Beyond Genetics: What is Next?

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No disclosures to report.

2003: Proposed Priority Areas for IBD Research

A presentation to the Digestive Diseases Interagency Coordinating Committee (NIH, FDA, CDC, DoD, DoA, etc.), April 30, 2003, John. D. Rioux

(N.B. NOD2 was the only IBD gene known at the time)

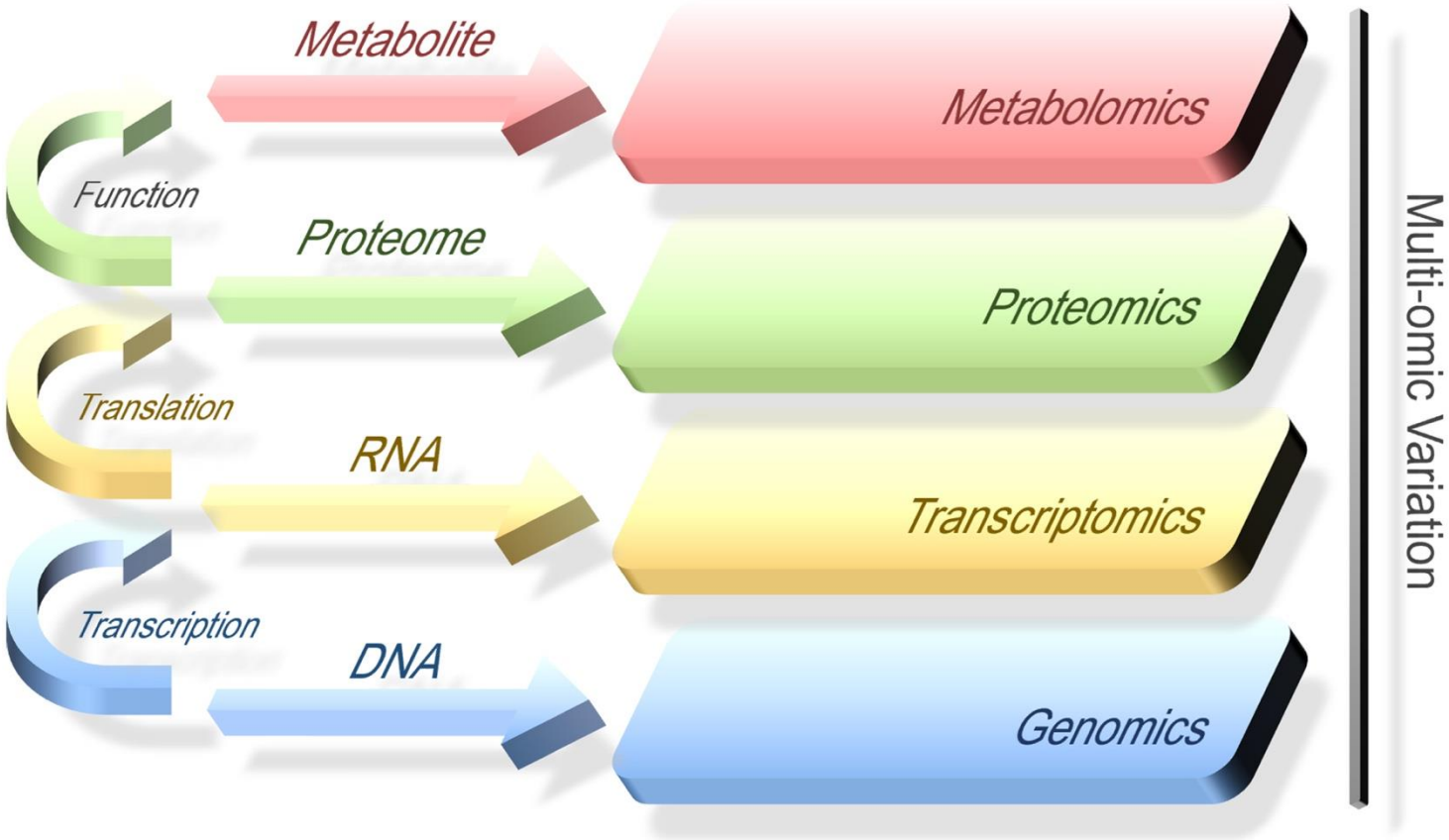
(1) Identification of IBD genes and causal variants

(2) *Idem* but in under-studied populations

(3) Understand role of IBD genes in **biology and in pathophysiology**

(4) Understand influence of genetic variation on **diagnosis and treatment**

Taking Advantage of the “Omics Cascade”



Dynamic

Integration =

Confirmatory/
Complementary
Evidence

Figure modified from: Cong & Endo (2022); OMICS: A Journal of Integrative Biology, 26:7

Bulk vs Single cell vs Spatial **Genomics**

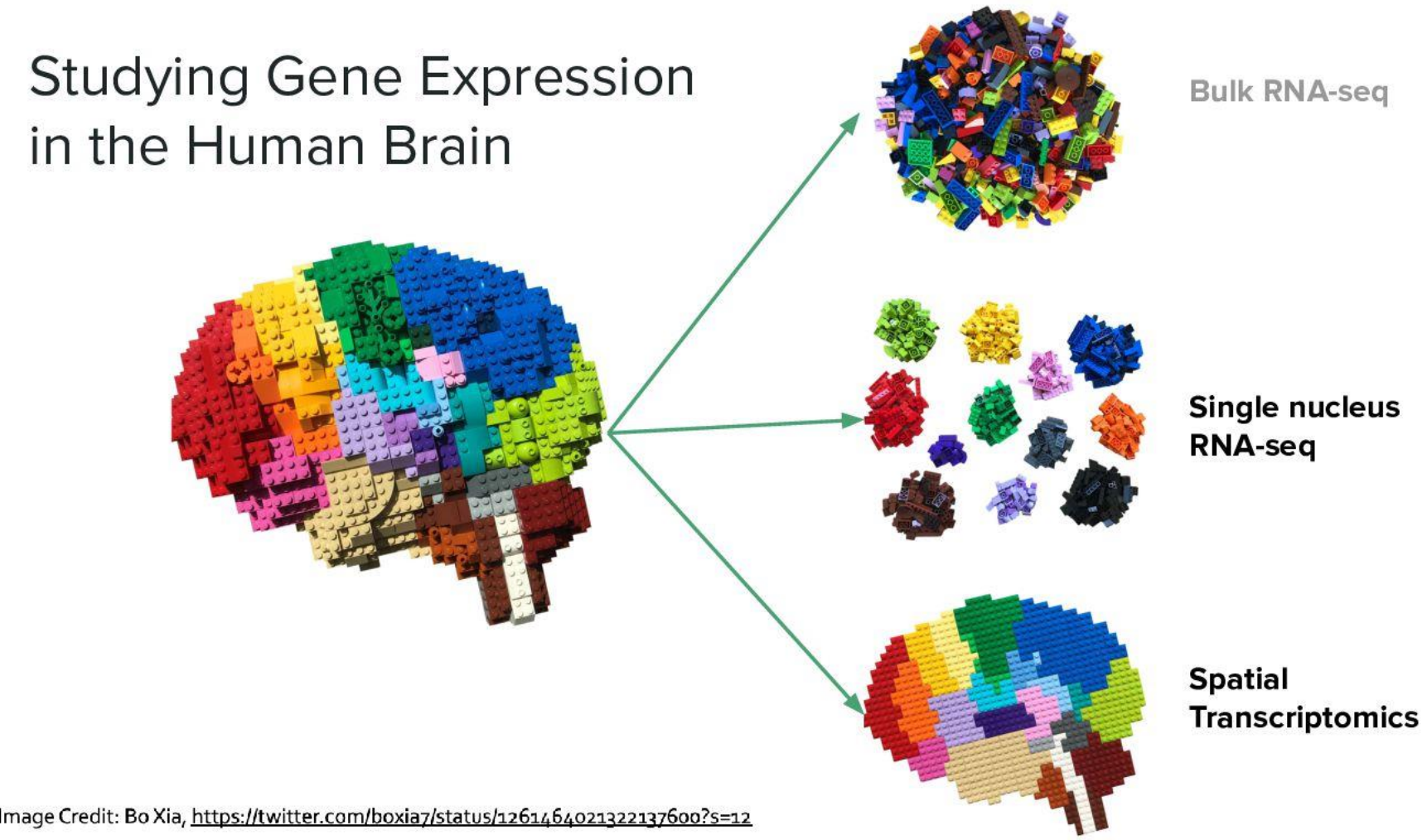


Image Credit: Bo Xia, https://twitter.com/boxia7/status/1261464021322137600?s=12

Multiple Uses/Applications

- Biological context for genes within GWAS loci → prioritization of genes and functional studies
- Study in detail dynamic processes in liquid and solid tissues
  (e.g. disease progression, healing, response to therapy, etc.)
- Identify potential biomarkers of clinical events & outcomes
- Etc.

