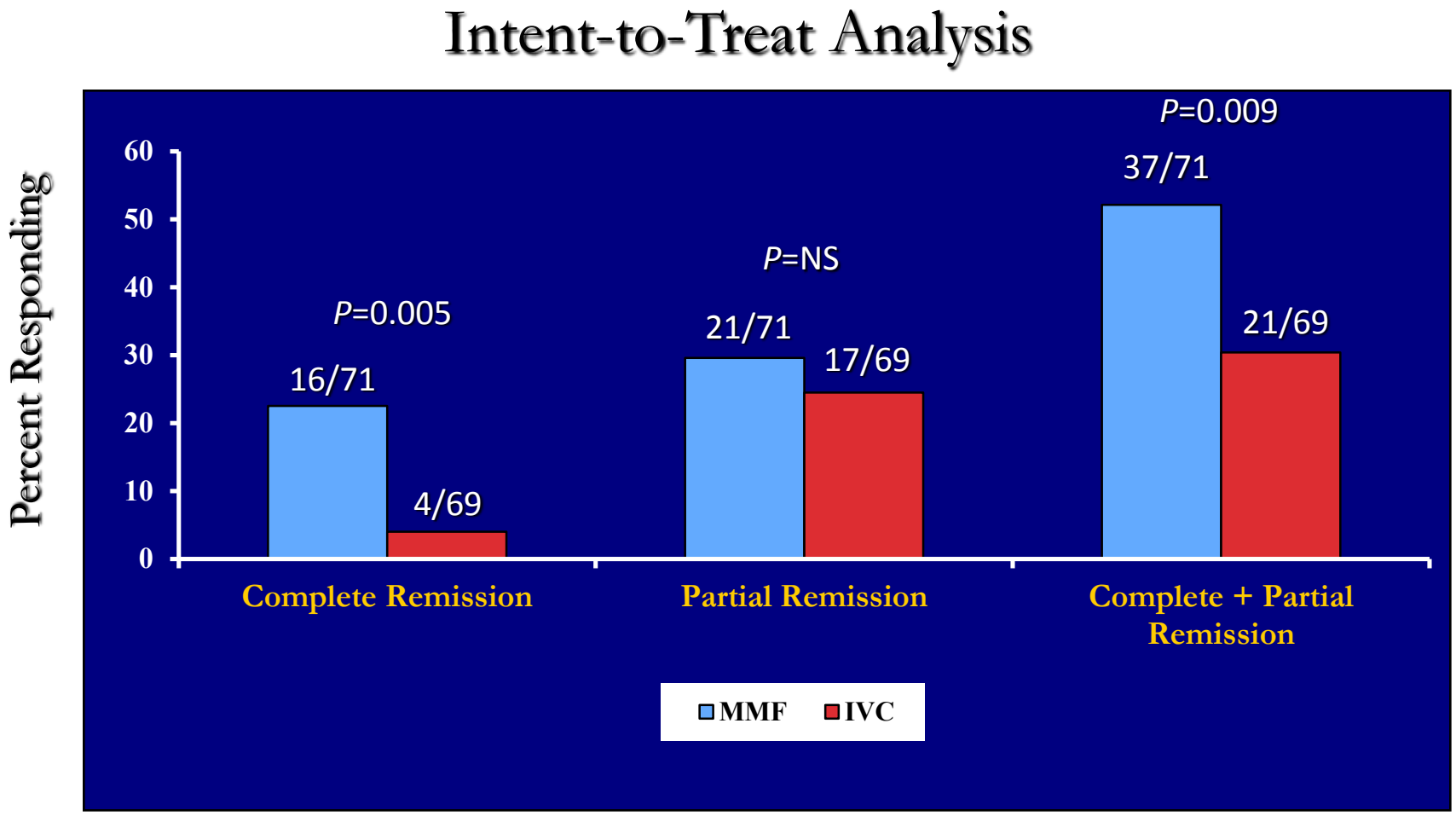
Advances in Lupus Research

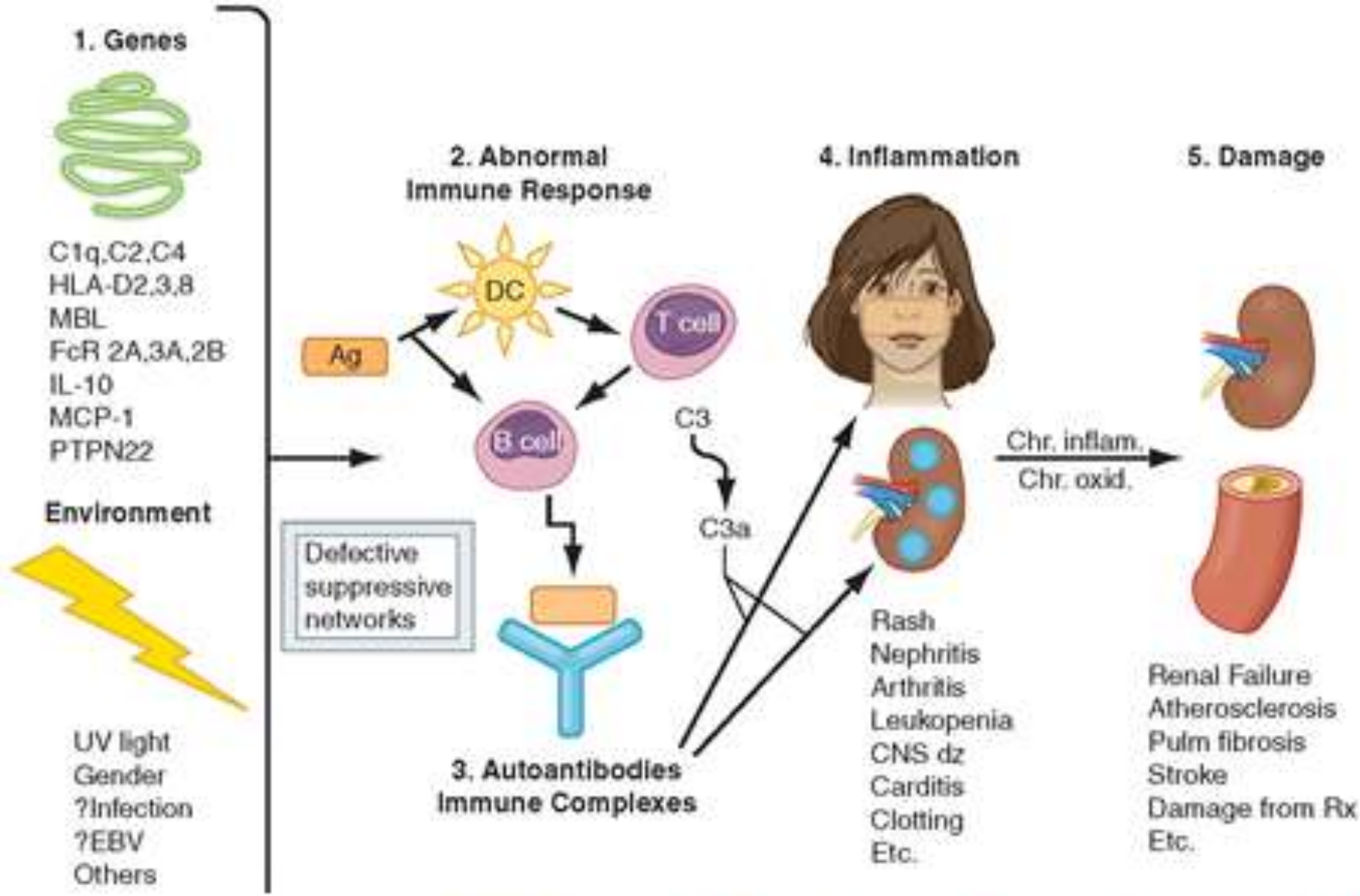
- Atlanta Lupus Summit
- Oct 14, 2017

Why we need new therapies in autoimmune diseases.



Ginzler et al: NEJM 2005

Systemic Lupus Erythematosus (SLE)



Fauci AS et al Harrison's Principles of Internal Medicine, 17th Edition

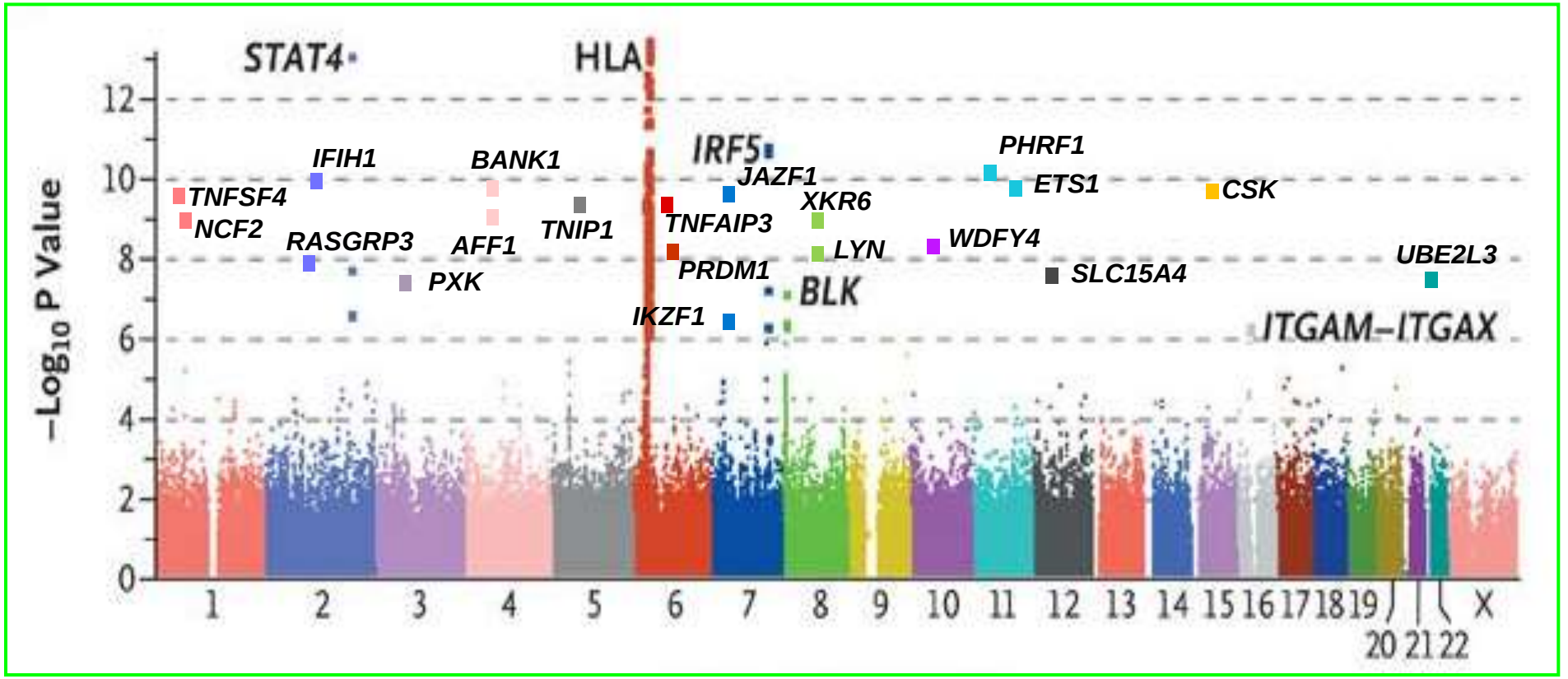
Why Study Genetics in SLE?

