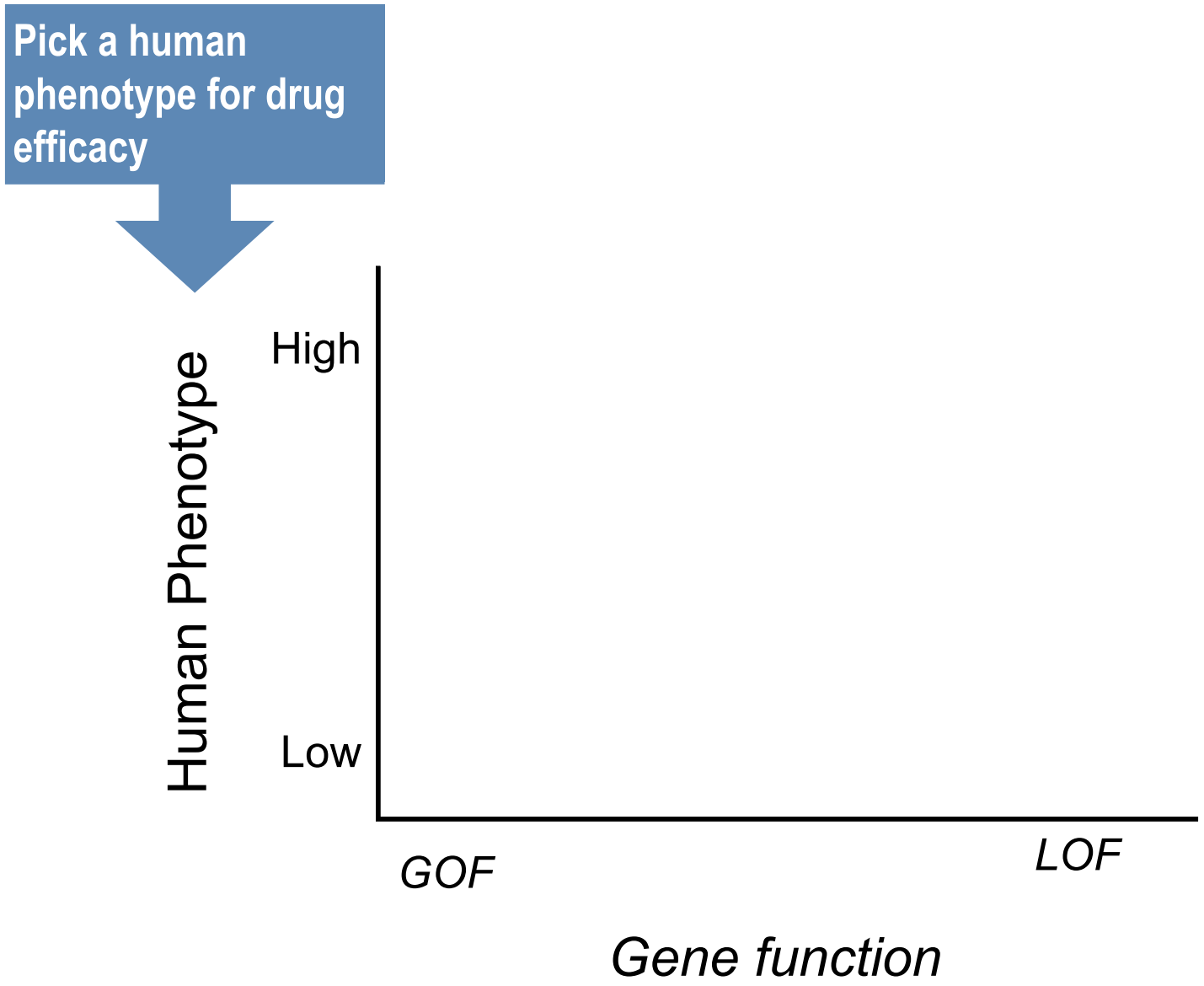
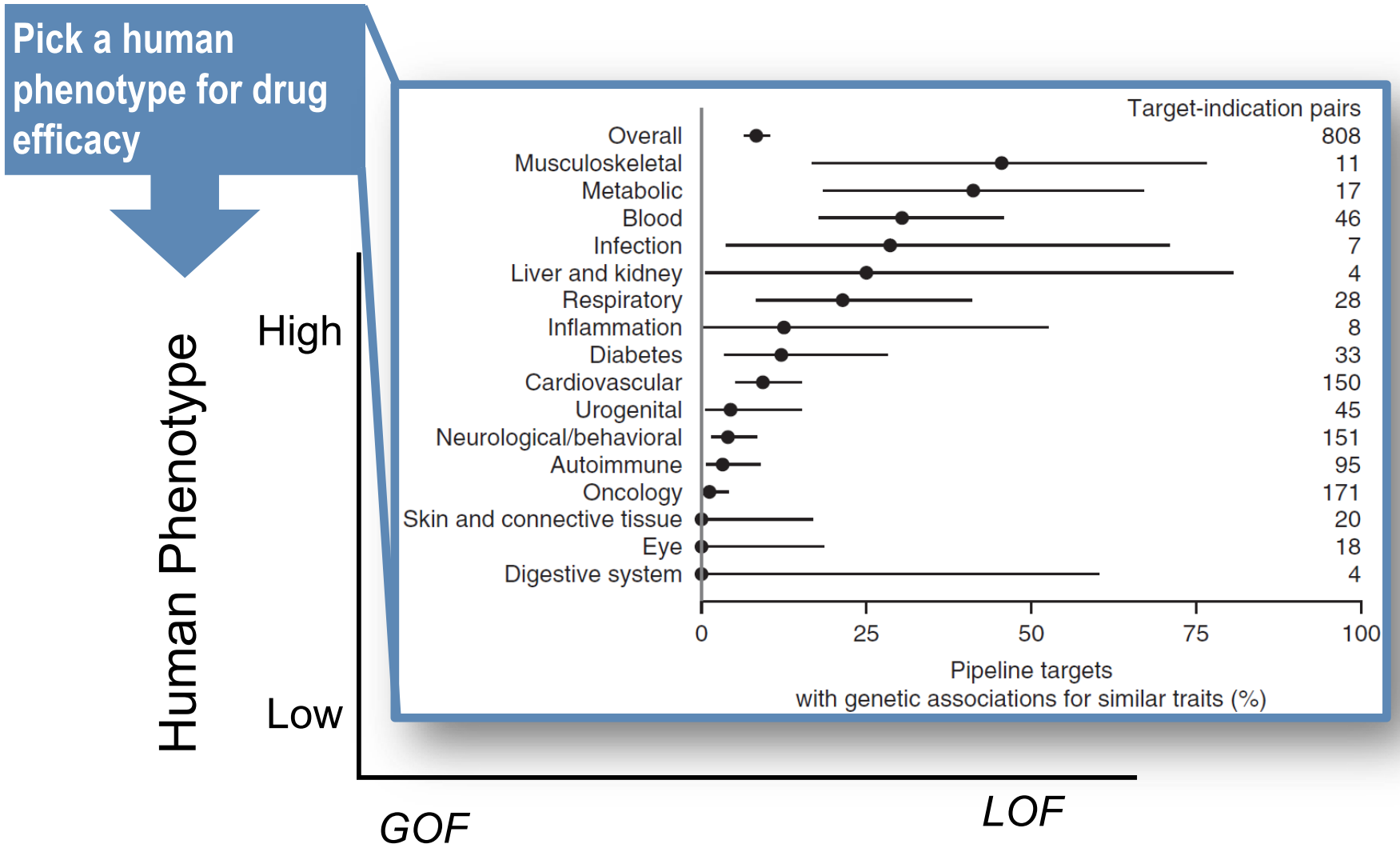
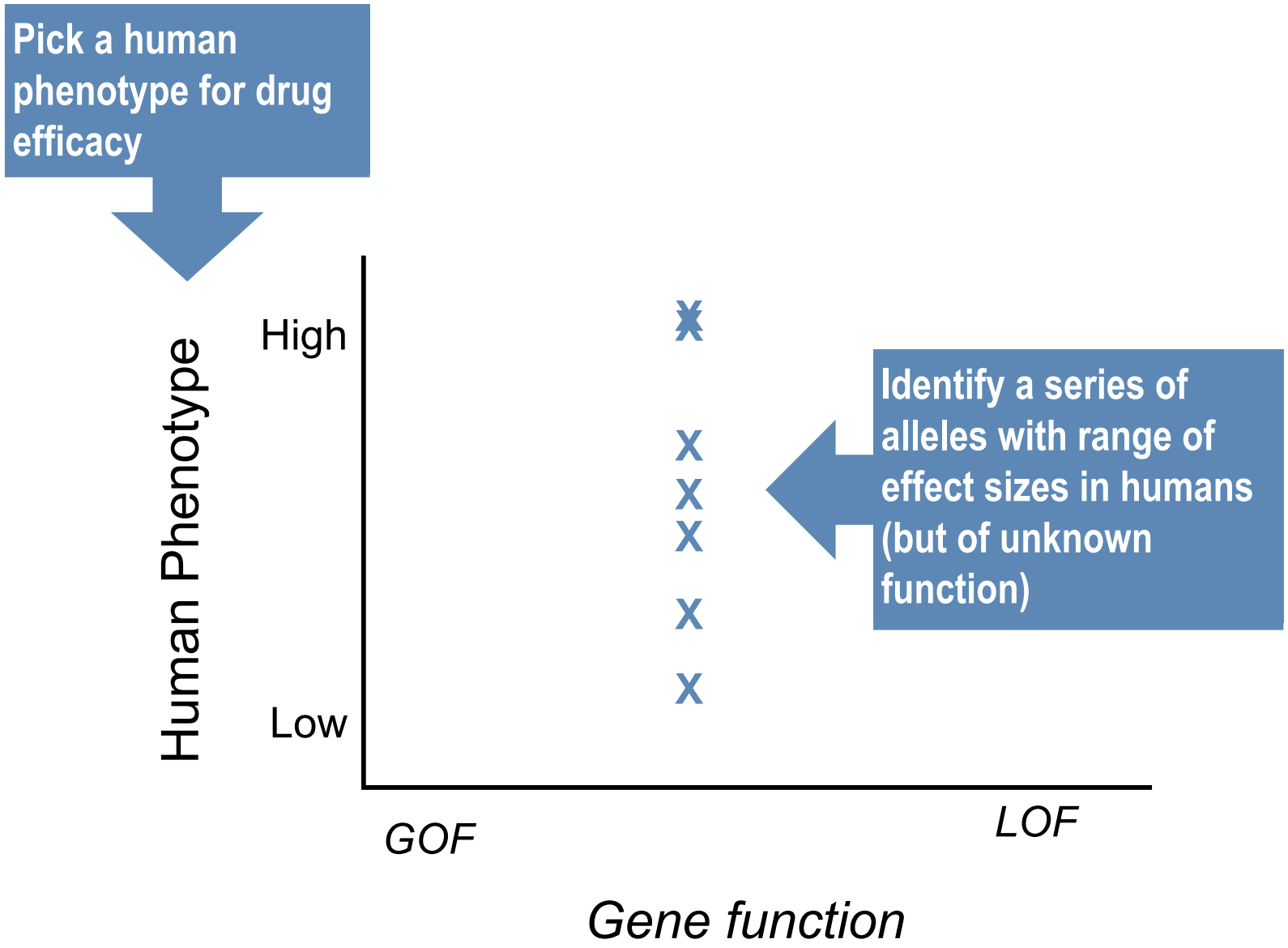
A model



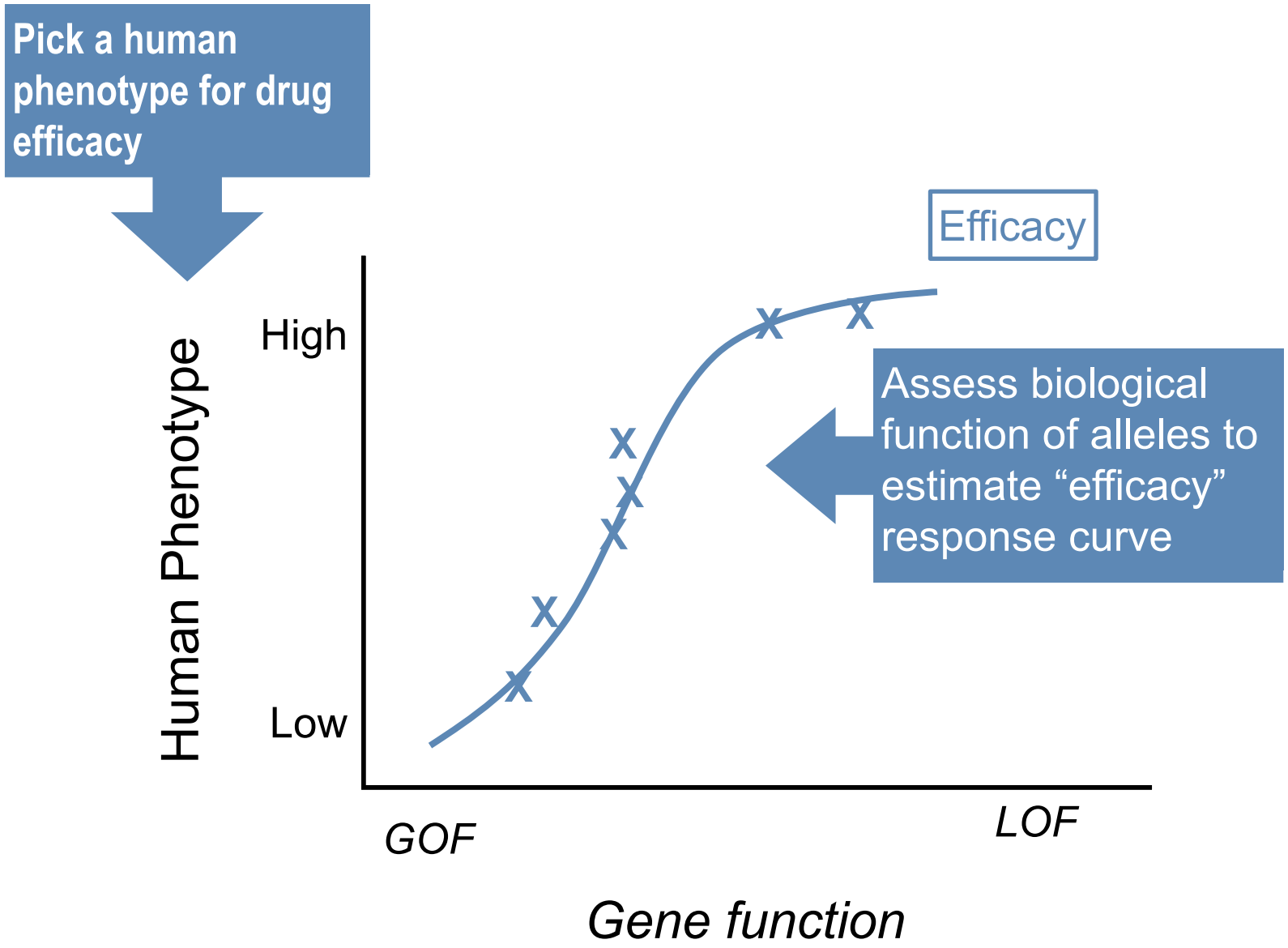
Plenge, Scolnick, Altshuler *Nature Reviews Drug Discovery* (2013)



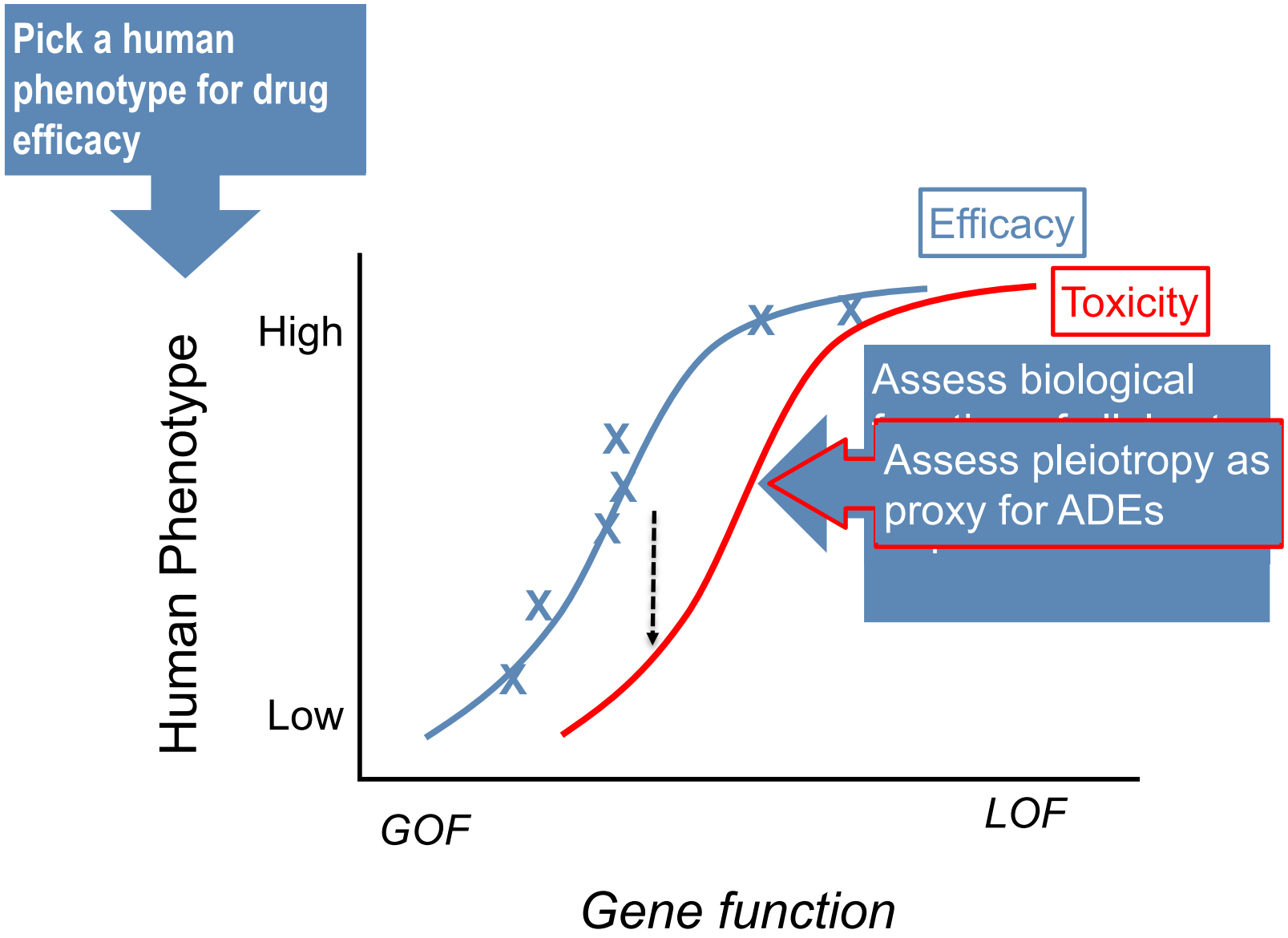
Nelson et al *Nature Genetics* 2015



Plenge, Scolnick, Altshuler *Nature Reviews Drug Discovery* (2013)



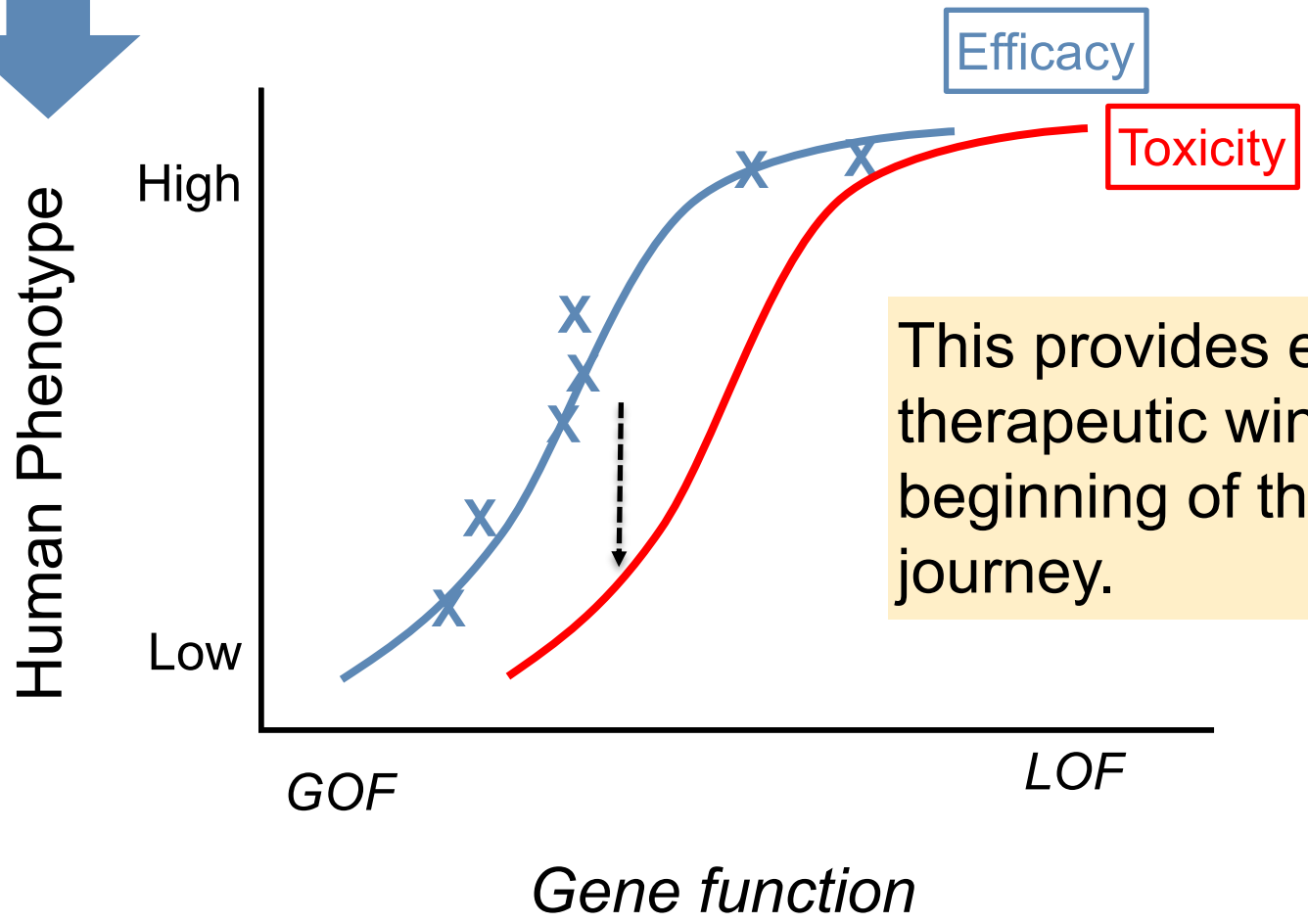
Plenge, Scolnick, Altshuler *Nature Reviews Drug Discovery* (2013)



Plenge, Scolnick, Altshuler *Nature Reviews Drug Discovery* (2013)

New target for drug screen!

Pick a human phenotype for drug efficacy



This provides evidence for the therapeutic window at the beginning of the drug discovery journey.

