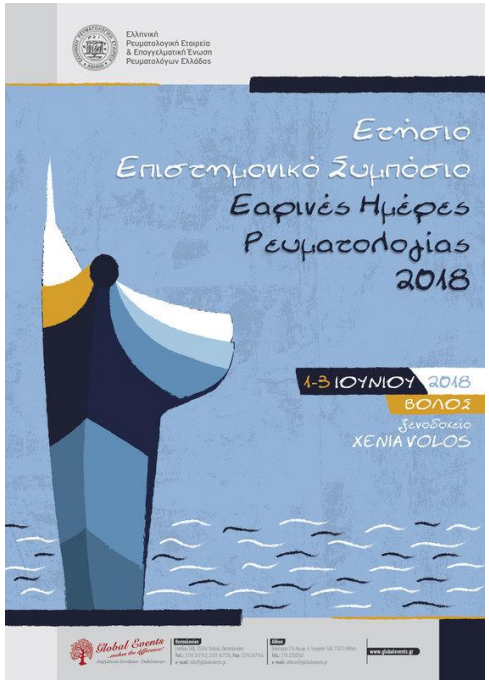


ΝΕΕΣ ΘΕΡΑΠΕΙΕΣ ΣΤΟ ΣΕΛ

Δ Τ ΜΠΟΥΜΠΑΣ

ΠΕΡΙΓΡΑΜΜΑ

Πολλοί δυνητικοί στόχοι στο ΣΕΛ *αλλά*

- Δυσκολίες στη νόσο
- Τα ερωτήματα:

  1.Ποιος θεραπευτικός στόχος? 2. Ποια εκδήλωση του ΣΕΛ (δηλ γενικά ΣΕΛ ή νεφρίτιδα)

  3. Σχεδιασμός προσθήκη, μη κατωτερότητα, με τι? 4. Καταληκτικά σημεία.
- Αποτυχίες:Τι μάθαμε από αυτές -Rituximab (anti-CD20 mAb). Άλλες αποτυχίες
- Επιτυχίες: Benlysta τι μάθαμε από αυτές ? Ο δείκτης SRI
- Νέες βιολογικές σε μελέτες φάσης III
- Νέα μόρια μικρά και μεγάλα σε αρχικές μελέτες
- Νέες χρήσεις και επανατοποθέτηση εγκεκριμένων φαρμάκων

Πολλοί δυνητικοί στόχοι στο ΣΕΛ

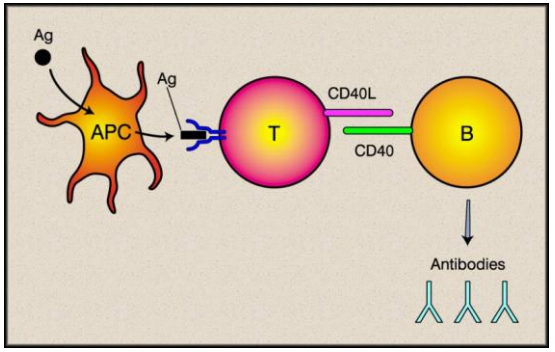
Πολλοί δυνητικοί στόχοι στο ΣΕΛ διαρκώς αυξανόμενοι
1999 : B and T cells and autoantibodies

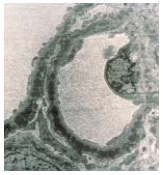
- **Antigen-driven, T cell dependent production of auto-antibodies. Known antigens (histonic proteins)**

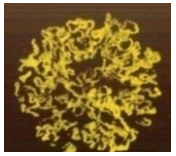
- Cytokines: IL-10, IFN-a and IFN-g play a role in the **pathogenesis**
- **Co-stimulation-T cell help: Role of co-stimulatory molecules: CD40L, CTLA4Ig**

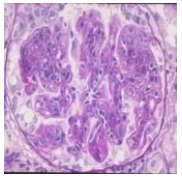
- **Formation of immune complexes and activation of the complement resulting in tissue injury**

- **Cytotoxicity-direct auto(ab)-mediated and cytokine –T cell mediated injury**









The role of innate immunity limited at complement and IFNa. TLRs and dendritic cells were not in our vocabulary. Genes?

Η επανάσταση των τεχνικών υψηλής απόδοσης

High-throughput technologies for identification of novel risk genes and targets in SLE

Level 1: gene polymorphisms

-***Genome-wide association studies and functional genomics***

Level 2: Gene expression

-***cDNA microarrays***

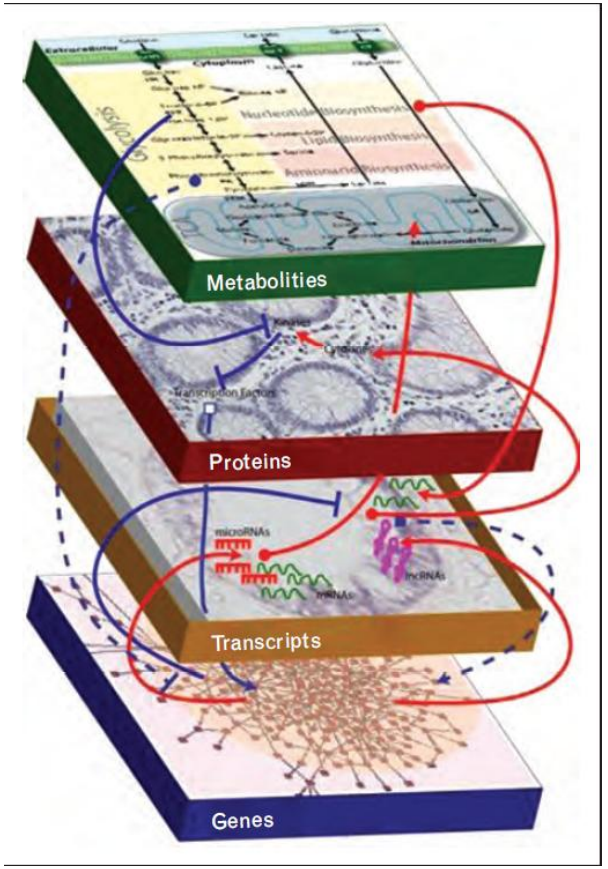
Level 3: Regulation of gene expression *(post-transcriptional, translational, post- translational)*

-***miRNAs***

Level 4: Proteomics and Metabolomics

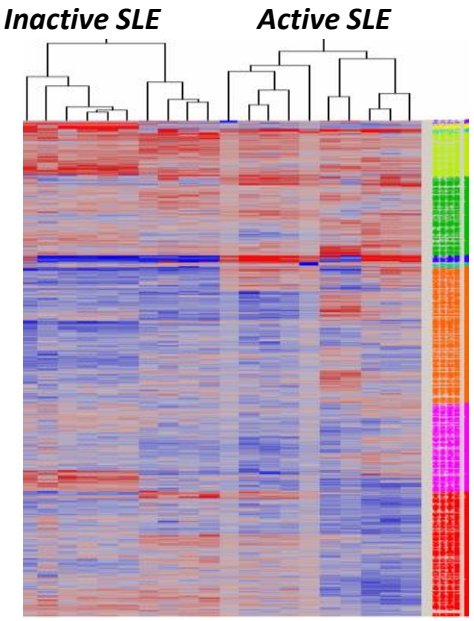
- ***serum and/or tissue***

Level 5: Next generation sequencing



Peripheral Blood and Bone Marrow cDNA Arrays

cDNA microarrays: In addition to the IFN signature active SLE patients express **a strong neutrophil and autophagy signature especially in the bone marrow**



cDNA microarray analysis (BM)

Nakou et al. Arthr Rheum 2008

Granulopoiesis & products of activated PMNs
ELA2
LYZ
CD24
DEFA
CTG
Apoptosis of granulocytes
Annexin
FOXO3A
CXCR4
LYN
Adhesion genes (Integrin family)
ITGβ2 (CD18)
MAP3K11
ACTB, ACTG1
ARPC3