*** Important to validate across multiple contexts, designs, studies (incl. archival samples)

Identification of Robust Profile of Serum Proteins Associated with CD

NIDDK IBD Genetics Consortium (IBDGC)

Retrospective
IBDGC Repository samples

- 300 CD
- 300 matched controls

		IBDGC-1 (B2B3) vs Controls			IBDGC-2 (B2B3) vs Controls			Combined (B2B3) vs Controls			Combined (ALL CD) vs Controls		
	ANALYTES	BETA	SE	*P*	BETA	SE	*P*	BETA	SE	*P*	BETA	SE	*P*
	CXCL9/MIG	0.364	0.137	8.40E-03	0.422	0.124	7.82E-04	0.395	0.092	1.68E-05	*0.441	*0.084	*1.40E-07
	CXCL1/GRO-α	0.360	0.162	2.79E-02	0.473	0.093	7.19E-07	0.445	0.081	3.73E-08	*0.437	*0.066	*4.78E-11
	IL-6	0.495	0.180	6.47E-03	0.386	0.115	9.34E-04	0.417	0.097	1.69E-05	*0.419	*0.082	*2.87E-07
	VEGF	0.411	0.248	1.00E-01	0.263	0.160	1.01E-01	0.307	0.135	2.28E-02	*0.359	*0.112	*1.39E-03
+	+ IL-2Rα	0.498	0.131	1.89E-04	0.215	0.100	3.16E-02	0.319	0.079	5.74E-05	*0.333	*0.069	*1.38E-06
	+ IL-10	0.245	0.250	3.29E-01	0.340	0.209	1.04E-01	0.301	0.160	6.02E-02	0.318	0.131	1.55E-02
	CXCL8/IL-8	0.275	0.077	4.32E-04	0.317	0.072	1.51E-05	0.297	0.052	1.50E-08	*0.293	*0.046	*1.62E-10
+	IL-7	0.470	0.165	4.87E-03	0.231	0.067	6.70E-04	0.265	0.062	2.06E-05	*0.280	*0.051	*3.11E-08
	HGF	0.229	0.120	5.68E-02	0.297	0.083	4.09E-04	0.275	0.068	5.56E-05	*0.265	*0.058	*5.14E-06
+	IL-18	0.191	0.120	1.15E-01	0.271	0.100	7.22E-03	0.238	0.077	1.99E-03	*0.261	*0.068	*1.11E-04
	+ CCL11/Eotaxin	0.261	0.097	7.71E-03	0.148	0.092	1.09E-01	0.201	0.067	2.53E-03	*0.245	*0.058	*2.61E-05
	MIF	0.046	0.151	7.59E-01	0.282	0.121	2.11E-02	0.189	0.095	4.56E-02	0.209	0.083	1.20E-02
	IL-1RA	0.097	0.133	4.66E-01	0.272	0.097	5.57E-03	0.211	0.079	7.26E-03	0.206	0.066	1.94E-03
	CXCL12/SDF-1α	0.153	0.066	2.24E-02	0.240	0.058	4.27E-05	0.202	0.044	3.37E-06	*0.192	*0.037	*2.80E-07
	IL-5	0.167	0.062	7.92E-03	0.140	0.071	5.14E-02	0.155	0.047	9.39E-04	*0.168	*0.041	*4.80E-05
+	CD40L	0.096	0.103	3.54E-01	0.112	0.092	2.25E-01	0.104	0.068	1.27E-01	0.134	0.059	2.24E-02
	G-CSF	0.064	0.071	3.68E-01	0.140	0.061	2.17E-02	0.108	0.046	1.94E-02	0.125	0.040	1.70E-03
	IL-16	0.434	0.171	1.18E-02	0.092	0.091	3.12E-01	0.167	0.080	3.68E-02	0.123	0.065	5.79E-02
+	+ IFN-γ	0.102	0.051	4.82E-02	0.119	0.050	1.80E-02	0.111	0.036	1.99E-03	*0.121	*0.031	*1.22E-04
	PDGF-ββ	0.143	0.086	9.72E-02	0.052	0.097	5.92E-01	0.103	0.064	1.09E-01	0.120	0.057	3.55E-02
	TNF-α	0.071	0.067	2.89E-01	0.107	0.085	2.10E-01	0.085	0.053	1.07E-01	0.114	0.047	1.63E-02
+	+ IL-1β	0.092	0.058	1.14E-01	0.078	0.048	1.02E-01	0.084	0.037	2.29E-02	0.091	0.031	3.91E-03

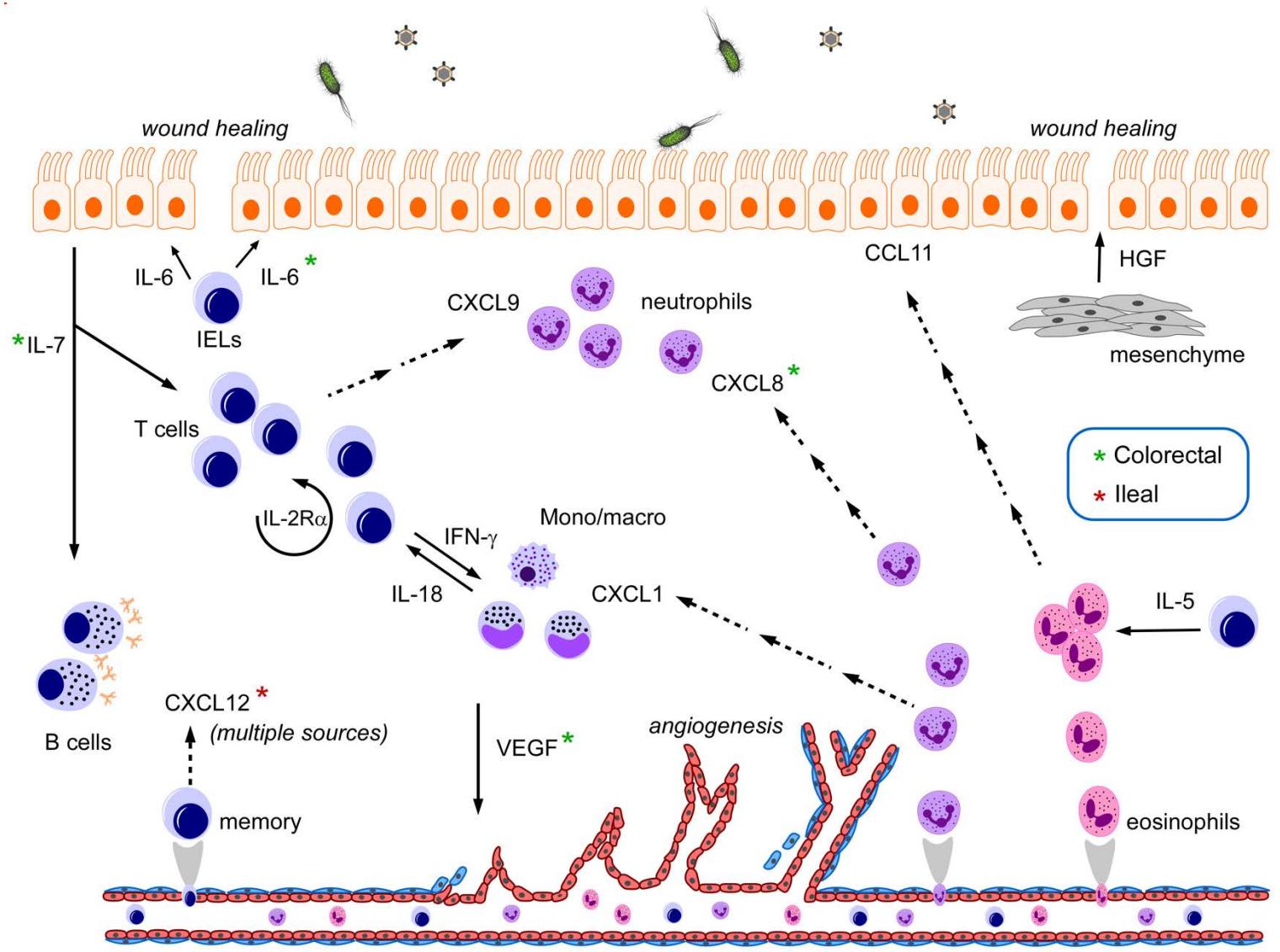
\+ Gene within GWAS locus

\+ Gene for its ligand/receptor within GWAS locus

Significant after Bonferroni correction (p<0.0015)

Serum Analyte Profiles Associated With Crohn's Disease and Disease Location.
Boucher G. et al. Inflamm Bowel Dis. 2022 Jan 5;28(1):9-20.