Population-based Familial Aggregation of SLE

- 23 million participants in Taiwan National Health Insurance database in 2010 (n=18,283 for SLE patients).
- 1st degree relatives of SLE pts have **RRs for SLE**: **>300** for twins, **24** for Sibs, 11 for parents, 14 for offspring, 4 for spouses.
- RRs for other autoimmune diseases: **6** for SS & SSc, 3 for RA, MG, IIM, MS, 1.7 for TID, 1.4 for IBD.
- Phenotypic variance of SLE: 44% for heritability, 26% for shared environmental factors, 30% for non-shared environmental factors.

Kuo C et al, JAMA intern Med 2015: 175, 1518

SLE Susceptibility Loci From 8 Initial GWAS ($P < 5 \times 10^{-8}$)



European: (1) *N.Engl.J Med.* 2008;358: 900-909. (2) *Nat.Genet.* 2008; 40: 204-210.
(3) *Nat.Genet*. 2008; 40: 211-216. (4) *Nat.Genet*. 2008; 40:1059-1061.

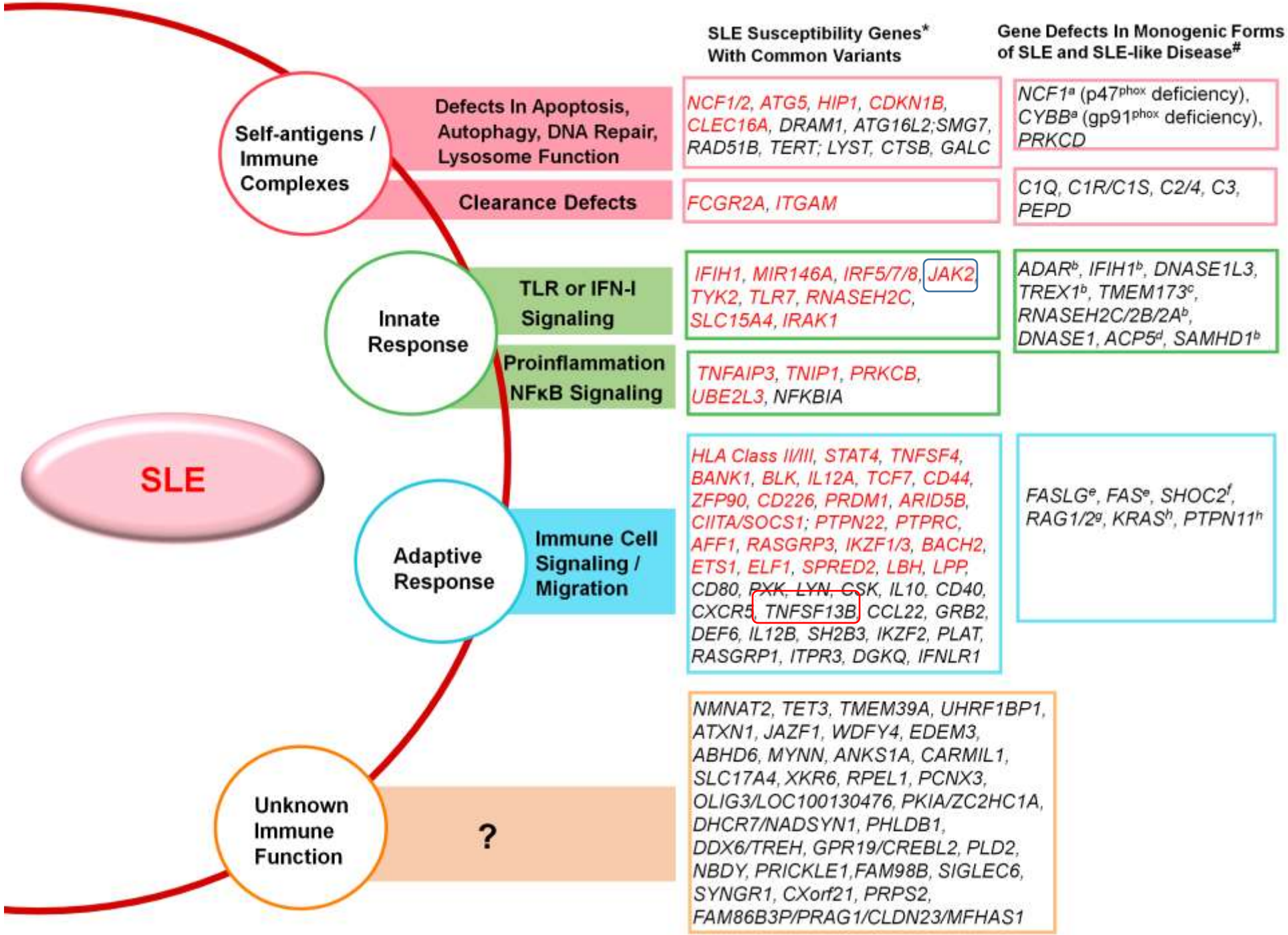
Asian: (1) *Nat.Genet*. 2009; 41:1234-1237. (2) *PLoS Genet*. 2010; 6: e1000841.
(3) PLoS Genet. 2012; 8:e1002455. (4) *Ann Rheum Dis*. 2014; 73: 1240-1245.

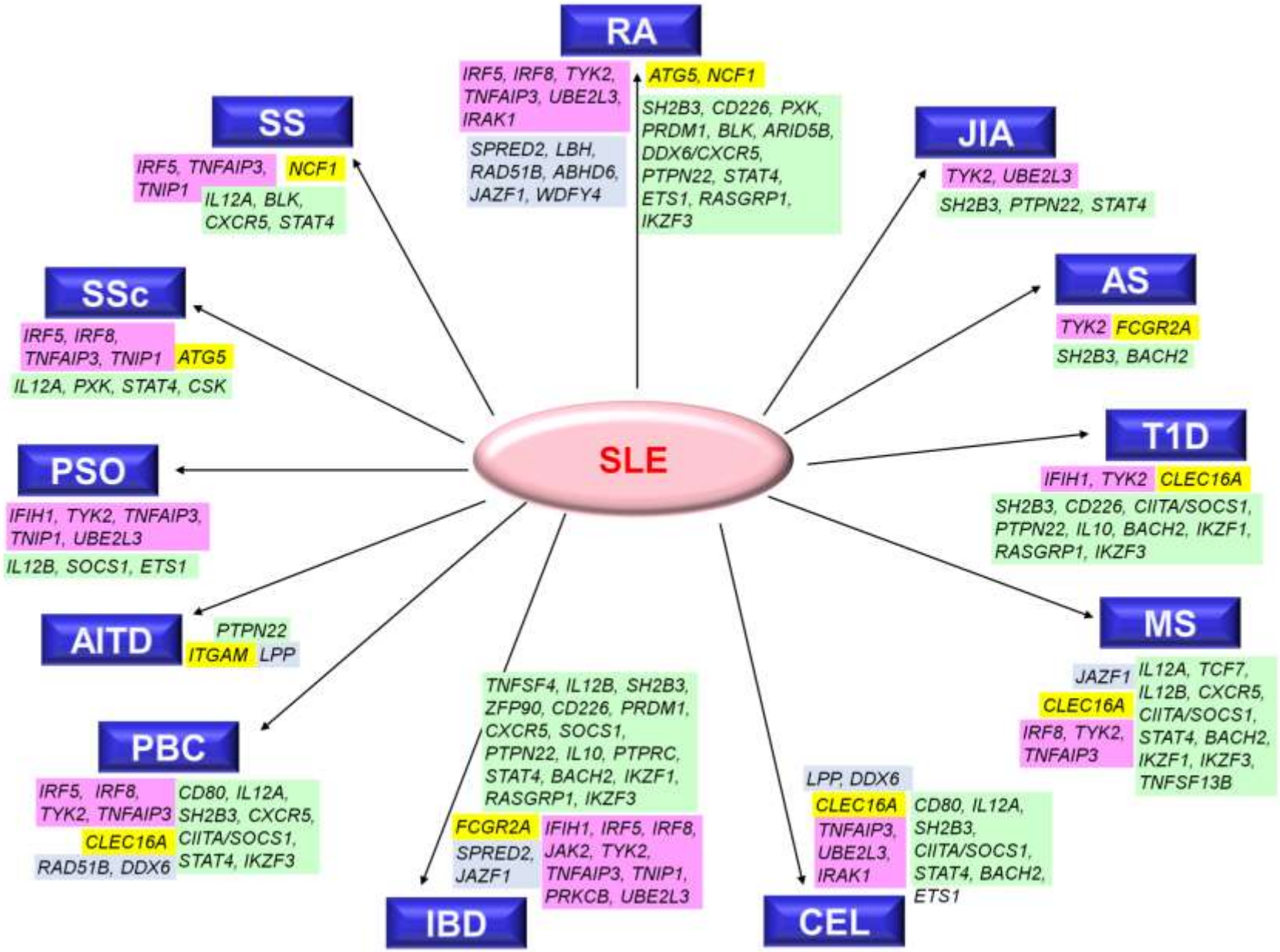
Illumina HumanHap300, 550, 610-Quad Bead-Chip arrays or
Affymetrix 100K, 5.0 SNP arrays

< 600,000 SNP markers

Approximately 100 SLE-associated Loci Identified (October 2017)

- The SLE susceptibility genes with common variants are identified through GWAS, meta-analysis, fine-mapping or replication studies yielding $p<5\times10\text{-}8$ in at least one ancestry.
- Genes labeled in **red** show association with SLE in multiple ancestries, while genes in **black** show association unique to one ancestry.


