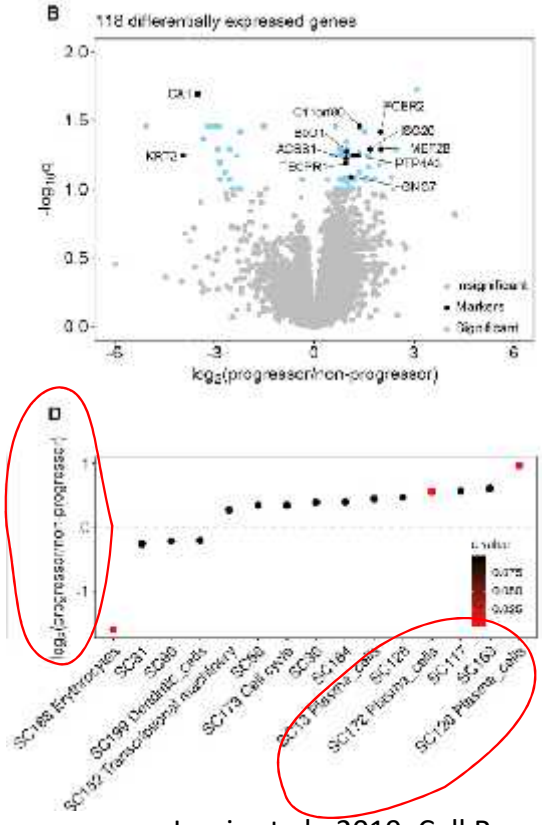
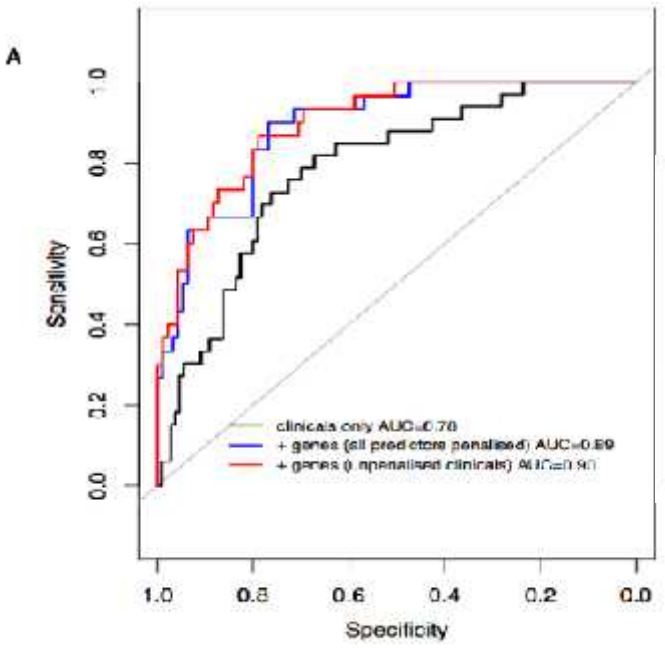
Single-cell RNA-seq-annotated WGCNA modular analysis shows that **increased plasma cell module** expression is predictive of **radiographic progression at 12 months**



Lewis et al., 2019, Cell Reports 28, 2455

Combined models based on gene expression and clinical characteristics predict better clincia outcomes (bDMARDs use % Rx progression)

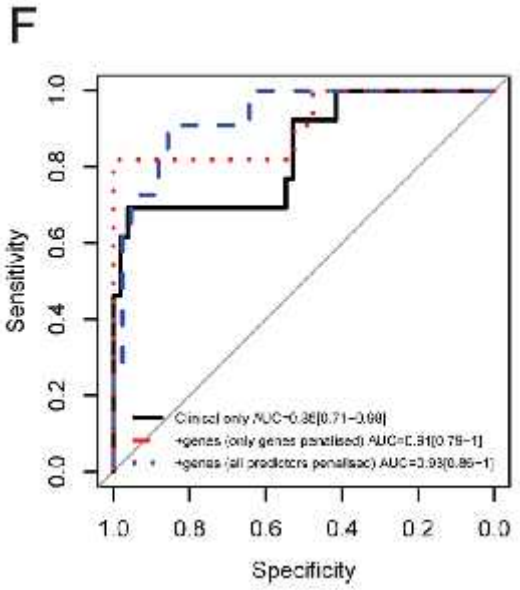
Optimal predictor of need for bDMARDs:
Model including both clinical covariates and genes



	All predictors penalised	Unpenalised clinicals
(Intercept)	-0.322	-3.572
Pathotype		-0.224
CRP	0.015	0.037
TJC		-0.061
DAS28	0.246	0.88
GP3L14	0.242	0.255
IL8	0.26	0.265
CSF1	-0.08	-0.084
MMP3	0.051	0.047
LTB	0.017	
HVEP1	-0.143	-0.181
IL20	-0.223	-0.239
UBASH3A	0.049	
MMP10	0.149	0.16
NOG		-0.038
IFNB1		-0.025

Ann Rheum Dis 2019;0:1–11

Optimal predictor of radiographic progression
A model incorporating RF titre, and the expression of 7 genes (SDC1, CSF2, DENND1C, CD180, UBASH3A, CXCL1, MMP10)



Ann Rheum Dis 2019;78:761–772.

PEAC early RA cohort and synovial RNAseq analysis

- RNA sequence analysis **advances the understanding of RA pathogenesis:**
  - ✓ revealing major differences in synovial gene expression across the histo-pathotype spectrum
  - ✓ identifying associated pathways and gene modules for each pathotype
- Synovial modules were **superior for predicting clinical outcomes**:
  - ✓ clinical response to DMARD therapy at 6 months
  - ✓ poor prognosis in terms of radiographic progression at 12 months.

Lewis et al., 2019, Cell Reports 28, 2455

Menu

- Clinical
  - Difficult to treat RA
  - JAKis / Cardiovascular risk

- Basic Science
  - Cellular characterization
  - Biomarkers for response / prognosis

Rituximab versus tocilizumab in anti-TNF inadequate responder patients with rheumatoid arthritis (R4RA): 16-week outcomes of a stratified, biopsy-driven, multicentre, open-label, phase 4 randomised controlled trial

R4RA trial:
TNFi – non responders
the first biopsy-driven randomised clinical trial in RA

Lancet. 2021 Jan 23;397(10271):305-317

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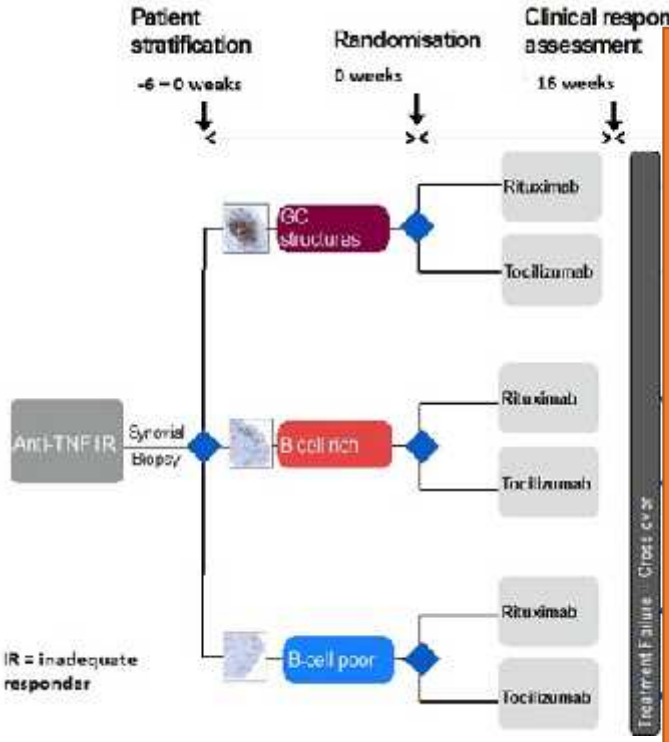