Serum Proteomics Can Detect Complex Pathophysiology



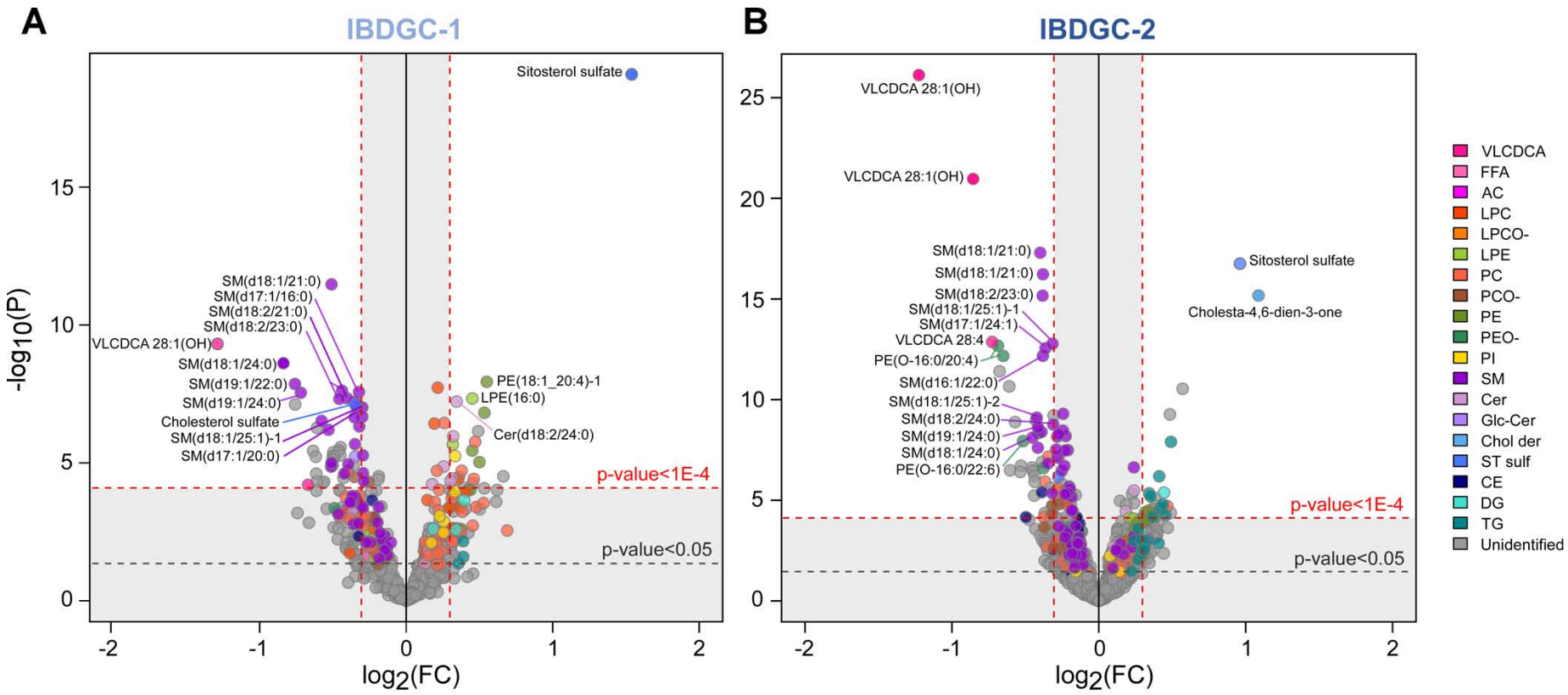
Serum Analyte Profiles Associated With Crohn's Disease and Disease Location.
Boucher G. et al. Inflamm Bowel Dis. 2022 Jan 5;28(1):9-20.

Untargeted Lipidomics (>1000 MS Features) Reveals Significant and Reproducible Differences between CD and Matched Controls

Retrospective
IBDGC Repository samples

- 300 CD
- 300 matched controls

>1000 lipid metabolites

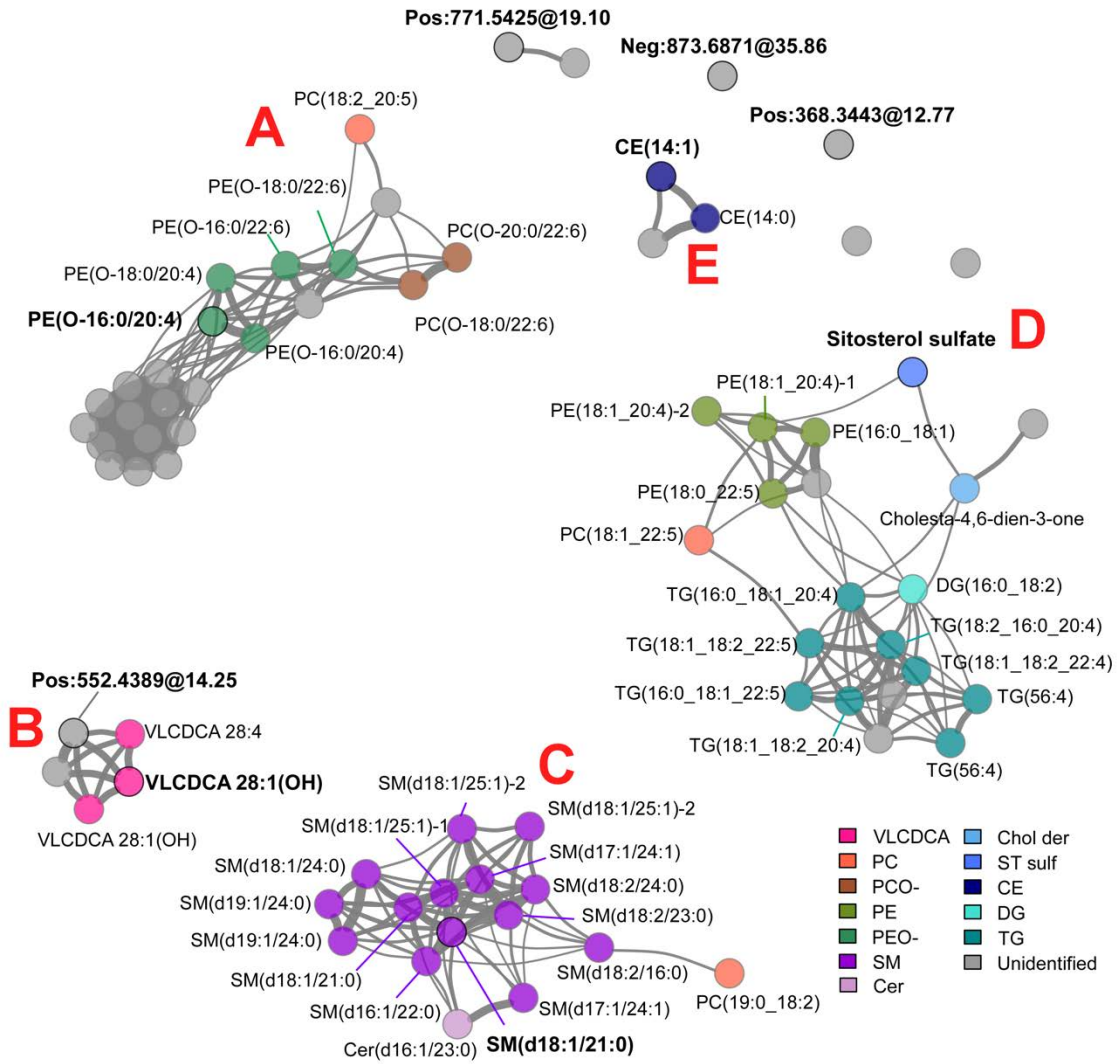


C

		Lipid sub-class																				Total
		VLCDCA	FFA	AC	LPC	LPCO-	LPE	PC	PCO-	PE	PEO-	PI	SM	Cer	Glc-Cer	Chol der	ST sulf	CE	DG	TG	ND	
# features	IBDGC-1 - p<0.05	2	1	3	23	3	11	40	9	10	1	6	47	11	3		2	11	3	3	132	**321**
	IBDGC-2 - p<0.05	3	1	3	11	5	9	51	18	14	9	3	60	24	7	1	2	36	5	20	502	**784**
	IBDGC-1 - p<1E-4 & | log_2(FC) | > 0.3	2			1		2	4	1	5		1	20	5	1		2	1			27	**72**
	IBDGC-2 - p<1E-4 & | log_2(FC) | > 0.3	3						3	2	4	5		14	1		1	1	2	1	8	28	**73**