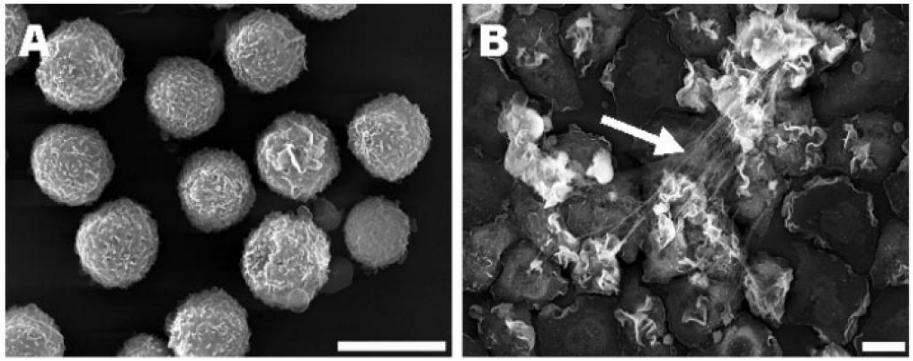
IFN SIGNATURE IN 70% OF PATIENTS

REPORTS

Neutrophil Extracellular Traps Kill Bacteria

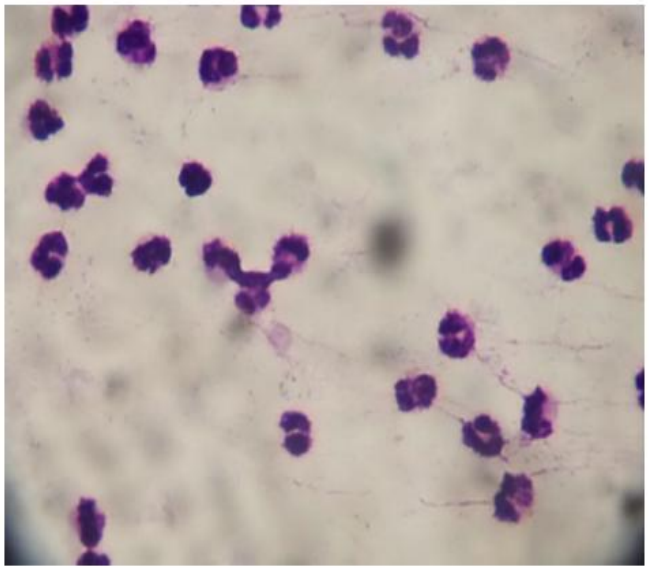
Volker Brinkmann,[1] Ulrike Reichard,[1,2] Christian Goosmann,[1,2]
Beatrix Fauler,[1] Yvonne Uhlemann,[2] David S. Weiss,[2]
Yvette Weinrauch,[3] Arturo Zychlinsky[2]*

Neutrophils engulf and kill bacteria when their antimicrobial granules fuse with the phagosome. Here, we describe that, upon activation, neutrophils release granule proteins and chromatin that together form extracellular fibers that bind Gram-positive and -negative bacteria. These neutrophil extracellular traps (NETs) degrade virulence factors and kill bacteria. NETs are abundant in vivo in experimental dysentery and spontaneous human appendicitis, two examples of acute inflammation. NETs appear to be a form of innate response that binds microorganisms, prevents them from spreading, and ensures a high local concentration of antimicrobial agents to degrade virulence factors and kill bacteria.



Science 2004; 303: 1532-35

Serum and PMNs isolation (double gradient centrifugation - Ficolls)
PMNs viability >95% (Trypan blue staining)
PMNs purity >95% (**Giemsa staining**)



Netting Insights into Fibrosis

N ENGL J MED 376;15 NEJM.ORG APRIL 13, 2017

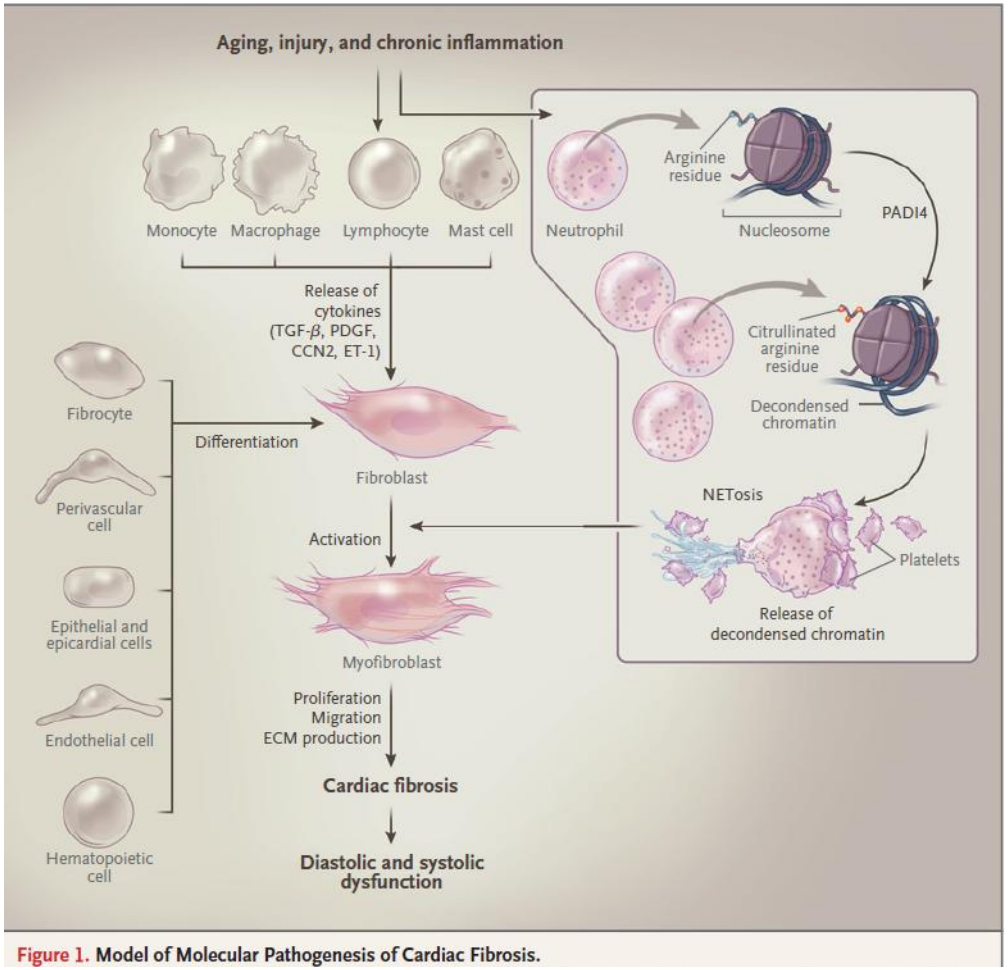
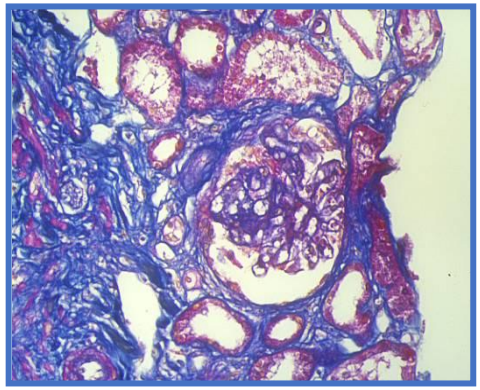
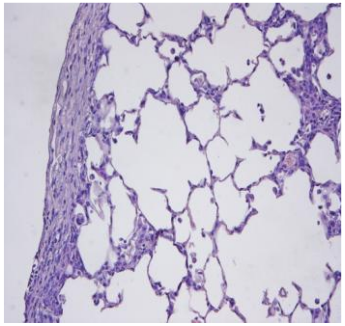


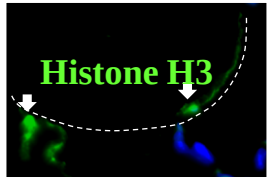
Figure 1. Model of Molecular Pathogenesis of Cardiac Fibrosis.

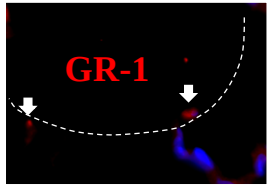
Renal Fibrosis



Lung Fibrosis E Synolaki et al

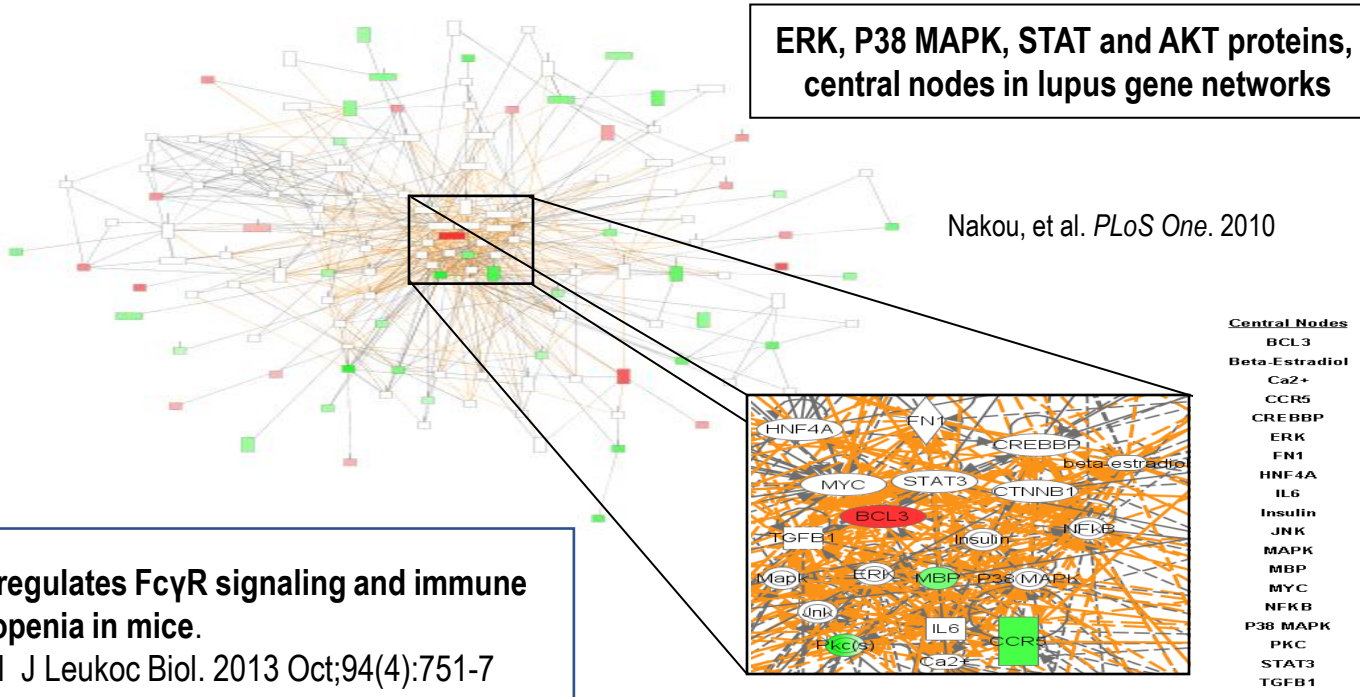






ERK, P38 MAPK, STAT and AKT proteins, central nodes in lupus gene networks

Nakou, et al. *PLoS One*. 2010



Tpl2 kinase regulates FcγR signaling and immune thrombocytopenia in mice.
Kyrmizi I et al J Leukoc Biol. 2013 Oct;94(4):751-7