Plenge, Scolnick, Altshuler *Nature Reviews Drug Discovery* (2013)

An example in immunology

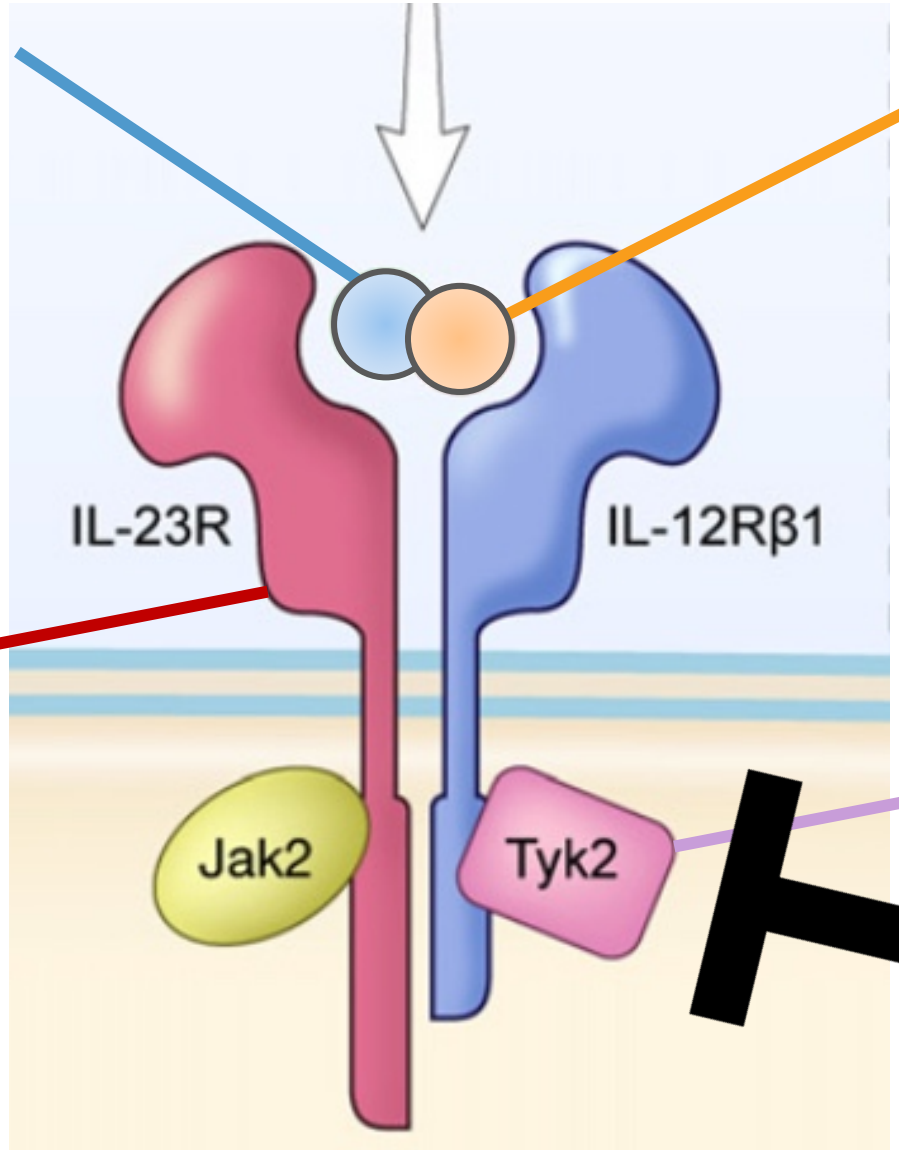
Example of allelic series model: *TYK2*

- TYK2 is an intracellular signaling molecule (next slide)
- Rare, complete human knockout is associated with immunodeficiency and risk of infection
- Common protein coding alleles reduce TYK2 function and protect from risk of autoimmune disease (*e.g.*, psoriasis, RA, SLE, IBD)
- Same common alleles do not increase risk of infection

IL23 signaling and psoriasis

IL23A (IL-23p19)

IL12B (IL-12p40)



IL23R

TYK2

?

O'Shea and Plenge *Immunity* (2012)

nature genetics

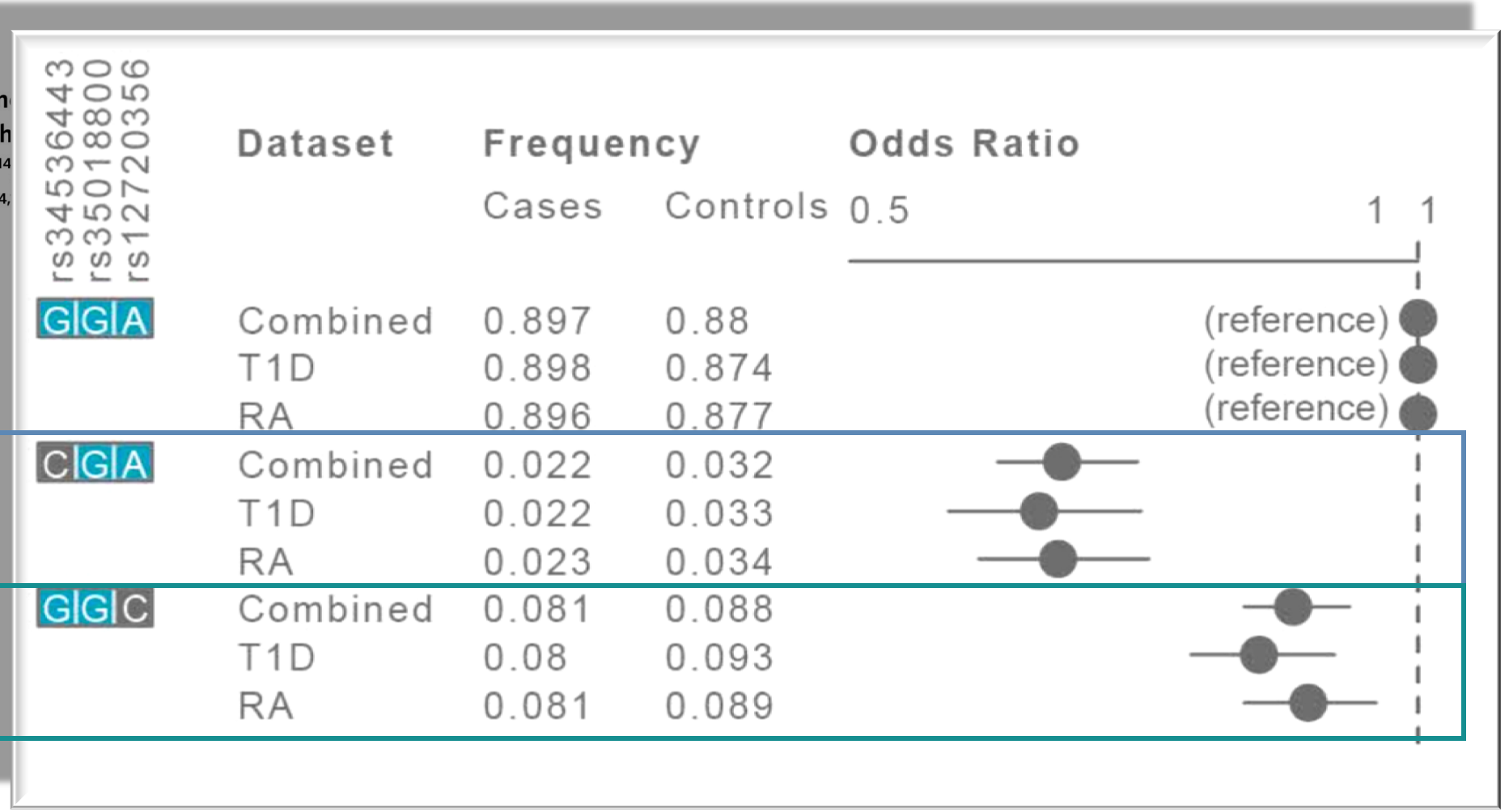
LETTERS

https://doi.org/10.1038/s41588-018-0216-7

TYK2 gene

Fine-mapping and functional studies highlight potential causal variants for rheumatoid arthritis and type 1 diabetes

Harm-Jan Westra[1,2,3,4,5,20], Marta Martínez-Bon
Yang Luo[1,2,3,4], Nikola Teslovich[1,2,3,4], Jane Worth
Lars Klareskog[13], Solbritt Rantapaa-Dahlqvist[14]
John A. Todd[17], Steve Eyre[9,10], Peter A. Nigrovic[4,
Soumya Raychaudhuri[1,2,3,4,9,19]*



P1104A

I684S

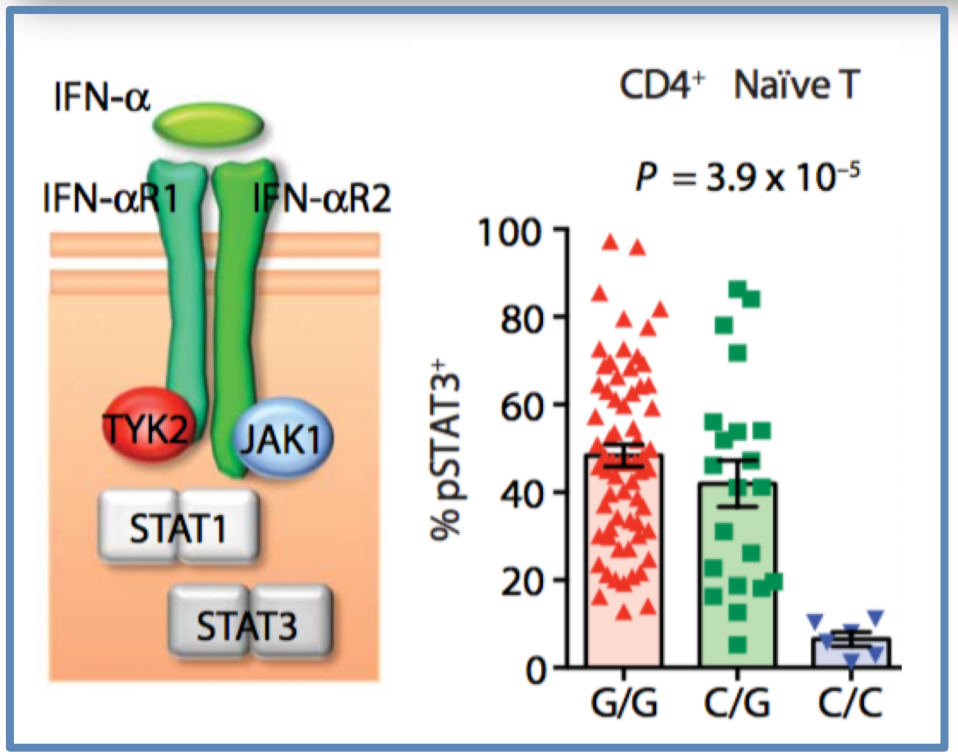
(low freq: A928V)

SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

AUTOIMMUNITY

Resolving *TYK2* locus genotype-to-phenotype differences in autoimmunity

Calliope A. Dendrou,[1] Adrian Cortes,[1,2] Lydia Shipman,[1] Hayley G. Evans,[1] Kathrine E. Attfield,[3] Luke Jostins,[2] Thomas Barber,[1] Gurman Kaur,[3] Subita Balaram Kuttikkatte,[3] Oliver A. Leach,[1] Christiane Desel,[1] Soren L. Faergeman,[1,4] Jane Cheeseman,[5] Matt J. Neville,[5,6] Stephen Sawcer,[7] Alastair Compston,[7] Adam R. Johnson,[8] Christine Everett,[8] John I. Bell,[9] Fredrik Karpe,[5,6] Mark Ultsch,[8] Charles Eigenbrot,[8] Gil McVean,[2] Lars Fugger[1,3,4]*



P1104A allele that protects from autoimmunity is associated with ~80% loss-of-function (LoF) in C/C homozygous state

Same LoF allele has no obvious increased risk of infection

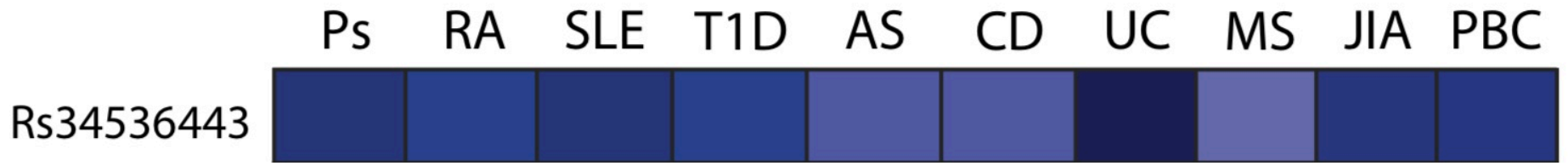
		Rs34536443 genotype			
		G/G	*G/C*	*C/C*	Total
normal	In U.K. Biobank	105,794 (90.63%)	10,689 (9.16%)	249 (0.21%)	116,732 (100%)
Infections	Mycobacterial	20 (86.96%)	3 (13.04%)	0 (0.00%)	23
	Specific bacterial (For example, *S. aureus*)	54 (90.00%)	5 (8.33%)	1 (1.67%)	60
	Specific viral (e.g. HSV, VZV, viral encephalitis)	93 (96.88%)	3 (3.12%)	0 (0.00%)	96
	Mucocutaneous candidiasis	46 (88.46%)	6 (11.54%)	0 (0.00%)	52
	Total	213 (92.21%)	17 (7.36%)	1 (0.43%)	231

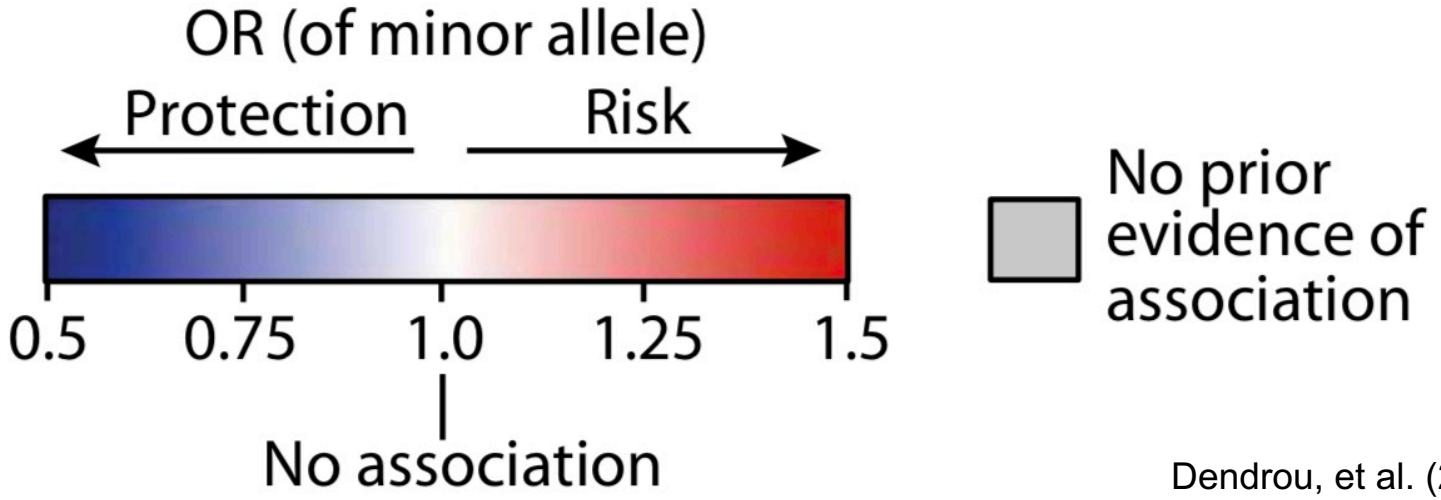
~80% LoF is *not* associated with increased infection

Dendrou, et al. (2016)
Science Translational Medicine

P1104A protects from multiple autoimmune diseases

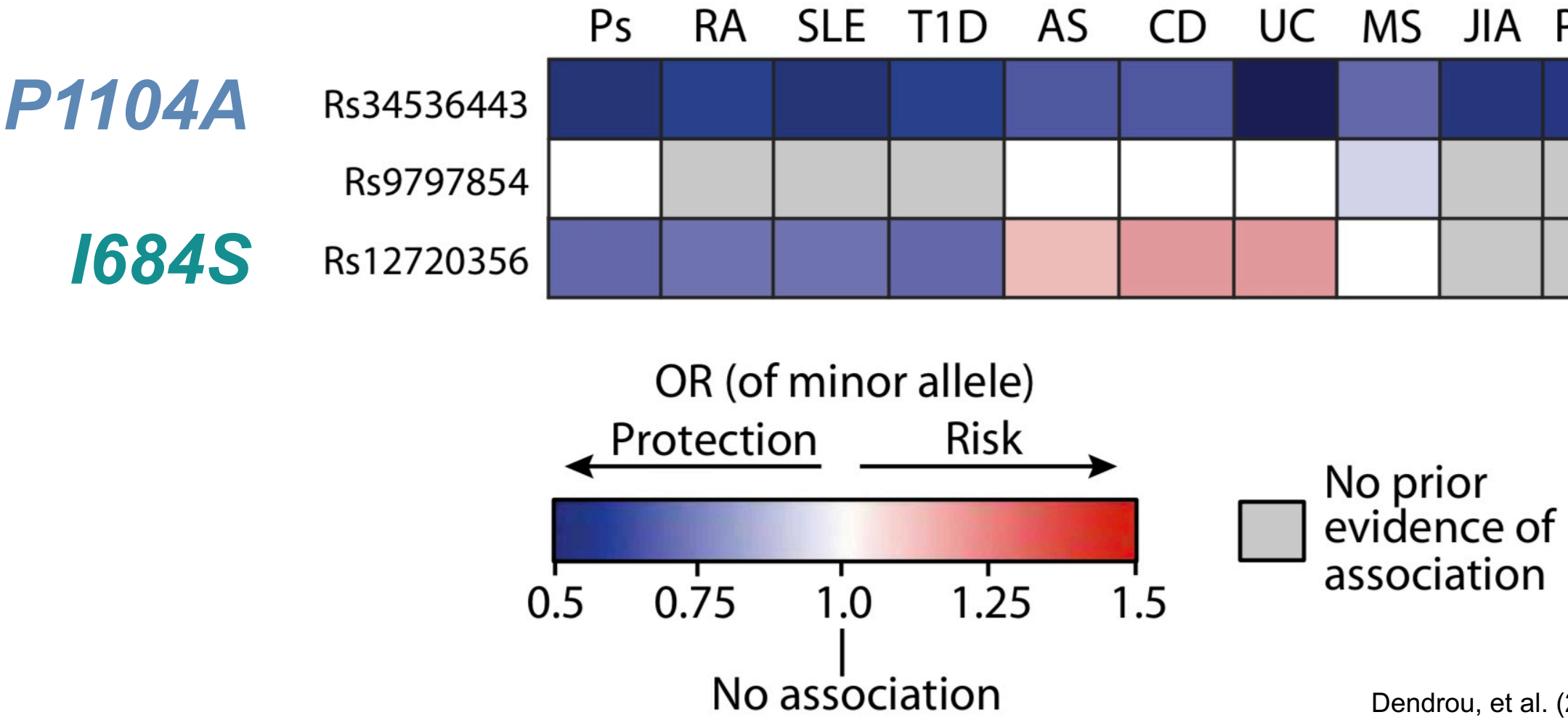
P1104A





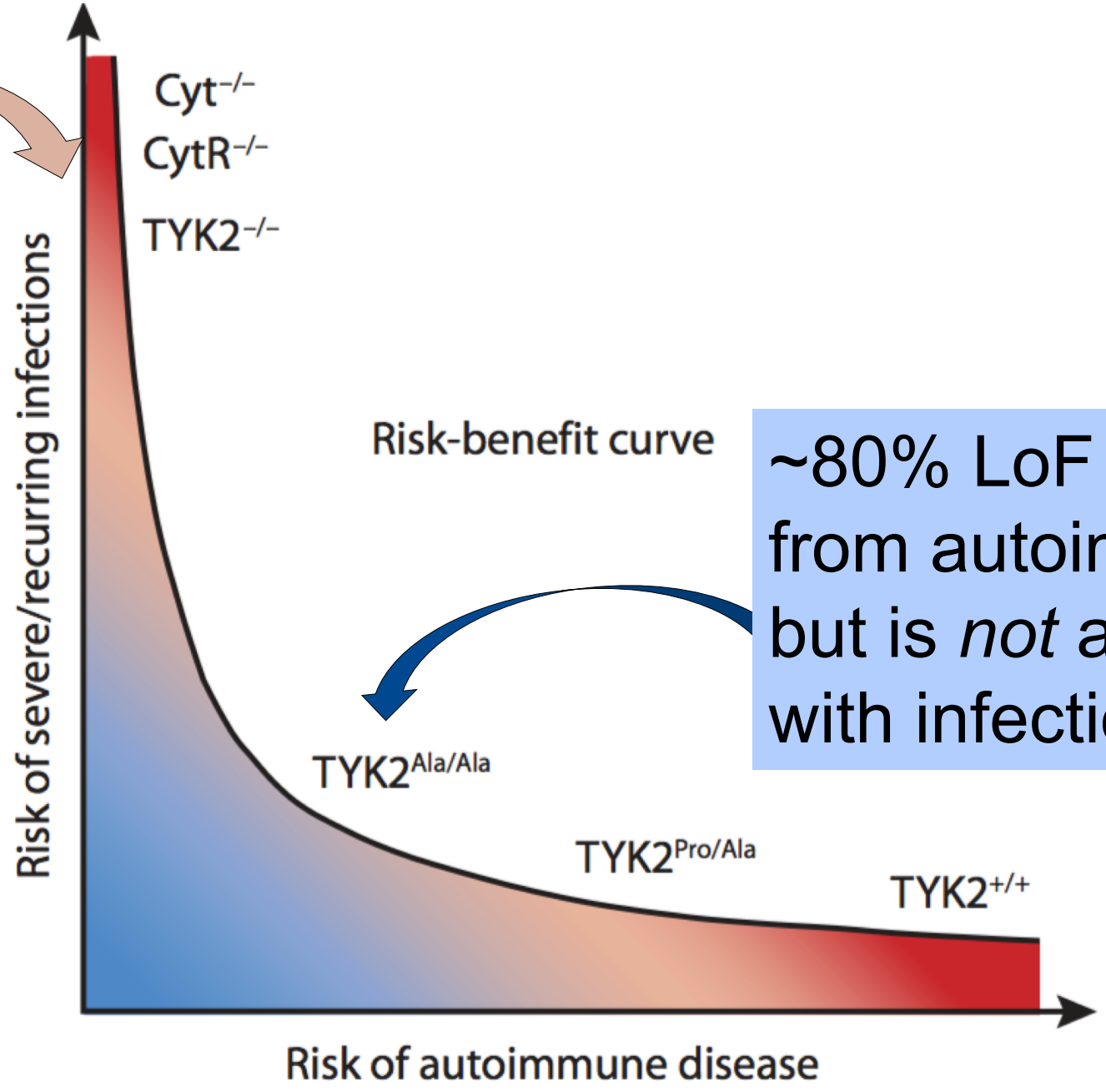
Dendrou, et al. (2016)
Science Translational Medicine

But *I684S* variant shows a more complicated pattern!