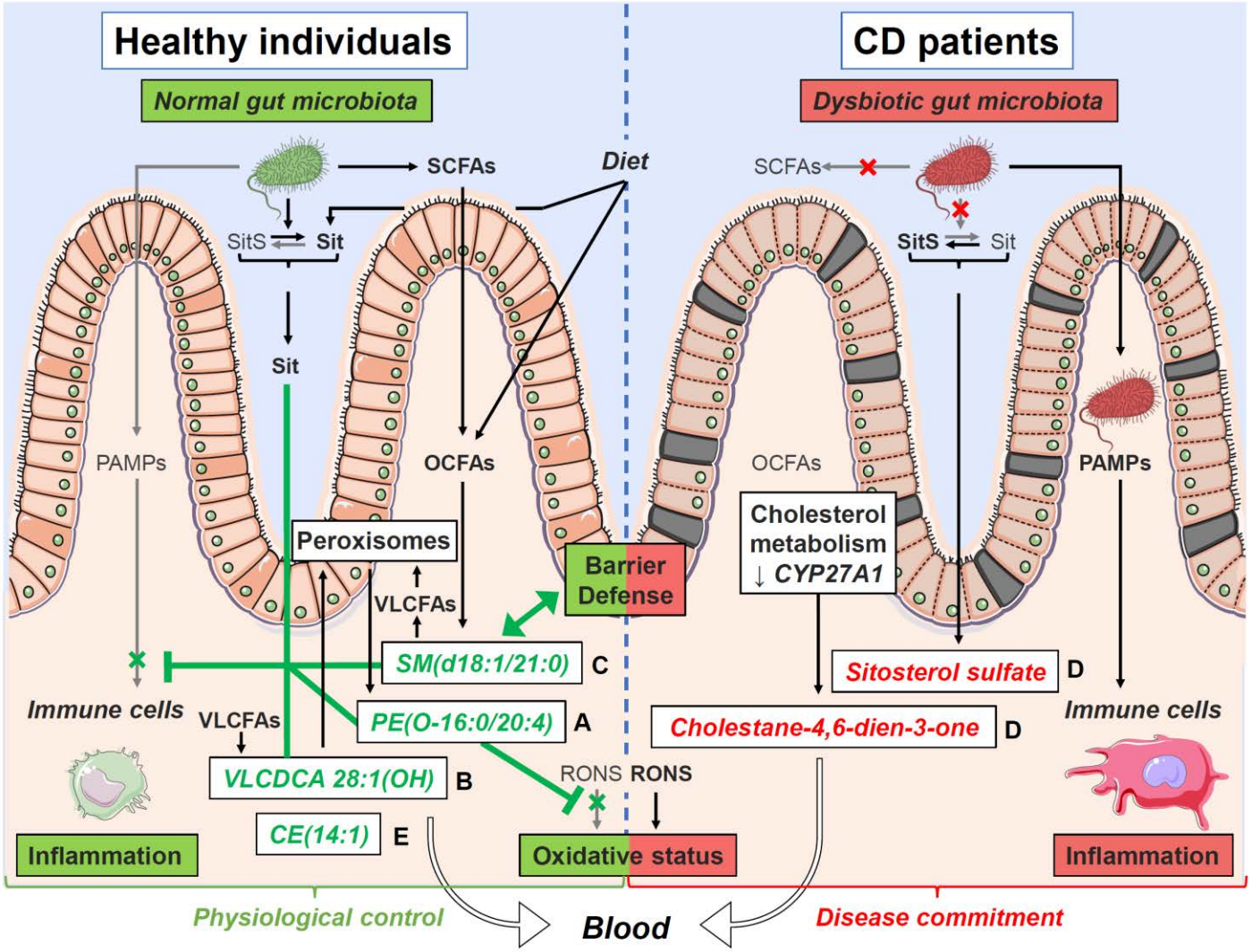
Serum Lipidomic Screen Identifies Key Metabolites, Pathways and Disease Classifiers in Crohn's Disease. Ferru-Clément et al. Inflamm Bowel Dis. 2023 Jul 5;29(7):1024-1037.

CD-Associated Lipid Metabolites Organize in Correlated Clusters



Serum Lipidomic Screen Identifies Key Metabolites, Pathways and Disease Classifiers in Crohn's Disease. Ferru-Clément et al. Inflamm Bowel Dis. 2023 Jul 5;29(7):1024-1037

Serum Lipidomic Profiles Reveal Biological Pathways (Host and Flora) Perturbed in CD



↓ PE(O-16:0/20:4) +5 cluster A
- PE essential neutrophil membranes
- Low levels reflection of dysregulated peroxisomal lipid metabolism

↓ VLCDCA 28:1(OH) +4 cluster B
- aka a Gastrointestinal Tract Acid (GTA)
- Anti-inflammatory effects (and structure) like SPMs

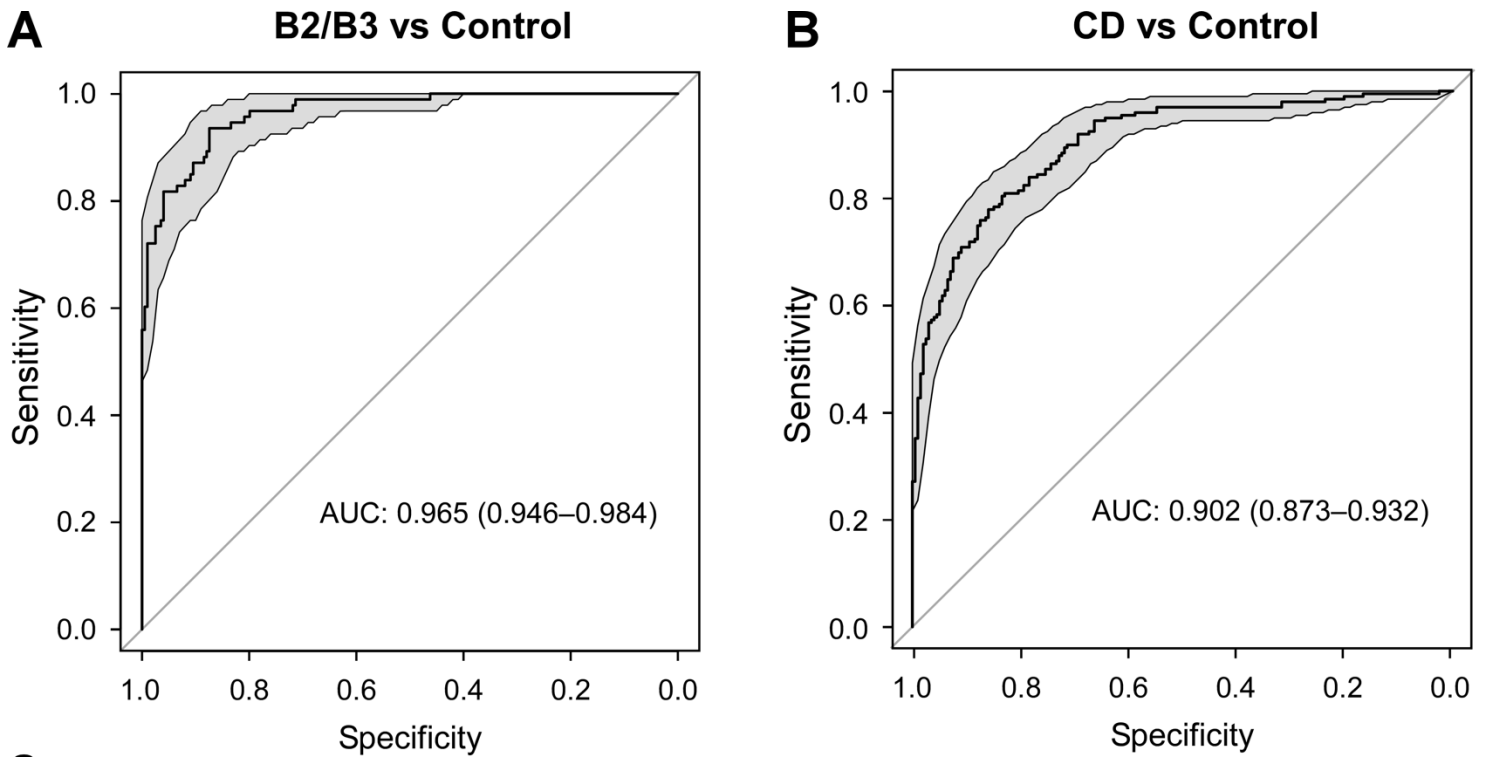
↓ SM(d18:1/21:0) +11 cluster C
- All 12 metabolites have OCFA or VLCFA
- OCFA from microbial (SCFA) or host (BCAA) metabolism
- VLCFA from dysregulated intestinal metabolism of SMs

↑ Sitosterol sulfate +15 cluster D
- Includes cholesta-4,6-dien-3-one
- Dysbiosis/Barrier dysfunction

↓ CE(14:1) +2 cluster E
- Impaired cholesterol metabolism - deficiency of plasmalogens
- Decreased ismA+ (chol dehydrogenase) microbial species

Serum Lipidomic Screen Identifies Key Metabolites, Pathways and Disease Classifiers in Crohn's Disease. Ferru-Clément et al. Inflamm Bowel Dis. 2023 Jul 5;29(7):1024-1037

Models Using 9 or Fewer Lipid Features are Strong Disease Classifiers



Currently in development as a Diagnostic tool for CD

Currently 50% of CD patients Diagnosis takes >2 years

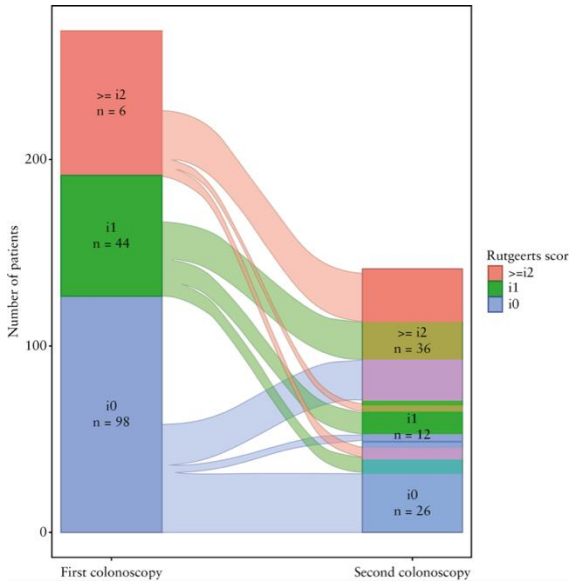
Serum Lipidomic Screen Identifies Key Metabolites, Pathways and Disease Classifiers in Crohn's Disease. Ferru-Clément et al. Inflamm Bowel Dis. 2023 Jul 5;29(7):1024-1037