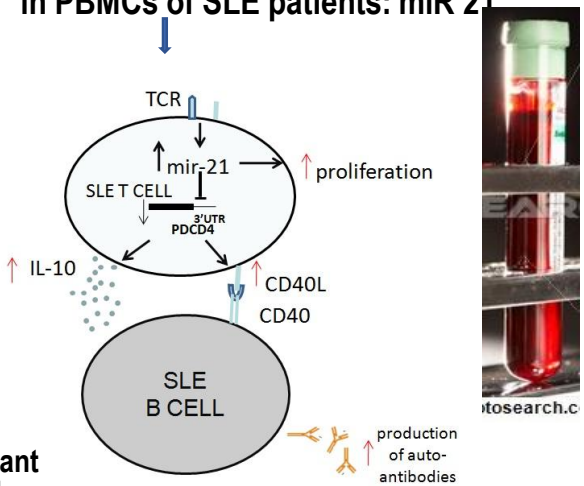
Gene Network Analysis Reveals Activation of Multiple Kinase Pathways

Τα miRNA ρυθμίζουν την εκφραση αλλων γονιδίων και επομένως το φαινότυπο

MicroRNAs in SLE and Lupus Nephritis

Deregulation of immune response in the periphery

27 differentially expressed miRNAs in PBMCs of SLE patients: miR 21

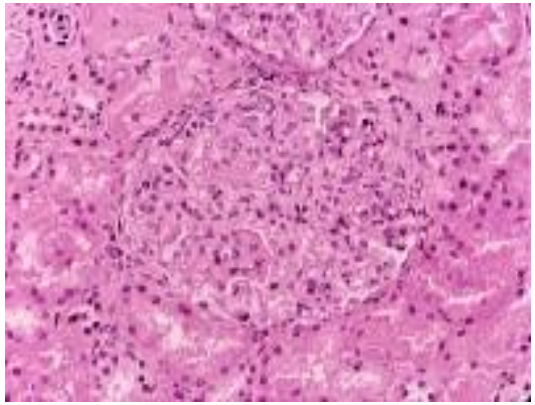


Inhibition of mir-21 affects PDCD4 and reverses the aberrant lupus T cell phenotype

(Stagakis et al, Ann Rheum Dis, 2011)

Genes controlling susceptibility of end organ to damage

Identify novel genes within the kidney



Proliferative /membranous nephritis

Μείωση προστατευτικών πρωτεϊνών λχ καλλικρείνη του νεφρού στο ΣΕΛ από υπερεκφραση του MiR 422a

MicroRNA Signature of Lupus Nephritis

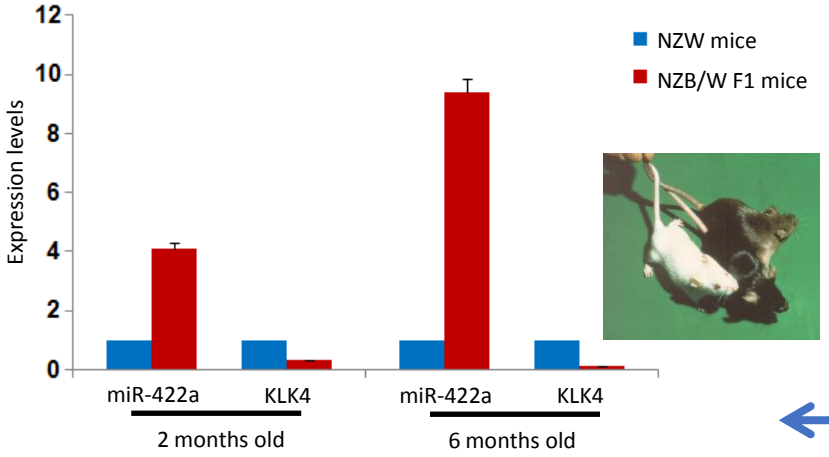
Krasoudaki et al NDT 2017

24 differentially expressed miRNAs in renal biopsy samples of LN patients vs healthy controls

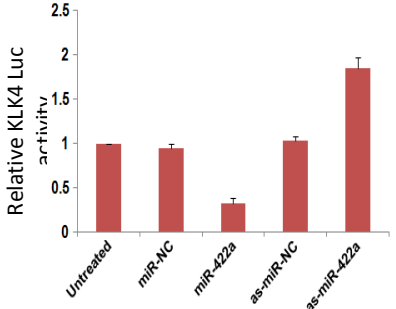
MicroRNA	Fold Change
miR-422a	17.25
miR-21	13.41

9 up-regulated

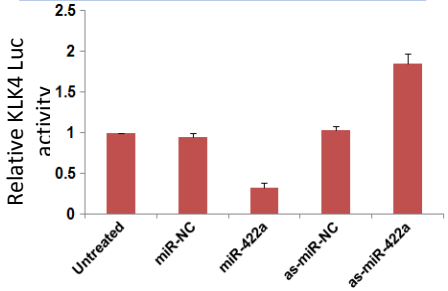
15 down-regulated



Decreased expression of KLK4



Decreased KLK4 luciferase activity



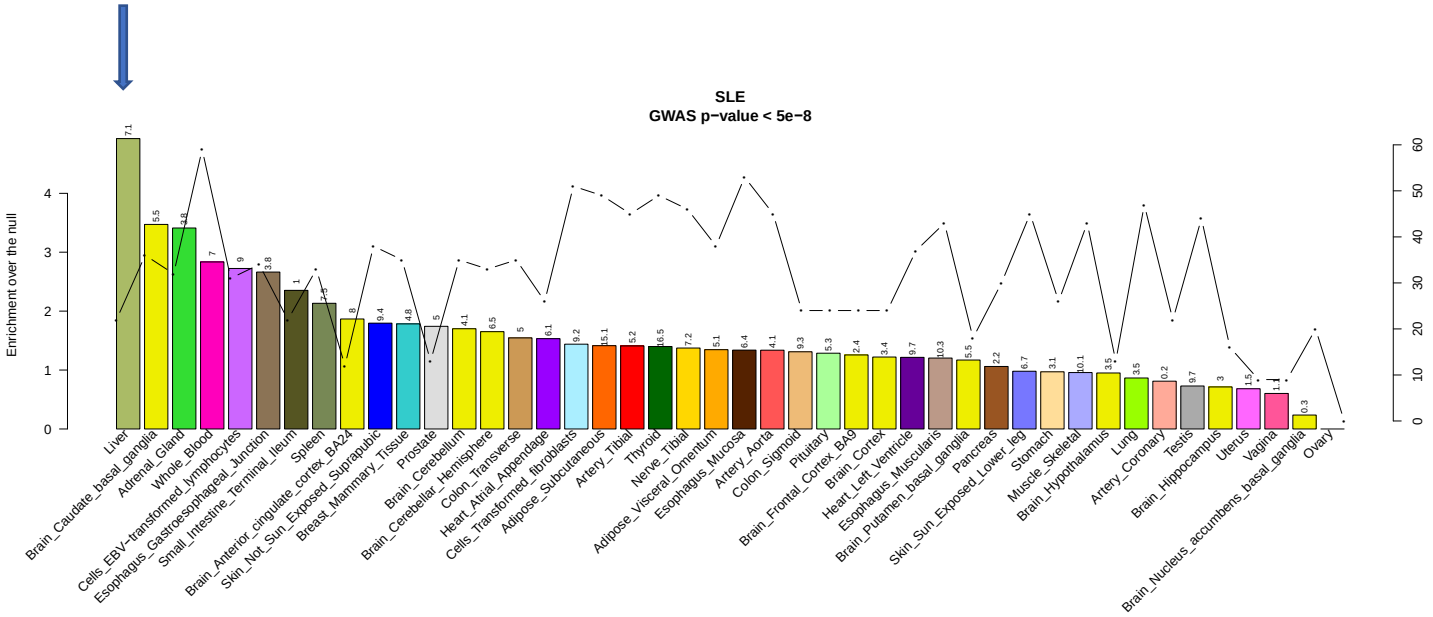
Administration of miR-422a antagomir in lupus prone mice