SLE Susceptibility Genes* With Common Variants
Gene Defects In Monogenic Forms of SLE and SLE-like Disease#
SLE
Self-antigens / Immune Complexes
Defects In Apoptosis, Autophagy, DNA Repair, Lysosome Function
NCF1/2, ATG5, HIP1, CDKN1B, CLEC16A, DRAM1, ATG16L2;SMG7, RAD51B, TERT; LYST, CTSB, GALC
NCF1[a] (p47[phox] deficiency), CYBB[a] (gp91[phox] deficiency), PRKCD
Clearance Defects
FCGR2A, ITGAM
C1Q, C1R/C1S, C2/4, C3, PEPD
Innate Response
TLR or IFN-I Signaling
IFIH1, MIR146A, IRF5/7/8, JAK2, TYK2, TLR7, RNASEH2C, SLC15A4, IRAK1
ADAR[b], IFIH1[b], DNASE1L3, TREX1[b], TMEM173[c], RNASEH2C/2B/2A[b], DNASE1, ACP5[d], SAMHD1[b]
Proinflammation NFκB Signaling
TNFAIP3, TNIP1, PRKCB, UBE2L3, NFKBIA
Adaptive Response
Immune Cell Signaling / Migration
HLA Class II/III, STAT4, TNFSF4, BANK1, BLK, IL12A, TCF7, CD44, ZFP90, CD226, PRDM1, ARID5B, CIITA/SOCS1; PTPN22, PTPRC, AFF1, RASGRP3, IKZF1/3, BACH2, ETS1, ELF1, SPRED2, LBH, LPP, CD80, PXK, LYN, CSK, IL10, CD40, CXCR5, TNFSF13B, CCL22, GRB2, DEF6, IL12B, SH2B3, IKZF2, PLAT, RASGRP1, ITPR3, DGKQ, IFNLR1
FASLG[e], FAS[e], SHOC2[f], RAG1/2[g], KRAS[h], PTPN11[h]
Unknown Immune Function
?
NMNAT2, TET3, TMEM39A, UHRF1BP1, ATXN1, JAZF1, WDFY4, EDEM3, ABHD6, MYNN, ANKS1A, CARMIL1, SLC17A4, XKR6, RPEL1, PCNX3, OLIG3/LOC100130476, PKIA/ZC2HC1A, DHCR7/NADSYN1, PHLDB1, DDX6/TREH, GPR19/CREBL2, PLD2, NBDY, PRICKLE1,FAM98B, SIGLEC6, SYNGR1, CXorf21, PRPS2, FAM86B3P/PRAG1/CLDN23/MFHAS1



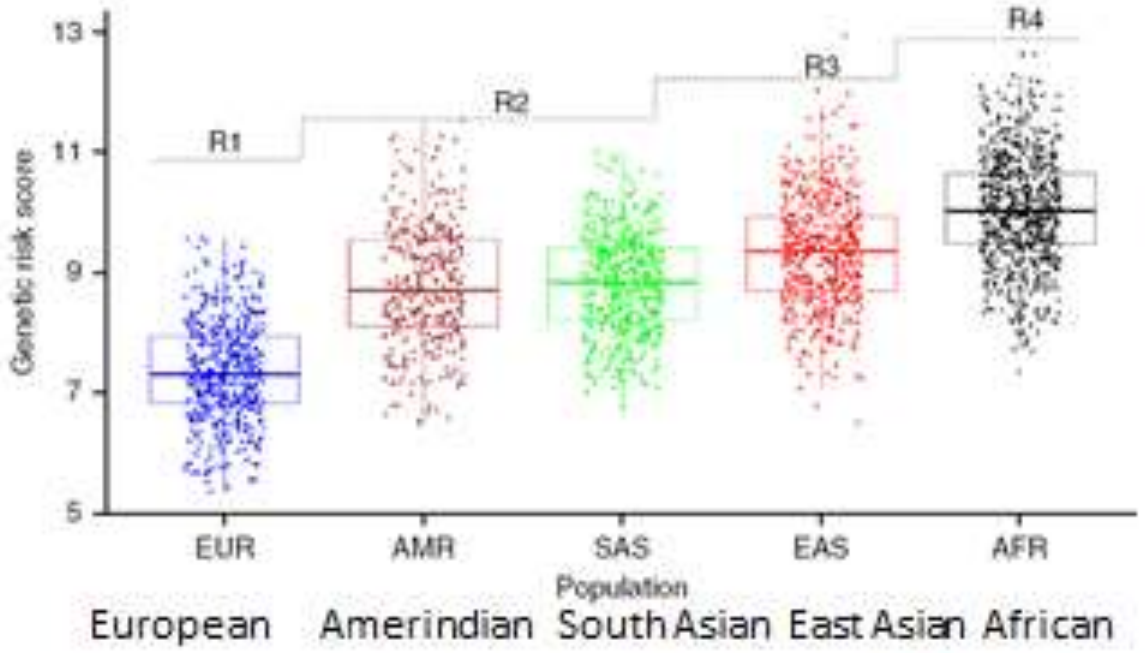
Deng Y & Tsao BP, Genetics of Human SLE, Dubois' Lupus Erythematosus, 2017

Genetic Load of each Individual

Genetic risk score of SLE is different for each ancestry

$$\text{Cumulative genetic risk score} = \sum_{i=1}^{m} \ln(\text{OR}_i)G_i$$

- M represents the number of SLE risk loci (m=63, including 52 previously reported and 10 novel autosomal GWAS loci). No MHC region and X chromosome.
- G is the number of risk alleles at a given SNP (0,1,2)



Healthy individuals in the 1000 genomes project

Morris DL et al, Nat. Genet 2016; 48:940-946

Could we apply Genetic Risk Scores to patient care?

$$\text{Cumulative genetic risk score} = \sum_{i=1}^{m} \ln(\text{OR}_i) G_i$$

Elevated Genetic Risk Score:

- younger age of SLE onset
- male gender
- ancestry populations (Africans > East Asians > S Asians and Amerindians > Europeans)

Could we use GRS to distinguish:

- 1st degree relatives at risk for disease?
- Patients who are likely to flare frequently?
- Treatment response?
- Accelerated Damage?

Lessons Learned from SLE Risk Loci

- SLE-associated gene products involved in type I IFN & NF-κB signaling, T & B cell signaling, immune clearance, and unknown pathways
- Approximately 30 loci with unknown immune functions could reveal novel insights to the disease pathogenesis.
- SLE and other autoimmune diseases share many risk loci.
- Most SLE-associated SNPs contained within the same region across **European**, **Asian**, Amerindian, and African derived populations. Many share the same risk allele.
- The SLE risk variant of TNFSF13B, that causes cytokine BAFF overexpression, is the FDA approved drug anti-BAFF mAb, Belimumab.
- A genetic basis (Genetic Risk Scores) of increased prevalence of SLE in non-Europeans, men affected with SLE, and patients with younger age at disease onset