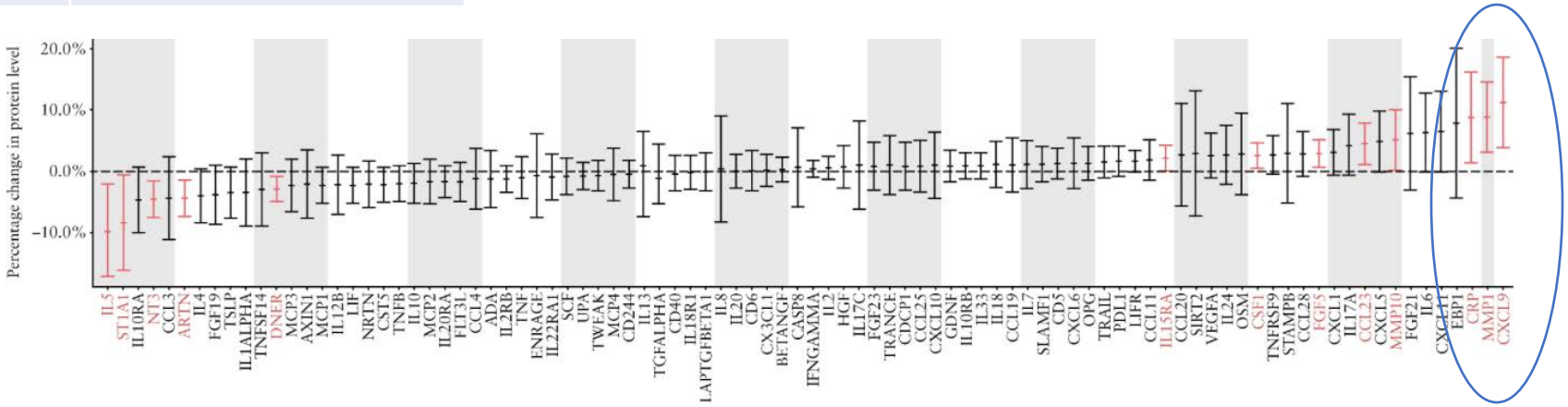
CXCL9 as a **Serum** Marker of Disease Recurrence in CD

CD recurrence following resection of terminal illeum (213 patients)

	Median time since surgery	Disease recurrence
1st colonoscopy	7 months (IQR 6-9)	60 (30%)
2nd colonoscopy	19 months (IQR 16-23)	36 (49%)

NIDDK IBD Genetics Consortium

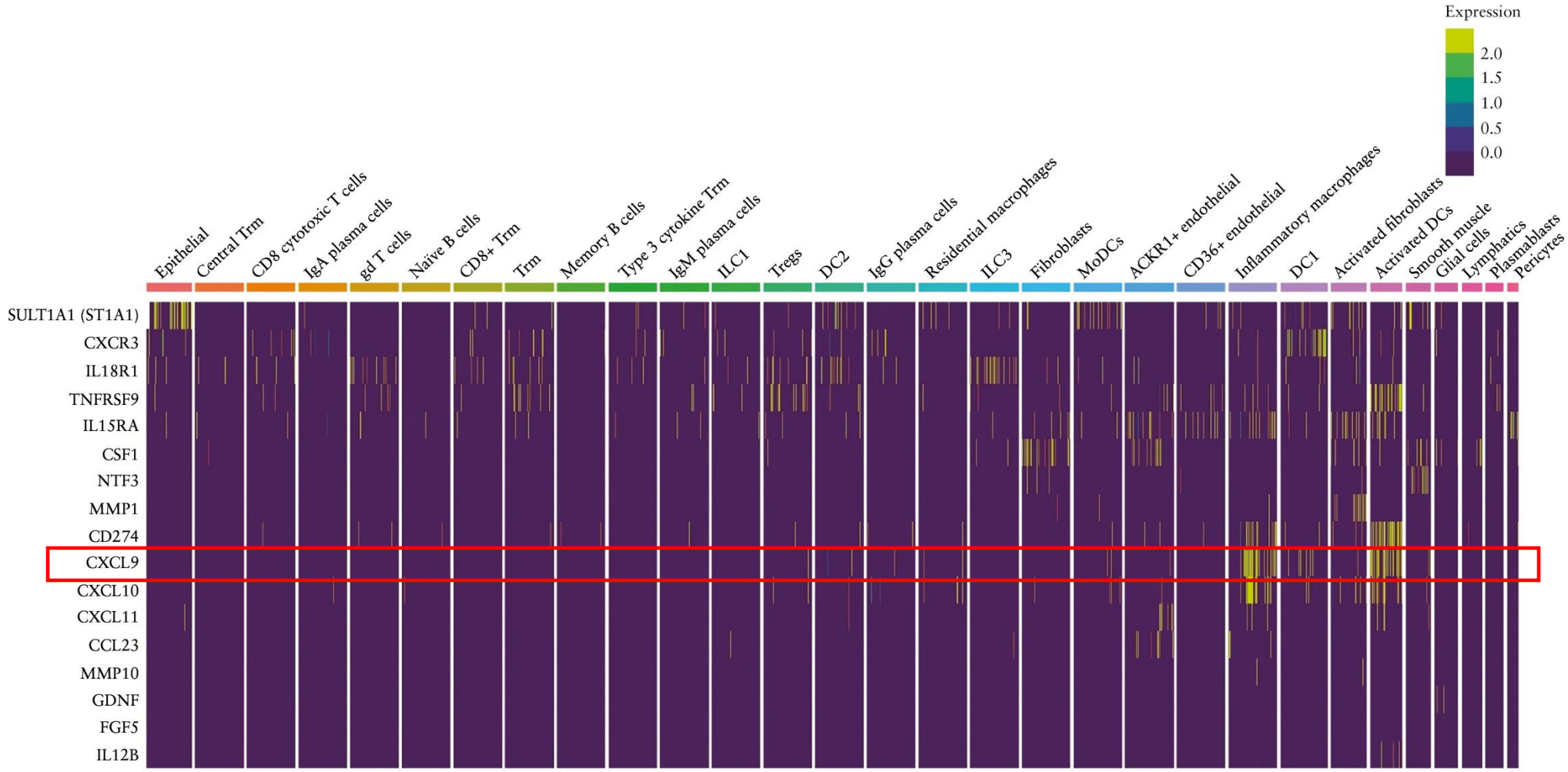




- CXCL9 most associated with disease recurrence
- CXCL9 & CXCL11 (two CCR3 ligands) among TNF subgroup

Walshe, M. et al. A Role for CXCR3 Ligands as Biomarkers of Post-Operative Crohn's Disease Recurrence. J Crohns Colitis. 2022 Jul 14;16(6):900-910.

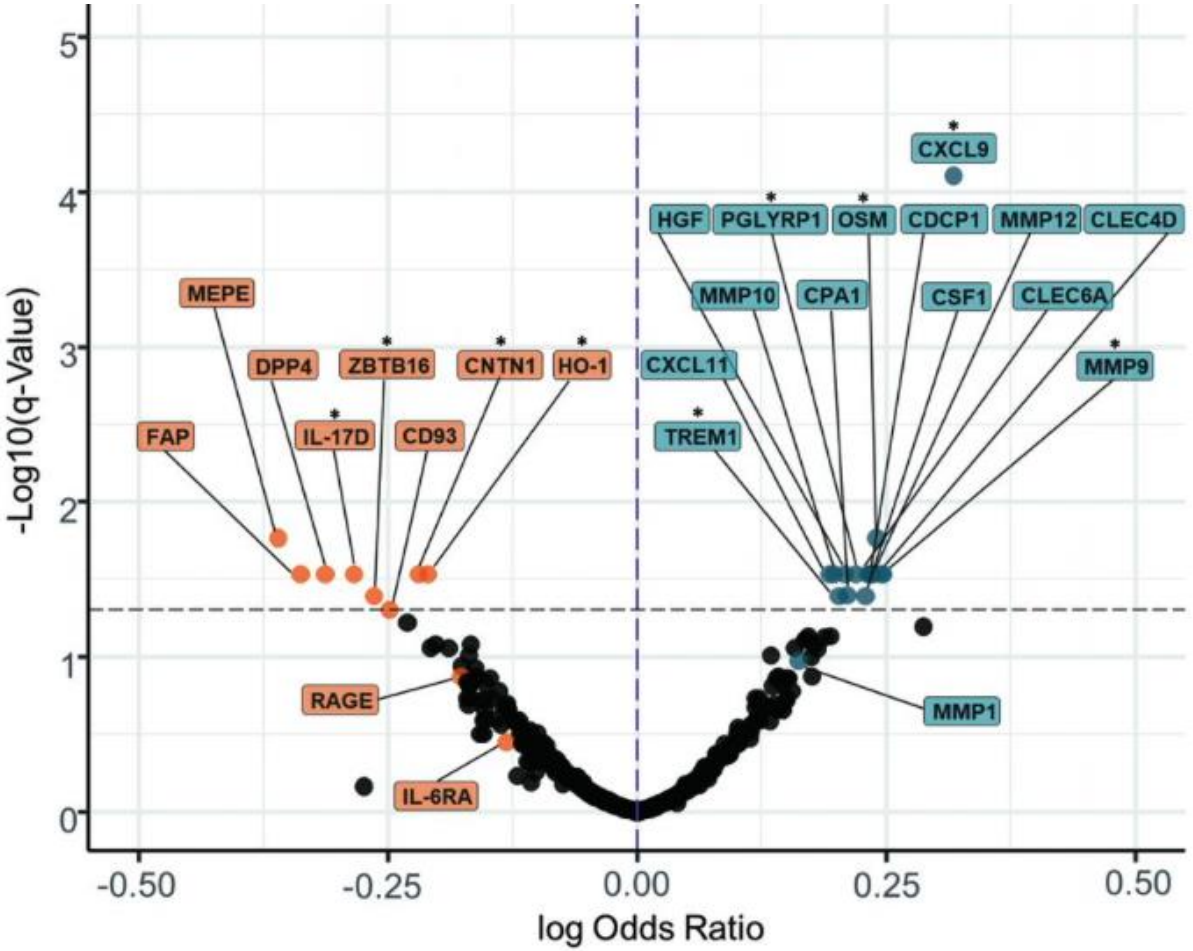
Single cell RNA sequencing expression of resected ileal tissues



Walshe, M. et al. A Role for CXCR3 Ligands as Biomarkers of Post-Operative Crohn's Disease Recurrence. J Crohns Colitis. 2022 Jul 14;16(6):900-910.