CHIP-Seq: upstream of KLK4 binding sites for IRF1 and RXRA

Kallikrein genes associated with lupus and glomerular basement membrane-specific antibody-induced nephritis in mice and humans. Liu et al JCI 2009

Ποιοι είναι οι πιο σημαντικοι ιστοί στο ΣΕΛ ? Αίμα και ήπαρ

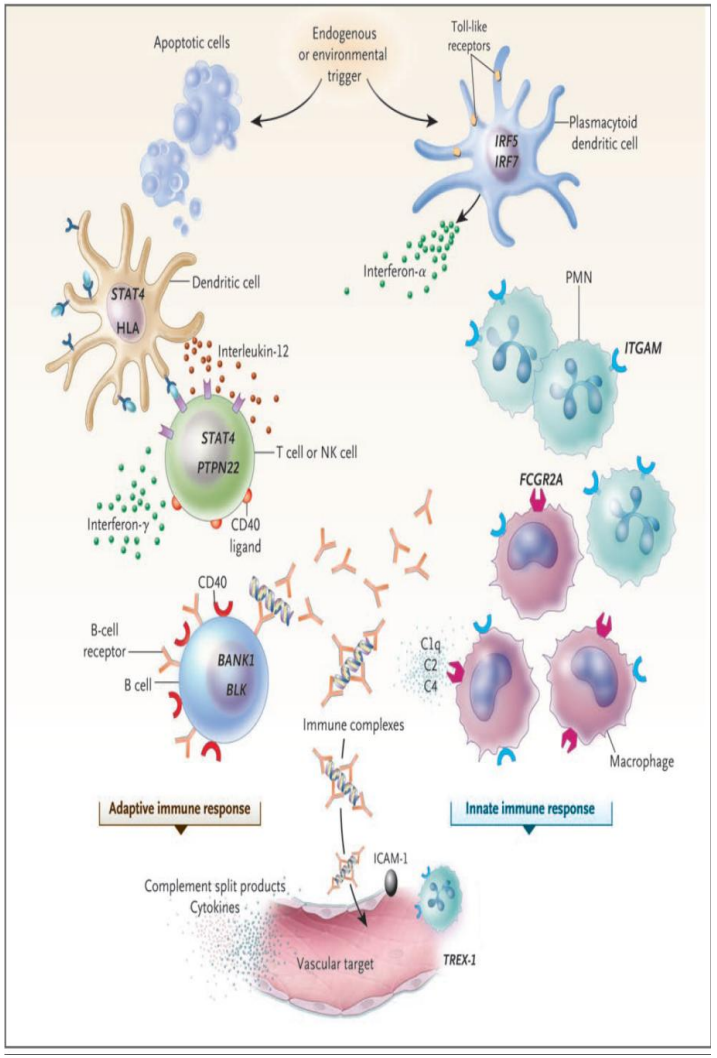
RNA seq and GWAS in SLE
Estimating causal tissue for GWAS variants G Bertsias, N Panousis et al



Ήπαρ: Μεταβολισμός, μικροβίωμα

Πολλοί δυνητικοί στόχοι στο ΣΕΛ διαρκώς αυξανόμενοι 2018

- Genes. At least 80.
  -Immune response genes
  - involved in endothelial function
  -Tissue response to injury.
- New cells macrophages, neutrophils, dendritic cells, T regs
- New cytokines IL-1, IFN-a, IL-12/23
- New survival factors for B cells: TRL- agonists, Blys, April
- New cytokines
- New molecular targets ie kinases



Crow. NEJM 2009

Better understanding of pathogenesis and the introduction of biologic therapies in RA has created an impetus for novel therapies in SLE

A. Targeting B cells	
• B cell depleting	Anti-CD20 mAb (rituximab, ocrelizumab)
• Modulating B cells	Anti-CD22 mAb (epratuzumab)
• Inhibiting B cell growth factors	Anti-BAFF (anti-BLyS) (belimumab) TACI-Ig (atacicept)
•Targeting plasma cells	Bosertan
B. Targeting T cells	
• Inhibiting costimulation	CTLA4-Ig (abatacept)
C. Targeting cytokines	Anti-IL-6 (tocilizumab) Anti-IL-10 Anti-TNF (infliximab) Anti-IFNα (MEDI-545) Anti-IL-12/23 (Ustekinumab)

D. Small molecules targeting intracellular targets ie kinases, cAMP

Δυσκολίες στη νόσο

Η μεγαλύτερη δυσκολία όλων: η επιτυχία της IV-CY
(ευλογία και κατάρα ταυτόχρονα)

Δυσκολίες στη νόσο

- Ετερογένεια

  -μοριακή

  -φαινοτυπική λχ νεφρίτιδα, αιματολογικός, ΝΨ-ικος ΣΕΛ

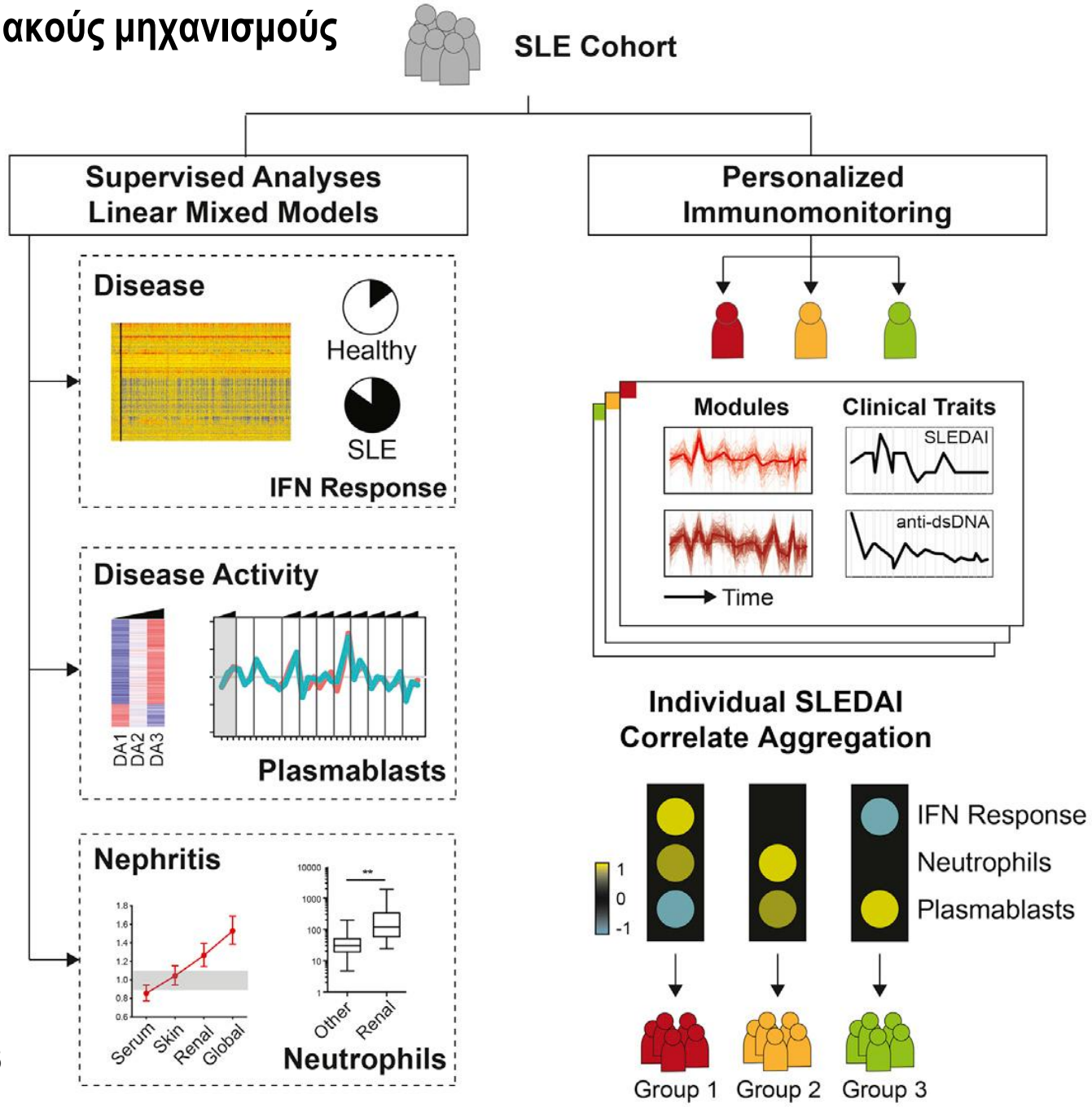
- Μικρότερος αριθμός ασθενών για μελέτες
- Σχεδιασμός – προσθήκη στη συνήθη θεραπεία, μη κατωτερότητας και εάν ναι έναντι τίνος?
- Το πρόβλημα των στεροειδών και της συνήθους θεραπείας λχ ριτουξιμάμπη
- Καταληκτικά σημεία όχι γενικώς αποδεκτά