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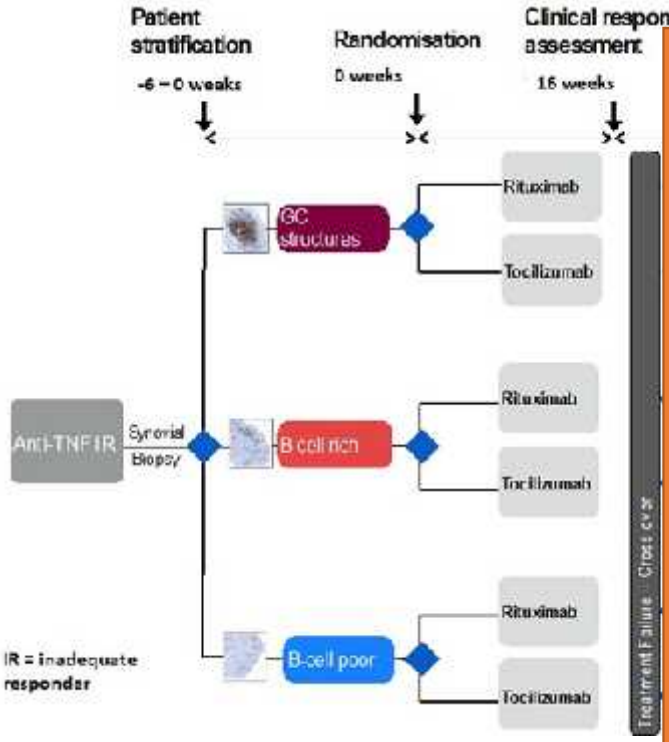

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Patients were classified as B-cell poor or B-cell rich according to:

A. HISTOLOGY: Semi-quantitative scoring to determine expression of CD20 B cells, CD3 T cells, CD138 plasma cells, and CD68 lining and sublining macrophages.

B. RNAseq: B-cell specific gene module, derived from analysis of FANTOM5 gene expression data.

Lancet. 2021 Jan 23;397(10271):305-317

Synovial tissue RNAseq analysis better predicts response compared to histology: ONLY 12% of B-cell poor ST respond to RTX vs 50% in TCZ

Clinical outcomes at 16 weeks in the intention-to-treat **B-cell poor population**

	Histological classification				RNA sequencing classification			
	Rituximab (n=38)	Tocilizumab (n=41)	Treatment effect	Unadjusted p value	Rituximab (n=33)	Tocilizumab (n=37)	Treatment effect	Unadjusted p value
Primary endpoint*								
CDAI ≥50% improvement at week 16	17 (45%)	23 (56%)	11% (−11 to 33)	0·31	12 (36%)	20 (63%)	26% (3 to 50)	0·035
Supplementary endpoint*								
CDAI >50% improvement and CDAI <10·1 at week 16	9 (24%)	19 (46%)	23% (7 to 43)	0·035	4 (12%)	16 (50%)	38% (17 to 59)	0·0012

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Clinical outcomes at 16 weeks in the intention-to-treat **B-cell rich population**

	Histological classification				RNA sequencing classification			
	Rituximab (n=33)	Tocilizumab (n=31)	Treatment effect	Unadjusted p value	Rituximab (n=30)	Tocilizumab (n=29)	Treatment effect	Unadjusted p value
Primary endpoint*								
CDAI ≥50% improvement at week 16	13 (39%)	16 (52%)	12% (−12 to 37)	0·33	15 (50%)	14 (48%)	−2% (−27 to 24)	0·89
Supplementary endpoint*								
CDAI ≥50% improvement and CDAI ≤10·1 at week 16	5 (15%)	11 (36%)	20% (−1 to 41)	0·085	7 (23%)	9 (31%)	8% (−15 to 30)	0·51

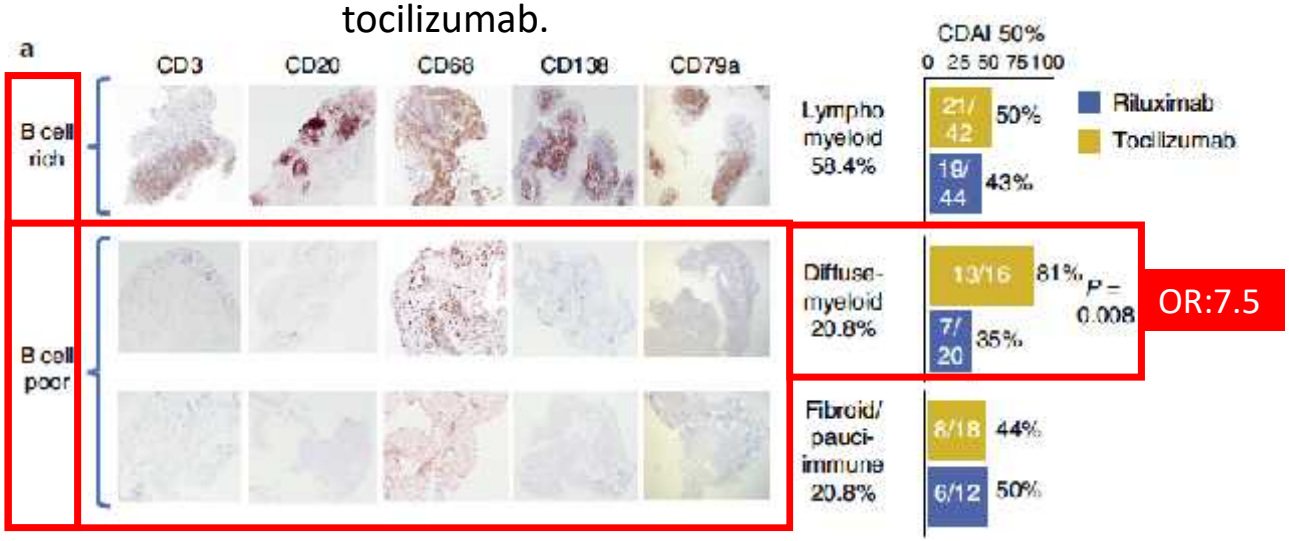
Lancet. 2021 Jan 23;397(10271):305-317

Synovial tissue and B cell signature (RNAseq) may guide treatment choices

- B cell poor histology predict low response to RTX
  - TCZ vs RTX: X2 (46% vs 24%)
- Molecular signature of low B cell better differentiates responses:
  - TCZ vs RTX: X4 (50% vs 12%)

Biomarkers for response to therapy: cellular subtype analysis predicts response, OR:16!!!

Synovial histological markers (**semiquantitative immunohistochemistry (IHC) scores**) at baseline associate with response to rituximab and tocilizumab.