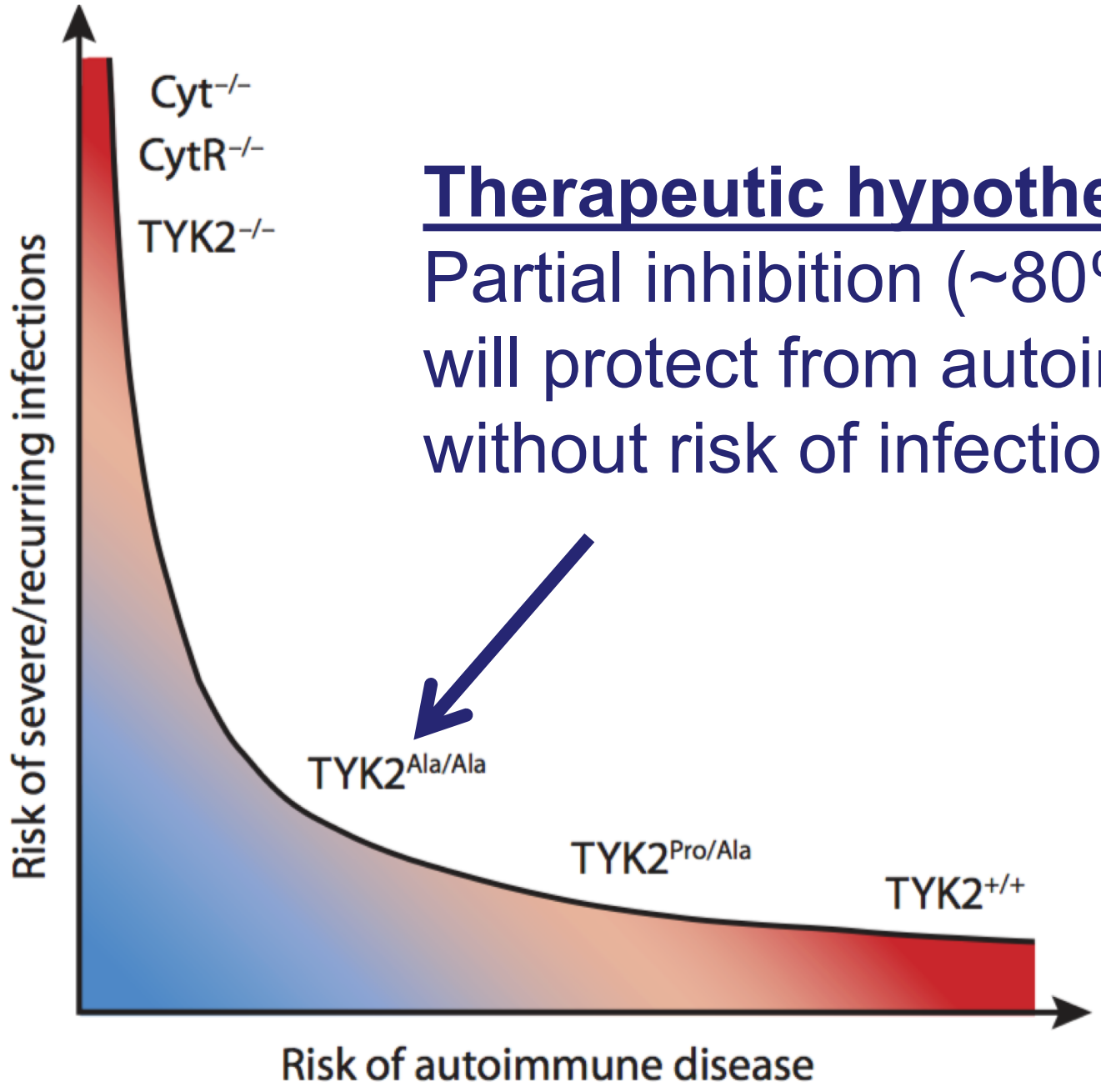


Dendrou, et al. (2016)
Science Translational Medicine

Complete TYK2 knockout increases risk of infection

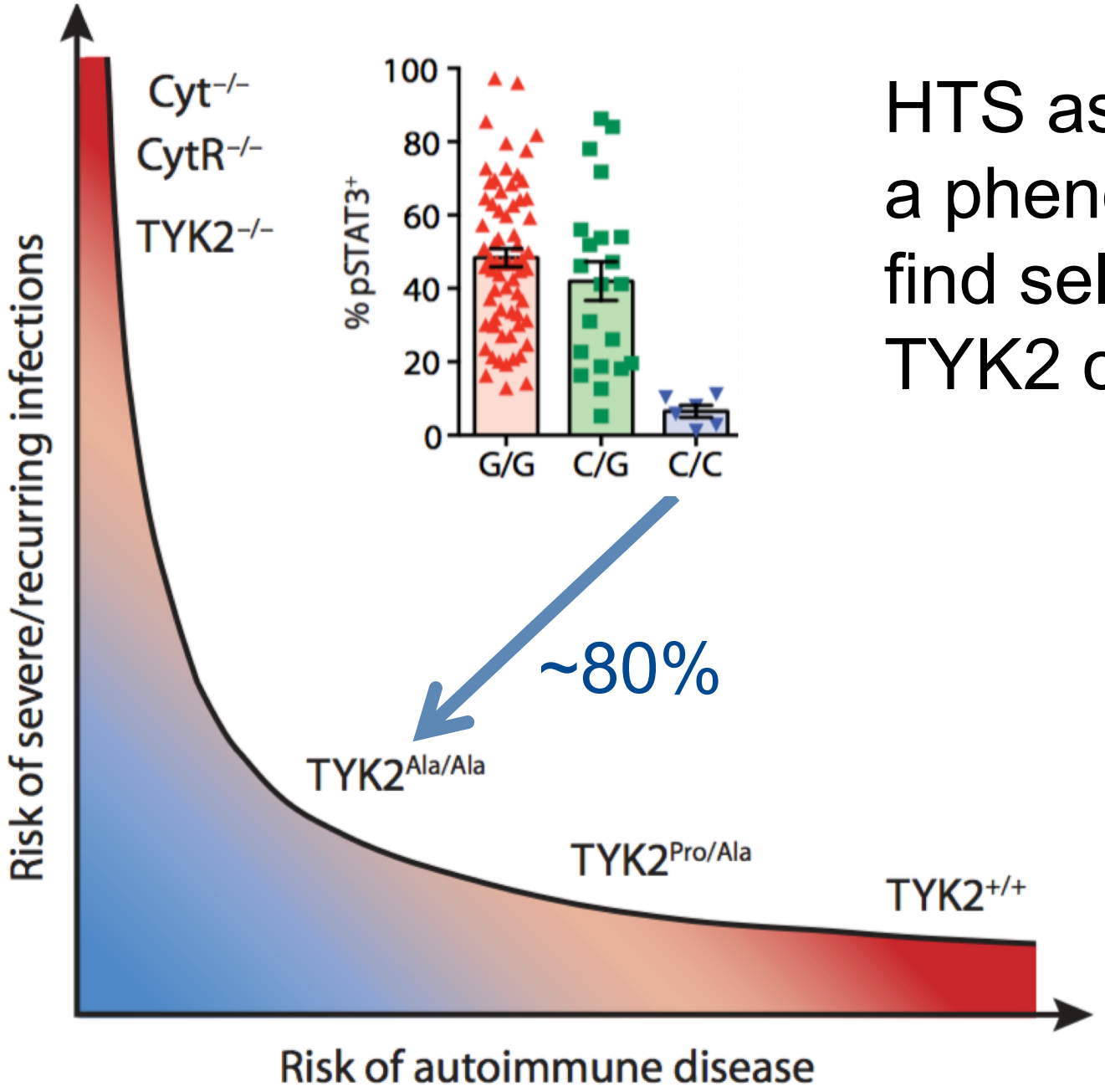


~80% LoF protects from autoimmunity but is *not* associated with infection

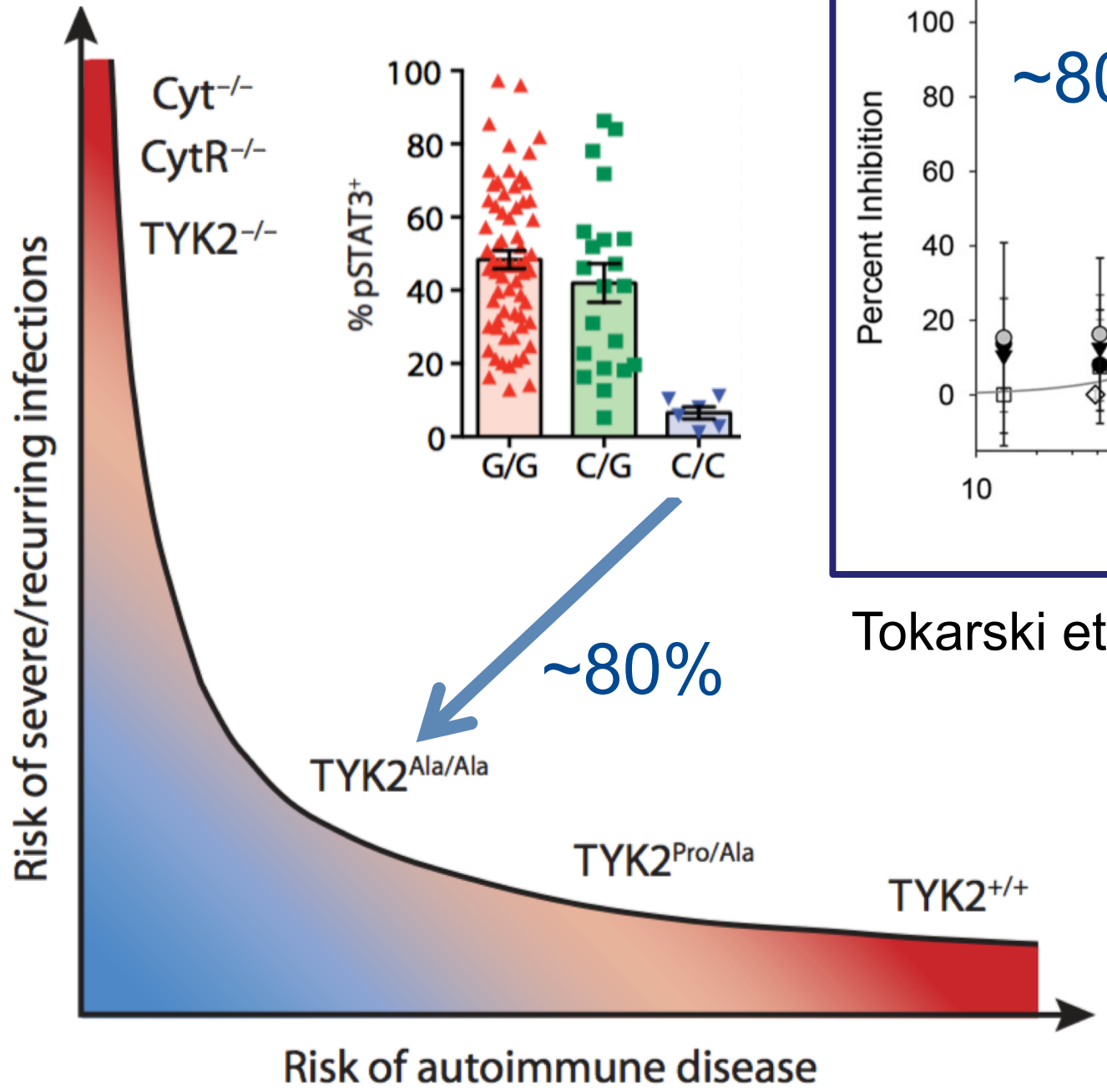


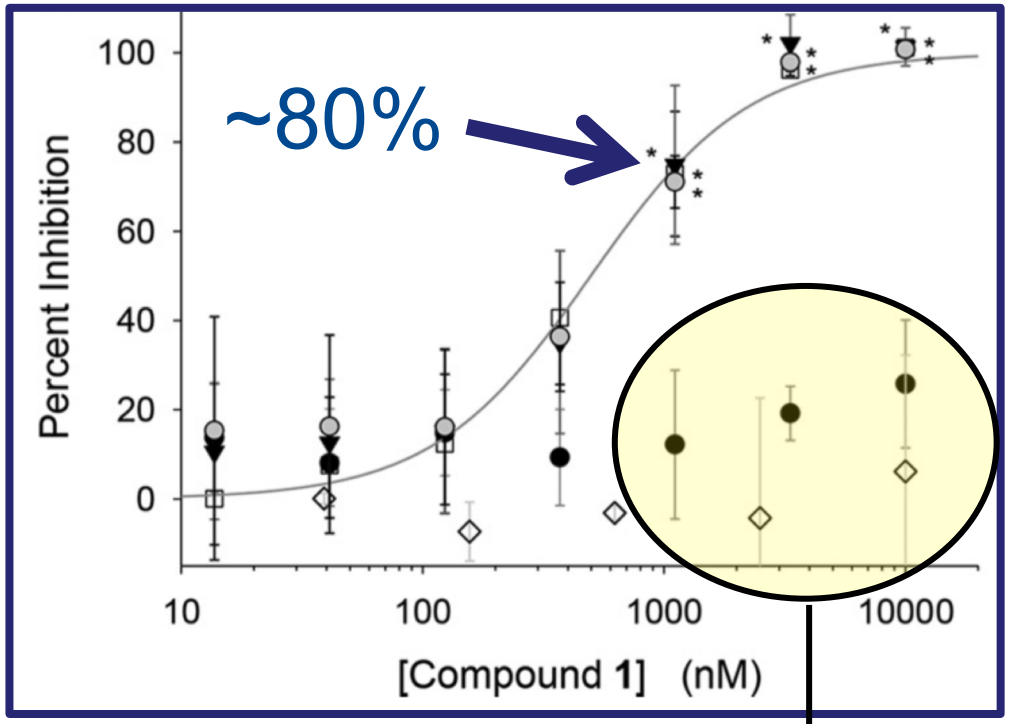
<u>Therapeutic hypothesis</u>:
Partial inhibition (~80%) of TYK2 will protect from autoimmunity without risk of infection

But matching *modality with mechanism* is challenging, especially selectivity over JAKs



HTS assay was used in a phenotypic screen to find selective inhibitors of TYK2 over other JAKs





Tokarski et al (2015) *JBC*

Retains selectivity over JAKs

Matching modality and mechanism: *"pseudokinase stabilizers" recapitulate human genetic mutations at functional level*

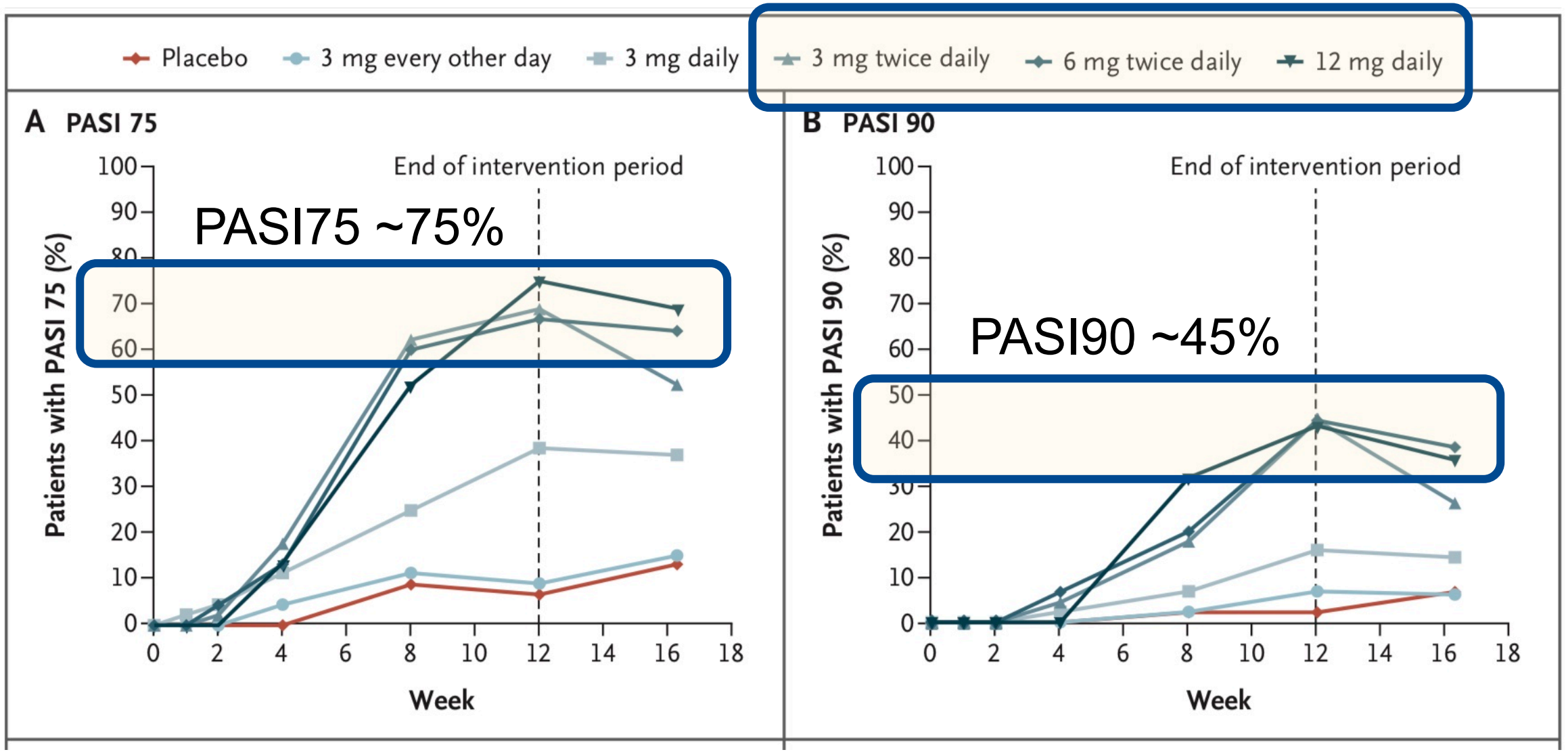
Allosteric inhibitor
(pseudokinase domain)

Orthosteric inhibitor
(kinase domain)

P1104A

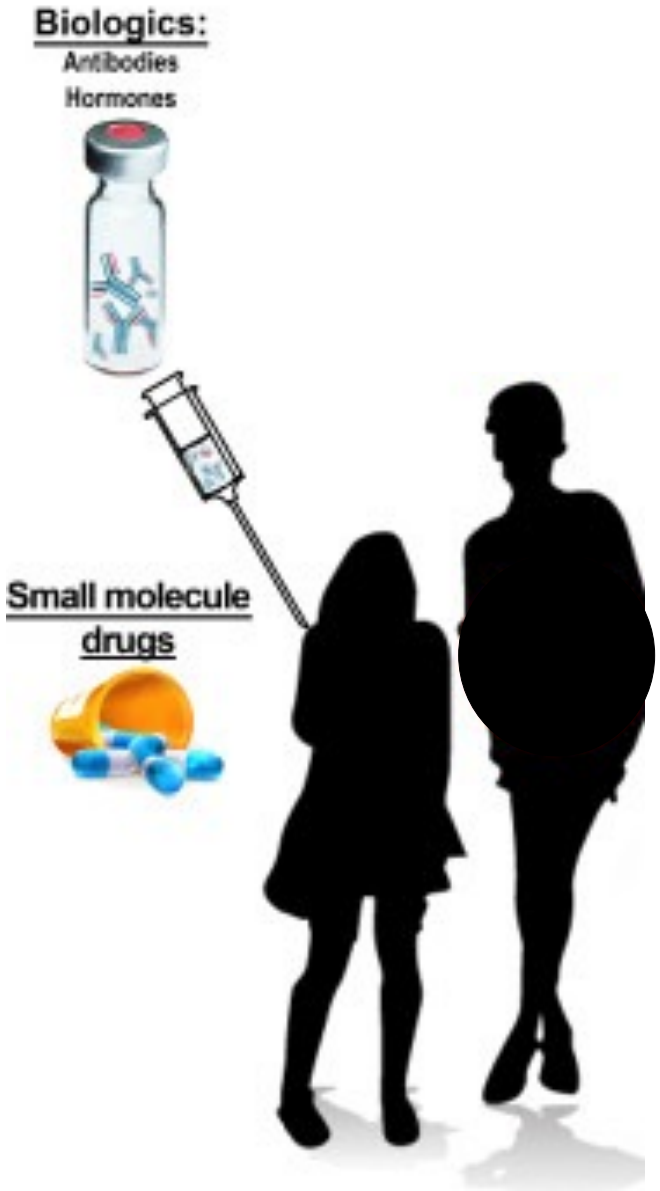
Tokarski et al *JBC* (2015)
Lupardus et al *PNAS* (2014)

50-80% TYK2 inhibition safe and effective in Phase 2 (psoriasis)



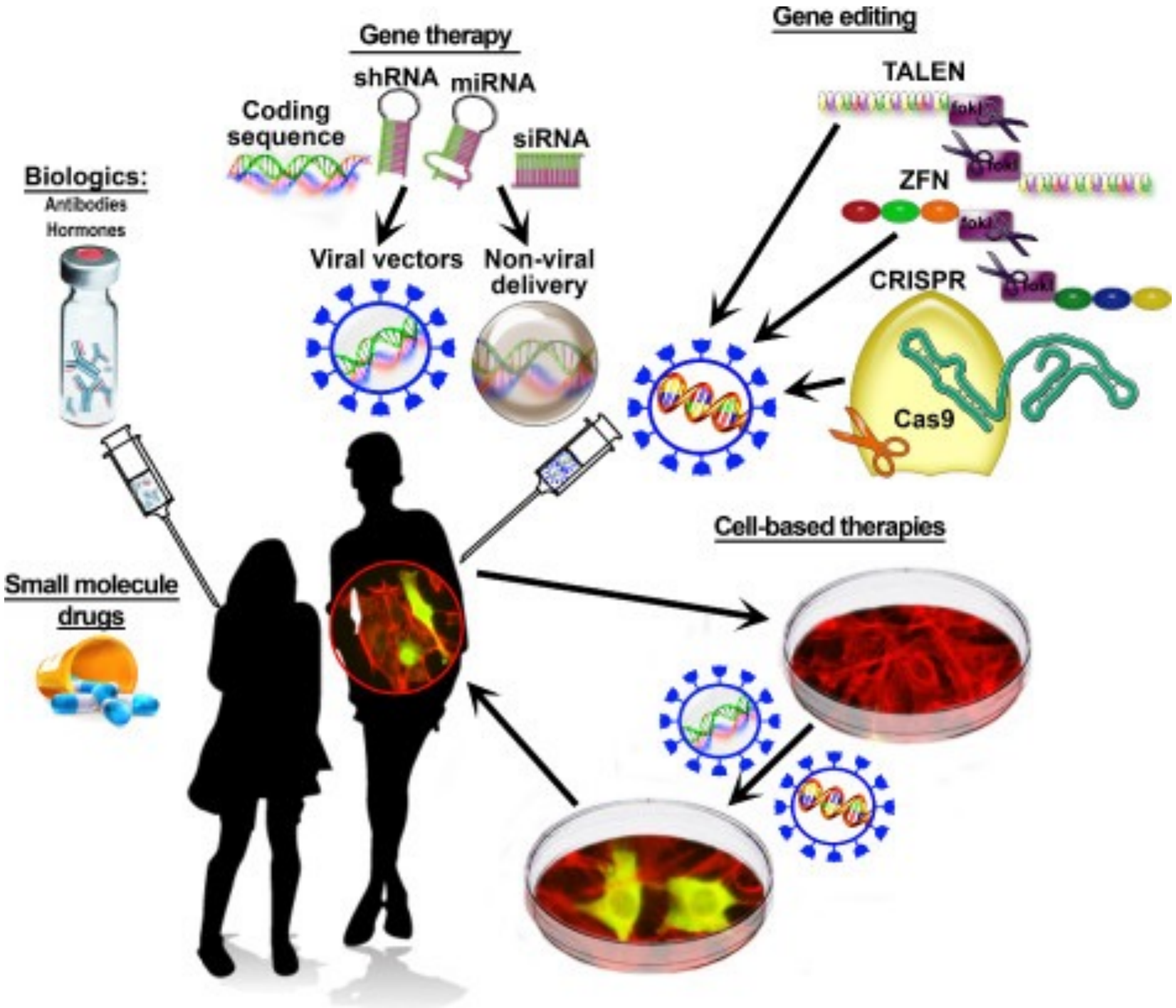
Papp et al (2018) *NEJM*

Matching *modality with mechanism* is a rate-limiting event in drug R&D



While we often first think of "conventional" small molecule and monoclonal antibodies…