Environmental factors in lupus

EBV

Silica

Pesticides/heavy metals

Smoking- current not past

Sunlight- flares

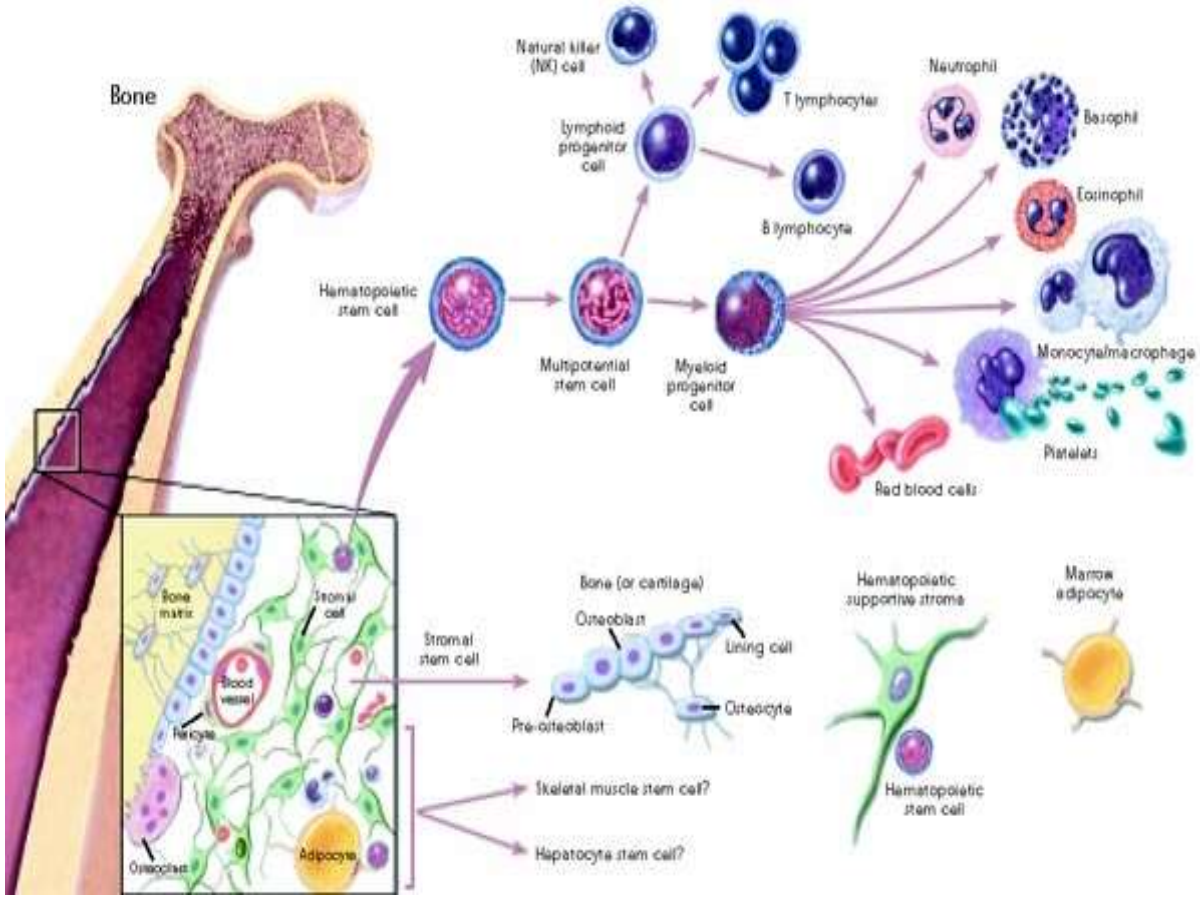
Drugs- anti-TNFs, hydralazine

Vitamin D deficiency

Cellular Therapies in Autoimmune Diseases

- Hematopoietic Stem Cell Transplants
  - Allogeneic
  - Autologous
- Mesenchymal Stem Cell Transplants
  - Allogeneic
  - Autologous
- Expansion of Individual Cell Subsets and reinfusion
  - Tregs
- Induced pluripotent stem cells
  - Differentiate into cell of choice

All are adult stem cells, not fetal stem cells



Comparison of adult stem cell therapies

	Hematopoeitic SC	Mesenchymal SC
HLA matching	Autologous	Not required
Preconditioning	Yes (XR, ATG, CTX)	No
Treatment mortality	Yes (3-12%)	No
Relapse	Yes (60+%)	Yes (?)
Retreatment	+/-	Yes
Recommended Tx	Severe scleroderma	??
Hospitalization	Yes	No
Cost	????	??
Mechanism	Marrow ablation	MSCs themselves
Availability	Limited to specialized sites	None now but can be rapidly expanded

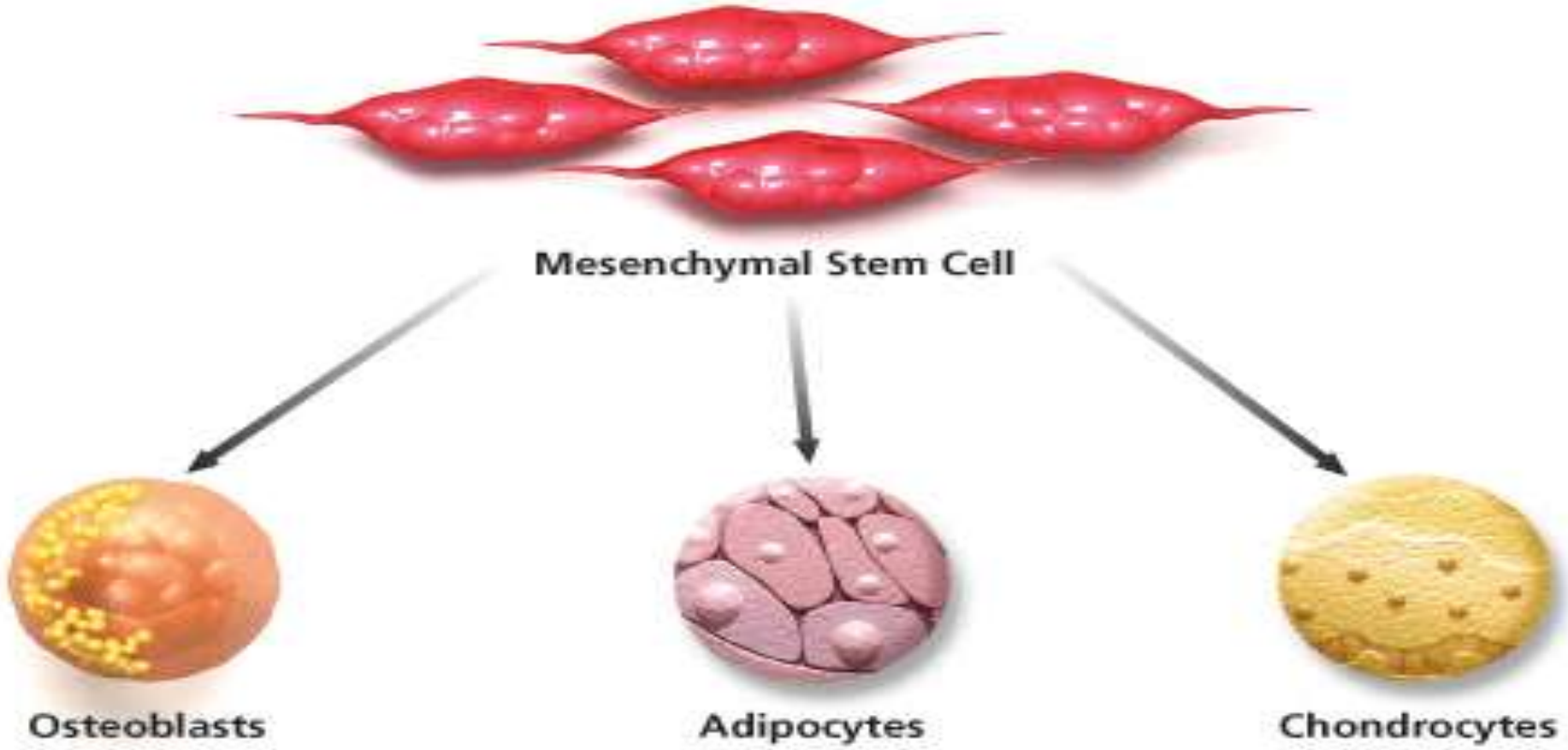
Table 1
Published experience with autologous hematopoietic stem cell transplant in SLE

		*Patients		Mortality				
Centre/source	**Reference**	**N**	**Conditioning**	**Overall N (%)**	**Transplant related N (%)**	**SLE related N (%)**	**Overall survival**	**Relapse-free survival**
EBMT registry (35 centres)†	**19 25**	85	Various	18 (21)	11 (13) (95% CI 5 to 17)	5 (6)	79% At 5 years (95% CI 66 to 86)	44% At 5 years (95% CI 32 to 56)
Northwestern University, USA	**20**	50	CY+ATG	8 (16)	2 (4)	4 (8)	84%	50% at 5 years
Zhengzhou, China	**29**	18	TLI+CY+ATG	NR	0 (0)	NR		72% (13/18) At median 12 (3–26) months' follow-up
Seoul, South Korea	**30**	7	CY+ATG	0 (0)	0 (0)	0 (0)		100% At median 13 (3–26) months' follow-up
Berlin, Germany	**23**	7	CY+ATG	2 (29)	1 (14)	1 (14)	71% (5/7)	72% At 60 months (range, 24–96 months)
National Institutes of Health, USA	**24**	8	CY+fludarabine +rituximab	2 (25)	2 (25)	0 (0)	75%	75% At a median 54 months (range, 36–60 months)

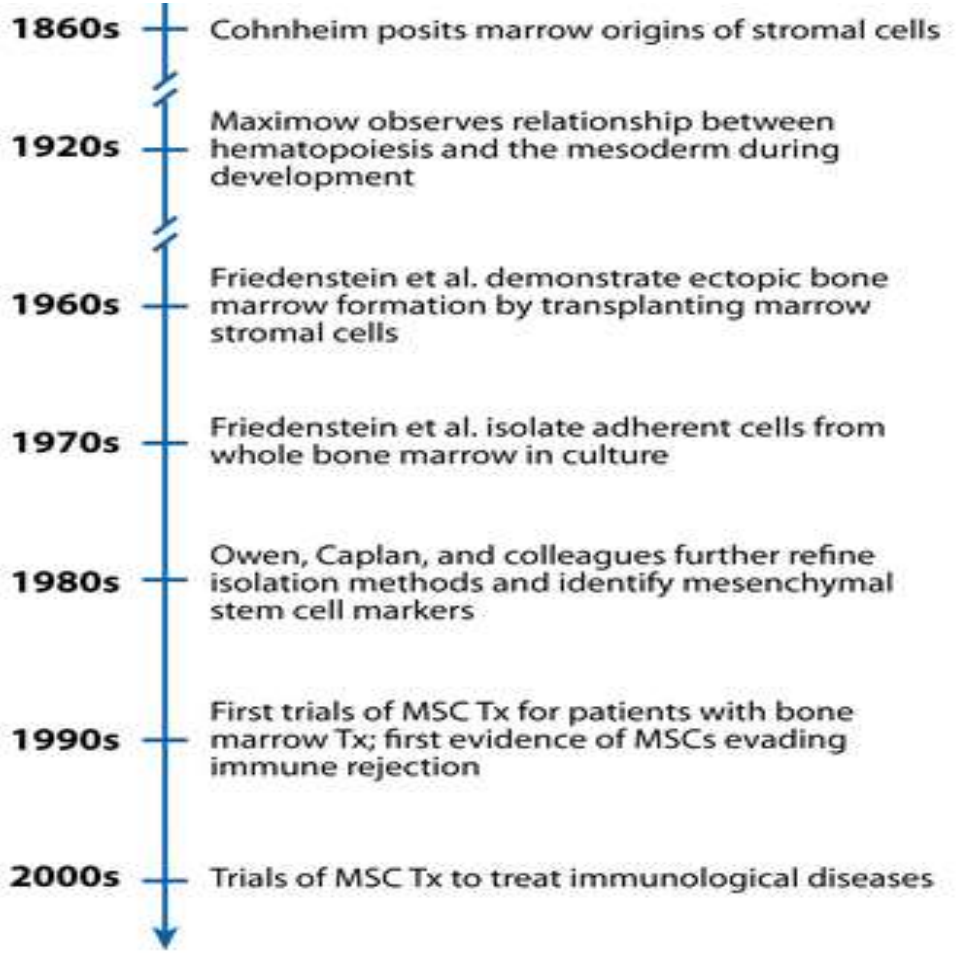
Why might autologous HSC not be as effective in lupus?