CXCL9 as a Pre-clinical Marker of Disease

2023



GEM Project

CXCL9 had the highest OR with future risk of CD (OR=2.07 per SD, 95% CI 1.58 to 2.73, q=7.9e-5)

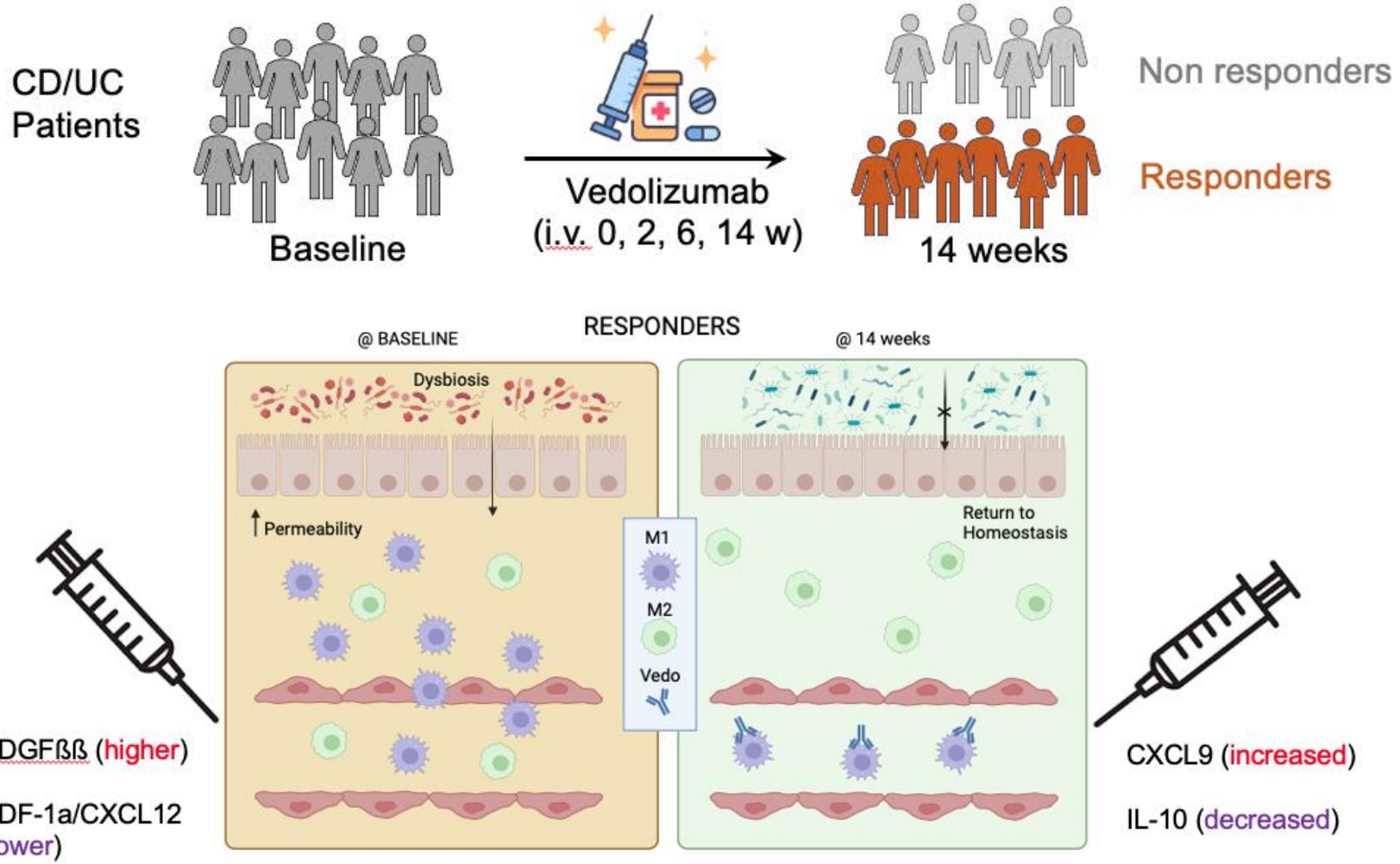
Leibovitzh H, et al. Immune response and barrier dysfunction-related proteomic signatures in preclinical phase of Crohn's disease highlight earliest events of pathogenesis. Gut. 2023 Feb 14:gutjnl-2022-328421. doi: 10.1136/gutjnl-2022-328421.

CXCL9 as a Marker of Response to Vedolizumab

iGENOMED
IBD GENOMIC MEDECINE CONSORTIUM

&

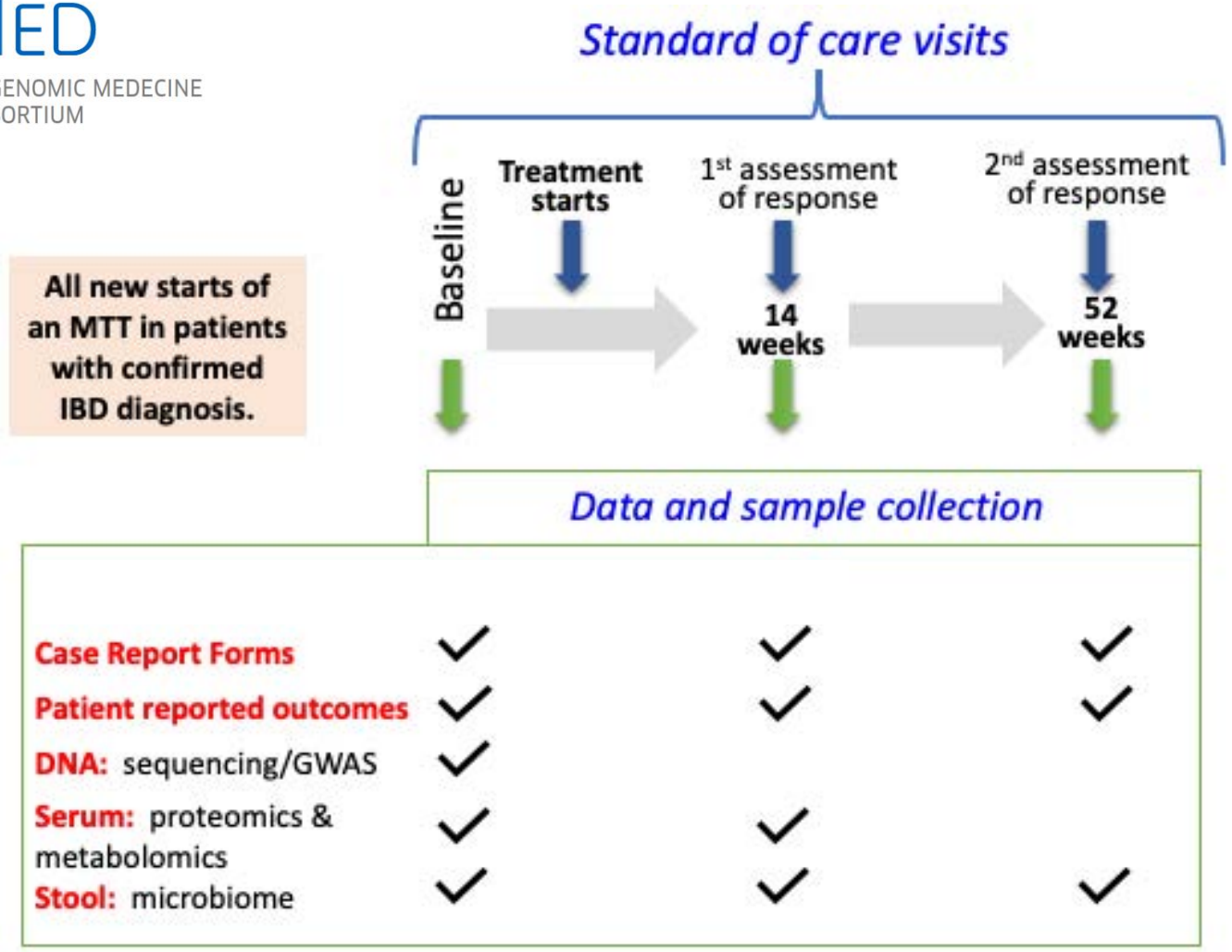
R. Xavier, MGH
A. Ananthakrishnan MGH



A pilot study to identify blood-based markers associated with response to treatment with Vedolizumab in patients with Inflammatory Bowel Disease
Rioux, JD. et al. medRxiv, doi: https://doi.org/10.1101/2024.09.19.24314034