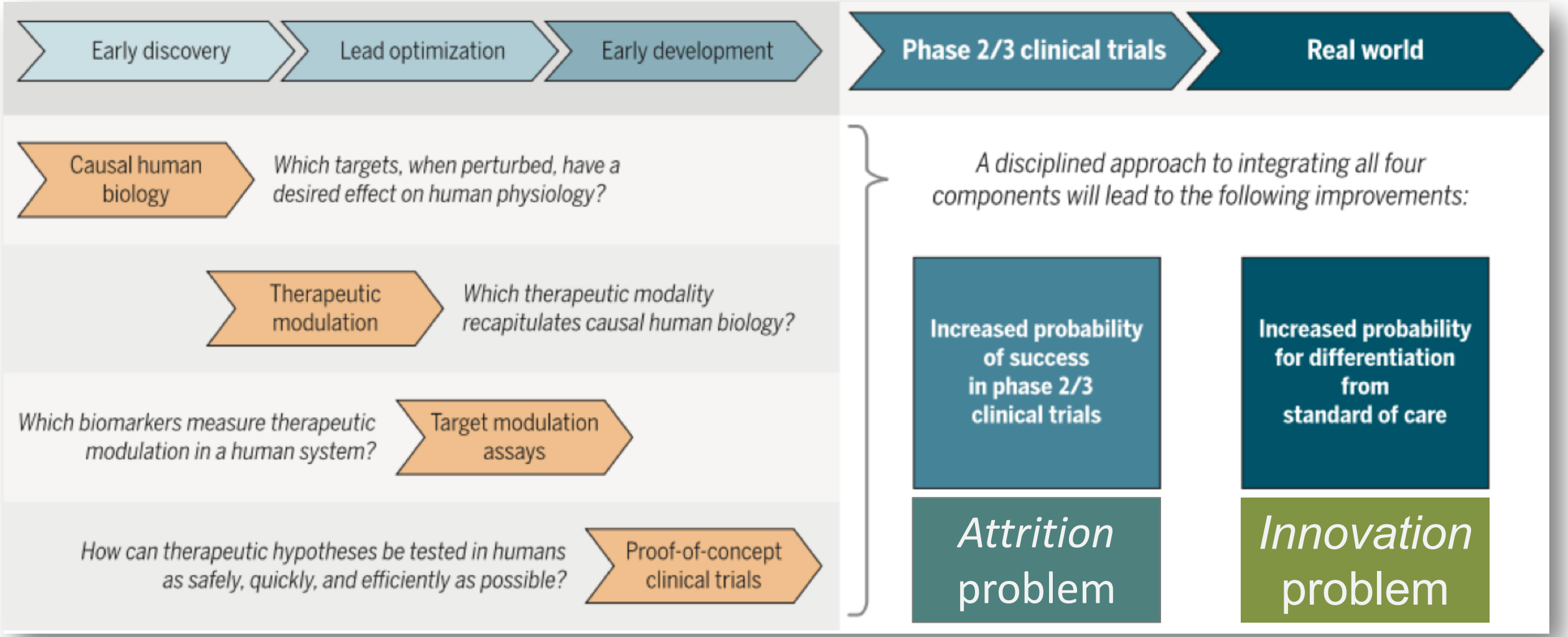
…there are many burgeoning therapeutic modalities



Go to examples

Other

mRNA replacement
protein degradation
macrocyclic peptides
microbiome
…*and more to come!*



Plenge *Science Translational Medicine* (2016)

What does the future hold?

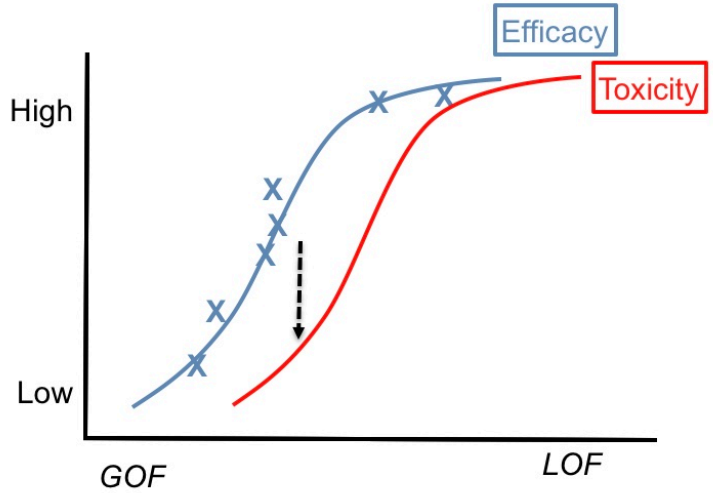
What would be *transformational*?

Five key areas to realize this future state

- **Genetic dose-response portal** – *beyond GWAS*
  - Continued genetic discovery with function / phenotype integrations
- **Causal human biology** – *beyond germline genetics*
  - Human pharmacology and human immunology
- **Matching modality & mechanism** – *beyond "conventional" medicines*
  - Cell and gene therapy for "living" therapeutics
- **Programmable therapeutics** – *beyond linear drug R&D*
  - Approved platform for all but final registrational trials
- **Digital confluence** – *beyond wearables and AI*
  - When discovery, development, and the real-world collide

How to build a genetic dose-response portal

- Genetic architecture of human disease
  - continued sequencing of rare, Mendelian diseases
  - human knockout project (e.g., dbLoF)
  - exome sequencing in case-control cohorts
- Functional interrogation
  - high-throughput assessment of mutations
  - single cell analyses in disease tissues at population scale
- Pleiotropy
  - integrated population-based biobanks with genotype / phenotype data
  - quantitative traits as biomarkers
- Data analysis
  - statistical methods to model dose-response
  - data integration and visualization



Go to PheWAS

Go to pQTL and MR

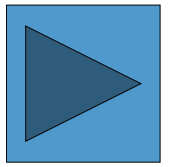
Go to *IL6R*

Blog on plengegen.com

Causal human biology – *beyond genetics*

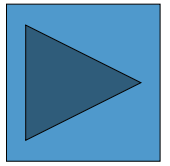
- Human pharmacology
  - Effective pharmacology (e.g., low-dose IL2 in autoimmunity)
  - Rationale combinations (e.g., apremilast + other MoA)
  - Non-responder populations (e.g., resistance to checkpoint inhibitors)
  - Bi-specifics (e.g., tissue targeting, T cell engagers)

Go to apremilast

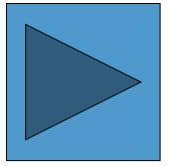


- Human immunology
  - Mapping peptide epitopes for autoantigens in autoimmunity
  - TCR and BCR sequencing of pathogenic lymphocytes in autoimmunity
  - Single-cell profiling is disease tissue (e.g., NIH-funded AMP)
  - Cloning therapeutic antibodies from elite responders
  - Cloning TCRs and BCRs for cell therapy
  - Characterization of neoantigens for cancer immune therapy

Go to vasculitis, celiac



Go to neoantigens

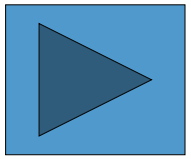


Blog on plengegen.com

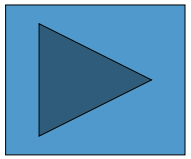
New therapeutic modalities – *beyond conventional molecules*

- Nucleic acid-based therapies
  - Cell therapies (e.g., CAR-T)
  - *ex vivo* gene replacement (e.g., PID)
  - mRNA gene replacement
  - Genome editing (e.g., CRISPR)
  - Anti-sense oligonucleotides (ASOs)
  - Small interfering RNA (siRNA)
- Non-nucleic acid-based therapies
  - Protein degradation
  - Microbiome
  - Peptides (e.g., tolerizing antigens)

Go to cell therapy



Go to modalities



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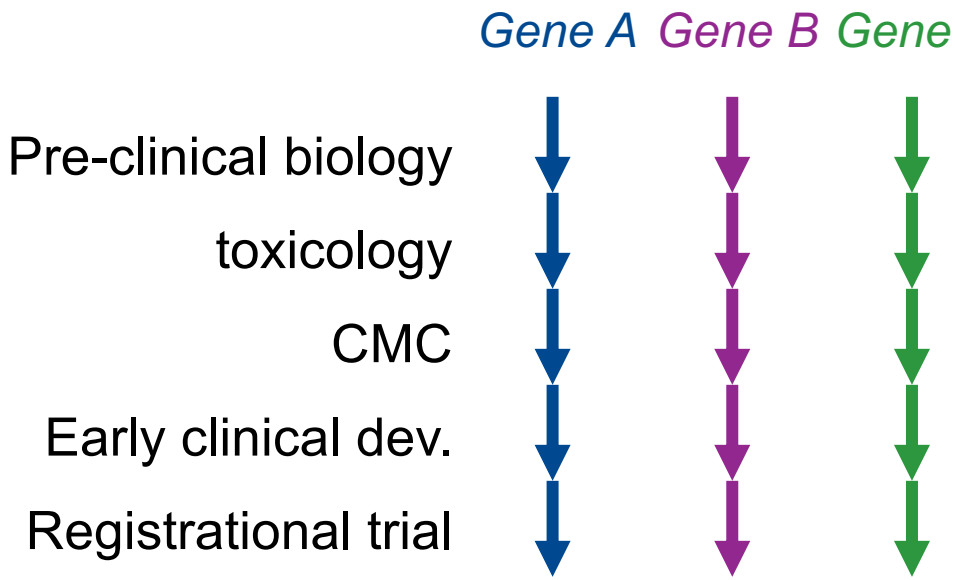
Programmable therapeutics for gene replacement

Conventional approach: one gene, one disease, one linear regulatory path to approval (e.g., pre-clinical biology, toxicology, CMC, early clinical dev, registrational trials)

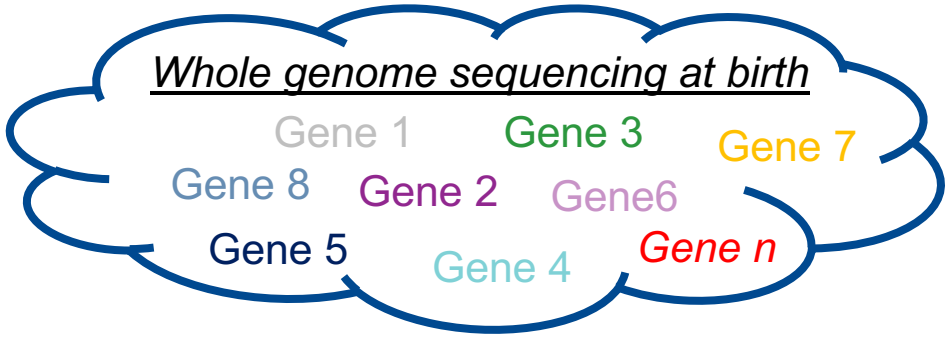


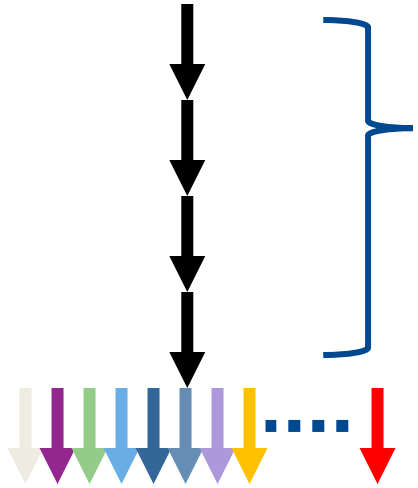
[Blog on plengegen.com]

Go to PID

Go to mRNA

Programmable approach: introduce a new gene into a "programmable" platform that has been approved for all pre-registrational trial activities



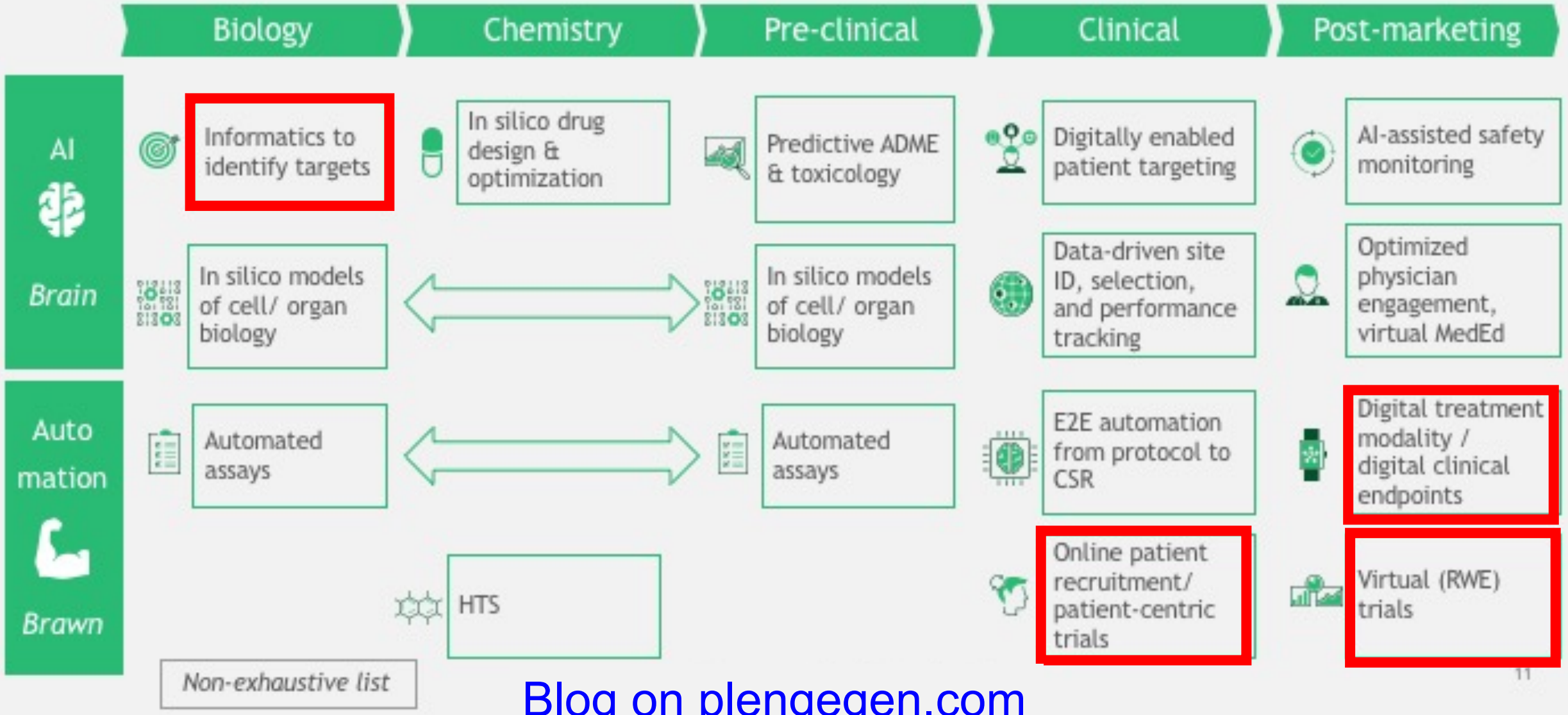


Programmable platform

Registrational trial w/ virtual cohorts

Regulatory approval

Digital confluence

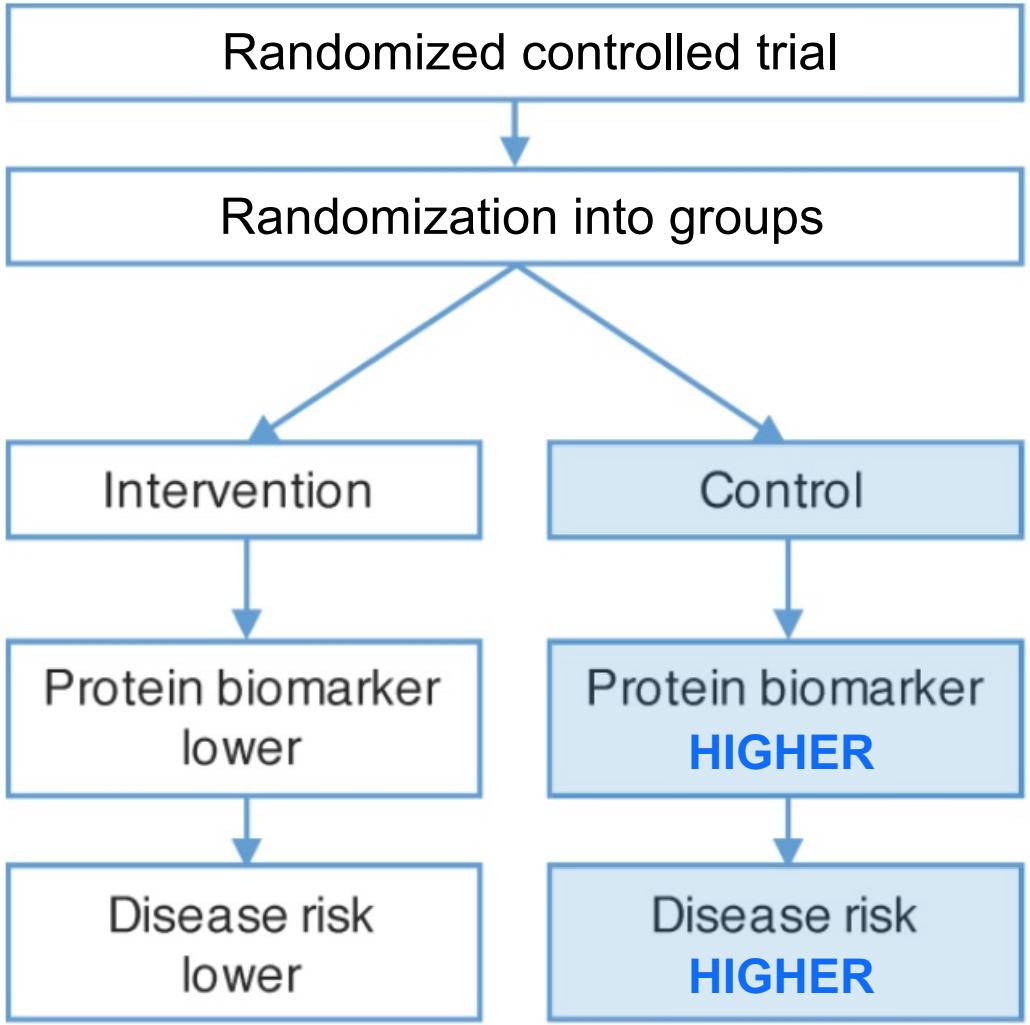


[Blog on plengegen.com]