- You are putting back into the patient the same cells that cause lupus in the first place.
- You have not changed their genetics
- Maybe without a new environmental insult it might work. If trigger is infection, you are markedly predisposing the patient to viral reactivation or new bacterial infection.
- Relapses of at least 50-60% at 5 years, given the risk of the procedure and other treatment options, makes HSC less attractive as a treatment.

Pluripotent MSCs

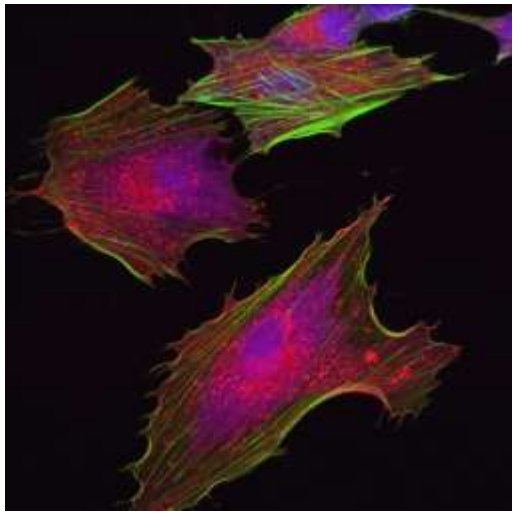


History of MSCs



Parekkadan B, Milwid JM. 2010.
Annu. Rev. Biomed. Eng. 12:87–117

Mesenchymal Stromal Cells



- Multi-potent progenitor cell
- Differentiate into osteocytes, chondrocytes, and adipocytes.
- Isolated from adult bone marrow, adipose tissue, and umbilical cords.
- No exclusive surface markers have been identified for MSC.
  - Negative:CD11b, CD14, CD31, CD34, CD45
  - Positive:HLA-I,CD105, CD73, CD29, CD90
- Can be expanded rapidly *in vitro*.
- Immune privileged- do not express Class II or costimulatory molecules CD80/CD86
- Can be given without matching. Can be given cross species
- Do not require preconditioning of patient or marrow ablation

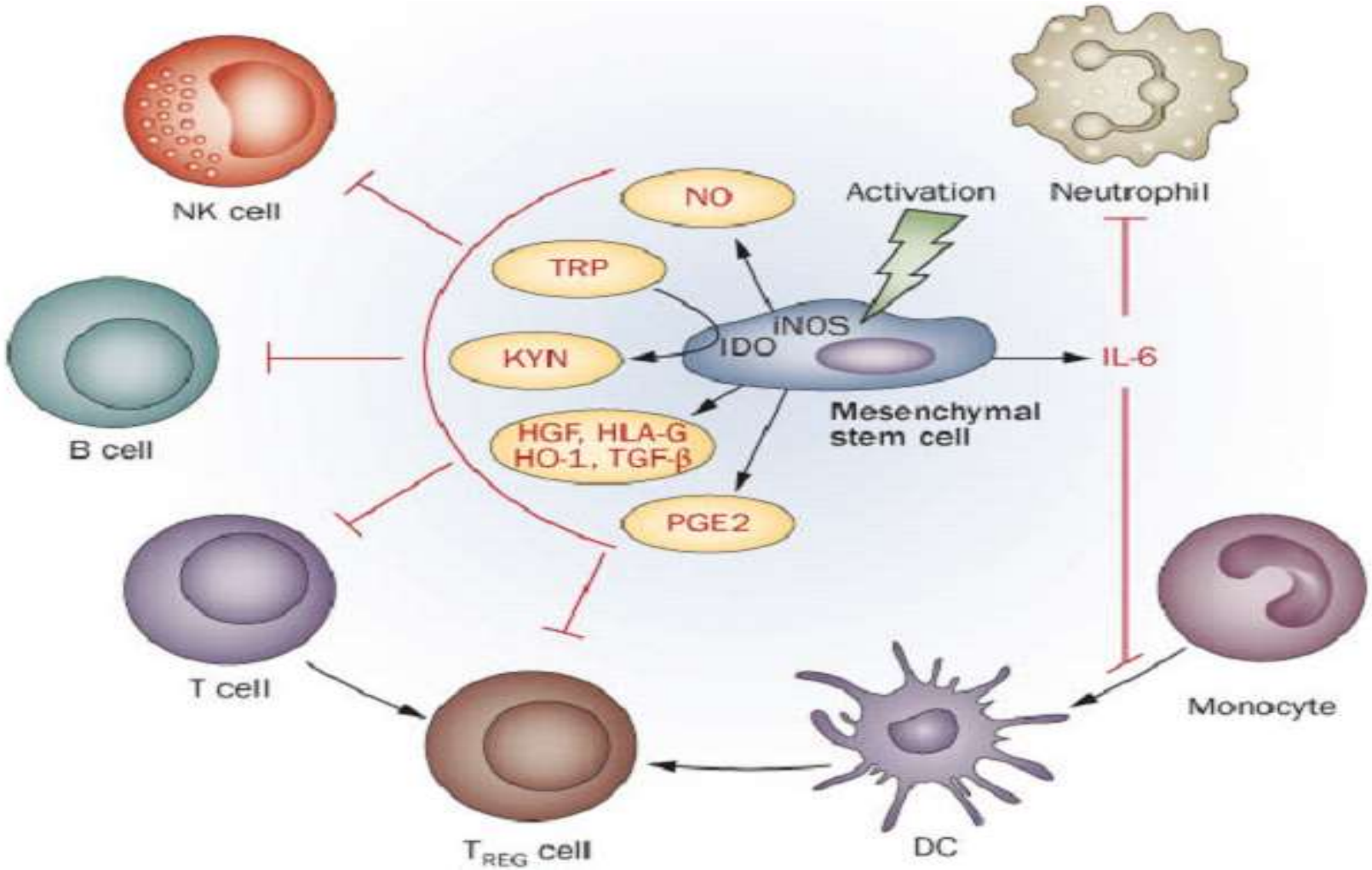
MSC are Migratory

- Circulating MSC pool in blood are increased under hypoxic conditions or post trauma.

- Several chemokines and growth factors are chemotactic stimuli for MSC.
  - stromal cell-derived factor-1α (SDF-1α)
  - Platelet derived growth factor (PDGF)
  - hepatocyte growth factors (HGF)
  - monocyte chemoattractant protein (MCP)
  - basic fibroblast growth factor (BFGF)

- MSC migrate across endothelial cell monolayers and through the underlying extracellular matrix.

Mesenchymal Stem Cells (MSCs)



MSC Therapy

- Mouse Models: EAE, Type 1 Diabetes, RA, SLE
- Human Disease:
  - GvHD
  - Type 1 Diabetes
  - Cardiac Disease
  - Multiple Sclerosis
  - Inflammatory Bowel Disease
  - Rheumatoid Arthritis
  - Parkinson's Disease
  - Sjögren's Syndrome

Early trials with MSC from various sources showed anti-inflammatory effect with little evidence of toxicity

http://www.clinicaltrials.gov

GVHD

- US
  - Prochymal by Osiris 2 or 8x6 MSC/kg 2 times
  - 32 Patients
  - 94% of patients had initial response to prochymal
    - 77% complete response, 16% partial response
- Europe
  - Phase 2; 55 patients
  - .4-9x10^6 MSC/kg ; 27 one dose, 22 two dose, and 6 three to five dose
  - 30 patients had a complete response and 9 showed improvement

Approved in Europe for treatment of steroid refractory GVHD

Kebriaei et al. Biol Blood Marrow Transplant, 2009
Le Blanc et al Lancet, 2004
Le Blanc et al Lancet, 2008

Rheumatoid Arthritis

- Allogeneic AD-MSC
  - 3 infusions
  - $1x10^6$, $2x10^6$ or $4x10^6$ MSC/kg
- 53 Refractory RA patients
- Results
  - Patient and disease characteristics were comparable for all three dose groups.
  - ACR20/50/70 were observed in 45/20/5% of cohort A vs. 28/14/5% on placebo at one month.
  - At 3 months 25/15/5 cohort A and 0/0/0 placebo

Alvaro-Gracia, et al. ACR 2013

Clinical Trials – Inflammatory Diseases

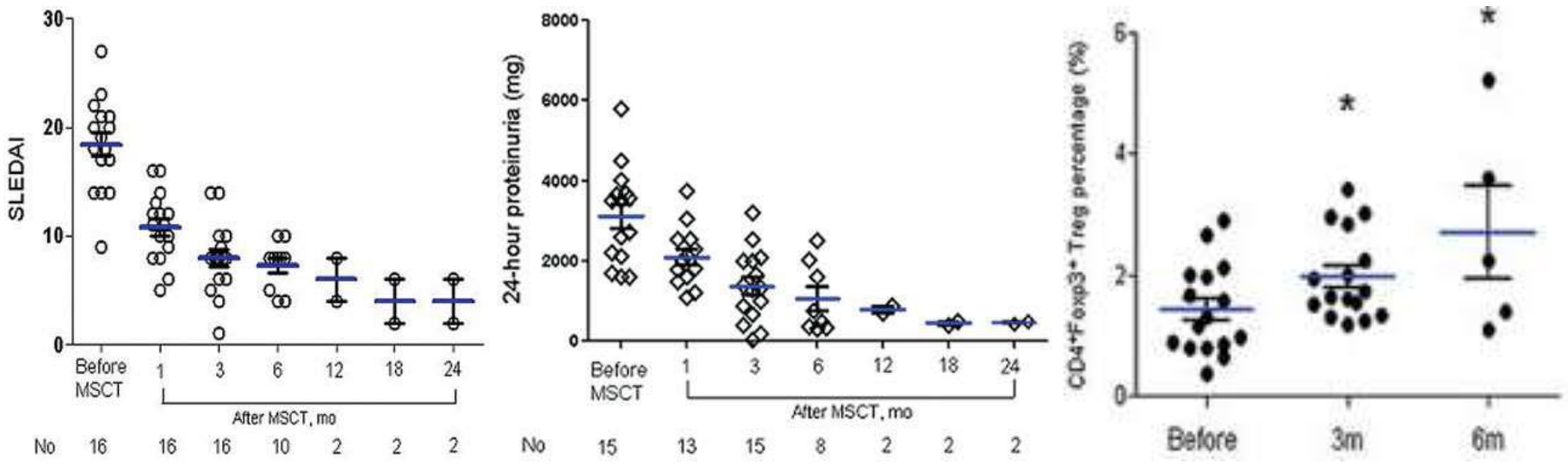
Disease	US	Europe	Published Results
Rheumatoid Arthritis	0	1	Yes
Systemic Sclerosis	0	1	Yes
Type 1 Diabetes	1	2	No (Prochymal)
Crohn's	5	3	Yes (Prochymal)
GVHD	12	11	Yes (Prochymal
SLE	0	0	Only China (6)