Nat Med. 2022 Jun;28(6):1256-1268

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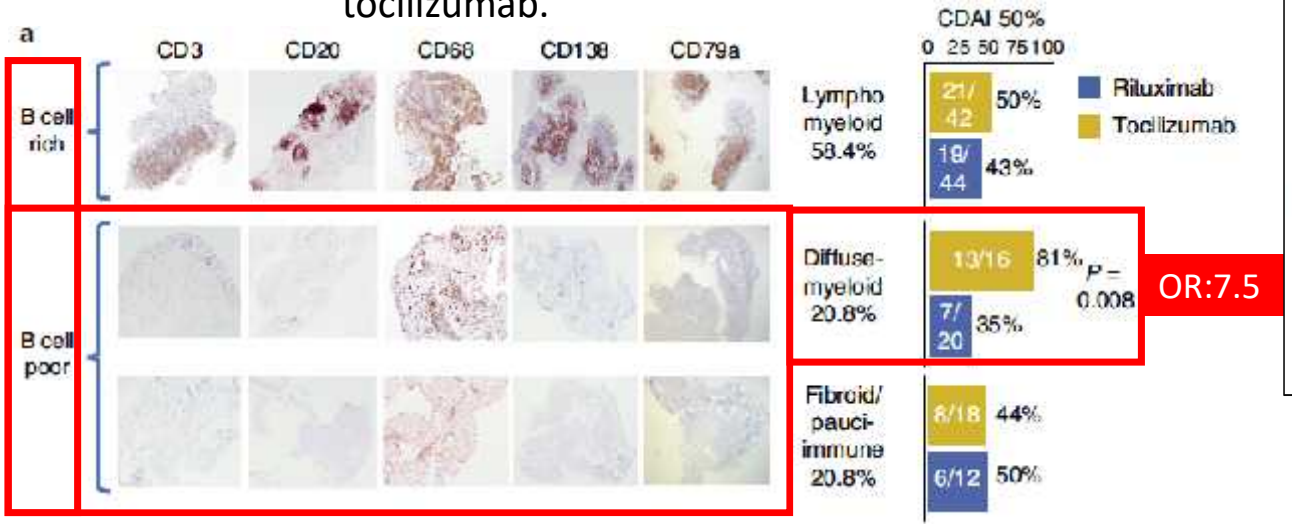
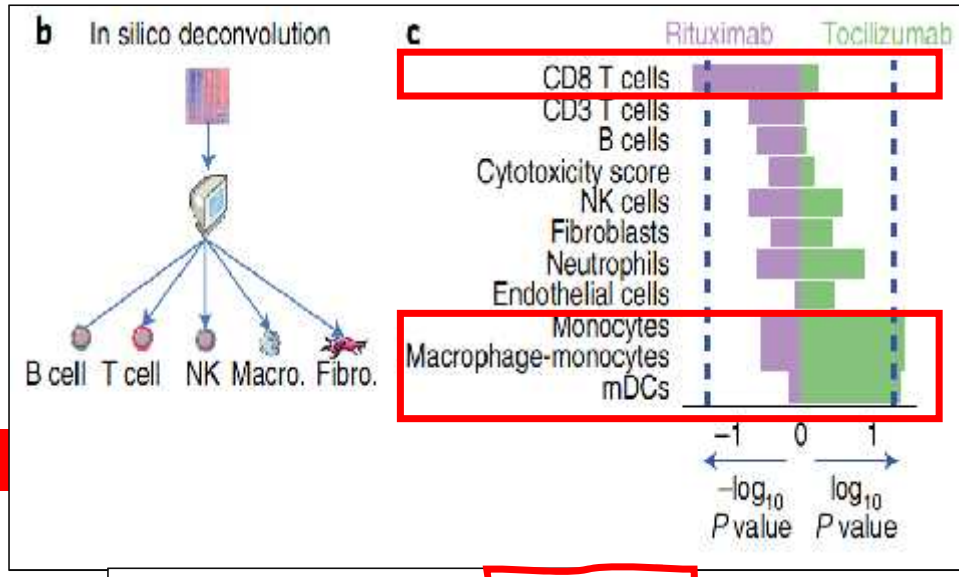
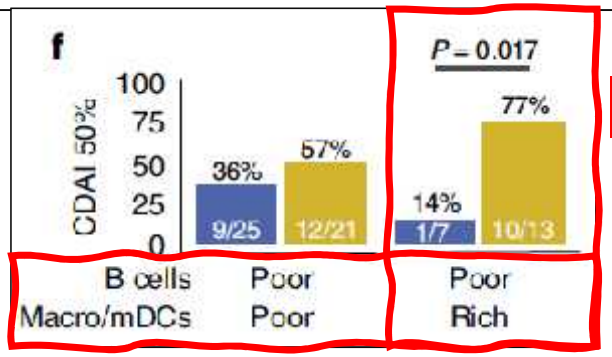


OR:7.5

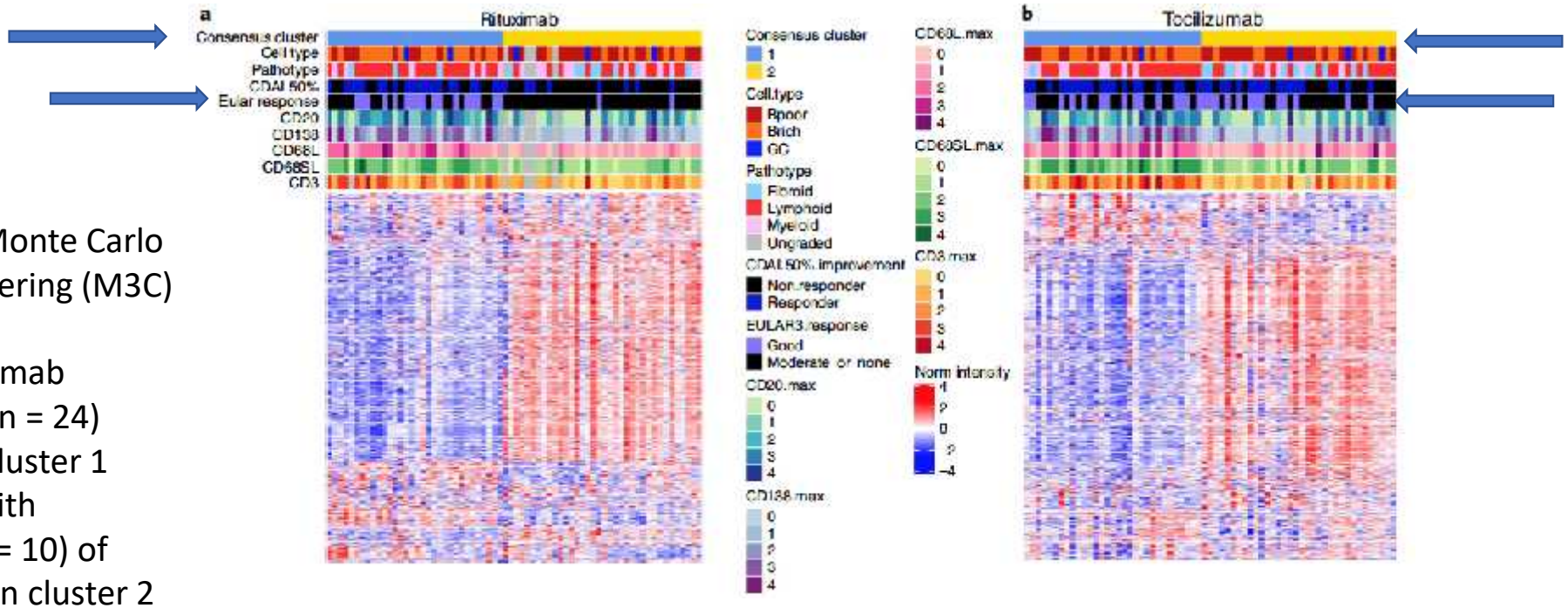




OR:16

Nat Med. 2022 Jun;28(6):1256-1268

Synovial RNAseq signature may classify patients for rituximab response better but not tocilizumab



Unsupervised Monte Carlo consensus clustering (M3C) showed:

- ✓ 71% of rituximab responders (n = 24) grouped in cluster 1 compared with
- ✓ only 29% (n = 10) of tocilizumab in cluster 2 (P = 0.0004)

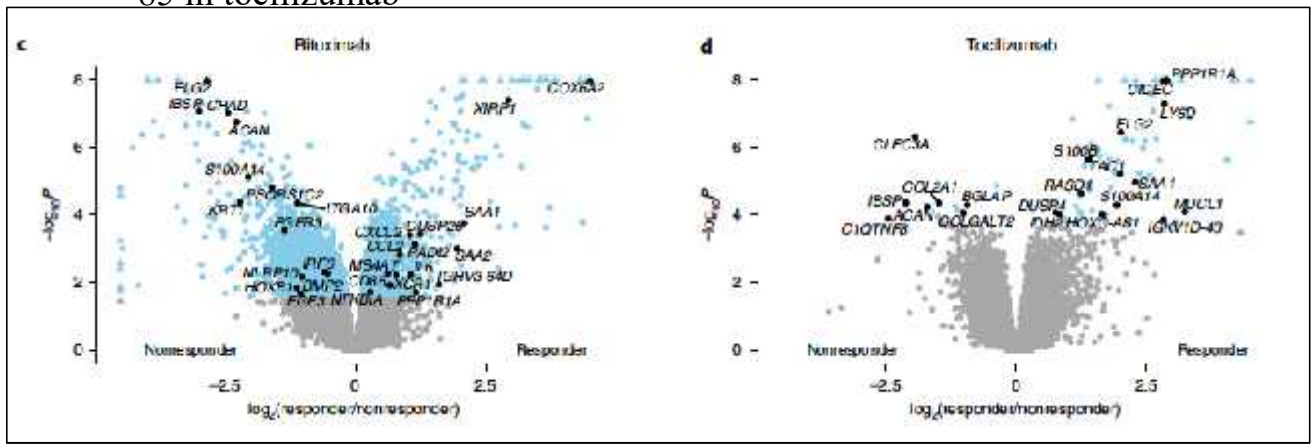
Nat Med. 2022 Jun;28(6):1256-1268

Treatment responses may be differentiated based on synovial molecular signatures at baseline