  -Γενικού λύκου λχ SELENA –SLEDAI, BILAG , SRI, ύφεση, χαμηλή ενεργότητα

  -Νεφρίτιδα –Τα σκληρά λχ Νεφρική ανεπάρκεια ή θάνατος είναι σπάνια ή καθυστερούν

  -Έμμεσα ενδιάμεσα καταληκτικά σημεία λχ πρωτεϊνουρία, 50% αύξηση κρεατινίνης

Ετερογένεια στους μοριακούς μηχανισμούς



Banchereau et al., 2016, Cell 165, 1–15
April 21, 2016 ª2016 Elsevier Inc.
http://dx.doi.org/10.1016/j.cell.2016.03.008

SLE Responder Index (SRI)

≥4-point improvement in SS score

and

No new BILAG 1A/2B flares

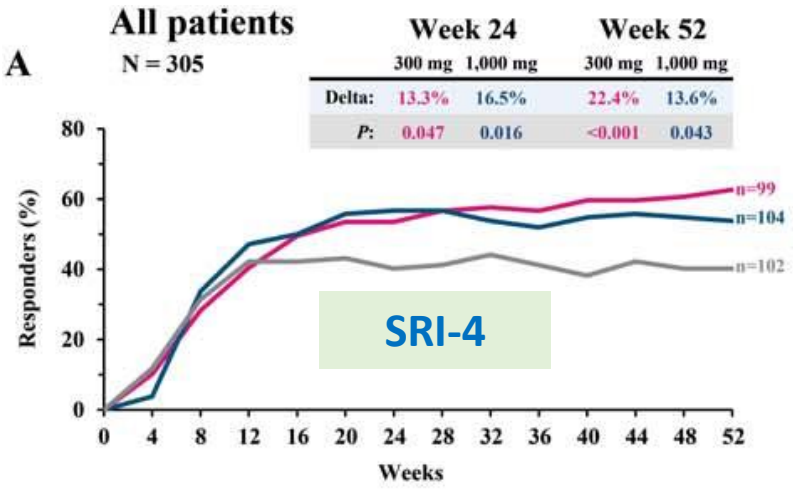
and

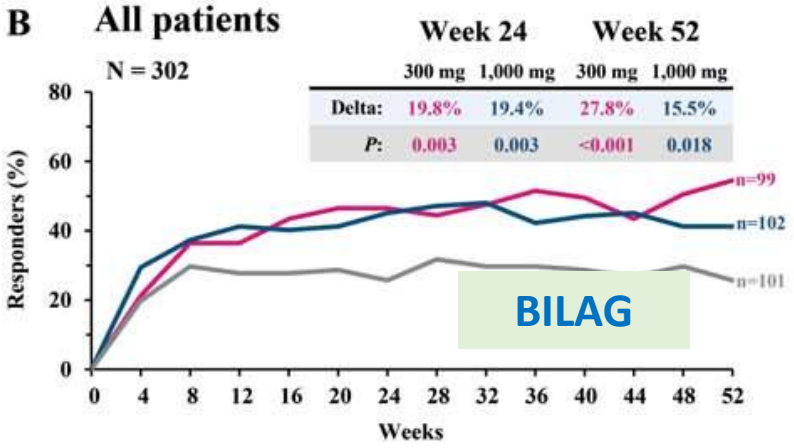
No worsening in PGA (<0.3-point increase)

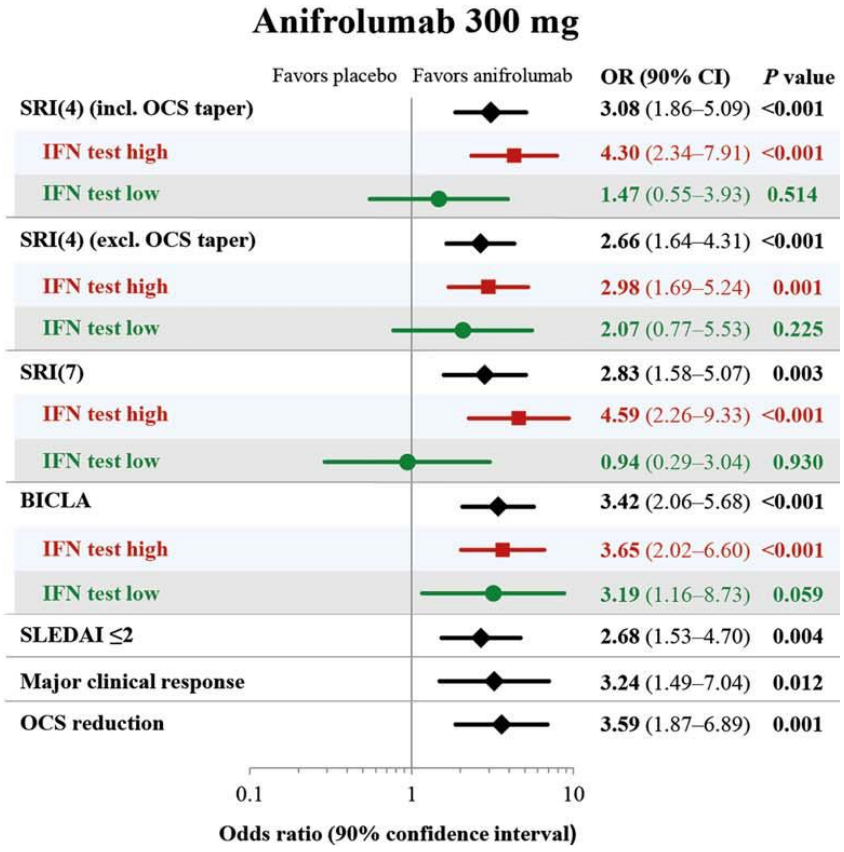
Biologics failing to meet the primary endpoint

- **Atacicept (+/- MMF): deaths due to infections!**
- **Epratuzumab (anti-CD22 mAb): lack of efficacy**
- **Sirukumab (anti-IL6 mAb): no efficacy in lupus nephritis**
- **Ocrelizumab (anti-CD20 mAb) + CYC or MMF: no (added) efficacy in lupus nephritis**
- **Tabalumab (anti-BAFF mAb): no efficacy**

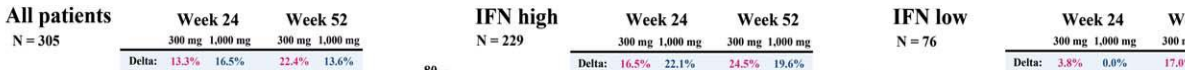
Anifrolumab (anti-IFNα receptor mAb) in SLE





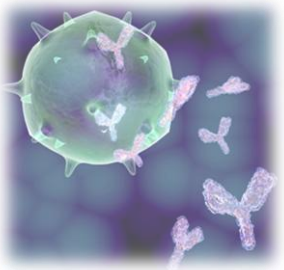


- Improvements in skin and joints, serology
- No significant change in fatigue



Furie R., *et al. Arthritis Rheumatol*. 2017;69(2)

Novel therapies under development/evaluation



Belimumab in active nephritis	Ongoing (phase III)
Blisibimod (anti-BAFF)	Ongoing (phase III)
IFNα blockade (anifrolumab)	Ongoing (phase III) Efficacy in several organs; steroid-tapering effect
Anti-IL6 (PF-04236921)	Evidence for efficacy (reduction in flares)
Abatacept in active nephritis	Ongoing
Anti-CD40 (BI 655064) in active nephritis	Ongoing (phase II)
Anti-CD40L pegylated (dapirolizumab)	Ongoing

Borba HH, et al. *Curr Rheumatol Rep*. 2016; 18: 44; Furie R, et al. *Arthritis Rheumatol*. 2017; 69: 376-386; Stohl W, et al. *Arthritis Rheumatol*. 2017; doi: 10.1002/art.40049; Wallace DJ, et al. *Ann Rheum Dis*. 2017; 76: 534-542; Narain S, et al. *Curr Opin Rheumatol*. 2016; 28: 477-87

Εξυπνότερη χρήση
Επανατοποθέτηση-νέες ενδείξεις γνωστών φαρμάκων στο ΣΕΛ

- Κορτικοειδή
- MMF
- New calcineurin inhibitors
- Συνδυασμός –Multitarget
- Tocilizumab
- Ustekinumab
- Kinase inhbitors: Tofacitinib, baritinib
- Otesla
- Low dose IL-2

Low prednisone

MyLupus trial

24-week, multicenter, open-label study
EC mycophenolate sodium
Standard vs reduced dose of prednisolone

	Time	Standard GC 1mg/kg/d N=42	Reduced GC 0.5mg/kg/d N=39
Serum creatinine (µmol/L; mean SD)	Baseline	74.8±35.6	75.7±27.1
	Week 24	73.3±35.0	68.2±20.7
uP/C ratio (g/g; mean ± SD)	Baseline	2.0±1.3	1.8±1.5
	Week 24	0.8±0.8	0.9±1.4
CR (%)	Week 24	19.0	20.5
Infections (%)		57.1	35.9*
Herpes zoster (%)		16.7	0**