*only allogeneic MSC

http://www.clinicaltrials.gov

SLE

- UC-MSC
- 16 Patients
- Improved SLEDAI, ↓ANA, ↓anti-dsDNA antibodies, ↓serum albumin, ↑renal function, ↑Treg

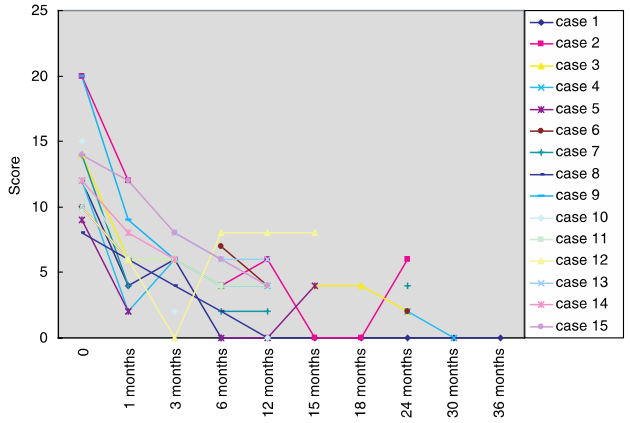


Sun et al Arth Rheum, 2010

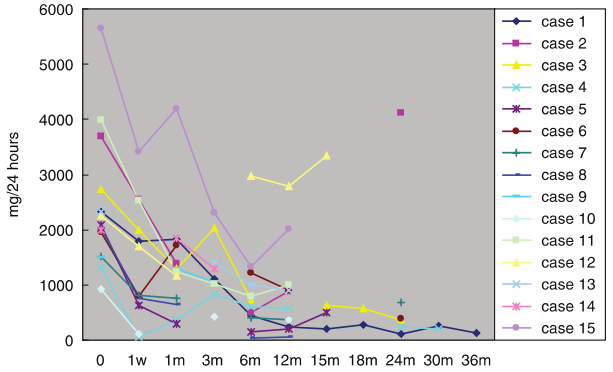
SLE

- Healthy donor BM-MSC
- 15 Patients
- ↓ SLEDAI score, ↓proteinuria, ↓anti-dsDNA antibodies

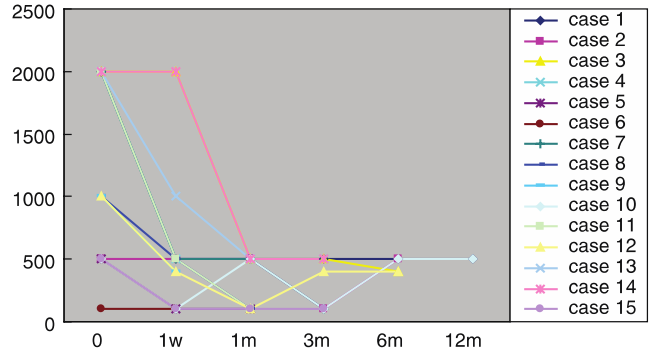
SLEDAI score



24-h Proteinuria



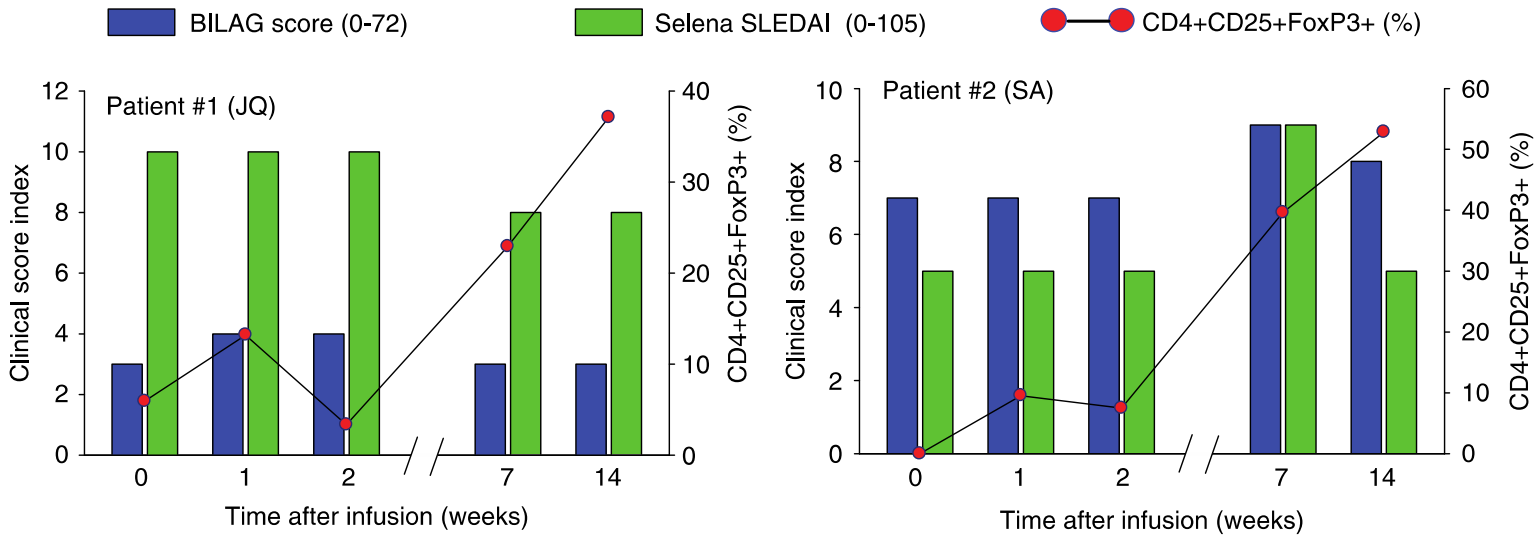
Anti-dsDNA antibodies



Liang et al Ann Rheum Dis, 2010

SLE

- Autologous BM-MSC
- 2 Patients
- No change in disease activity, ↑Treg



Carrion et al Lupus, 2010

MSCs and Lupus

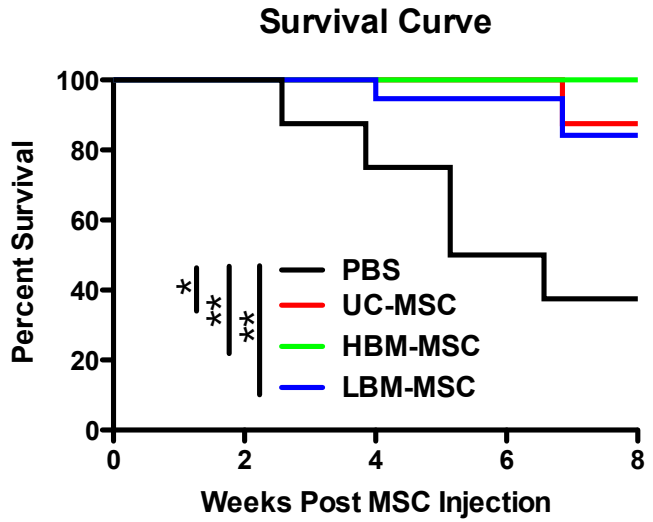
- Allogeneic MSC:
  - Treatment refractory SLE patients receiving BM-MSC from healthy donors experienced clinical improvement of disease.
  - Healthy donor and SLE-MSCs are capable of increasing regulatory T cells and reducing PBMC proliferation.
  - Treatment of SLE patients receiving UC-MSC from healthy donors had improved disease activity scores and increased peripheral Tregs.
- Autologous MSC:
  - BM-MSC from SLE patients caused an increase in circulating regulatory T cells but no beneficial effects on disease severity.

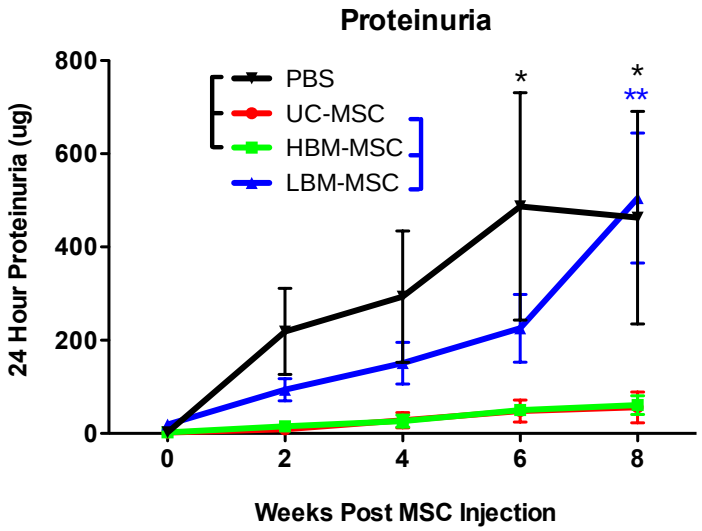
Question Remains

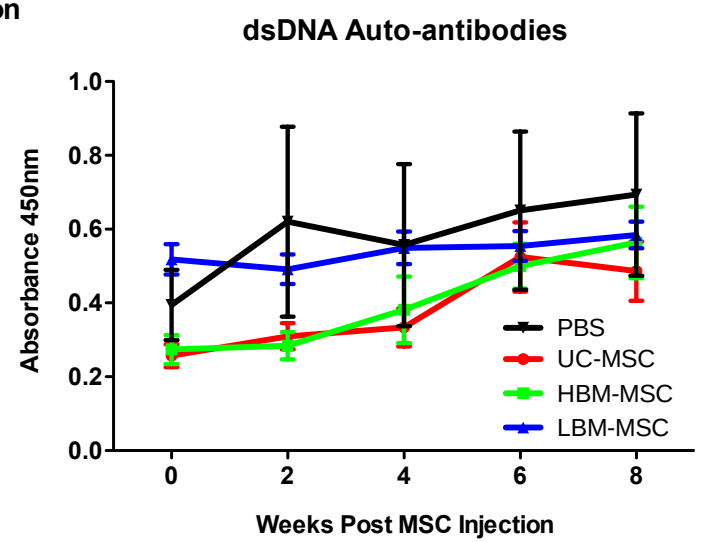
Which MSC source is best for SLE?