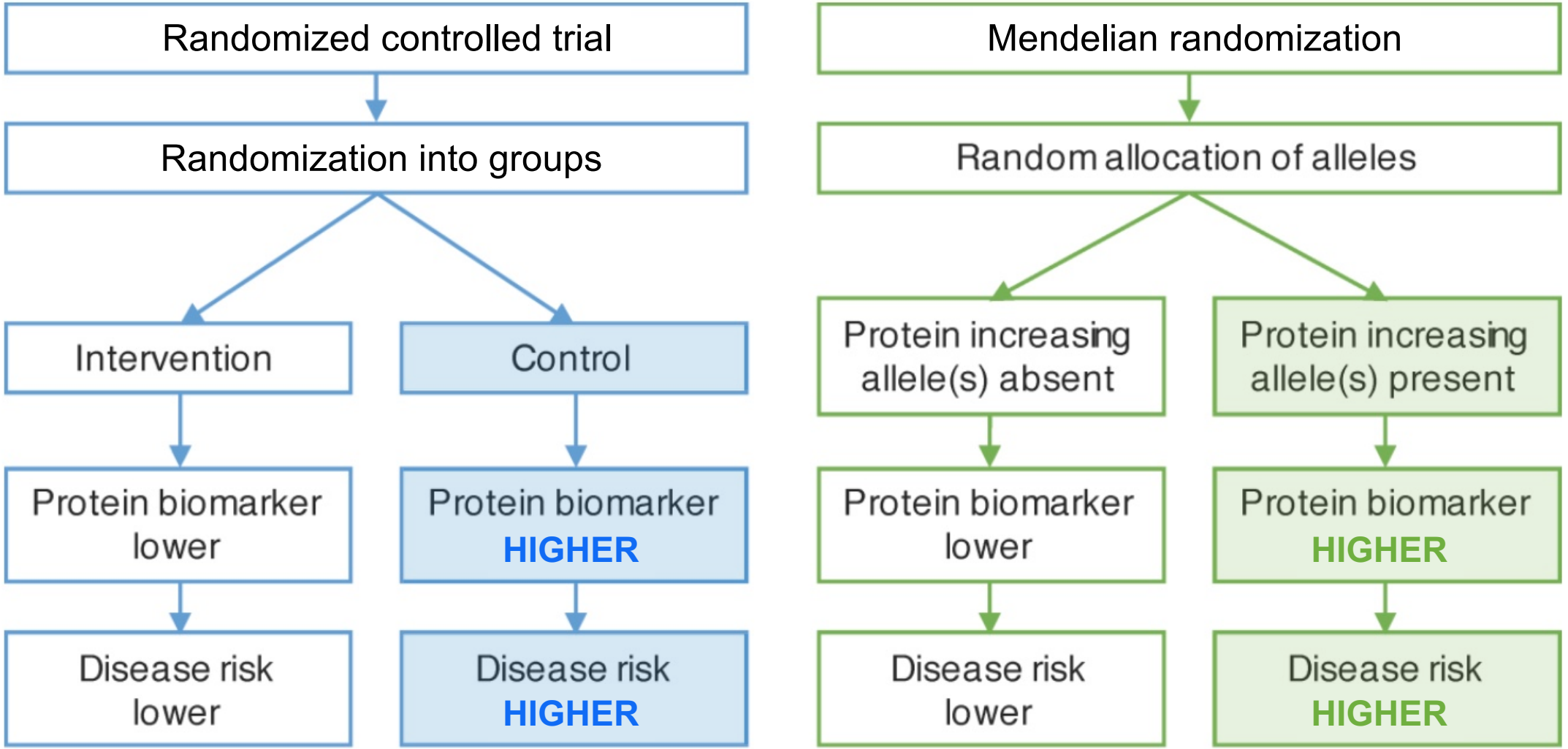
Back-ups

Mendelian randomization

Mendelian randomization: *nature's clinical trial*

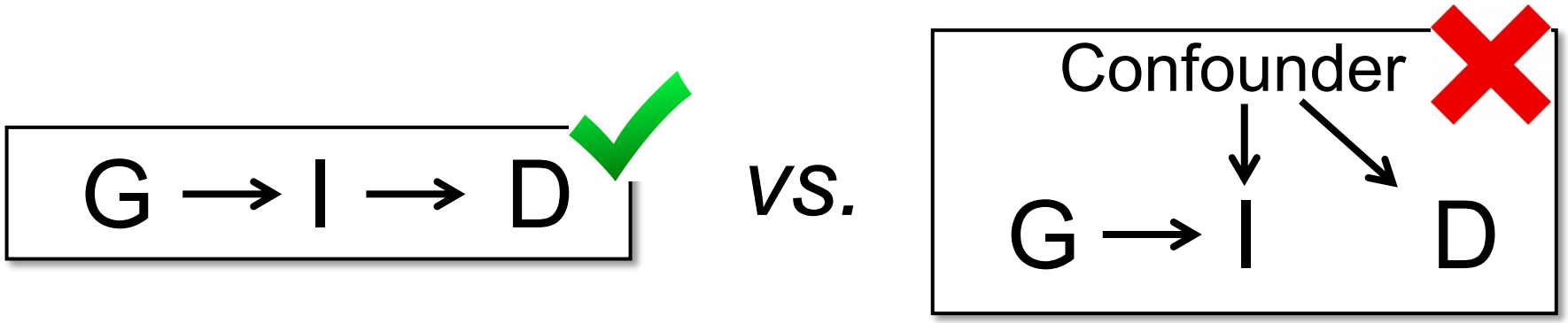


Mendelian randomization: *nature's clinical trial*

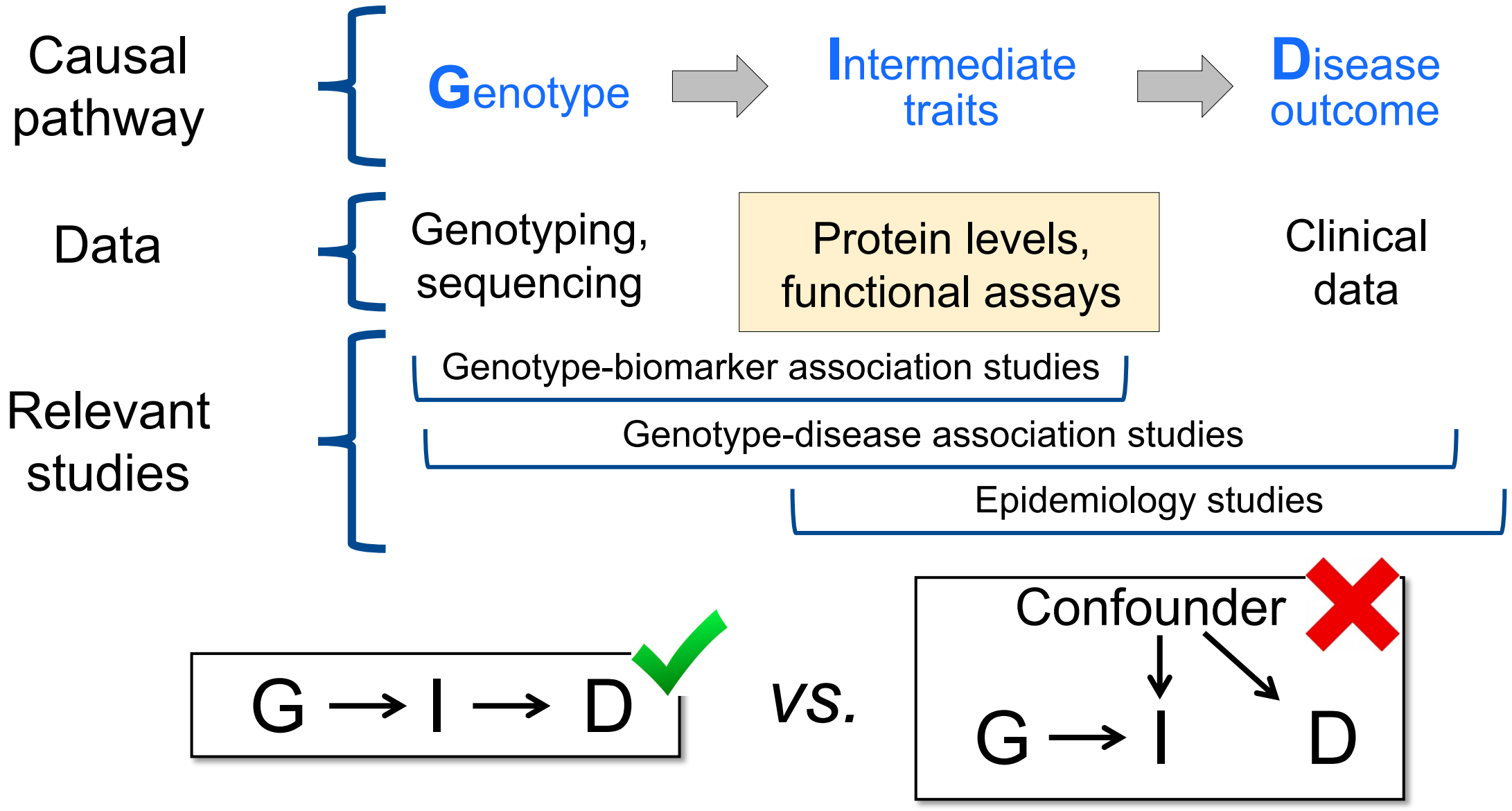


Genetics can bridge biomarker with clinical data, establishing a causal link for drug discovery

Causal pathway	**G**enotype →	**I**ntermediate traits →	**D**isease outcome
Data	Genotyping, sequencing	Protein levels, functional assays	Clinical data
Relevant studies	Genotype-biomarker association studies		
	Genotype-disease association studies		
	Epidemiology studies		



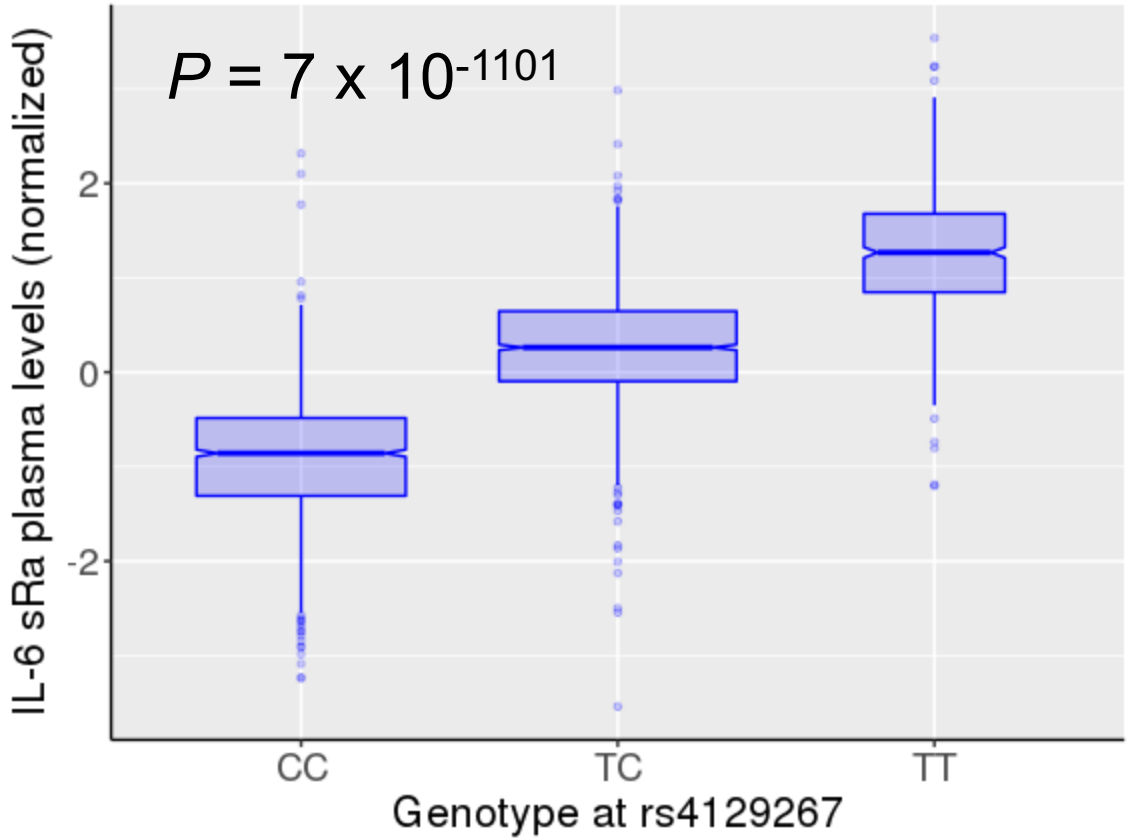
Large-scale proteomic databases are limiting



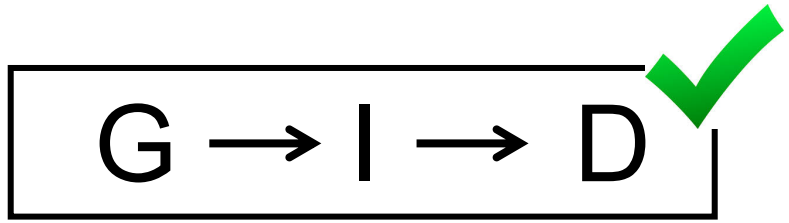
Emerging resource of pQTLs for MR

- Tested 3,622 plasma proteins in 3,301 healthy individuals from INTERVAL population cohort
- Identified 1,927 genetic associations with 1,478 proteins
- **Example**: *IL-6R* RA protective allele increases sIL-6R levels (see figure) but decreases membrane-bound IL6R
- **Therapeutic hypothesis**: preventing IL-6 signaling through IL-6R via blocking antibodies should treat RA symptoms

Sun, Maranville *et al (*2018) *Nature*



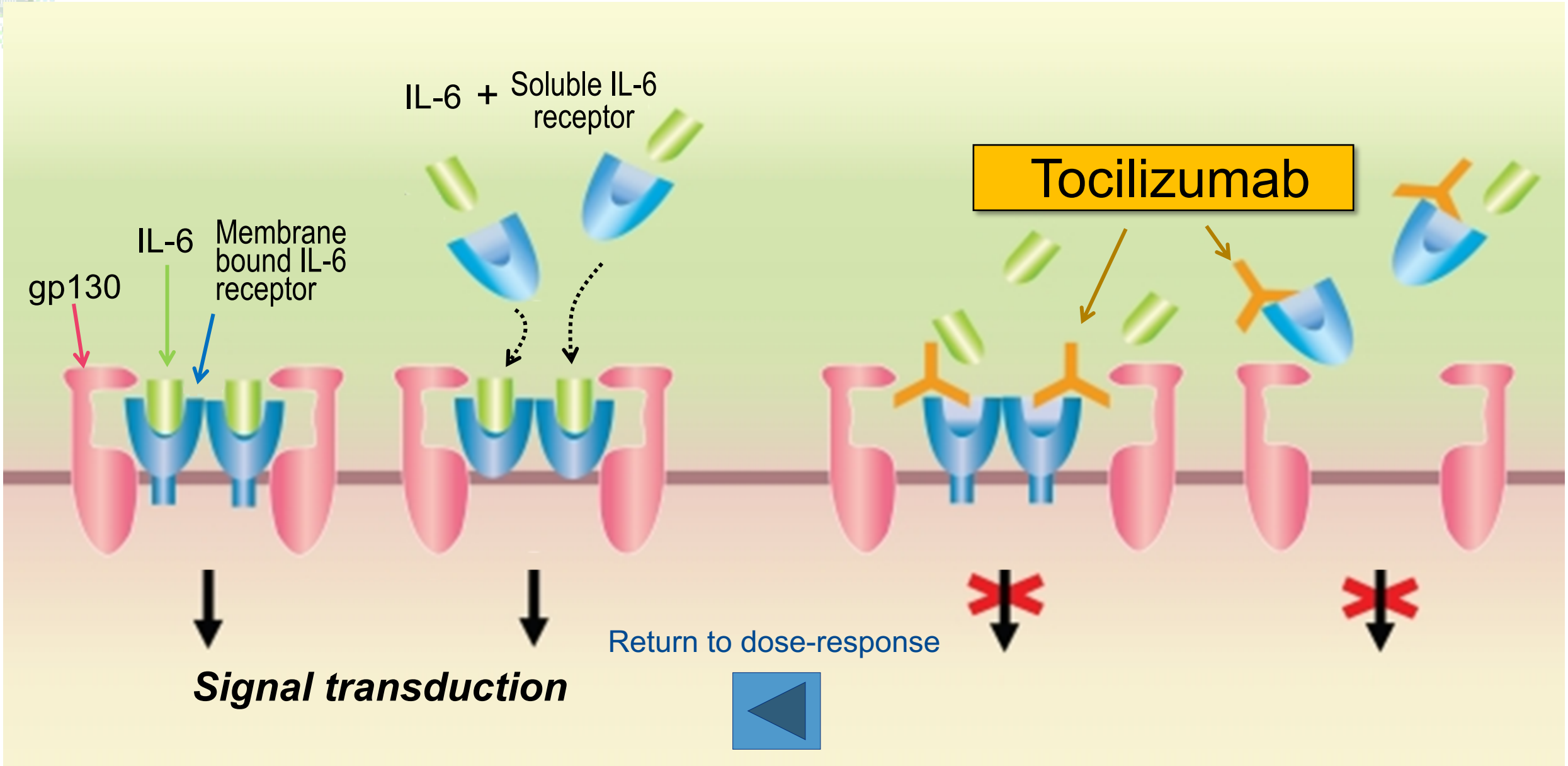
Mendelian randomization establishes a causal link between IL-6 pathway and risk of rheumatoid arthritis



IL-6R minor variant → *Less membrane IL-6R and less signaling* → *Protection from RA*

Thus, therapeutic targeting of IL-6R should be beneficial in treating RA patients

Tocilizumab mimics mutation by reducing IL-6R signaling



Phenome-wide association study (PheWAS)

Phenome-wide association studies (PheWAS)

Disease-Agnostic cohort

EHRs, Claims, Questionnaires, etc.

Clinical data

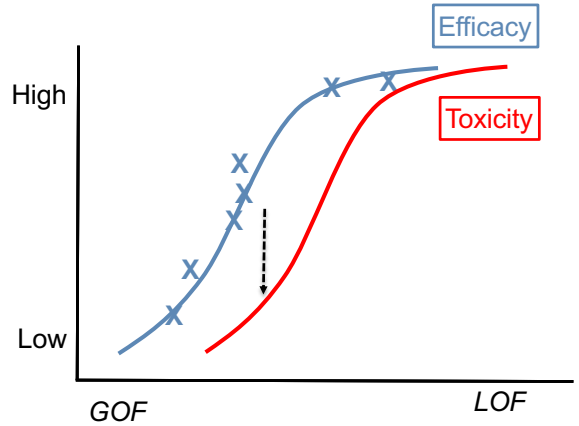
Genetic data

GWAS, exome sequencing, etc.

LoF SNP

Test association of selected SNP with clinical endpoints

- PheWAS code 1 — risk — surrogate for efficacy
- PheWAS code 2
- PheWAS code 3
- PheWAS code 4
- PheWAS code 5 — risk — surrogate for toxicity
- ⋮
- PheWAS code 500



nature genetics

LETTERS

https://doi.org/10.1038/s41588-018-0216-7

IFIH1 gene

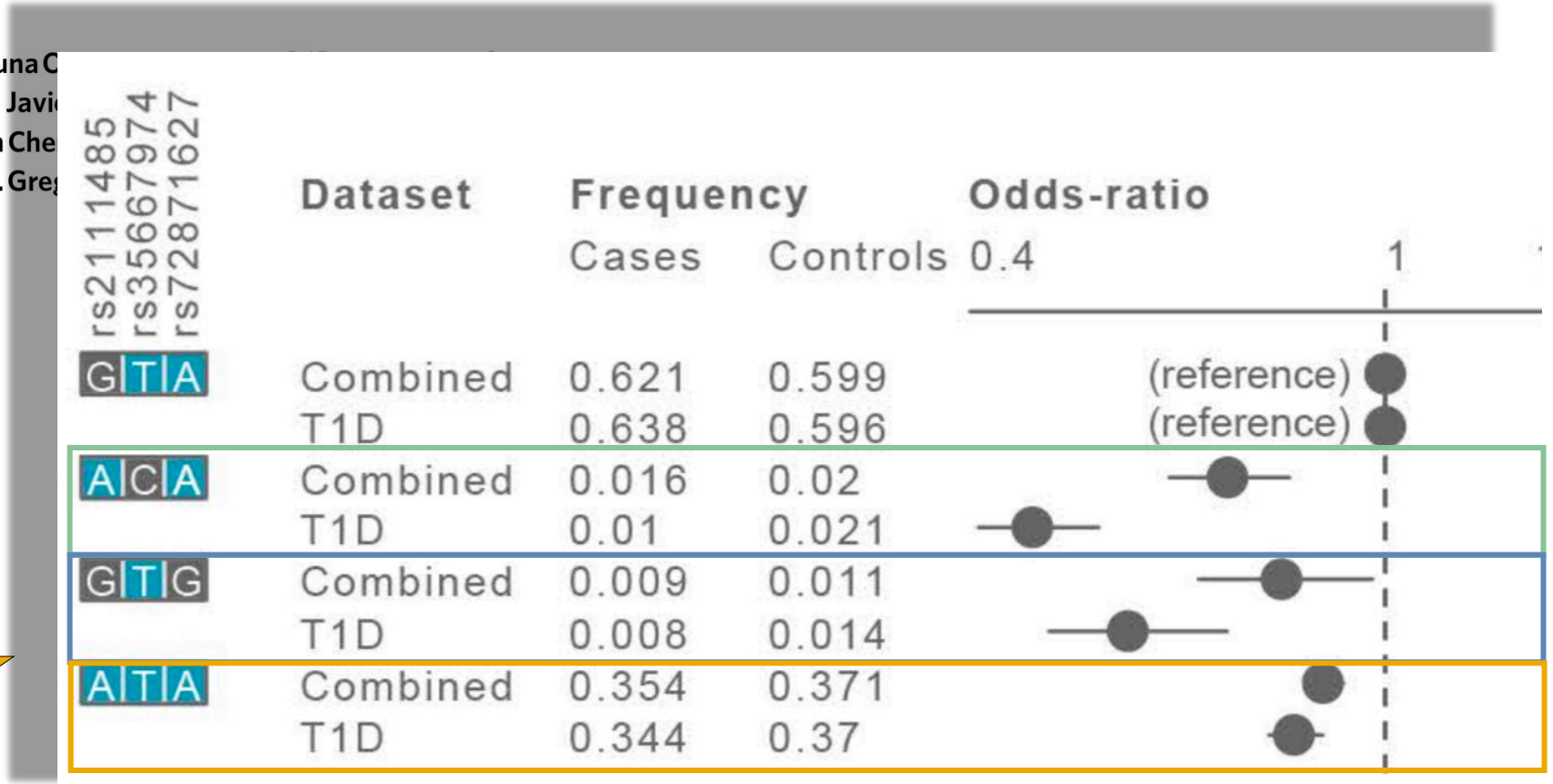
Fine-mapping and functional studies highlight potential causal variants for rheumatoid arthritis and type 1 diabetes