- Molecular signatures of treatment response.
- DEG analysis to identify genes associated with treatment response

Differentially expressed genes in responders vs nonresponders

- ✓ 6,625 genes in rituximab
- ✓ 85 in tocilizumab



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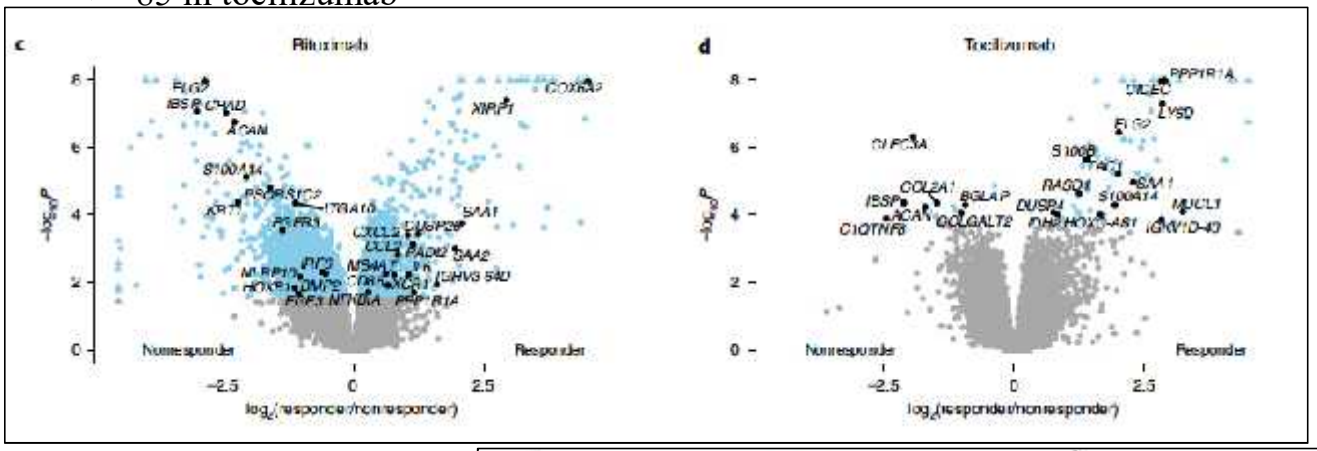
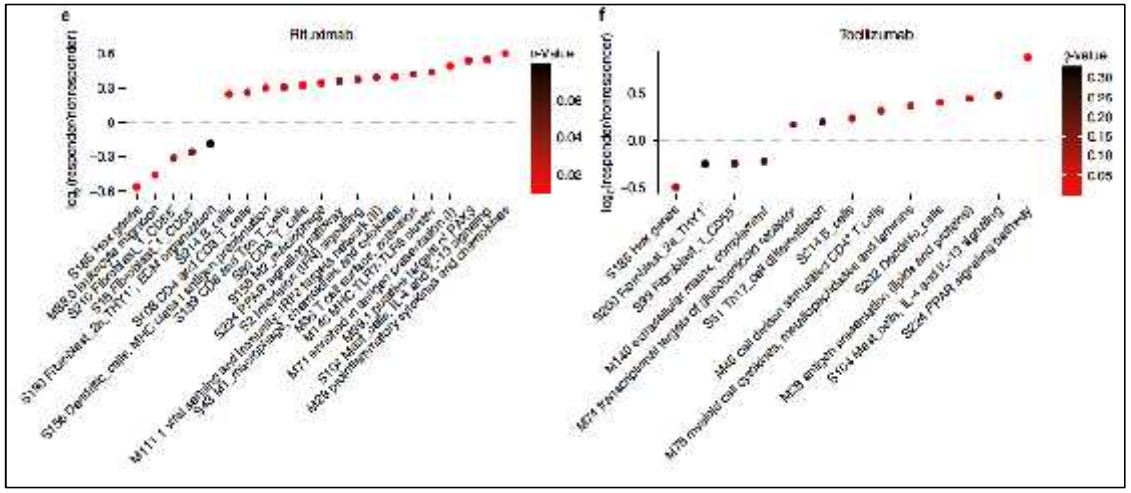


<u>Functional role of the DEGs (quantitative set analysis for gene expression (QuSAGE) modular analysis)</u>

- ✓ Increased in rituximab responders
  - ✓ Antigen presentation, T and B cell-related modules and interferon signaling
- ✓ Increased in rituximab nonresponders
  - ✓ Hox gene and fibroblast modules
- ✓ Increased in tocilizumab responders
  - ✓ Myeloid cell cytokine, peroxisome proliferator-activated receptor (PPAR) and metabolic

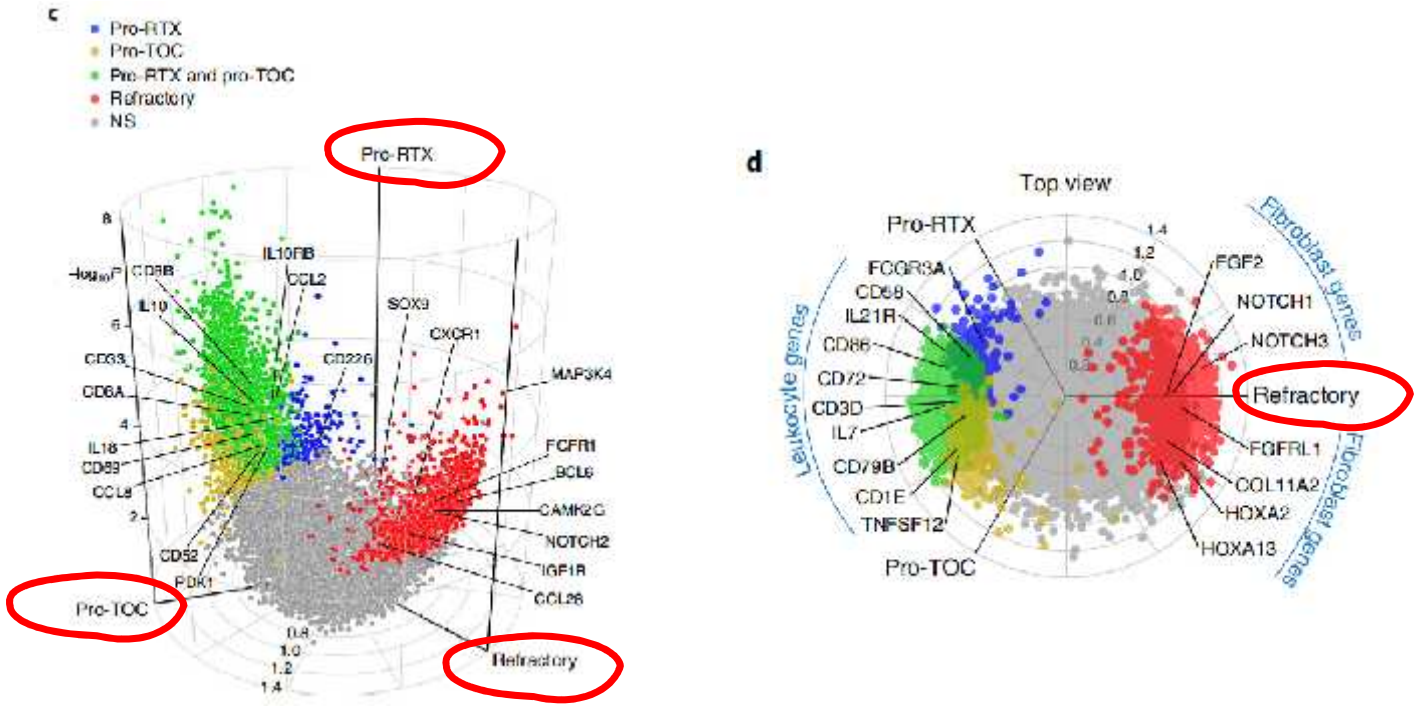


Synovium gene signature predictive multidrug resistance (tocilizumab/rituximab) and Difficult to treat RA