Sun LY, et al. Lupus 2007
Sun LY, et al. Stem Cell 2009
Zhang Z, et al. Cell Research 2008
Liang J, et al. Ann Rheum Dis 2010
Sun LY, et al. A&R 2010
Tyndall A, et al. Rheum 2007
Mok MY, et al. Lupus 2010
Carrion F, et al. Lupus 2009

Improved survival of lupus prone mice receiving human MSCs

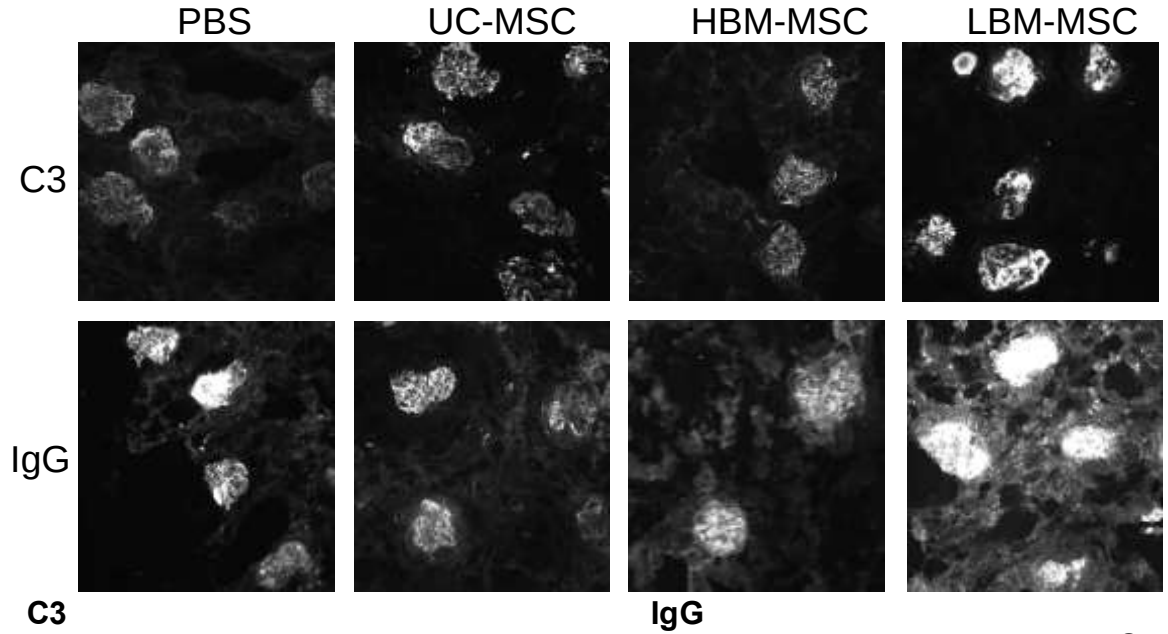


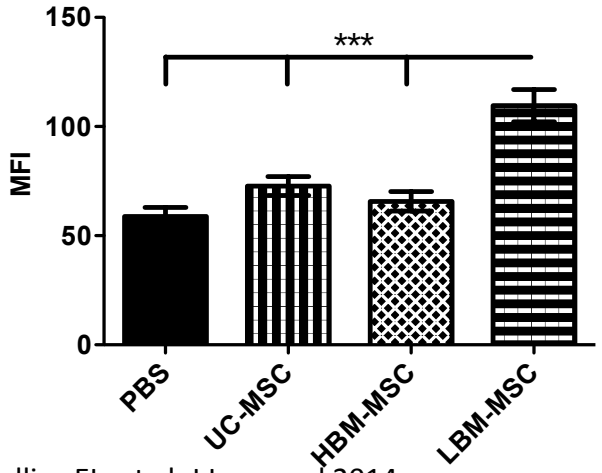


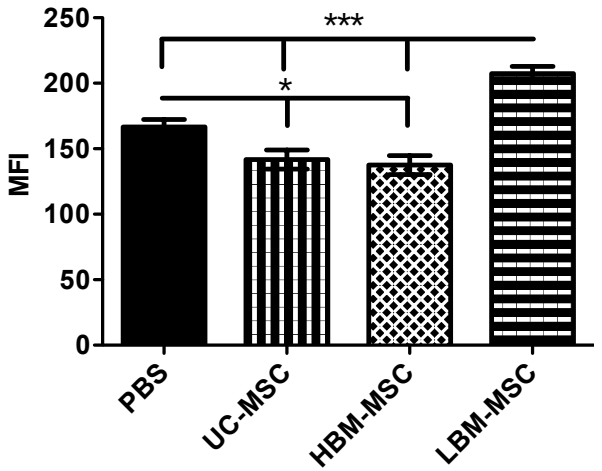


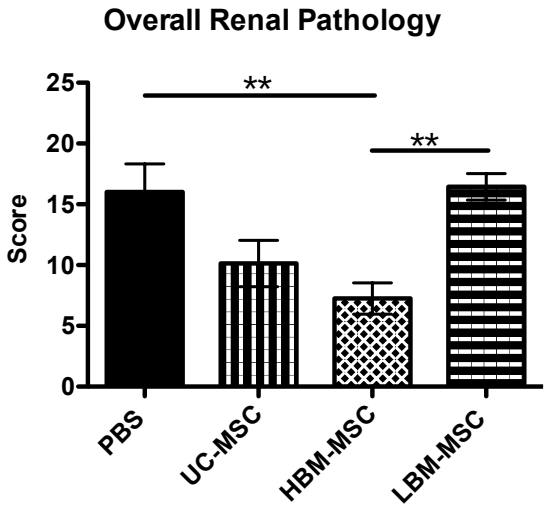
Collins EL, et al. J Immunol 2014

Renal Pathology is not prevented in mice receiving LBM-MSC



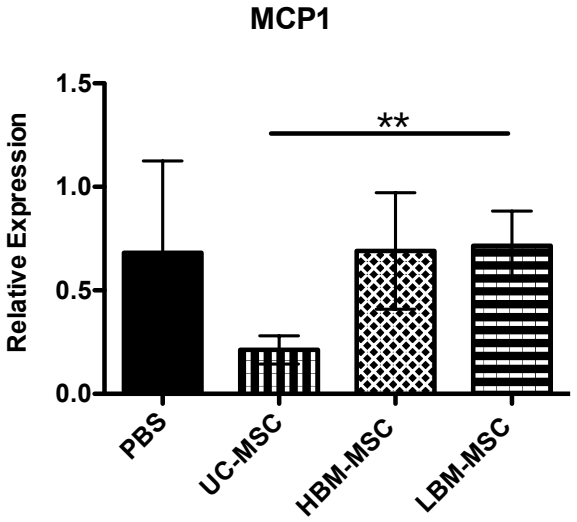


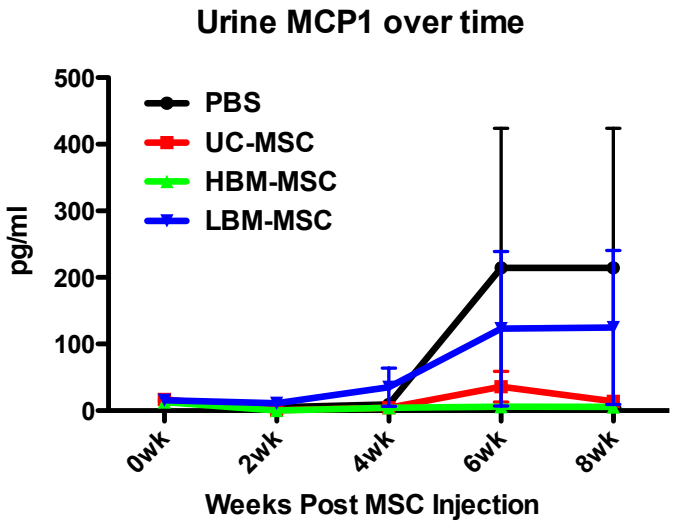


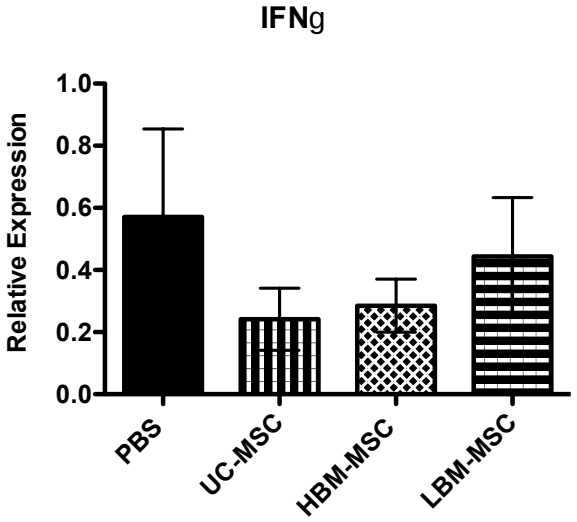


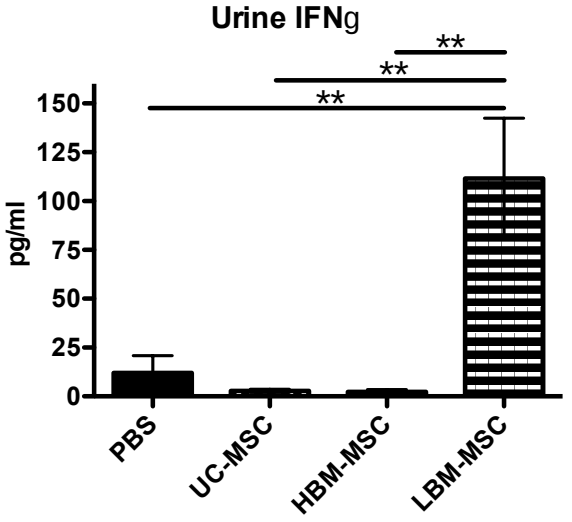
Collins EL, et al. J Immunol 2014

LBM-MSC not effective in reducing inflammatory markers

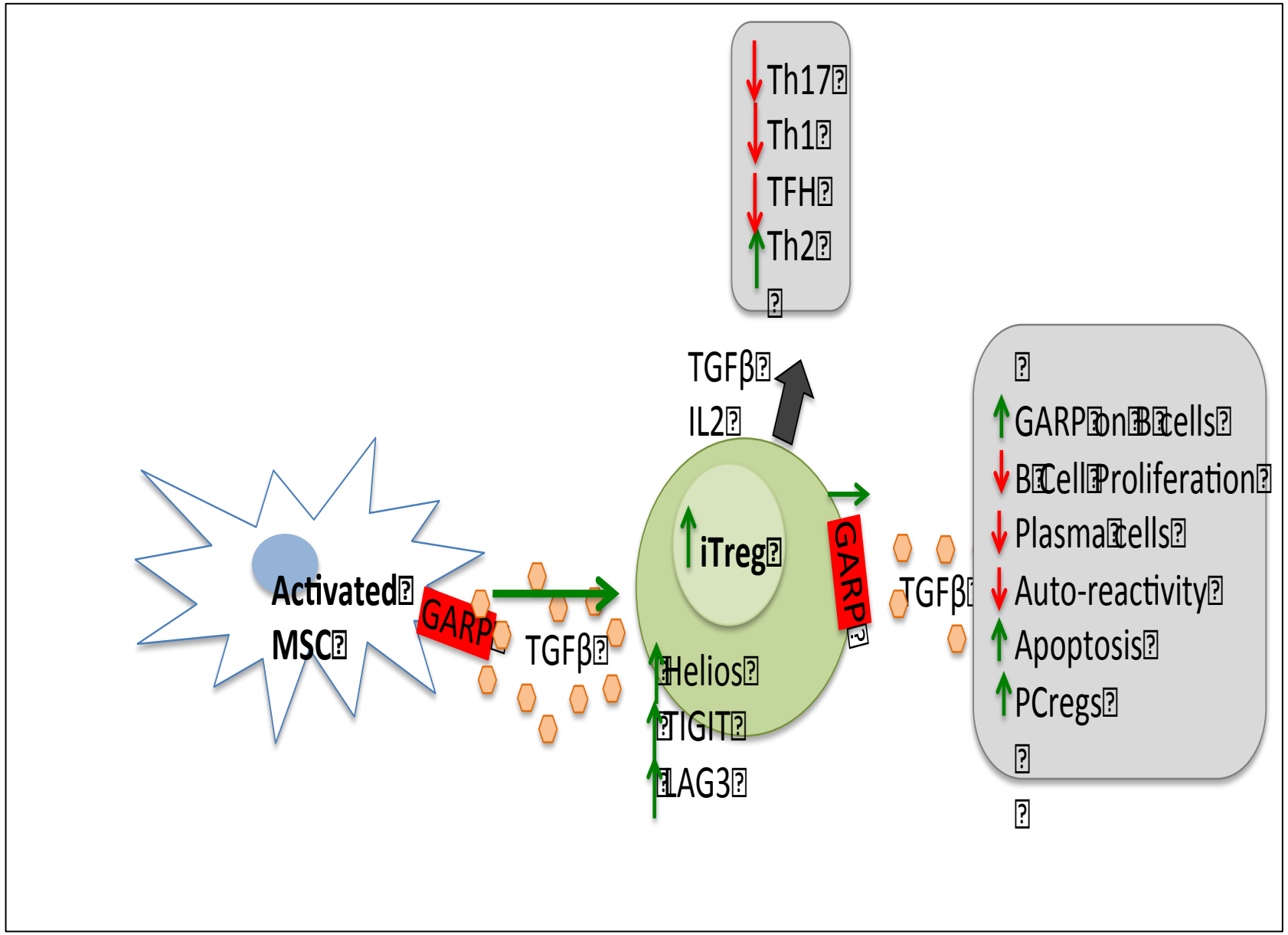








Collins EL, et al. J Immunol 2014


Th17
Th1
TFH
Th2
TGFβ
IL2
GARP on B cells
B Cell Proliferation
Plasma cells
Auto-reactivity
Apoptosis
PCregs
Activated MSC
GARP
TGFβ
iTreg
Helios
TIGIT
LAG3
GARP
TGFβ

Potential Toxicities

- Can induce embolization if give cells incorrectly
- Potential for tumor formation but not reported to date