*: 0.056
**: 0.012

Zeher M *et al*. Lupus 2011; 20: 1484

EXTENDED REPORT

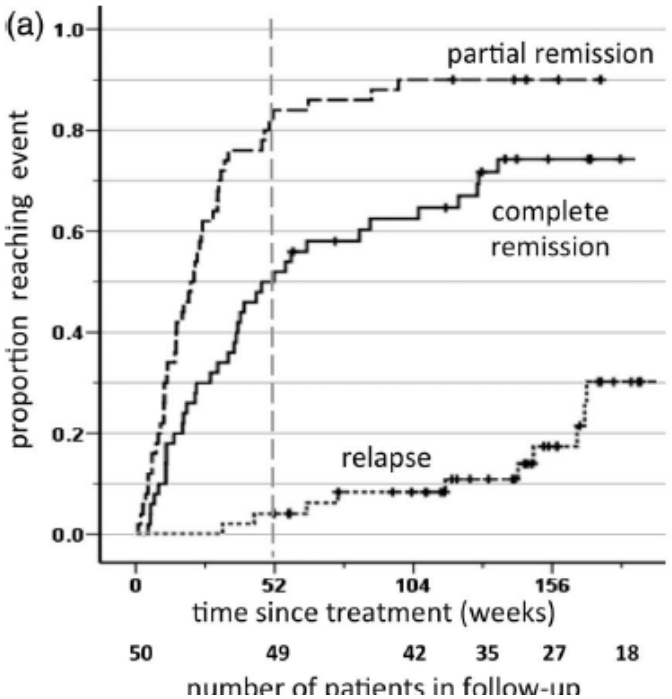
Ann Rheum Dis 2013;**72**:1280–1286. doi:10.1136/annrheumdis-2012-202844

Prospective observational single-centre cohort study to evaluate the effectiveness of treating lupus nephritis with rituximab and mycophenolate mofetil but no oral steroids

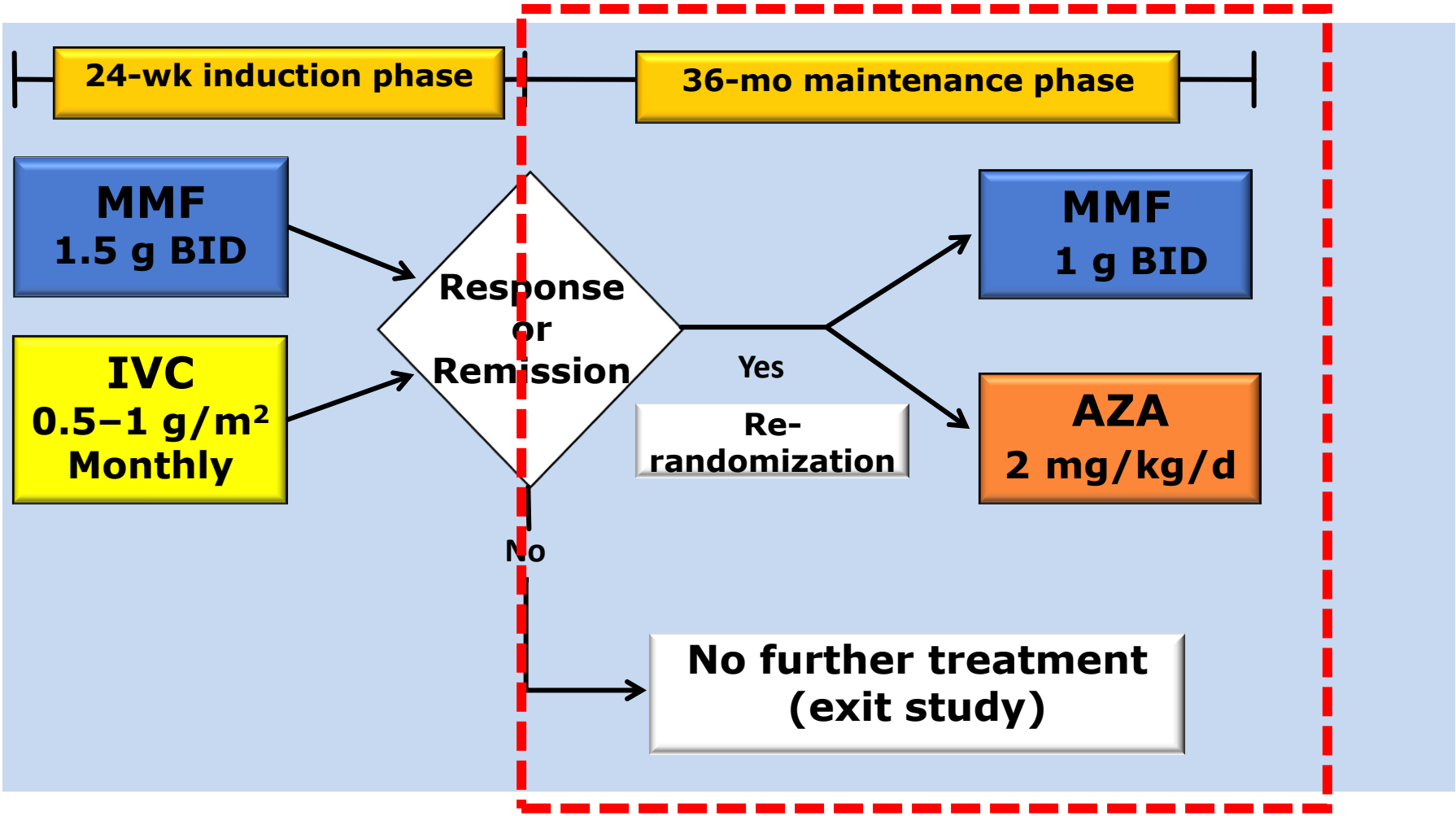
Marie B Condon,[1] Damien Ashby,[1] Ruth J Pepper,[1] H Terence Cook,[1,2] Jeremy B Levy,[1] Megan Griffith,[1] Tom D Cairns,[1] Liz Lightstone[1,2,3]

- **STEROID FREE RX!!!!**
- **Renal predominant-lupus**
- **Need confirmation**

- **50 patients. 2 doses of rituximab (1 g) and IV-MP (500 mg) on days 1 and 15;maintenance MMF**
- **Exclusions: Patients on maintenance steroids or with life-threatening SLE or requiring dialysis**
- **40%, active class IVor class IV+V LN, 22 (44%) patients had pure class V LN.**
- **58% had extrarenal involvement at presentation.**
- **Renal predominant-lupus-Need confirmation**



“Maintenance” treatment in lupus nephritis: ALMS trial



*Oral corticosteroids administered in induction and maintenance phases

AZA, azathioprine; IVC, intravenous cyclophosphamide; MMF, mycophenolate mofetil

New calcineurin inhibitors : Voclosporine

Voclosporine AURA trial PHASE 2 LANDMARK STUDY

- Voclosporine: new generation CNI, more potent-*less toxicity*
- 3- to 5-fold increase in potency compared to CsA. Faster elimination of metabolites
- There is less diabetes, hirsutism, gum disease and possibly hypertension with voclosporin.
- **Renal toxicity is similar** and infection risk probably similar also to ciclosporin/tacrolimus
- Stucturally similar to CsA (modification on amino acid 1 residue)
- Binds more tightly to calcineurin
- Well studied in psoriasis

2 x 250-500 IV MP
20-25 mg/d oral pred (5 mg/d at w12)
MMF: 2 g/d

Placebo
Voclosporin (2 doses)

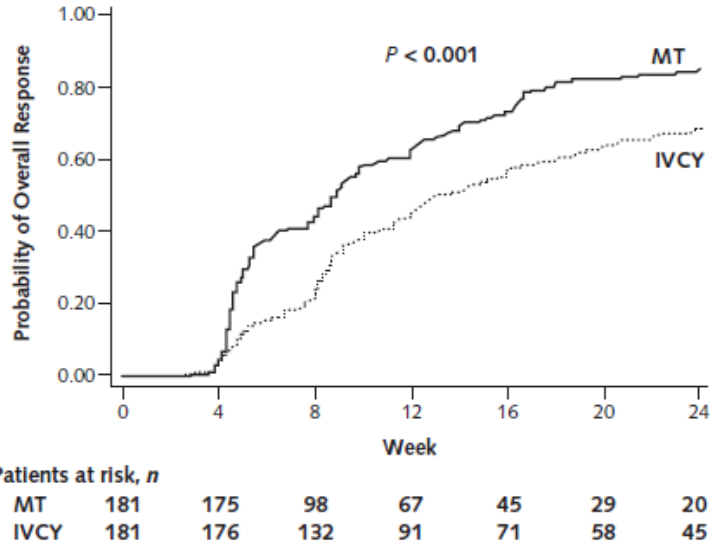
Press Release AUGUST 23 2016
X2 REMISSIONS, 13 DEATHS

Primary endpoint: uP/C ratio ≤ 0.5 mg/mg and no decrease eGFR of ≥20%

Συνδυασμός –Multitarget therapy:
Προσθήκη αναστολέων καλσινευρίνης (tacrolimus) σε στεροειδή και MMF

- 26 renal centers in China.
- Tacrolimus, 4 mg/d, and mycophenolate mofetil, 1.0 g/d, versus intravenous cyclophosphamide with a starting dose of 0.75 (adjusted to 0.5 to 1.0) g/m2 of body surface area every 4 weeks for 6 months.
- Both groups received 3 days of pulse methylprednisolone followed by a tapering course of oral prednisone therapy.
- Multitarget therapy provides superior efficacy compared with intravenous cyclophosphamide as induction therapy for LN
- Short term, China

Figure 3. Probability of achieving overall remission (complete remission and partial remission) in patients treated with the MT regimen or IVCY.