1,277 significant genes were unique to multi-drugresistant/refractory patients including (difficult to treat RA):

✓ fibroblast and extracellular matrix-encoding genes (fibroblast growth factor (FGF), homeobox (HOX) and NOTCH family genes)

✓ multiple cell-adhesion-molecule- and collagen-encoding genes

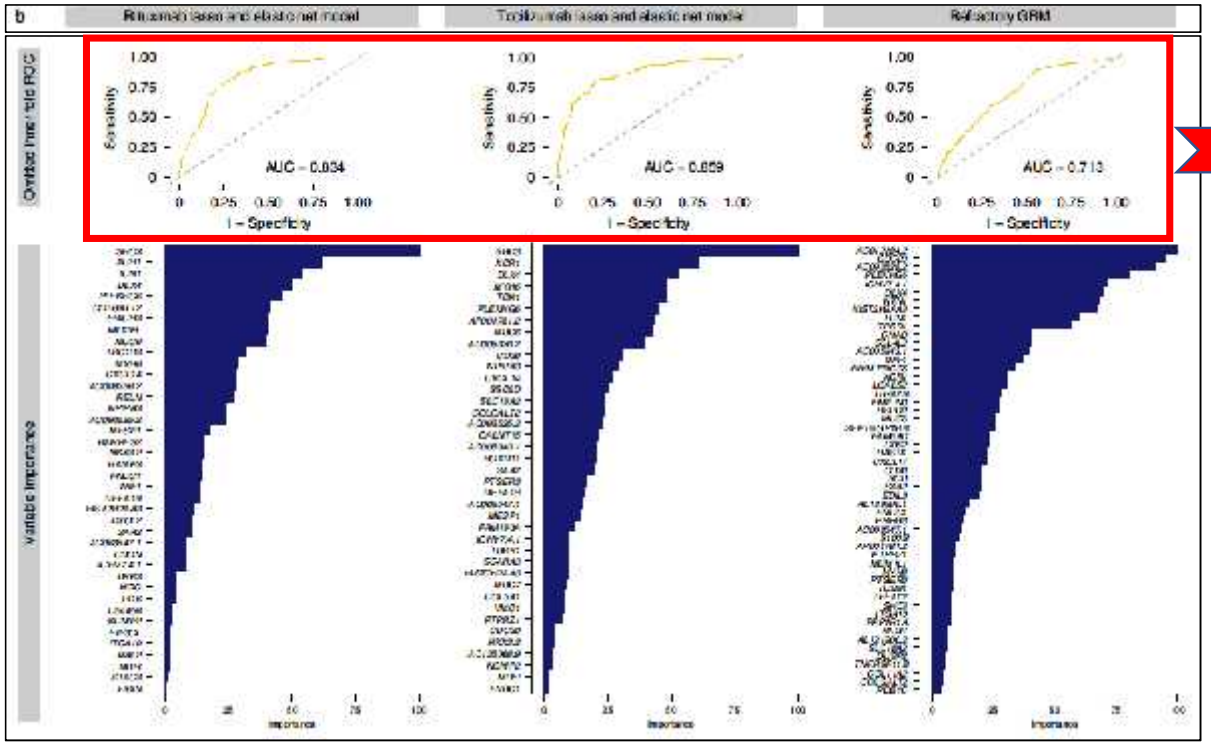


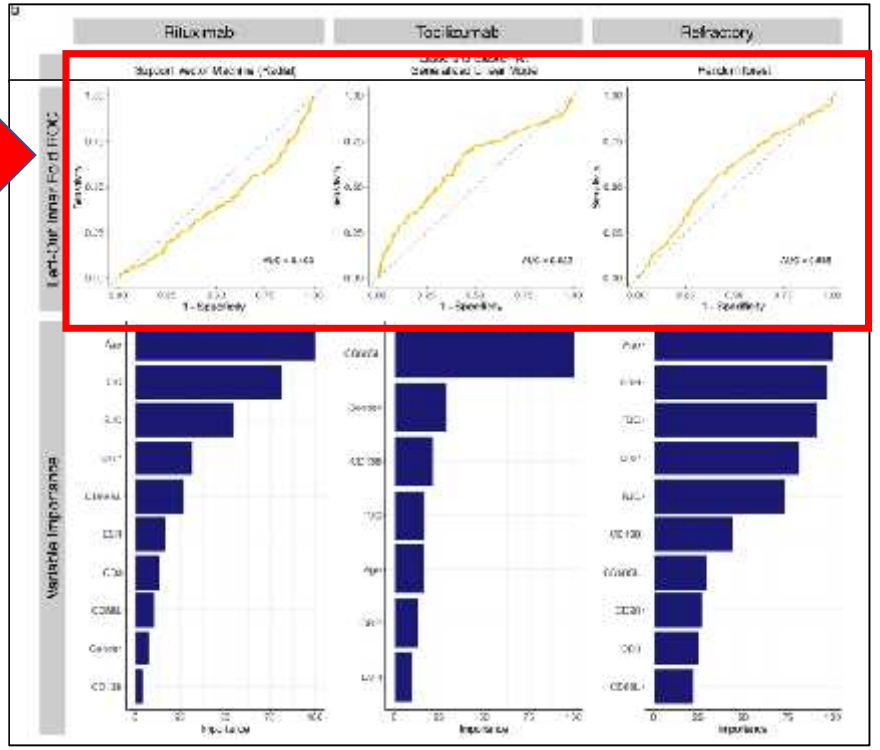
Nat Med. 2022 Jun;28(6):1256-1268

Models based on molecular synovial signatures are superior to predict response compared to clinical + histology models

40 genes for rituximab

39 genes for tocilizumab





Nat Med. 2022 Jun;28(6):1256-1268

“First biopsy-driven randomized trial in RA”

- Baseline histological and molecular signatures are associated with response to individual drugs
- Nonresponse to multiple biologics is linked to a specific pretreatment signature associated with fibroblasts
- Underscores the importance of integrating predictive molecular pathology signatures into clinical algorithms to optimize the usage of existing drugs

Nat Med. 2022 Jun;28(6):1256-1268

“PRIME” cells:
Predictors of flare

Peripheral blood biomarkers of RA relapse

Aim of the study: To look for molecular changes in blood that predict clinical flares