Harm-Jan Westra[1,2,3,4,5,20], Marta Martínez-Bonet[4,20], Suna C
Yang Luo[1,2,3,4], Nikola Teslovich[1,2,3,4], Jane Worthington[9,10], Javi
Lars Klareskog[13], Solbritt Rantapaa-Dahlqvist[14], Wei-Min Che
John A. Todd[17], Steve Eyre[9,10], Peter A. Nigrovic[4,18], Peter K. Greg
Soumya Raychaudhuri[1,2,3,4,9,19]*

rs35667974 – protects from T1D

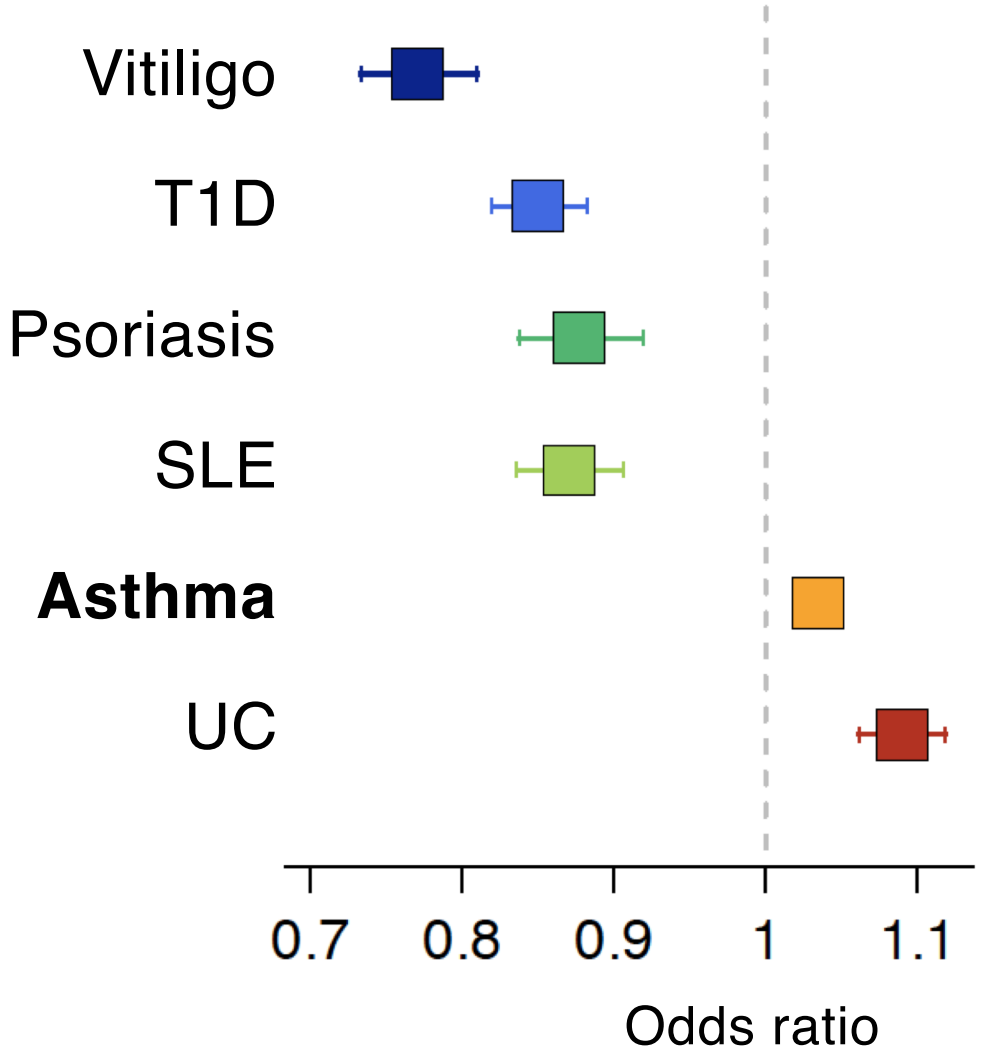
rs72871627 – protects from T1D

ATA haplotype – protects from T1D



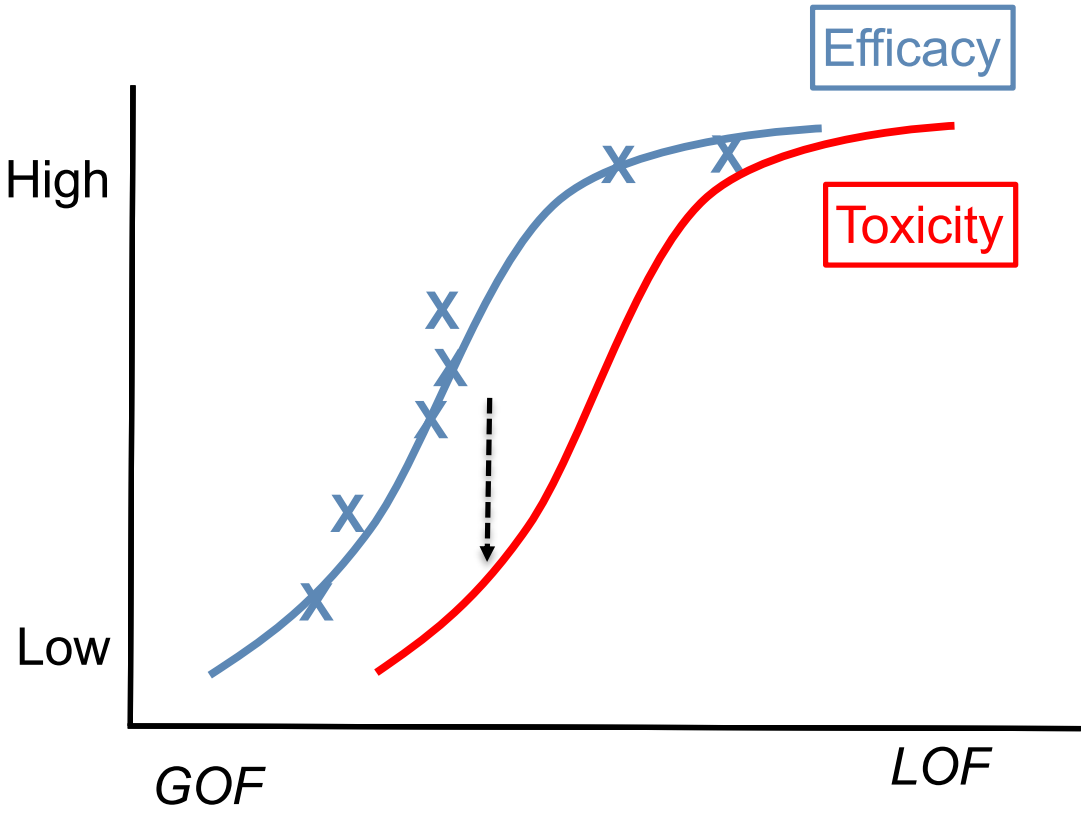
PheWAS example: *IFIH1*, autoimmunity, asthma

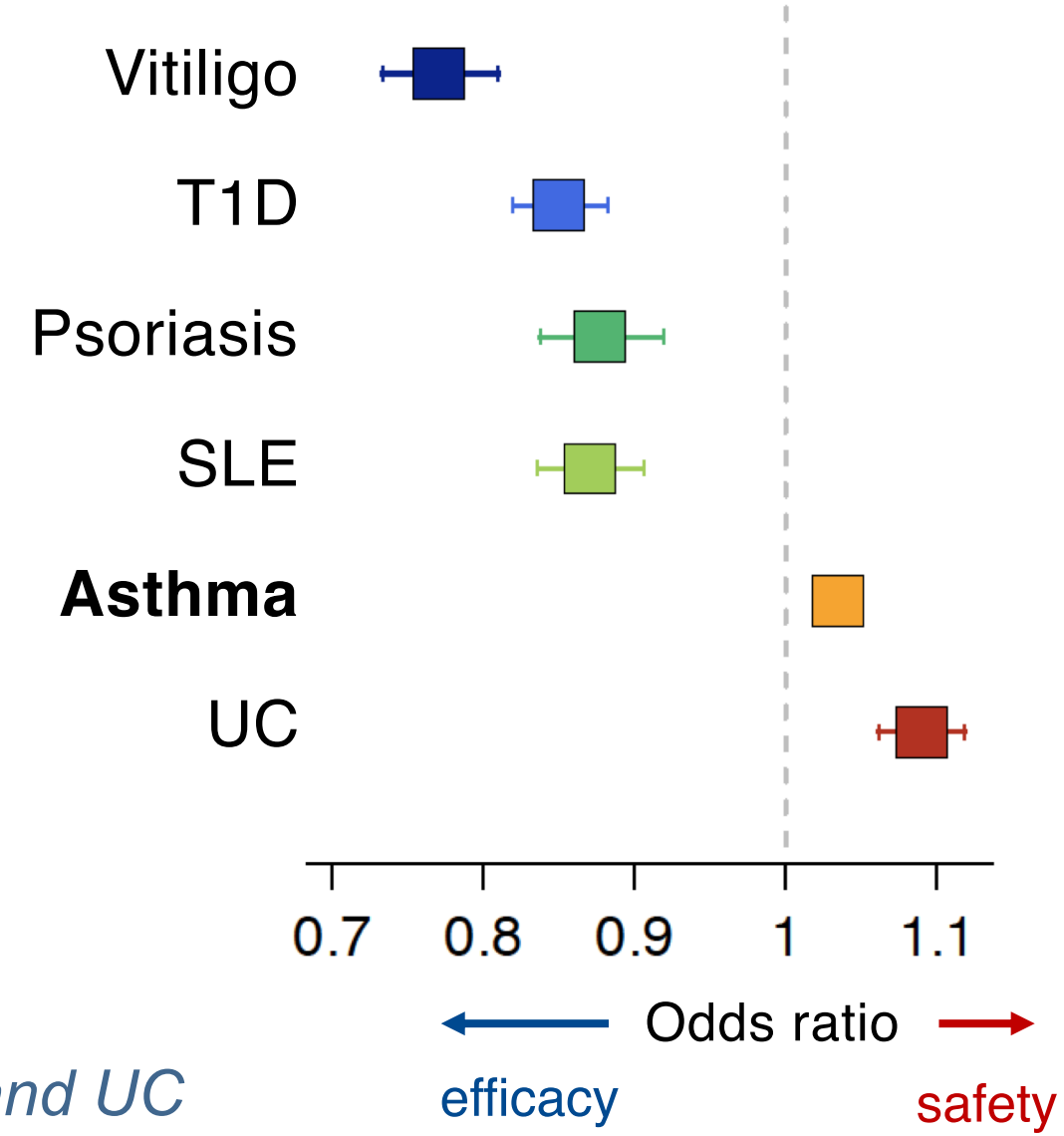
- PheWAS in ~800,000 individuals from four population cohorts
- Tested 25 SNPs for association with 1,683 clinical endpoints
- 10 novel associations discovered
- Example: *IFIH1* LOF allele protects from autoimmunity (known) but increases risk of asthma (novel finding)
- Therapeutic hypothesis: inhibiting IFIH1 may be effective in some autoimmune diseases but may make asthma worse



Diogo *et al* (2018) *Nature Communications*

Predicted impact of therapeutic inhibition of IFIH1





Beneficial effect for some autoimmune diseases, but increase risk of asthma and UC

FinnGen is a unique PheWAS resource

500,000 individuals (~10% of population)

National Registries, EHRs, etc.

Clinical data

Genetic data

Genome sequencing, Axiom array, imputation

SNP

Test association of selected SNP with clinical endpoints

PheWAS code 1

PheWAS code 2

PheWAS code 3

PheWAS code 4

PheWAS code 5

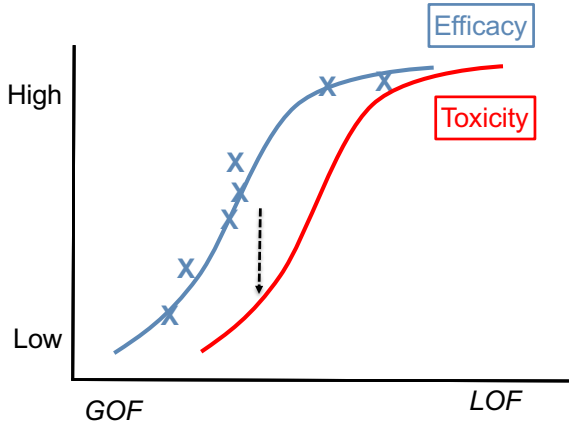
PheWAS code 500

risk

surrogate for efficacy

risk

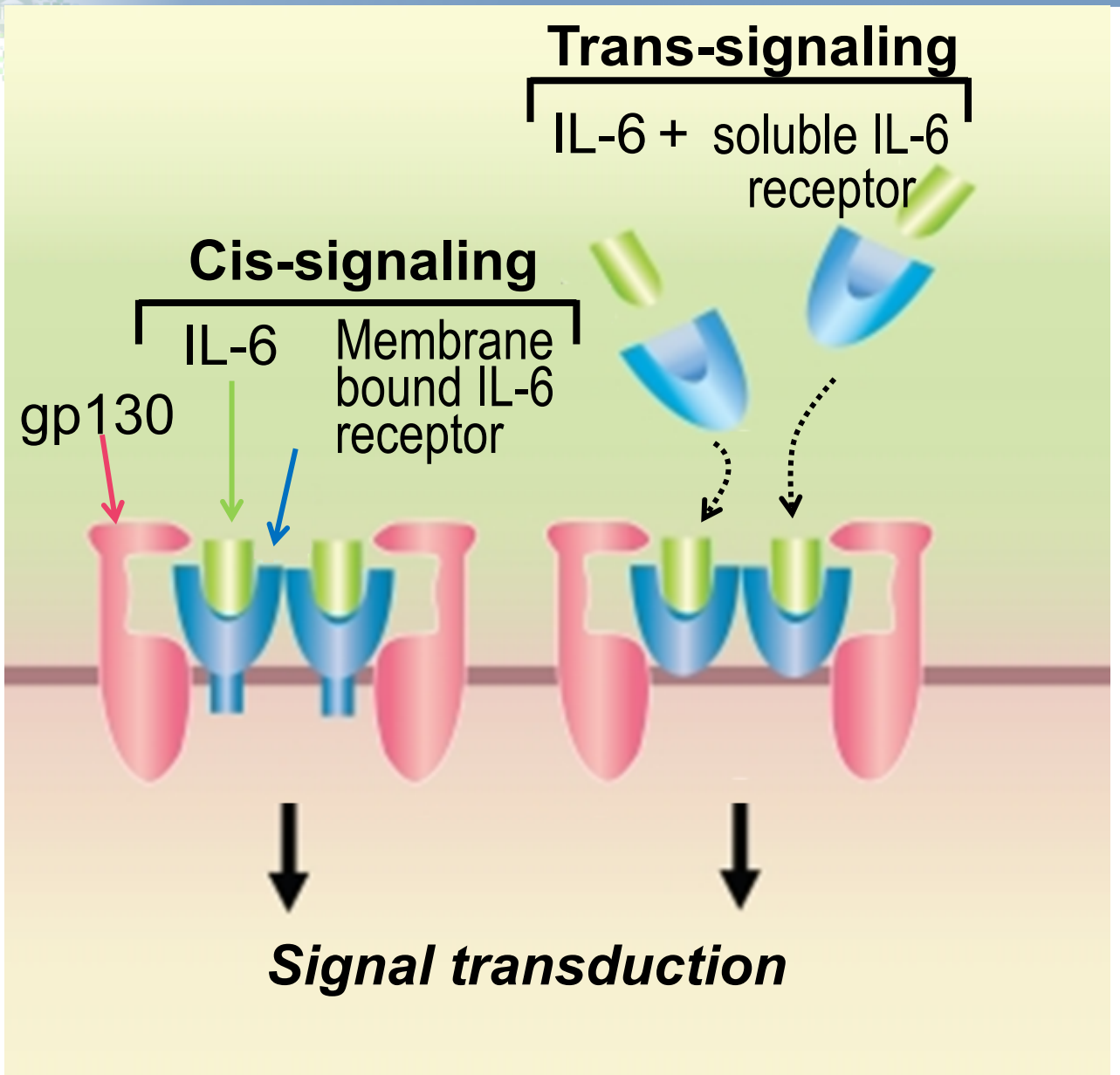
surrogate for toxicity



Return to dose-response

IL6R as a validated target

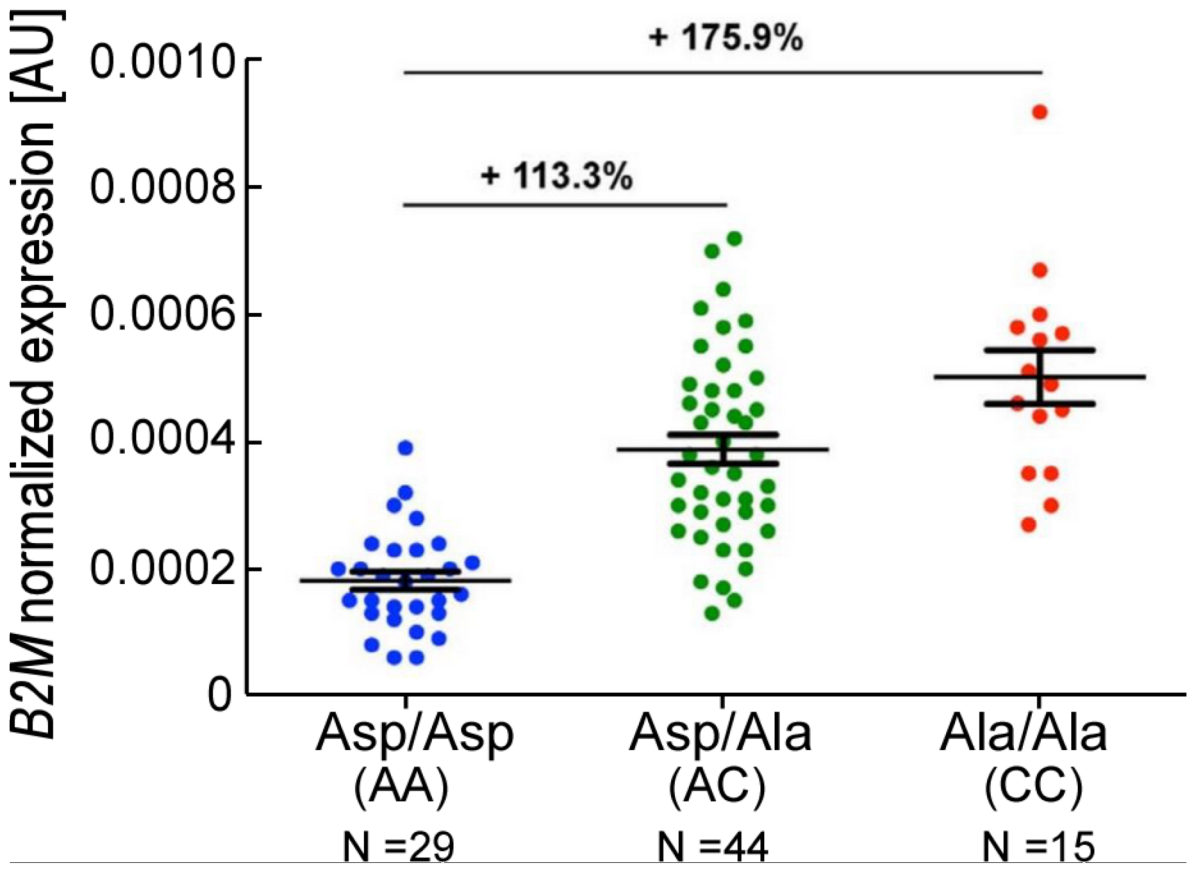
Two forms of IL-6R: *soluble and membrane-bound*



- *Cis-signaling* via membrane bound IL-6 receptor complex
- *Trans-signaling* via soluble IL-6 receptor
- Levels are inversely related: *more sIL-6R, less mIL-6R*
- ***What is the effect of the protective allele on IL-6R?***

Initial observations confusing: *protective RA allele appeared was associated with more circulating IL-6R*

More *soluble* IL-6R, but…

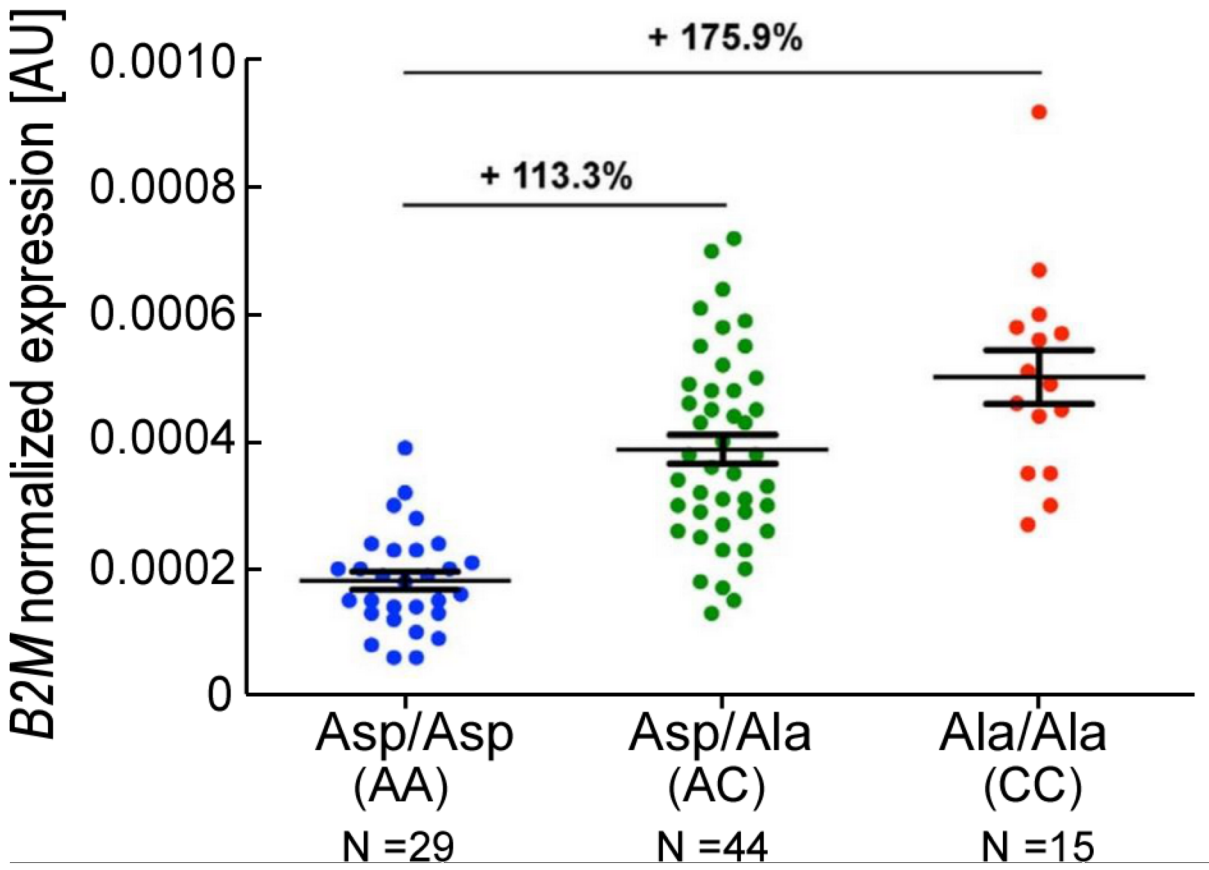


A (Asp) = risk allele C (Ala) = protective allele

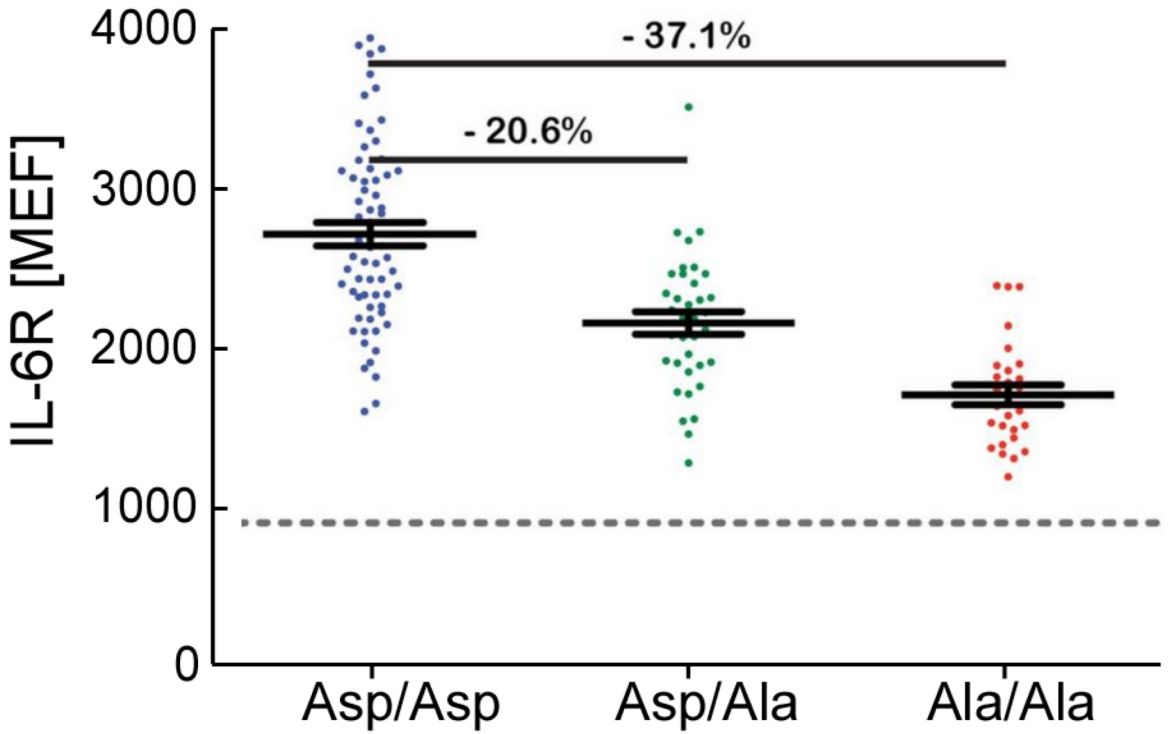
Ferreira *et al.* (2013) *PLoS Genetics*