MT = multitarget; IVCY = intravenous cyclophosphamide.

Annals of Internal Medicine

Multitarget Therapy for Maintenance Treatment of Lupus Nephritis

Haitao Zhang,* Zhengzhao Liu,* Minlin Zhou,* Zhangsuo Liu,† Jianghua Chen,‡ Changying Xing,§ Hongli Lin,‖ Zhaohui Ni,¶ Ping Fu,** Fuyou Liu,†† Nan Chen,‡‡ Yongcheng He,§§ Jianshe Liu,‖‖ Caihong Zeng,* and Zhihong Liu*

Patients who had undergone multitarget induction therapy continued to receive multitarget therapy (tacrolimus, 2–3 mg/d; mycophenolate mofetil, 0.50–0.75 g/d; prednisone, 10 mg/d), and patients who had received intravenous cyclophosphamide induction treatment received azathioprine (2 mg/kg per day) plus prednisone (10 mg/d). We

Similar relapses in both groups
Less side-effects in the multi-targeted group

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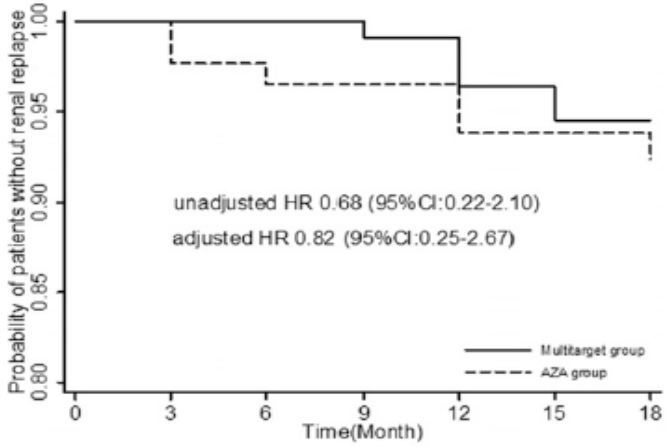
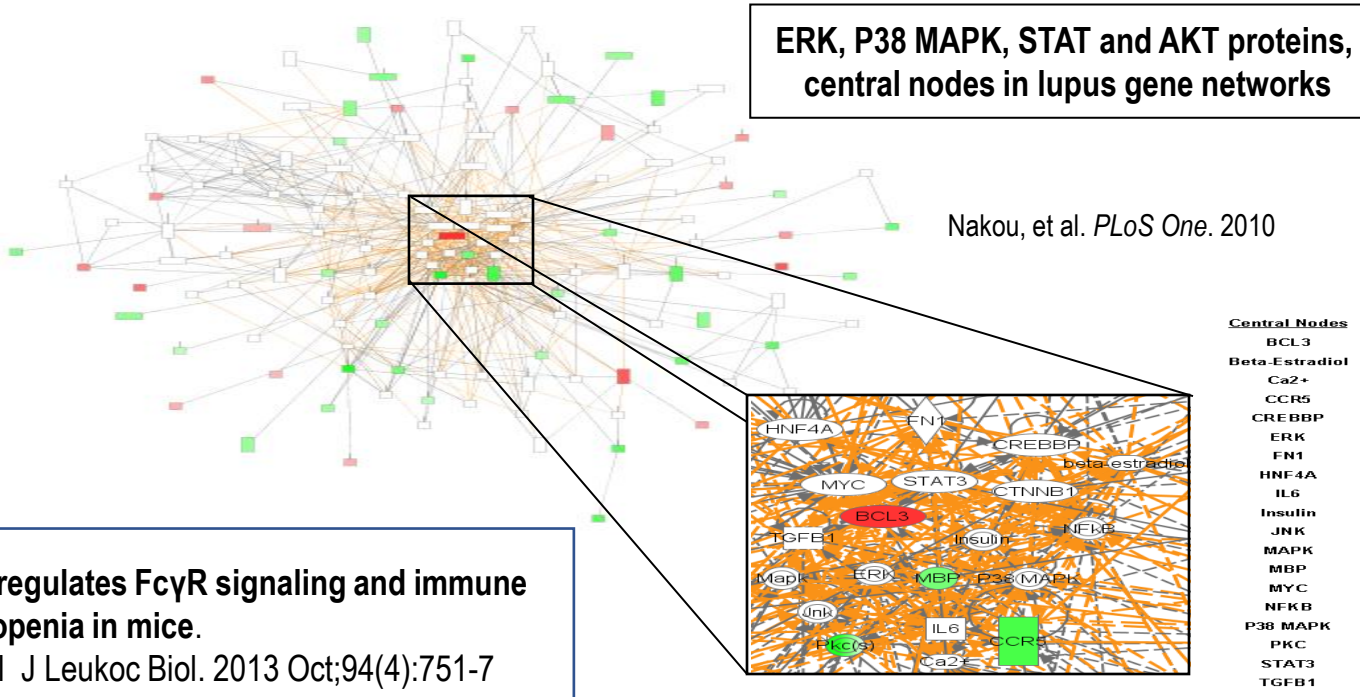


No. at risk							
Multitarget group	116	116	113	112	109	104	101
AZA group	90	87	83	76	72	66	64
No.of relapse							
Multitarget group	0	0	0	1	3	2	0
AZA group	0	2	1	0	2	0	1

Figure 2. Probability of patients without renal relapse during the maintenance treatment.

Repositioning: Tofacitinib and baricitinib for SLE

ERK, P38 MAPK, STAT and AKT proteins, central nodes in lupus gene networks

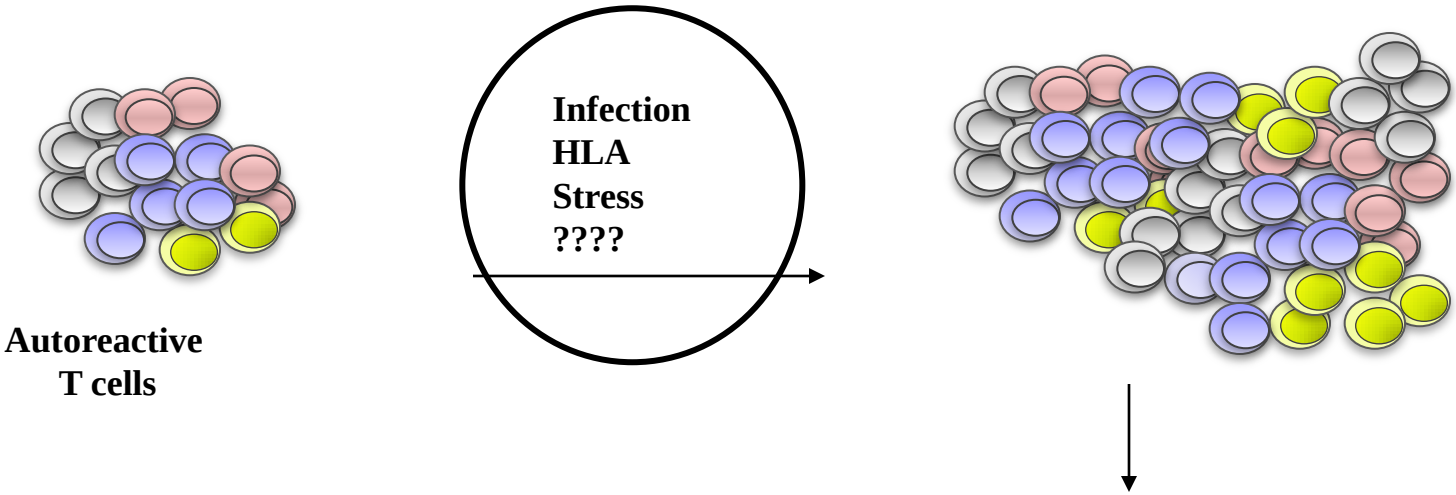


Nakou, et al. *PLoS One*. 2010

Tpl2 kinase regulates FcγR signaling and immune thrombocytopenia in mice.
Kyrmizi I et al J Leukoc Biol. 2013 Oct;94(4):751-7