N Engl J Med. 2020 Jul 16;383(3):218-228

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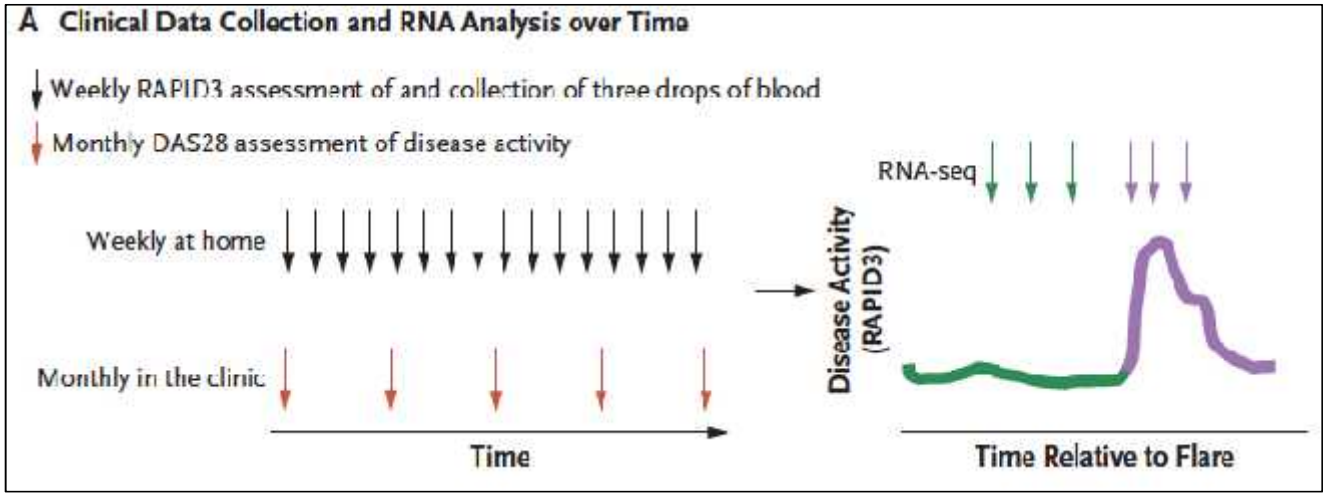
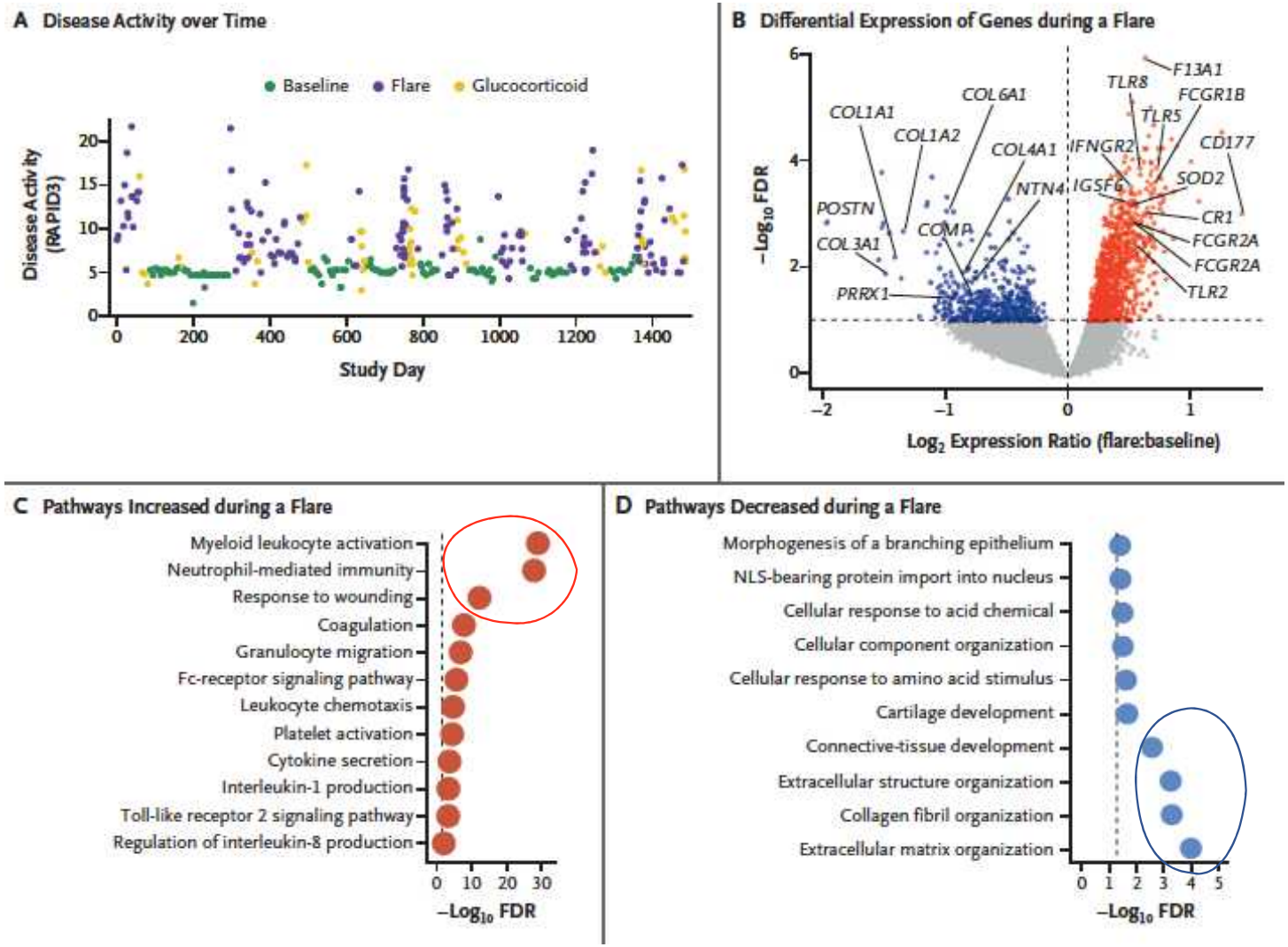


In the index patient, we assessed:

- 364 time points by RAPID3 during 8 flares over a period of 4 years
- 84 time points RNA-seq of peripheral blood specimens

N Engl J Med. 2020 Jul 16;383(3):218-228

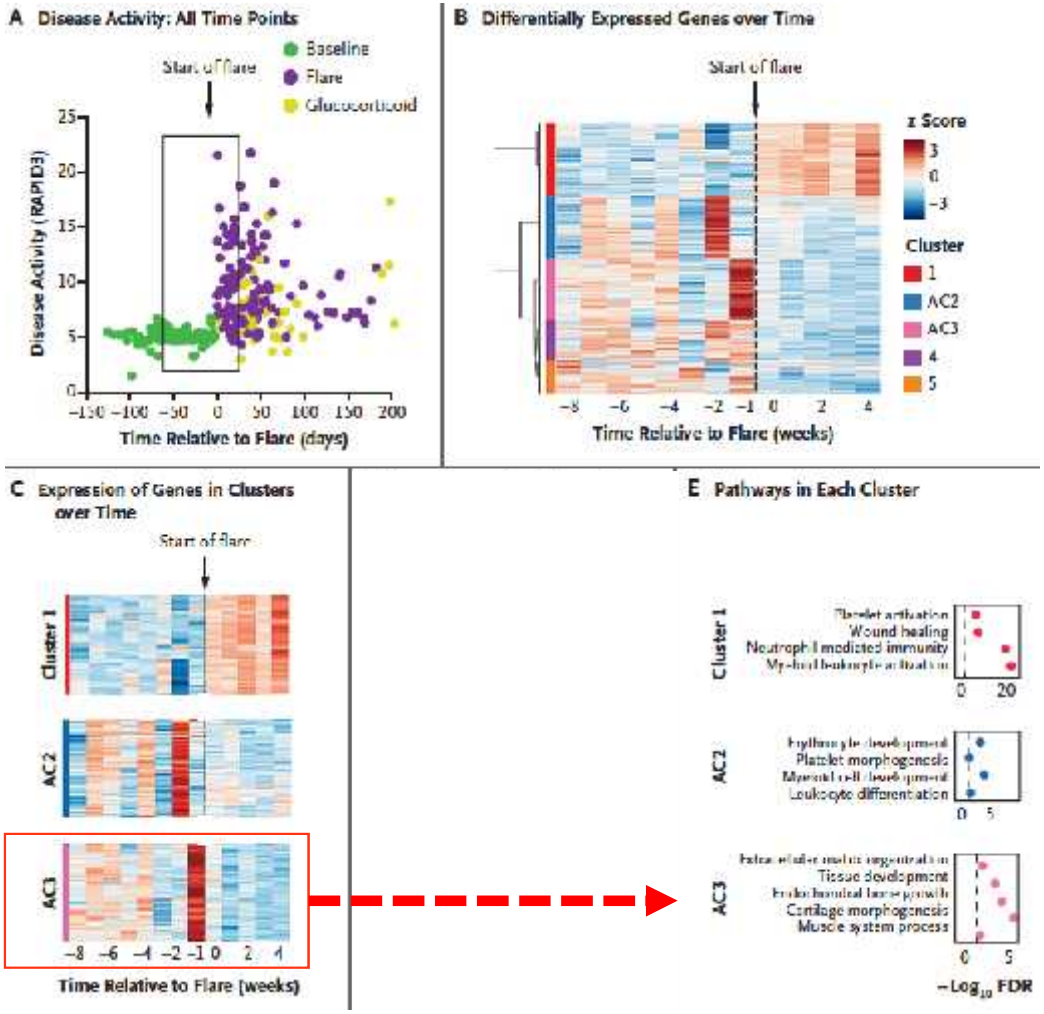
RNAseq analysis revealed genes and pathways up or downregulated during a flare