Protective *IL-6R* variant consistent w/ loss-of-function

More *soluble* IL-6R, but…



… less cell *surface* IL-6R

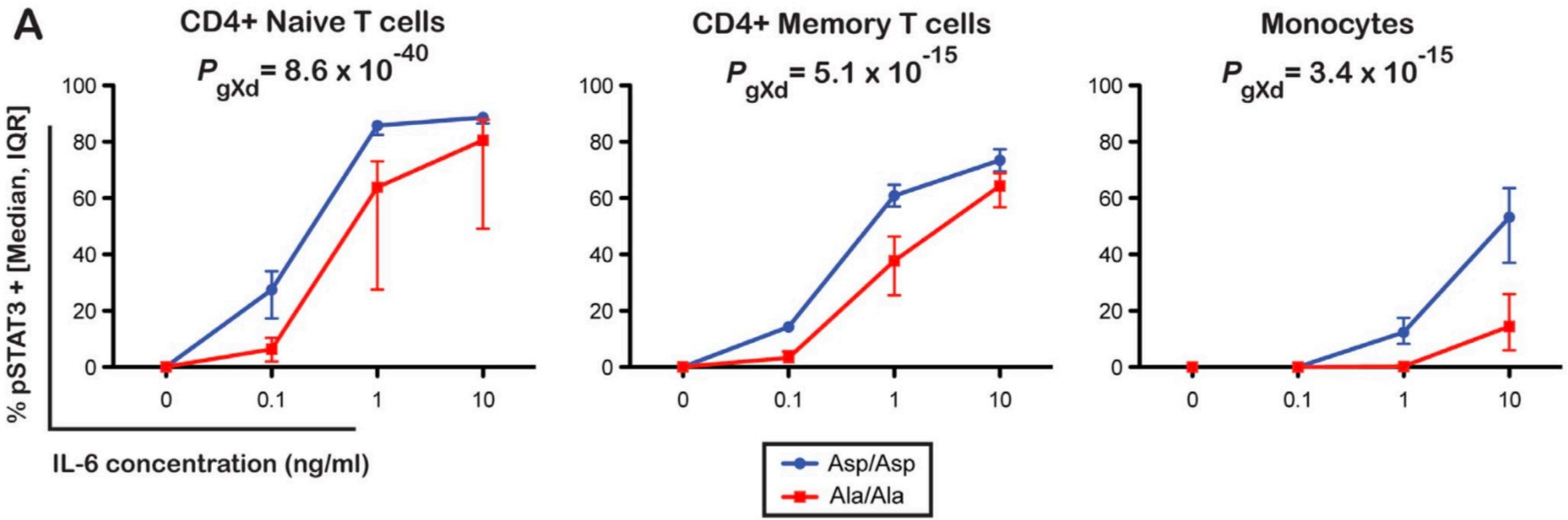


A (Asp) = risk allele C (Ala) = protective allele

Ferreira *et al.* (2013) *PLoS Genetics*

Protective *IL-6R* variant consistent w/ loss-of-function

Less cell surface IL-6R leads to less IL-6R signaling across cell types

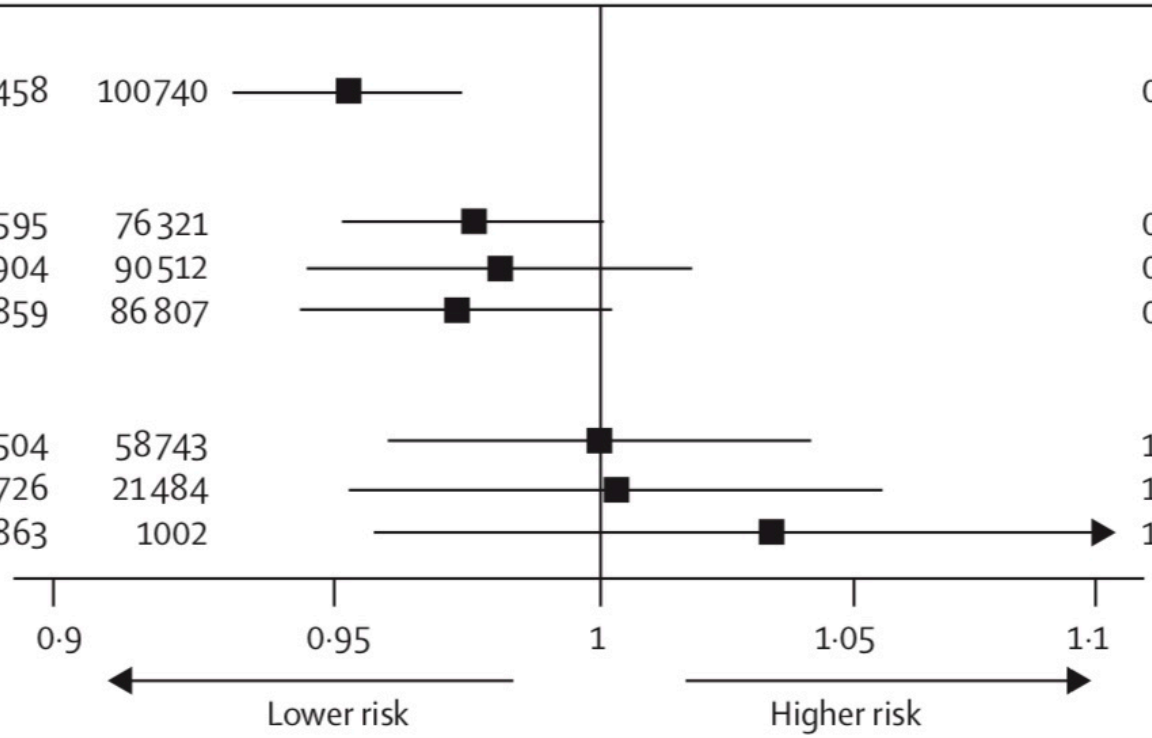


A (Asp) = risk allele C (Ala) = protective allele

Ferreira *et al.* (2013) *PLoS Genetics*

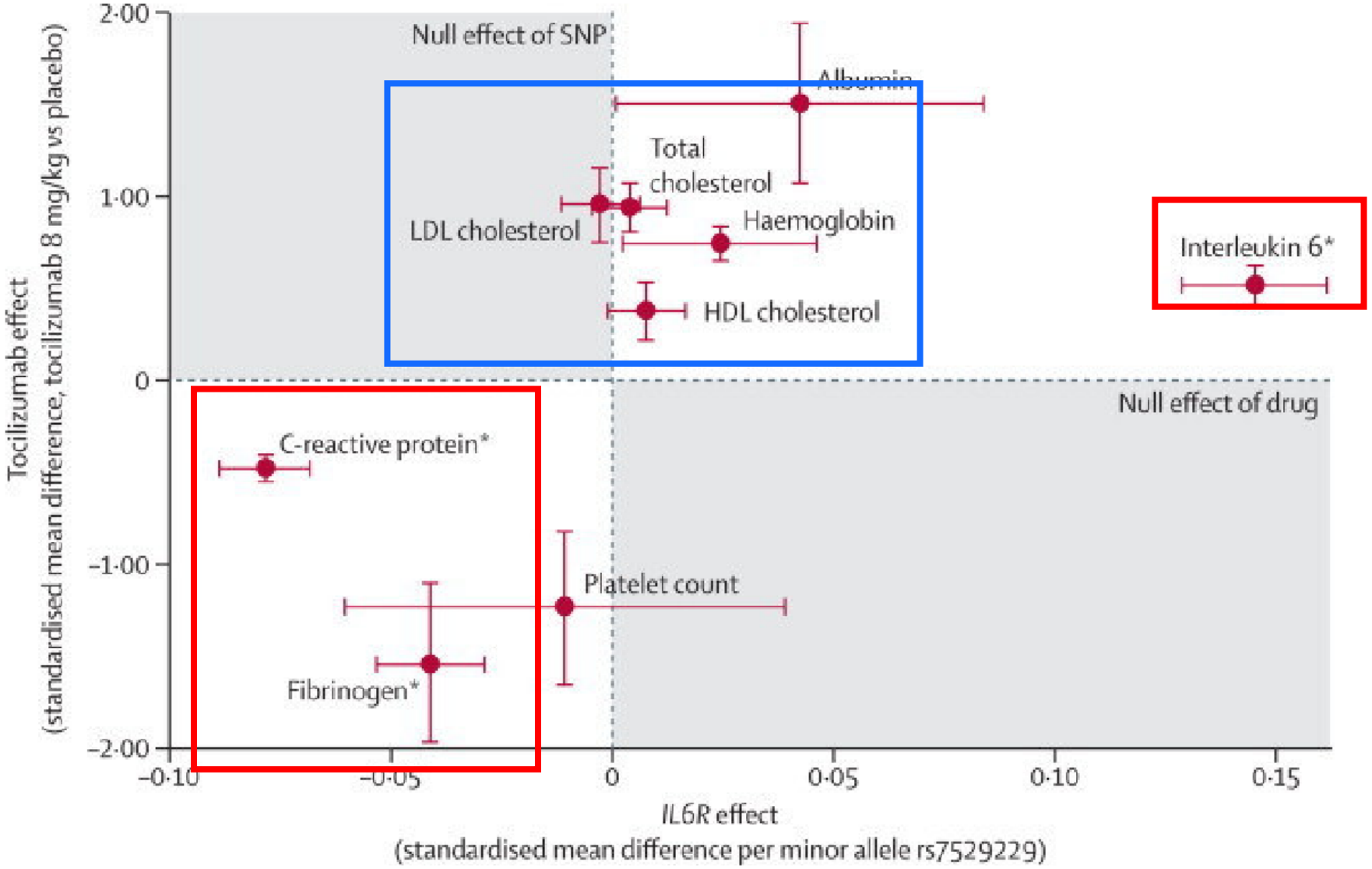
PheWAS-like approach predicts benefit of anti-IL6R therapy (tocilizumab) in coronary heart disease

	Number of studies	Cases	Controls	Odds ratio (95% CI)
Primary outcome				
All CHD (fatal and non-fatal)	34	25458	100740	0·95 (0·93–0·97)
Secondary outcomes: cardiovascular/metabolic				
All CVD (fatal and non-fatal)	26	17595	76321	0·98 (0·95–1·00)
All stroke (fatal and non-fatal)	27	6904	90512	0·98 (0·94–1·02)
Type 2 diabetes	28	12859	86807	0·97 (0·94–1·00)
Secondary outcomes: non-cardiovascular				
All cancer	12	22504	58743	1·00 (0·96–1·04)
Breast cancer	3	14726	21484	1·00 (0·95–1·06)
Colorectal cancer	2	1863	1002	1·03 (0·96–1·12)



Swerdlow *et al* (2012) *Lancet*

PheWAS-like approach can differentiate between on-target vs off-target effects of a drug



Drug but *not protective allele* has effect on cholesterol levels

➢ ***Off-target ADE***

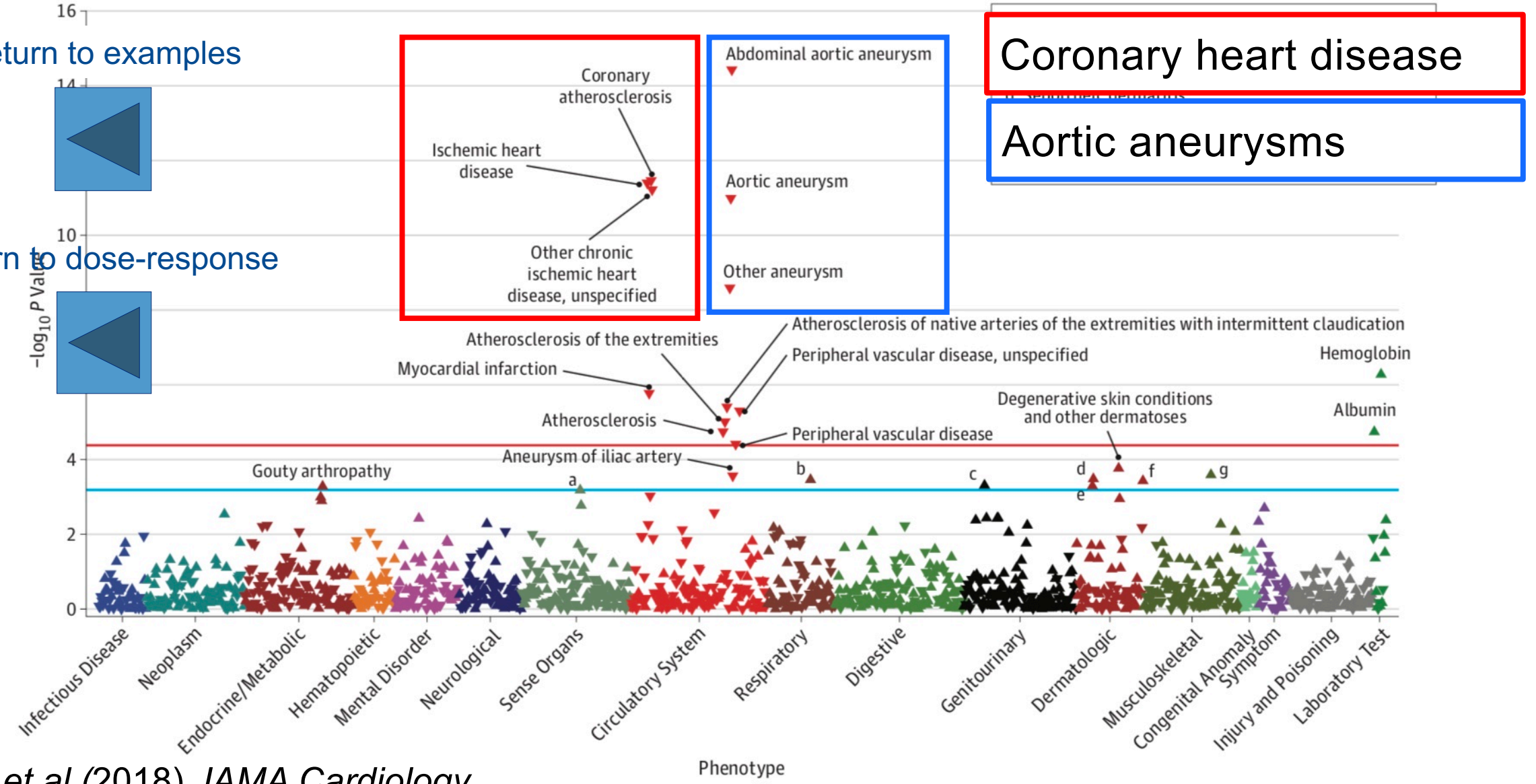
Protective allele *and drug* leads to more sIL-6 but less CRP and fibrinogen

➢ ***On-target effect***

PheWAS via electronic health records (EHR) can identify indications for drug repurposing

Return to examples

Return to dose-response



Cai *et al* (2018) *JAMA Cardiology*

Pre-clinical proof-of-concept in pemphigus vulgaris

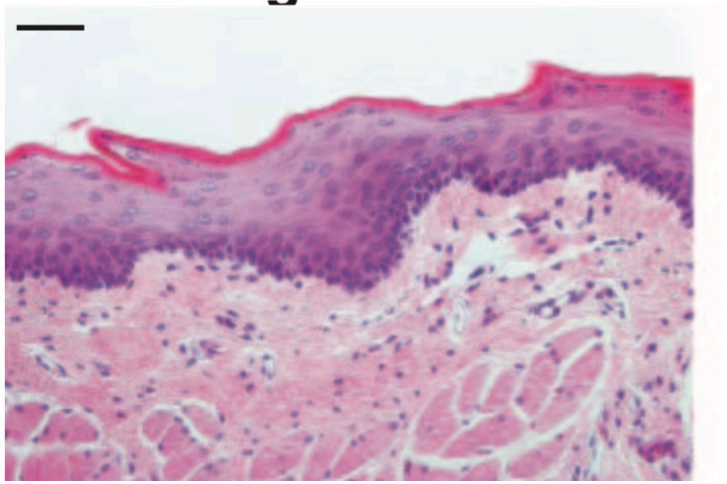
IMMUNOTHERAPY

Reengineering chimeric antigen receptor T cells for targeted therapy of autoimmune disease

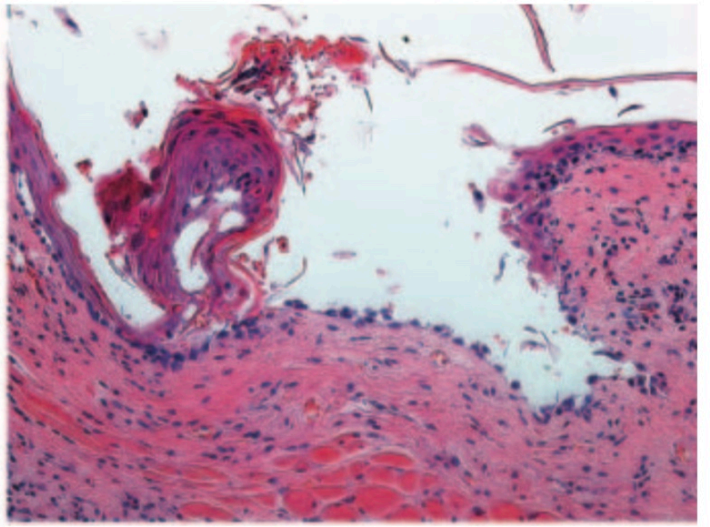
Christoph T. Ellebrecht,[1] Vijay G. Bhoj,[2] Arben Nace,[1] Eun Jung Choi,[1] Xuming Mao,[1] Michael Jeffrey Cho,[1] Giovanni Di Zenzo,[3] Antonio Lanzavecchia,[4] John T. Seykora,[1] George Cotsarelis,[1] Michael C. Milone,[2*†] Aimee S. Payne[1*†]

Ideally, therapy for autoimmune diseases should eliminate pathogenic autoimmune cells while sparing protective immunity, but feasible strategies for such an approach have been elusive. Here, we show that in the antibody-mediated autoimmune disease pemphigus vulgaris (PV), autoantigen-based chimeric immunoreceptors can direct T cells to kill autoreactive B lymphocytes through the specificity of the B cell receptor (BCR). We engineered human T cells to express a chimeric autoantibody receptor (CAAR), consisting of the PV autoantigen, desmoglein (Dsg) 3, fused to CD137-CD3ζ signaling domains. Dsg3 CAAR-T cells exhibit specific cytotoxicity against cells expressing anti-Dsg3 BCRs in vitro and expand, persist, and specifically eliminate Dsg3-specific B cells in vivo. CAAR-T cells may provide an effective and universal strategy for specific targeting of autoreactive B cells in antibody-mediated autoimmune disease.

Dsg3 CAAR

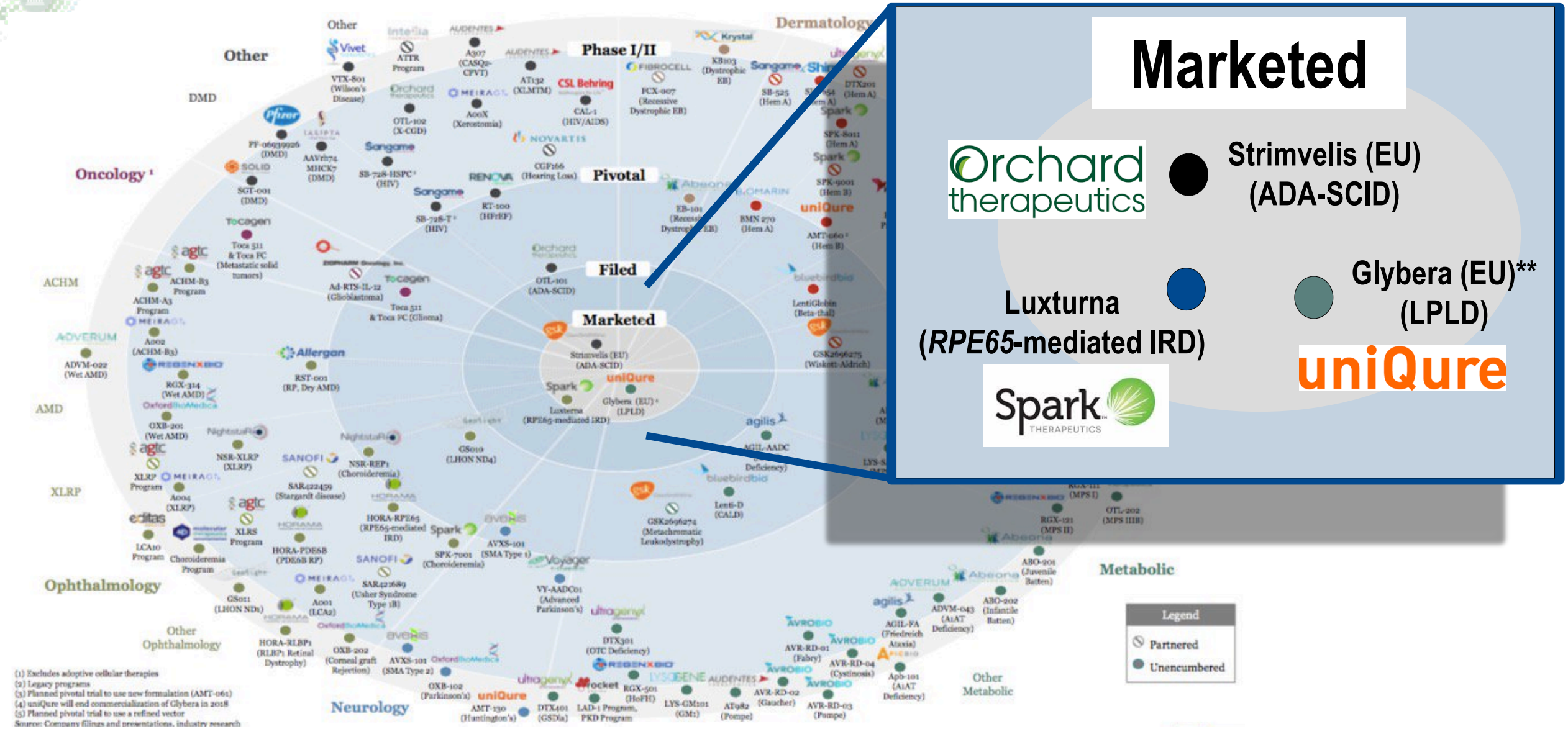


control CAR



Gene and mRNA therapy as programmable therapeutics

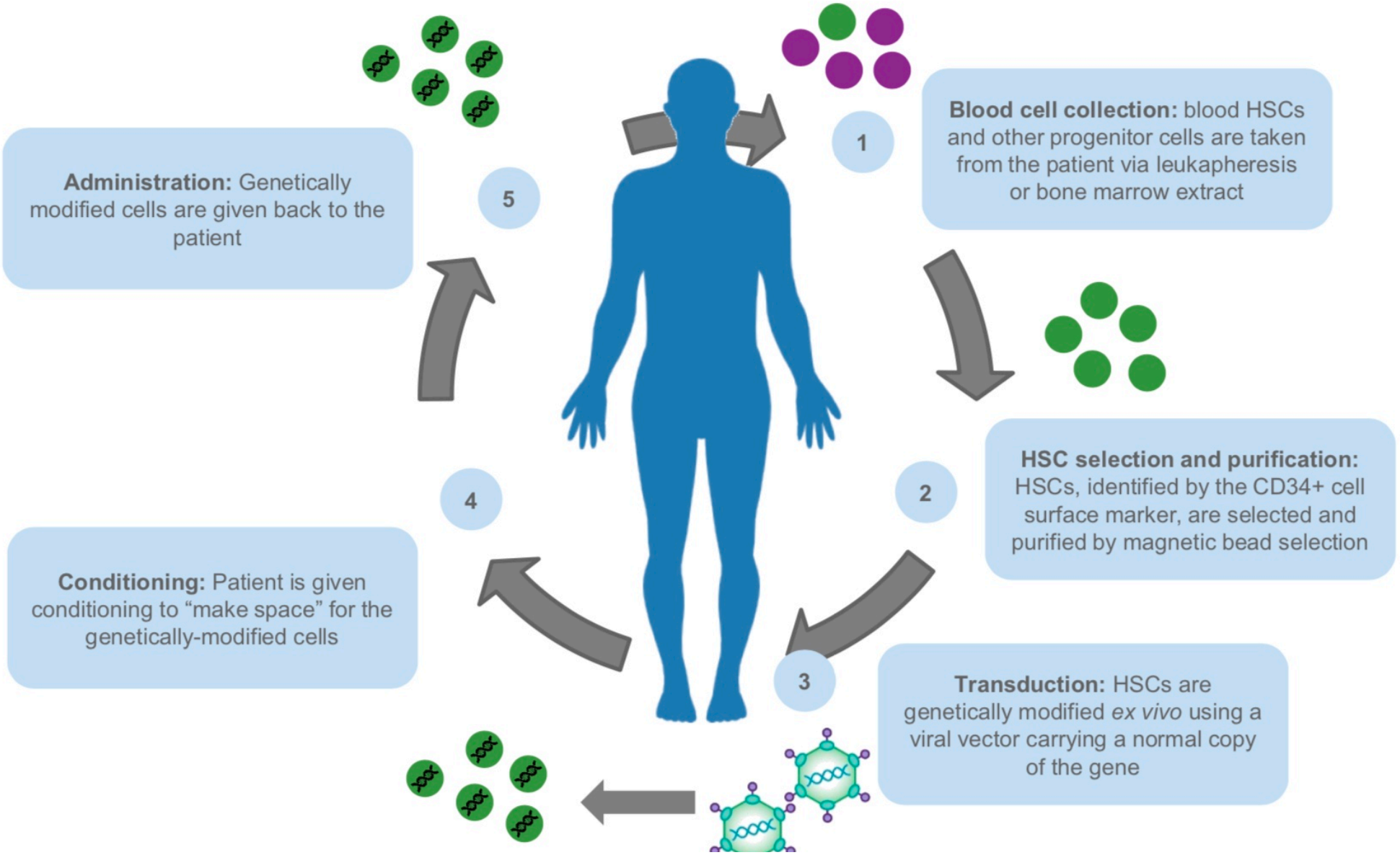
Gene therapy landscape – *three approvals, more to come*