- Potential for the cells to differentiate into tissue you don’t want
- Potential for allogenic reactions if use allogenic cells
- Immunosuppressive

Ongoing Studies of MSCs at MUSC

- SLE studies- Phase I in progress; Phase II pending funding
- Islet Cells in panceatectomy patients
- Scleroderma
- Type I diabetes in islet cell transplants

Case Report

- Patient maintained on mycophenylate and prednisone 10mg a day.
- She was screened successfully for Phase I trial of MSCs in lupus.
- She received an infusion of $1x10^6$ UC MSCs/kg one week ago.
- She tolerated the infusion with no adverse effects.
- She will be followed for the next 12 months to assess response to treatment.

Studies of MSCs in process elsewhere

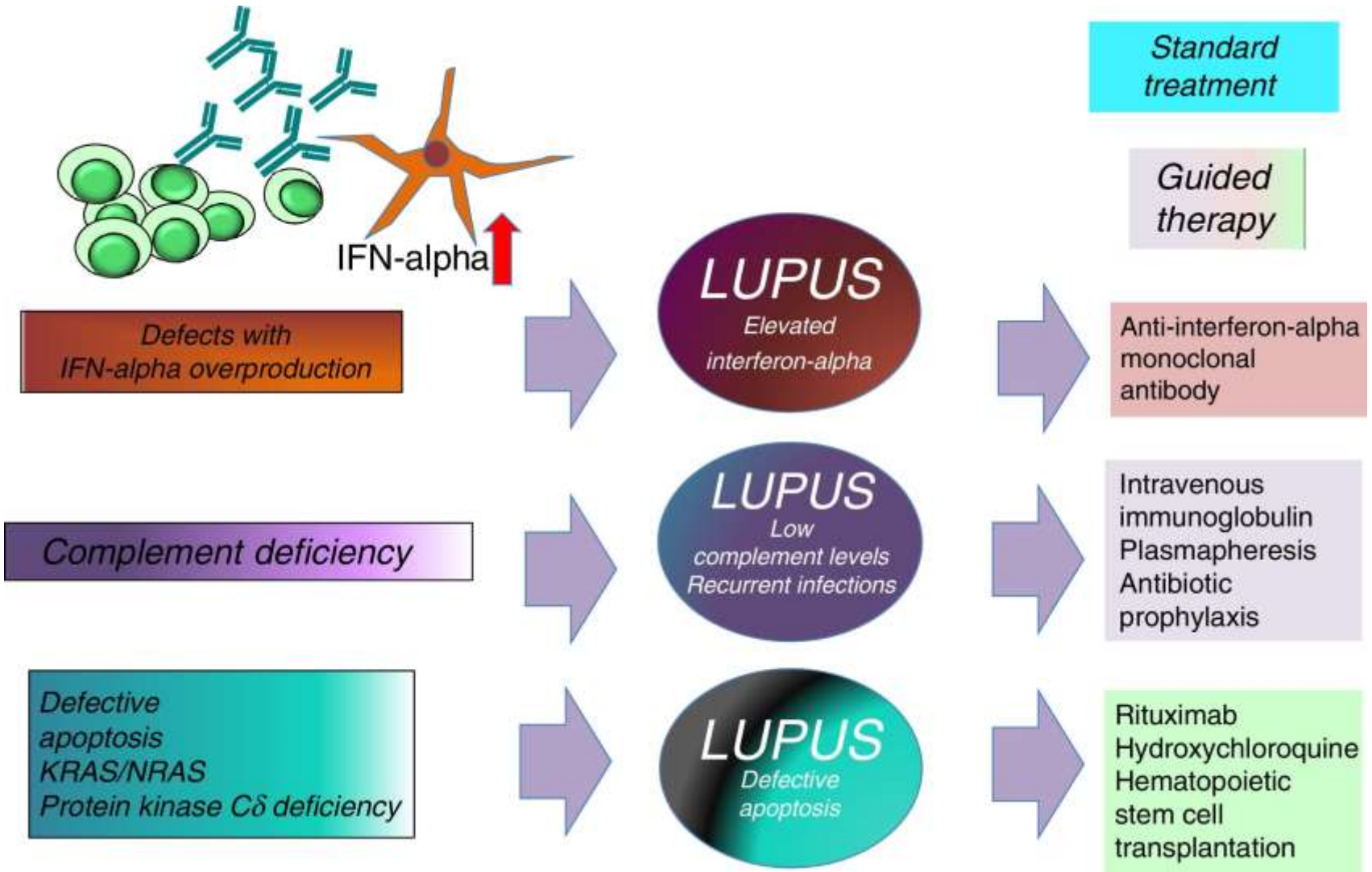
- Kidney transplants
- Inflammatory bowel disease
- JIA

MSCs in SLE Trial Protocol

- A Phase II sequential dose-escalation study evaluating the safety and feasibility of allogeneic umbilical cord derived mesenchymal stromal cells (MSC) for the treatment of adults with treatment refractory lupus
- IND 16377 approved (sponsor: Gary Gilkeson)

It Takes/Took A Village

Precision Treatment in Lupus



Questions?