1437 up-regulated
1176 down-regulated

“B cells” and “fibroblast” sequentially precede RA flare

AC2 was enriched with naive B-cell genes and AC3 was enriched with 3 sublining fibroblast genes (CD34+, HLA-DR+ and DKK3+)



- We focused the analysis on 65 specimens that were acquired 8 weeks before a flare and 4 weeks after flare initiation.
- We identified 2791 genes with significant differential expression over the time leading up to and during a flare (false discovery rate, <0.05),
- Hierarchical clustering of gene expression we identified **five clusters (1, AC2, AC3, 4, 5)**

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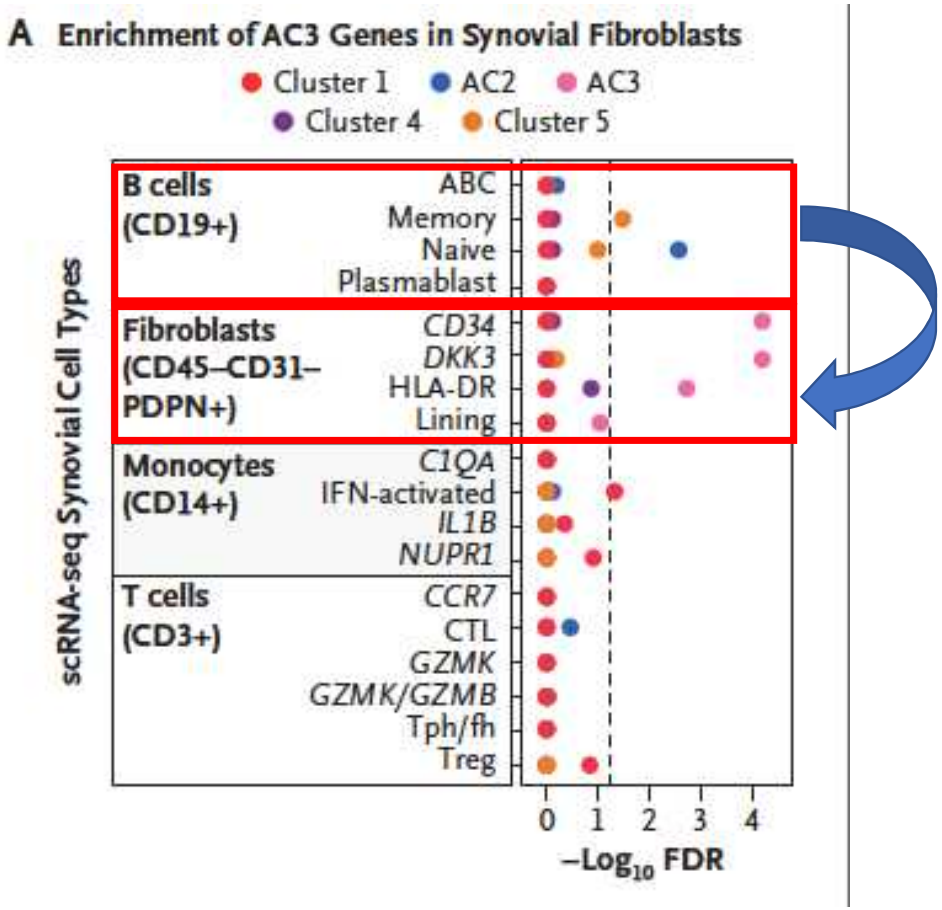
Proposed model for RA flare: sequential activation of B cells activates mesenchymal PRIME cells just before flares



- To better characterize the clusters identified by the time-series analysis of fingerstick blood and their relevance to synovitis we examined them for enrichment in **synovial cell subtypes characterized by scRNA-seq**
- This analysis of **5265 single synovial cells** from patients with rheumatoid arthritis and patients with osteoarthritis identified 4 fibroblast, 4 B-cell, 6 T-cell, and 4 monocyte subpopulations
- **Correlation to PB clusters:**
  - ✓ **AC2 was enriched with naive B-cell genes**
  - ✓ **AC3 was enriched with three sublining fibroblast genes (CD34+, HLA-DR+, and DKK3+)**

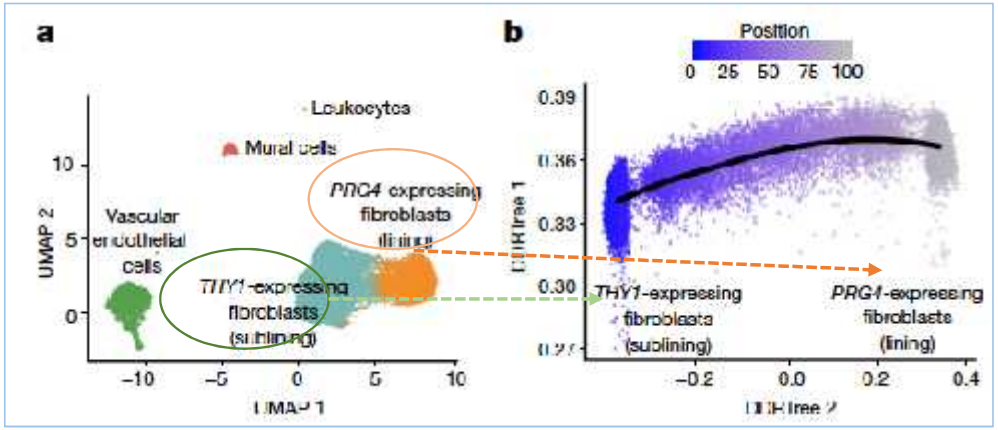
CD45–CD31–PDPN+ pre-inflammatory mesenchymal, or PRIME, cells may precede RA flare

- PRIME cells (preinflammatory mesenchymal) are increased in the blood during the period before a flare and suggested a model in which these cells become activated by B cells in the weeks before a flare and subsequently migrate out of the blood into the synovium

Single cell RNAseq (scRNA-seq) to identify novel therapeutic targets: synovium

Single cell RNAseq (scRNA-seq) to identify
✓ transcriptional gradient among synovial fibroblasts (PRG4, THY1+)
✓ positional identity.

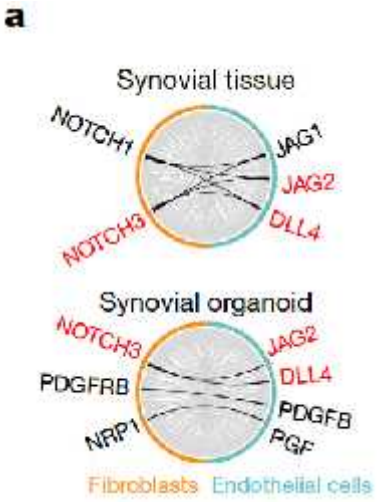
Signals derived from endothelial cells may differentiate **positional identity of FLS**



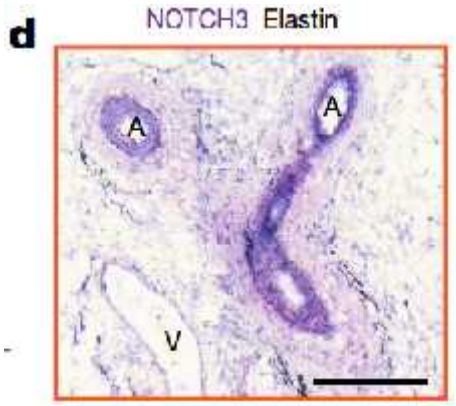
Nature. 2020 Jun;582(7811):259

NOTCH3 is a critical receptor in the differentiation and pathologic expansion of synovial fibroblasts in rheumatoid arthritis.