Prospective Multi ‘omic Study of Treatment Response

iGENOMED
IBD GENOMIC MEDECINE CONSORTIUM



Current recruitment sites:

- MUHC (PI: Bitton)
- CHUM (PI: Battat)
- Chicoutimi (PI: Tremblay)
- Johns Hopkins (PI: Lazarev)

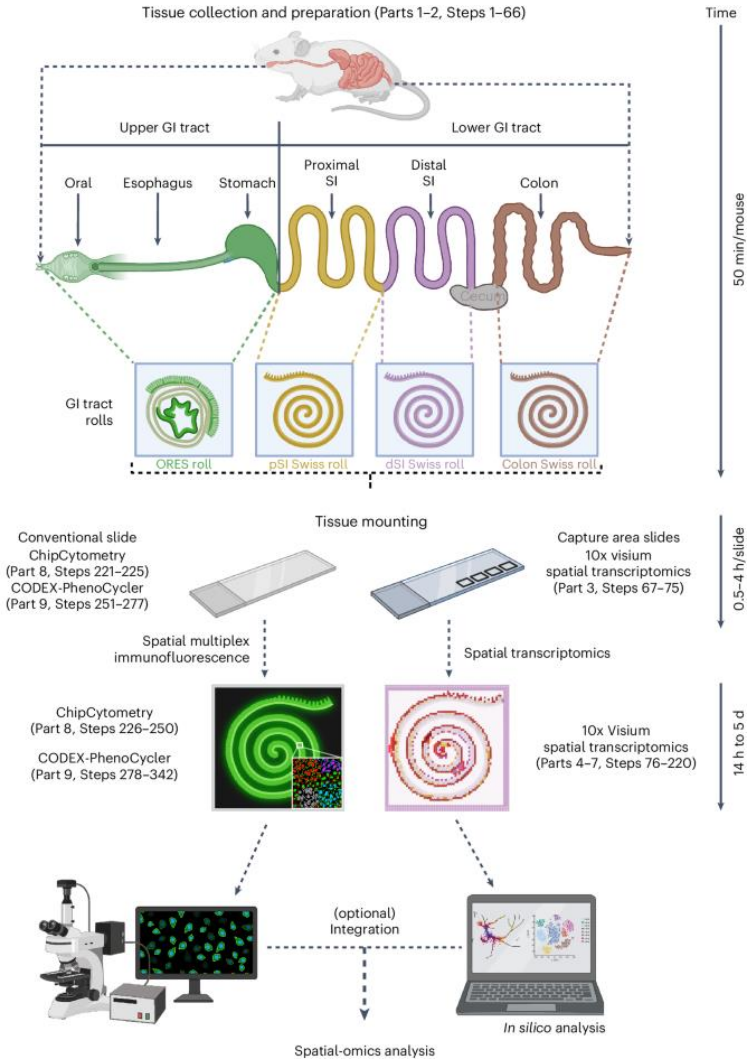
Pilot:
150 patients (completed)

Main study:
Additional 400+ patients (ongoing)

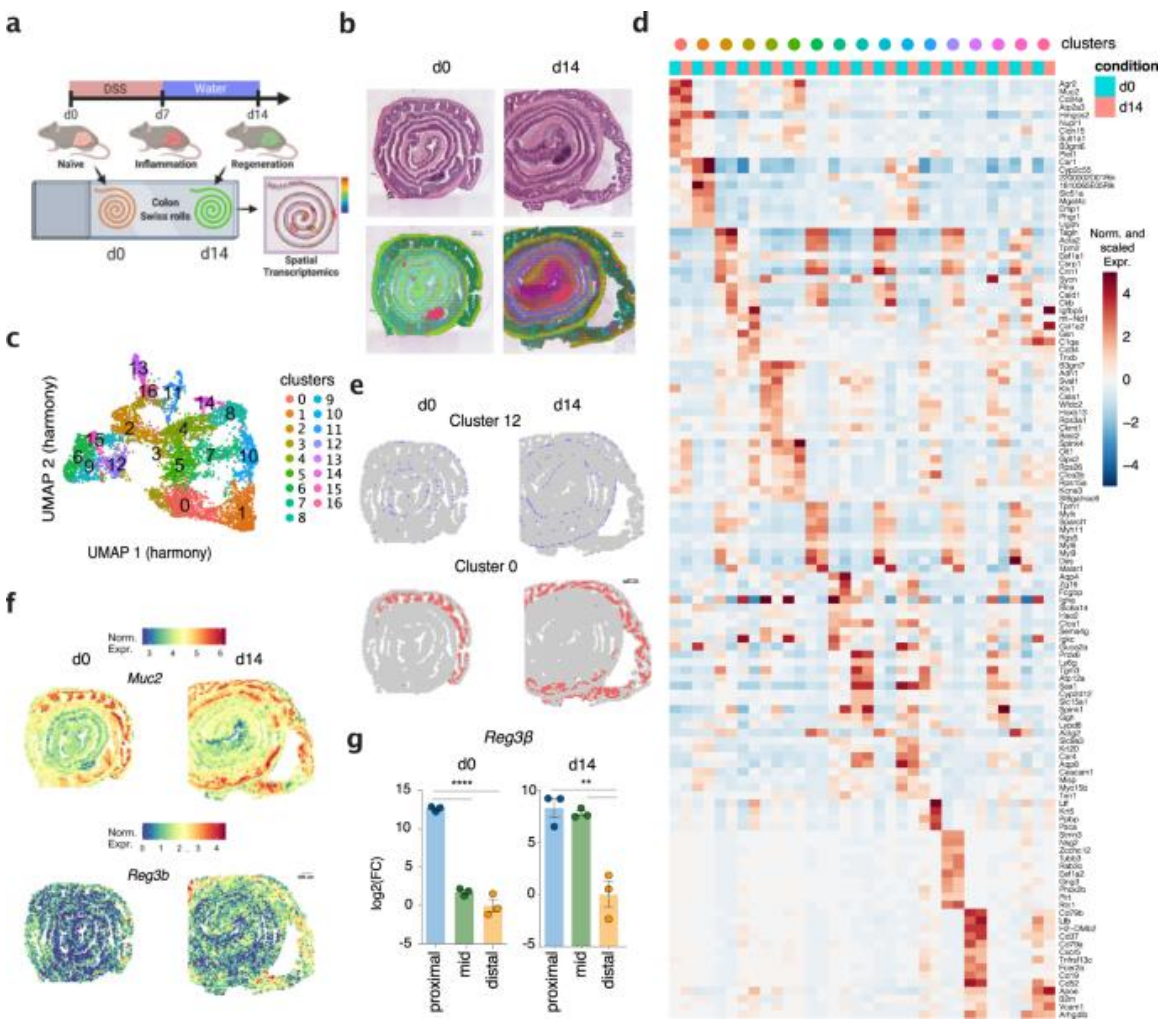
Substudies:

Immune cell status (FACS)
Immune cell responsiveness
scRNAseq of PBMC (ongoing)
spatial of intest. biopsies (planned)

A Spatial Transcriptomic Study of Wound Healing



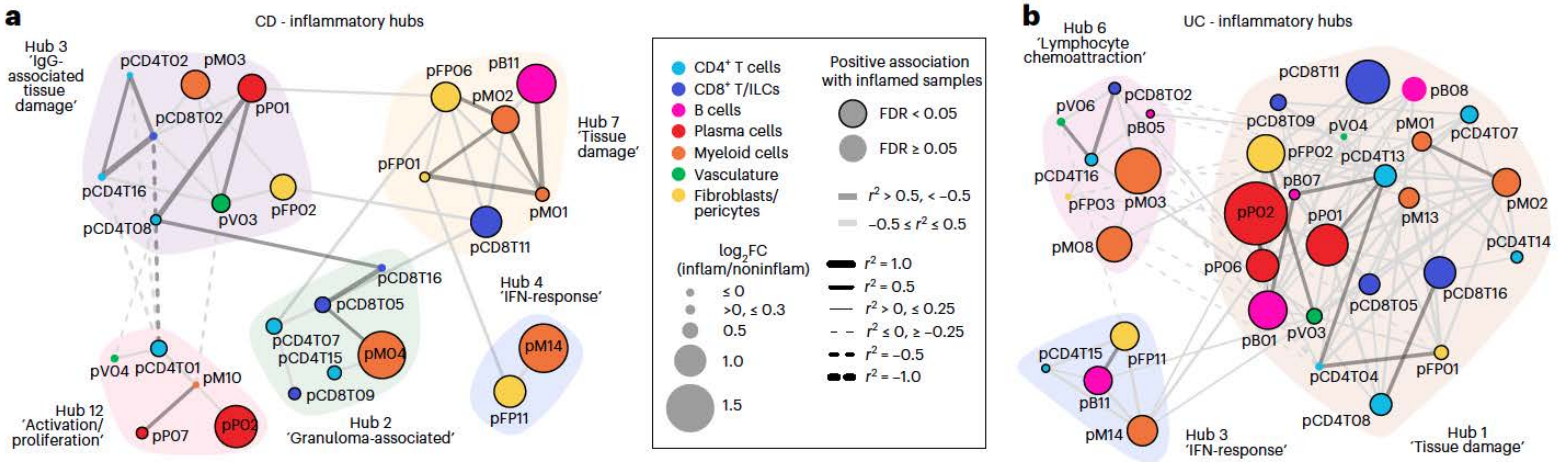
Monasterio, G., et al. A versatile tissue-rolling technique for spatial-omics analyses of the entire murine gastrointestinal tract. Nat Protoc 19, 3085–3137 (2024).