Primary immunodeficiency (PID) – *hundreds of genes implicated, but fewer than 5 in development*

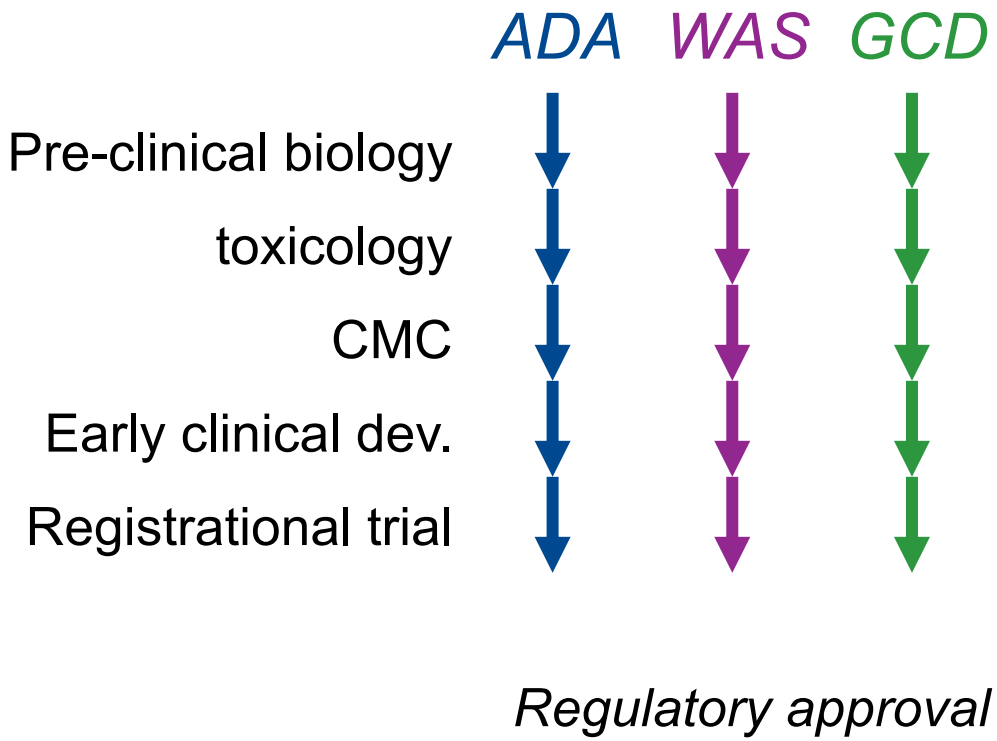
- Over 350 genes implicated in PID
- Range of phenotypes
  - Severe Combined Immune Deficiency (SCID): profound hypersusceptibility to infections and/or cancer
  - Moderate: increased risk of infection, chronic dermatologic conditions, persistent autoimmunity, inflammatory bowel disease, chronic lung conditions (e.g., fibrosis, asthma)
- Total incidence approaches 1% of the population
  - SCID estimated at 1 / 30,000 live births (or ~150 new cases in US each year)
- TREC assay for SCID now part of newborn screening in 50 US states (as of 2018)
- Foundations (e.g., JMF) actively involved in patient advocacy, research, more
  - 792 physicians at 358 institutions in 277 cities across 86 countries spanning 6 continents
- Approximately two-thirds of severe PID is amenable to cures via HSCT
- Gene therapy successful, but only 1 gene (out of 350) with a marketed product
- ***How will durable cures be achieved for the entire patient population?***

Autologous *ex vivo* gene replacement for PID leads to cure

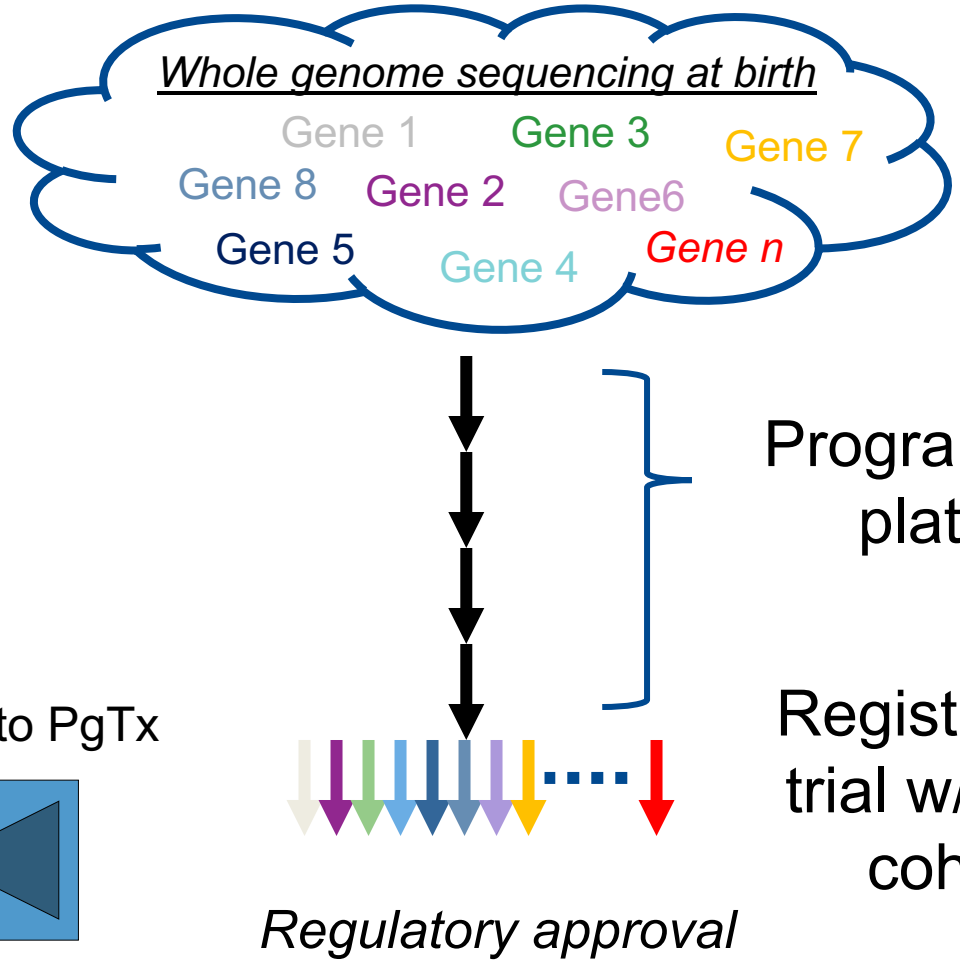


Programmable therapeutics for PID

Conventional approach: one gene, one disease, one linear regulatory path to approval (e.g., pre-clinical biology, toxicology, CMC, early clinical dev, registrational trials)



Programmable approach: introduce a new gene into a "programmable" platform that has been approved for all pre-registrational trial activities

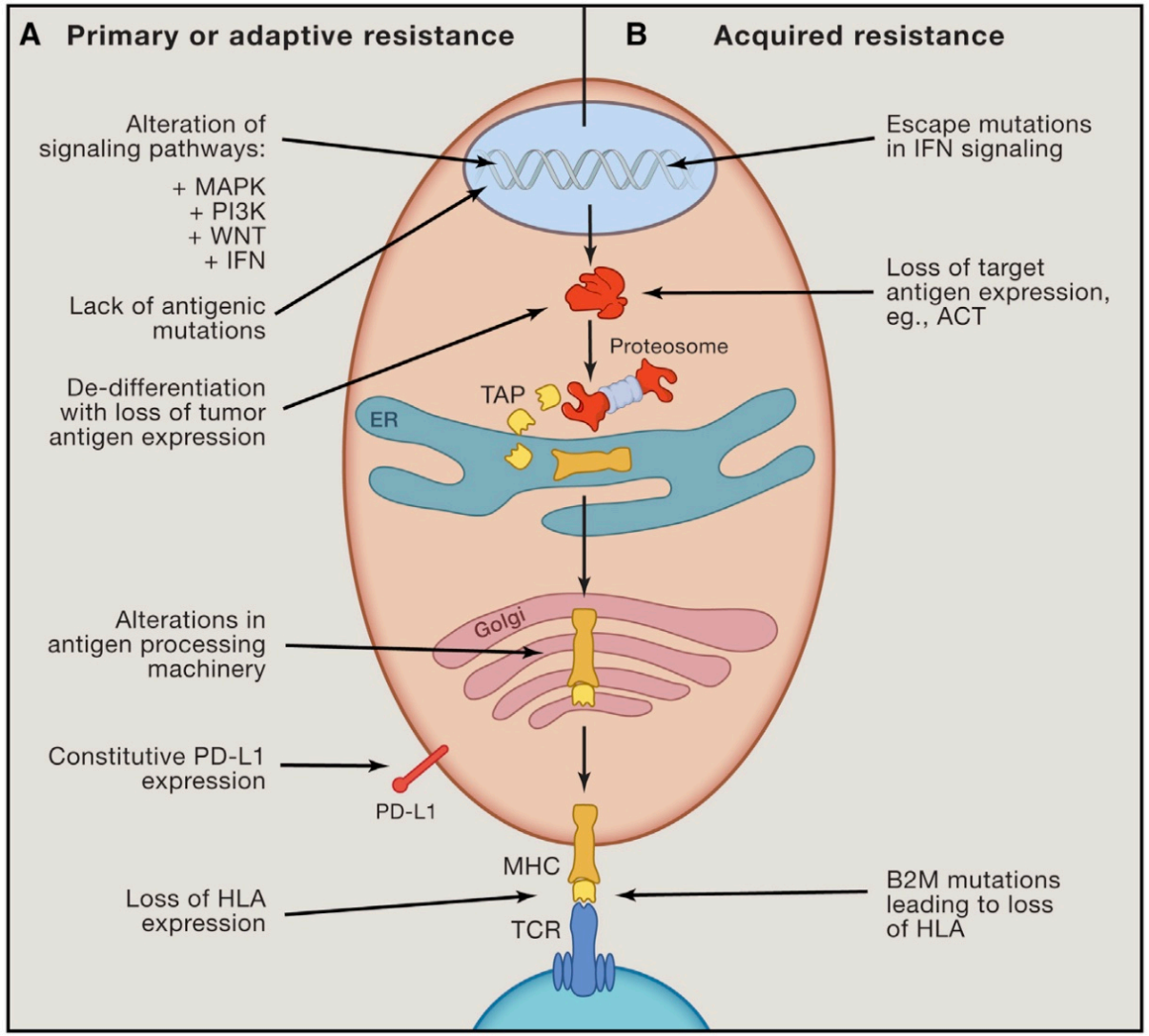


mRNA therapy as programmable therapeutic in oncology

Many mechanisms of resistance to checkpoint inhibitors

Table 2. Mechanisms of Primary and Adaptive Resistance to Immunotherapy

	Mechanism	Examples
tumor cell intrinsic	absence of antigenic proteins	low mutational burden lack of viral antigens lack of cancer-testis antigens overlapping surface proteins
	absence of antigen presentation	deletion in TAP deletion in B2M silenced HLA
	genetic T cell exclusion	MAPK oncogenic signaling stabilized b-catenin mesenchymal transcriptome oncogenic PD-L1 expression
	insensibility to T cells	mutations in interferon gamma pathway signaling
tumor cell extrinsic	absence of T cells	lack of T cells with tumor antigen-specific TCRs
	inhibitory immune checkpoints	VISTA, LAG-3, TIM-3
	immunosuppressive cells	TAMs, Tregs



Sharma et al Cell (2017)

High diversity of neoantigens requires programmable approach

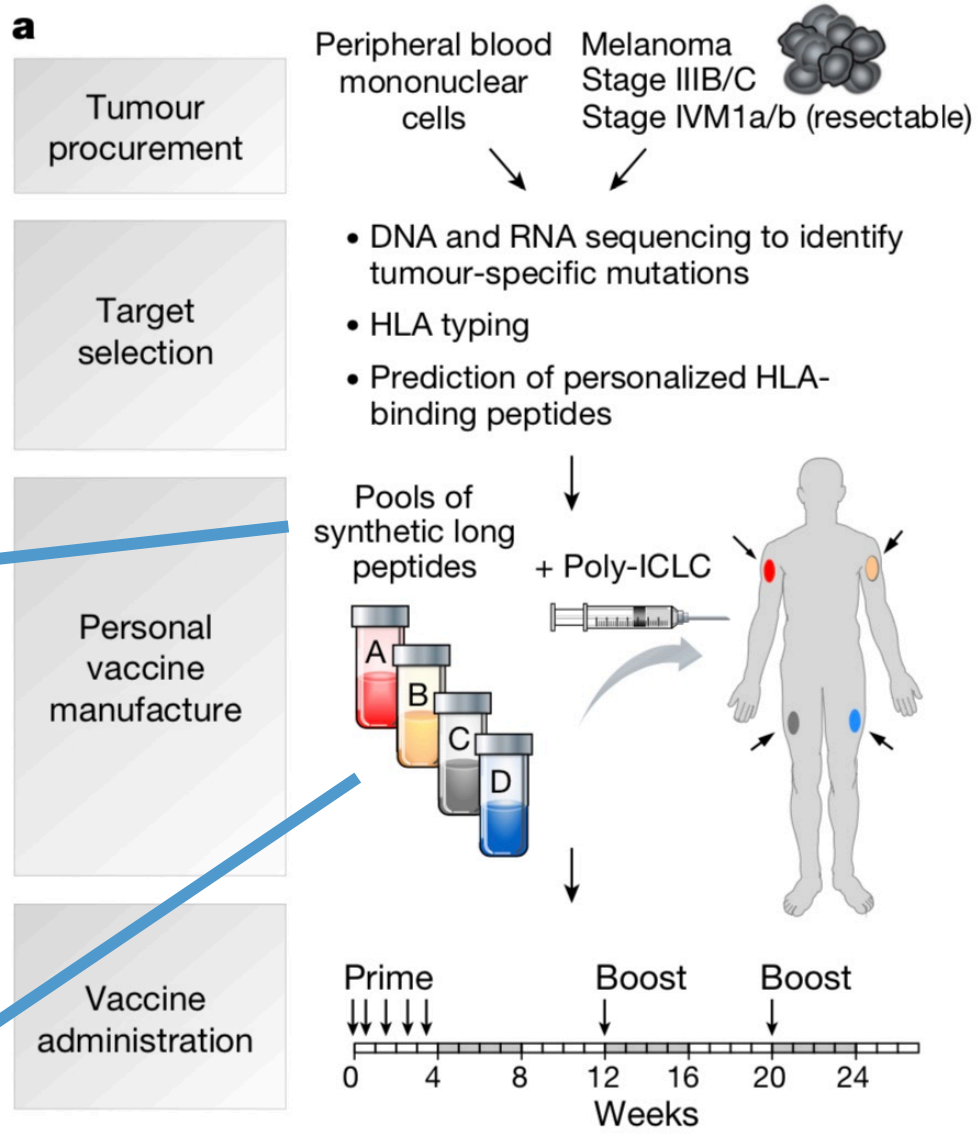
LETTER

doi:10.1038/nature22991

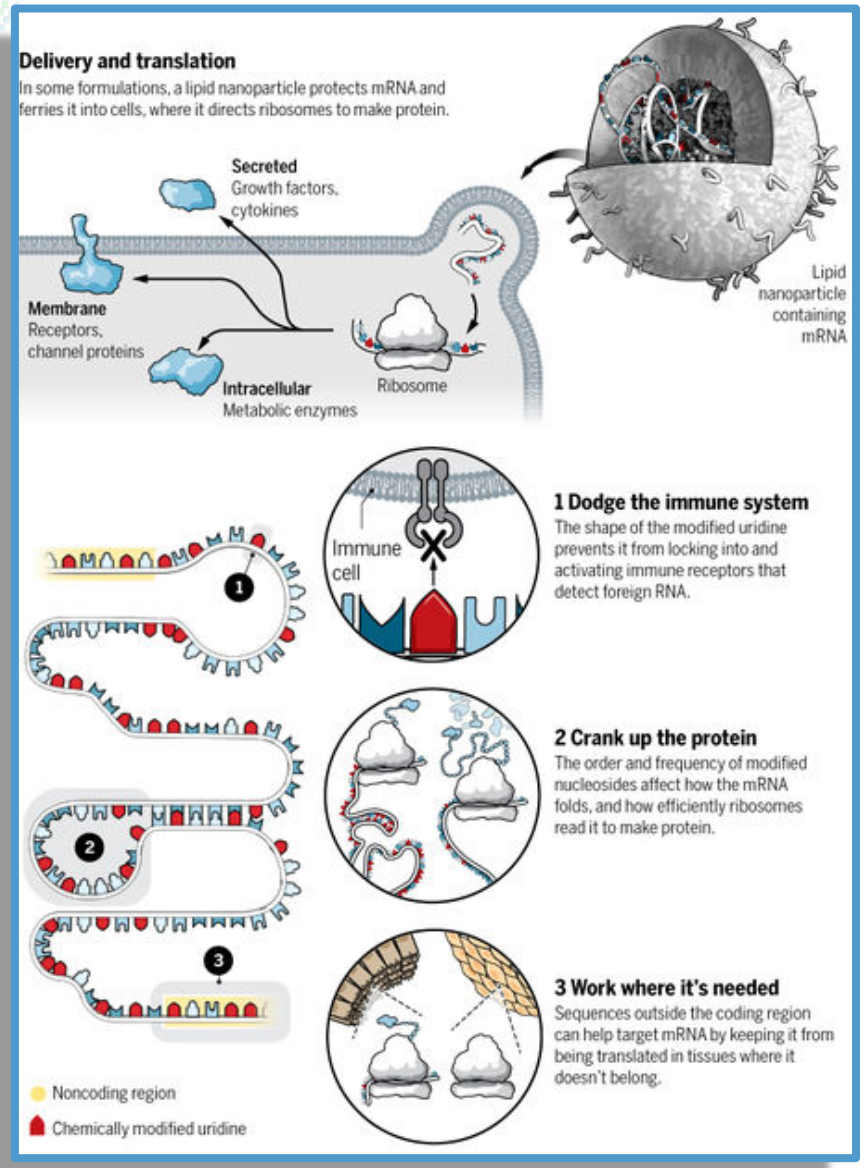
An immunogenic personal neoantigen vaccine for patients with melanoma

Patrick A. Ott[1,2,3]*, Zhuting Hu[1]*, Derin B. Keskin[1,3,4], Sachet A. Shukla[1,4], Jing Sun[1], David J. Bozym[1], Wandi Zhang[1], Adrienne Luoma[5], Anita Giobbie-Hurder[6], Lauren Peter[7,8], Christina Chen[1], Oriol Olive[1], Todd A. Carter[4], Shuqiang Li[4], David J. Lieb[4], Thomas Eisenhaure[4], Evisa Gjini[9], Jonathan Stevens[10], William J. Lane[10], Indu Javeri[11], Kaliappanadar Nellaiappan[11], Andres M. Salazar[12], Heather Daley[1], Michael Seaman[7], Elizabeth I. Buchbinder[1,2,3], Charles H. Yoon[3,13], Maegan Harden[4], Niall Lennon[4], Stacey Gabriel[4], Scott J. Rodig[9,10], Dan H. Barouch[3,7,8], Jon C. Aster[3,10], Gad Getz[3,4,14], Kai Wucherpfennig[3,5], Donna Neuberg[6], Jerome Ritz[1,2,3], Eric S. Lander[3,4], Edward F. Fritsch[1,4]†, Nir Hacohen[3,4,15] & Catherine J. Wu[1,2,3,4]

To generate a vaccine that targets personal neoantigens, we conducted whole-exome sequencing (WES) of matched tumour- and normal-cell DNA from individual patients, identified somatic mutations, orthogonally validated and assessed the expression of mutated alleles by RNA sequencing (RNA-seq) of the tumour, predicted which mutated peptides were likely to bind autologous HLA-A or HLA-B proteins of the patient, and synthesized clinical-grade long peptides[3] targeting up to 20 neoantigens per patient, admixed with the Toll-like receptor 3 (TLR3) and melanoma differentiation-associated protein 5 (MDA-5)



mRNA replacement as programmable therapeutic in oncology



Durable anticancer immunity from intratumoral administration of IL-23, IL-36γ, and OX40L mRNAs

Susannah L. Hewitt*, Ailin Bai*, Dyane Bailey, Kana Ichikawa, John Zielinski, Russell Karp, Ameya Apte, Kristen Arnold, Sima J. Zacharek, Maria S. Iliou, Khushbu Bhatt, Maija Garnaas, Faith Musenge, Ashley Davis, Nikhil Khatwani, Stephen V. Su, Graham MacLean, Samuel J. Farlow, Kristine Burke, Joshua P. Frederick†