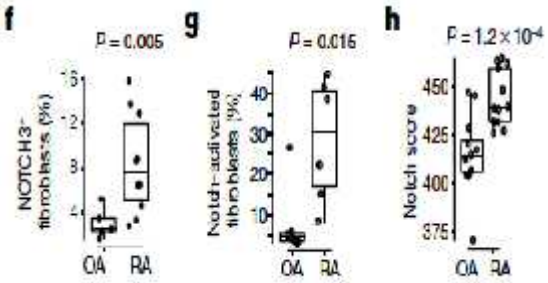
- ✓ Ligand–receptor analysis in synovial tissue (top) and organoid (bottom) scRNA-seq datasets
- ✓ Black lines indicate highly expressed ligands in endothelial cells (cyan) and receptors in fibroblasts (orange).



Immunohistochemistry staining of NOTCH3 (purple) and elastin (black) in synovial tissue



Fibroblast Notch activation in RA



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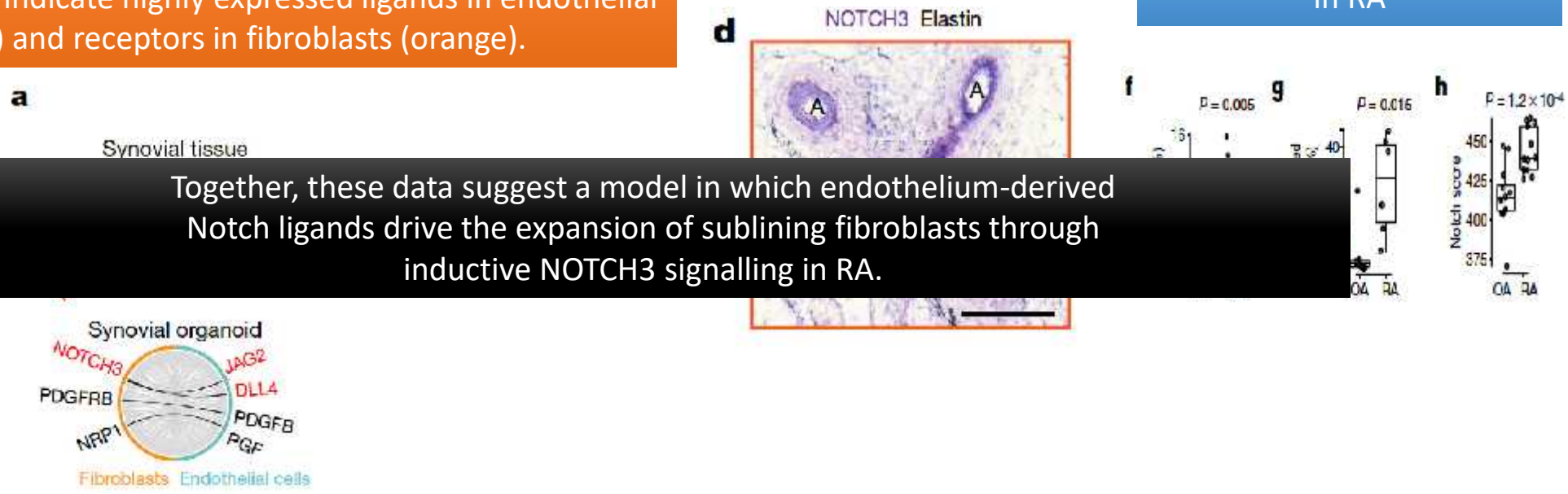
Fibroblast Notch activation in RA

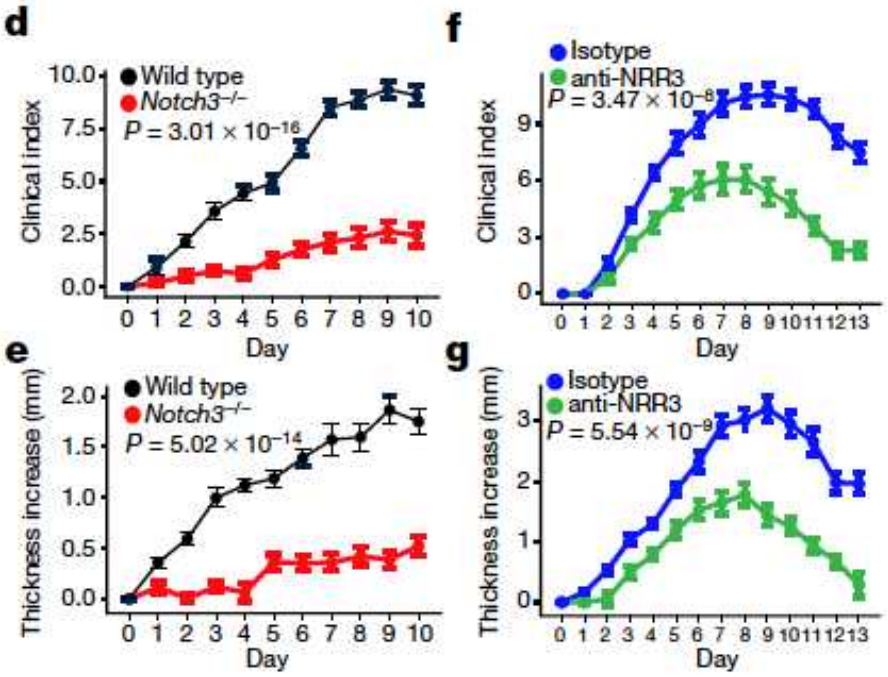


Together, these data suggest a model in which endothelium-derived Notch ligands drive the expansion of sublining fibroblasts through inductive NOTCH3 signalling in RA.

Nature. 2020 Jun;582(7811):259

NOTCH3 blockade attenuates inflammatory arthritis

K/BxN mice



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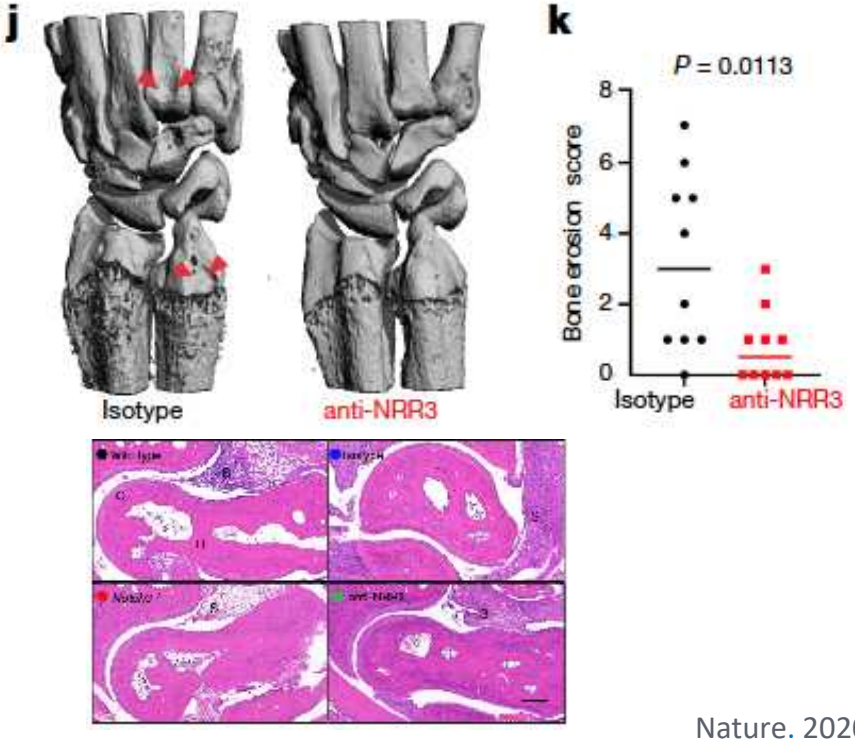

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RA 2022

- “Novel” clinical subgroups are characterized (DTT RA)
- ”Novel” treatment classes mature (JAKis)
- Molecular understanding of RA pathology may offer novel biomarkers of clinical responses and novel treatments