Gene Network Analysis Reveals Activation of Multiple Kinase Pathways

ΑΥΞΗΣΗ ΑΥΤΟΔΡΑΣΤΙΚΩΝ ΚΑΙ ΔΥΣΛΕΙΤΟΥΡΓΙΑ ΡΥΘΜΙΣΤΙΚΩΝ ΚΥΤΤΑΡΩΝ ΣΤΗΝ ΑΥΤΟΑΝΟΣΙΑ



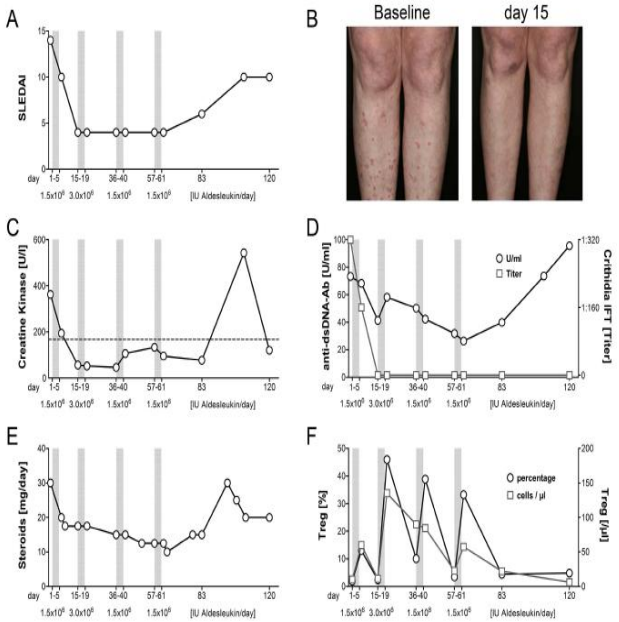
ΔΥΣΛΕΙΤΟΥΡΓΙΑ ΡΥΘΜΙΣΤΙΚΩΝ Τ ΚΥΤΤΑΡΩΝ ΣΤΟ ΣΕΛ

VLAHOU AR 2017

ΑΠΟΚΑΤΑΣΤΑΣΗ Τ ΡΥΘΜΙΣΤΙΚΩΝ ΜΕ ΧΑΜΗΛΕΣ ΔΟΣΕΙΣ IL-2

Rapid induction of clinical remission by low-dose interleukin-2 in a patient with refractory SLE

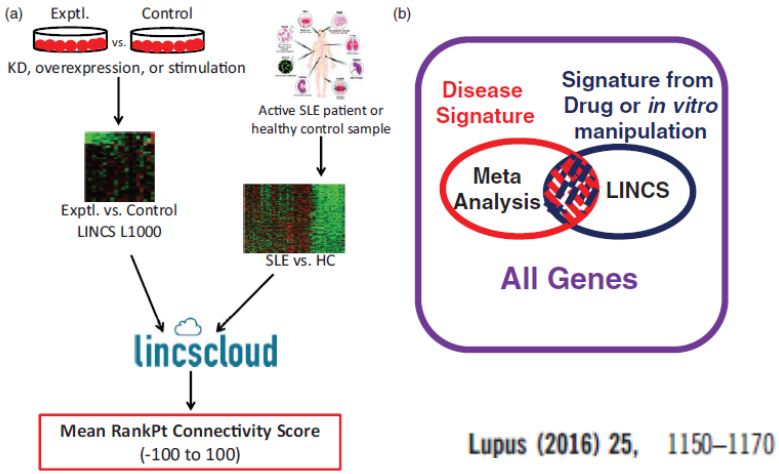
Low-dose interleukin-2 selectively corrects regulatory T cell defects in patients with systemic lupus erythematosus



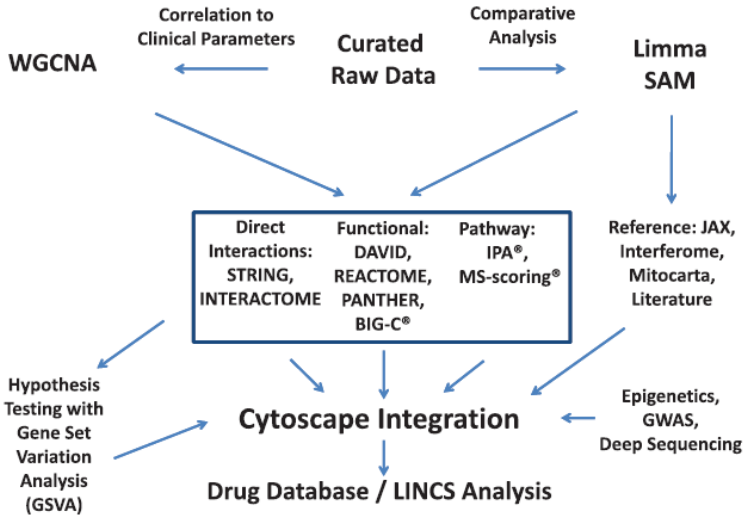
Conclusions Treg defects in patients with SLE are associated with IL-2 deficiency, and can be corrected with low doses of IL-2. The restoration of endogenous mechanisms of immune tolerance by low-dose IL-2 therapy, thus, proposes a selective biological treatment strategy, which directly addresses the pathophysiology in SLE.

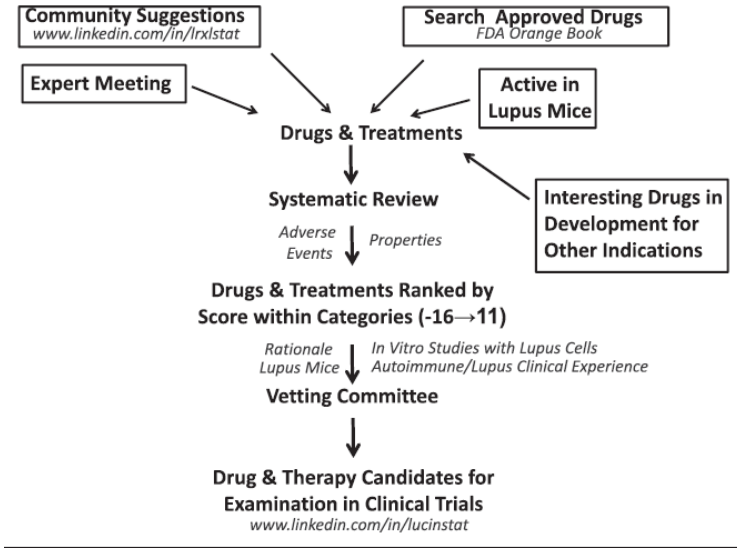
ARD Online First, published on January 21, 2015

Επανατοποθέτηση-νέες ενδείξεις γνωστών φαρμάκων

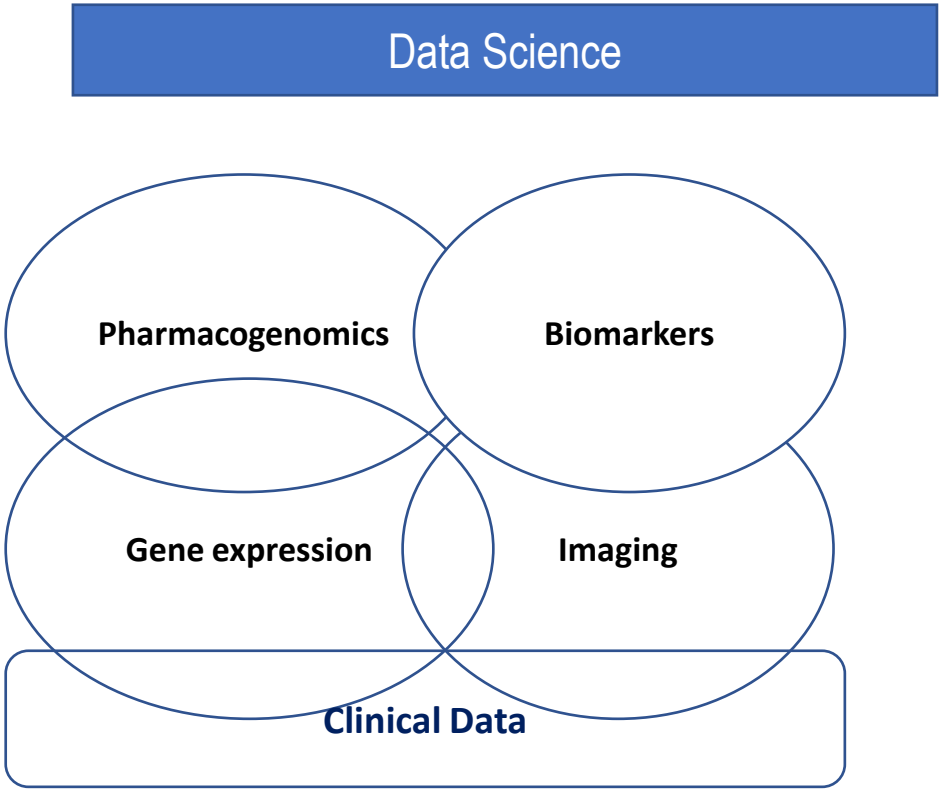


Lupus (2016) 25, 1150–1170





Πρωτοβουλία OBAMA


Data Science
Pharmacogenomics
Biomarkers
Gene expression
Imaging
Clinical Data

ΚΥΡΙΑ ΣΗΜΕΙΑ

- Πολλοί στόχοι- Δύσκολη νόσος
- Αποτυχίες:Τι μάθαμε από αυτές
- Επιτυχίες: Benlysta τι μάθαμε?
- Νέες βιολογικές σε μελέτες φάσης III
- Νέα μόρια μικρά και μεγάλα σε αρχικές μελέτες
- Νέες χρήσεις και επανατοποθέτηση εγκεκριμένων φαρμάκων