Parigi, S.M., et al. The spatial transcriptomic landscape of the healing mouse intestine following damage. Nat Commun 13, 828 (2022).

A longitudinal single-cell atlas of anti-tumour necrosis factor treatment in inflammatory bowel disease

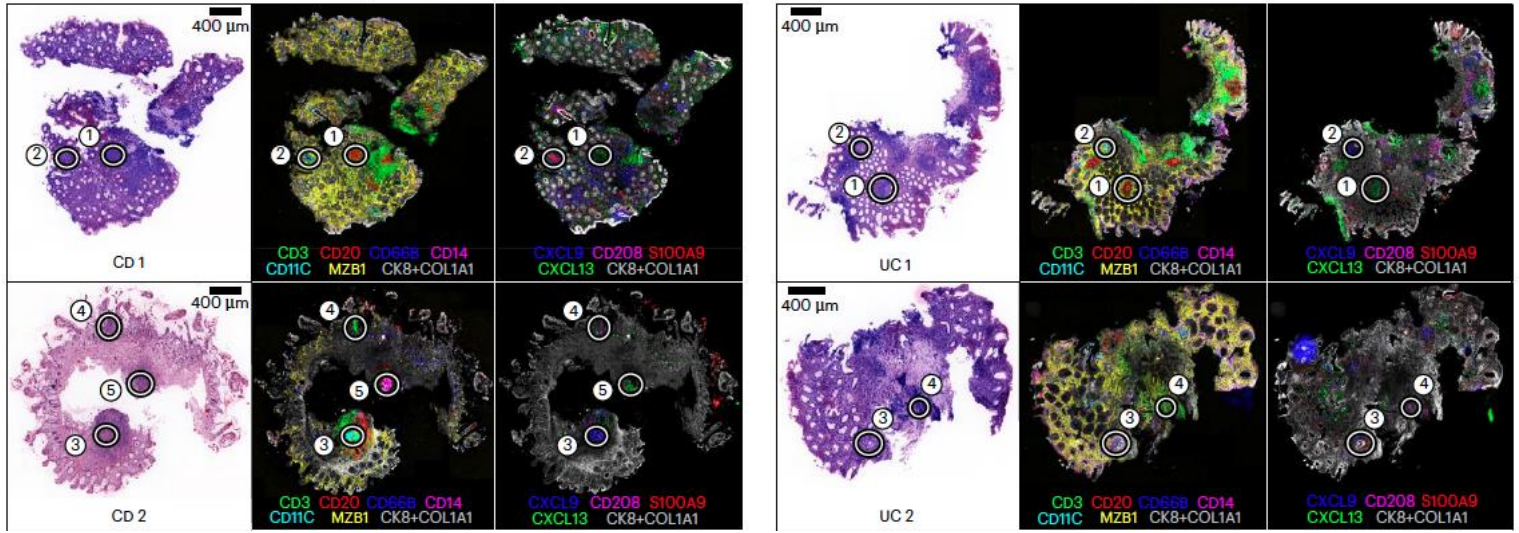
* 38 biologic-naïve patients with CD or UC and three healthy controls
* across five gut regions (terminal ileum, ascending colon, descending colon, sigmoid and rectum)
* before and after treatment with adalimumab/humira



Hub 2		Hub 3		Hub 4		Hub 7		Hub 12	
BATF	*HLA-DQB1*	*CCR7*	*RPL26*	*CXCL9*	*GBP1*	*AQP9*	*CXCL8*	*BTG2*	*MAF*
CTLA4	*HLA-DRB1*	*CXCR4*	*RPL28*	*CXCL10*	*ISG15*	*CCL3*	*FCER1G*	*DDX3X*	*MALAT1*
CTSH	*TNFRSF18*	*DERL3*	*RPL30*	*CXCL11*	*LAP3*	*CEMIP*	*FTH1*	*H3F3B*	*NEAT1*
GZMA	*TNFRSF4*	*IGFBP7*	*THY1*	*IDO1*	*STAT1*	*CTHRC1*	*GNLY*	*IER2*	*NFKBIZ*
NCR3	*TNFRSF9*	*MZB1*	*TIMP1*	*IFIT3*	*TNFSF10*	*CXCL6*	*SOD2*	*JUN*	

Hub 1				Hub 3		Hub 6	
BATF	*TNFRSF18*	*TIMP1*	*COL5A3*	*CXCL9*	*GBP1*	*CCR7*	*RSPO3*
CTLA4	*TNFRSF4*	*IL17A*	*FTH1*	*CXCL10*	*ISG15*	*CLIC3*	*S100A4*
GZMA	*TNFRSF9*	*CXCL13*	*CCL3*	*CXCL11*	*LAP3*	*CXCL12*	*S100A6*
GZMB	*TNFRSF1B*	*CD38*	*CCL20*	*IDO1*	*STAT1*	*RPL10*	*TCL1A*
GNLY	*HAVCR2*	*MKI67*	*MAF*	*IFIT3*	*TNFSF10*	*RPL12*	*TSPAN13*

CXCL9 expression pattern suggests IFN signaling is associated with inflammation and can be found in T cell aggregates and/or regions of epithelial damage in both diseases

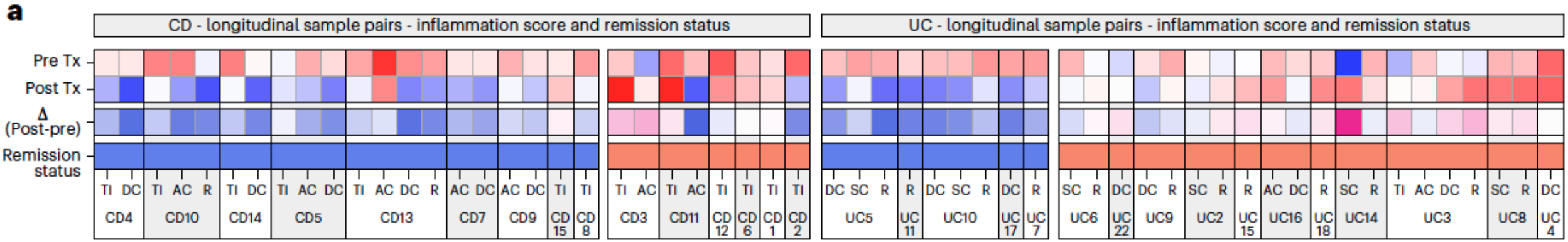


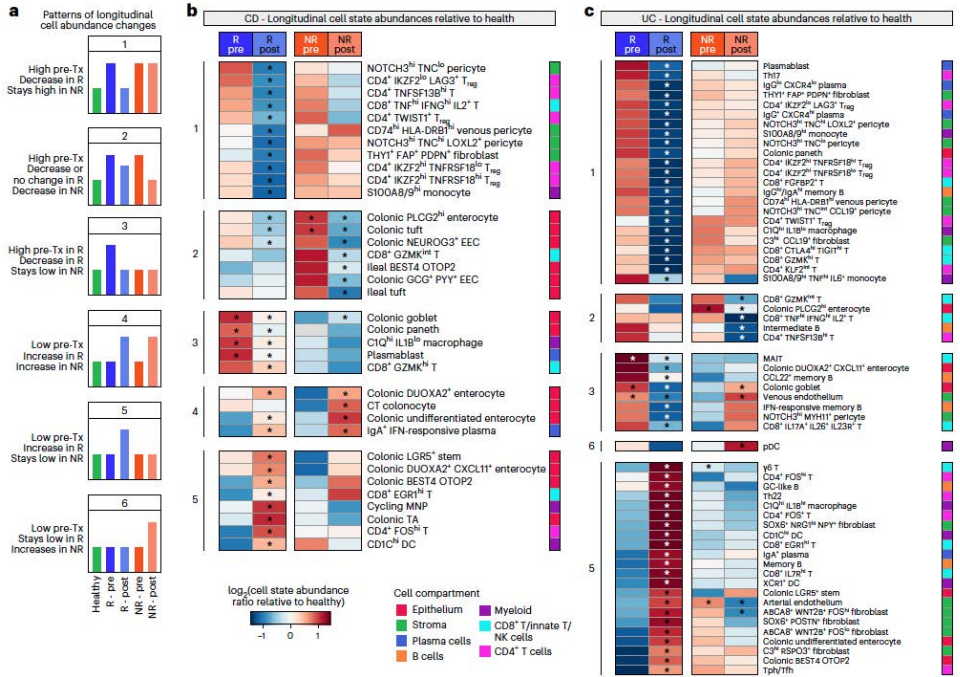
Thomas T. et al.
Nature Immunology volume 25, pages 2152–2165 (2024)

A longitudinal single-cell atlas of anti-tumour necrosis factor treatment in inflammatory bowel disease

9R; 6 NR

5R; 11 NR





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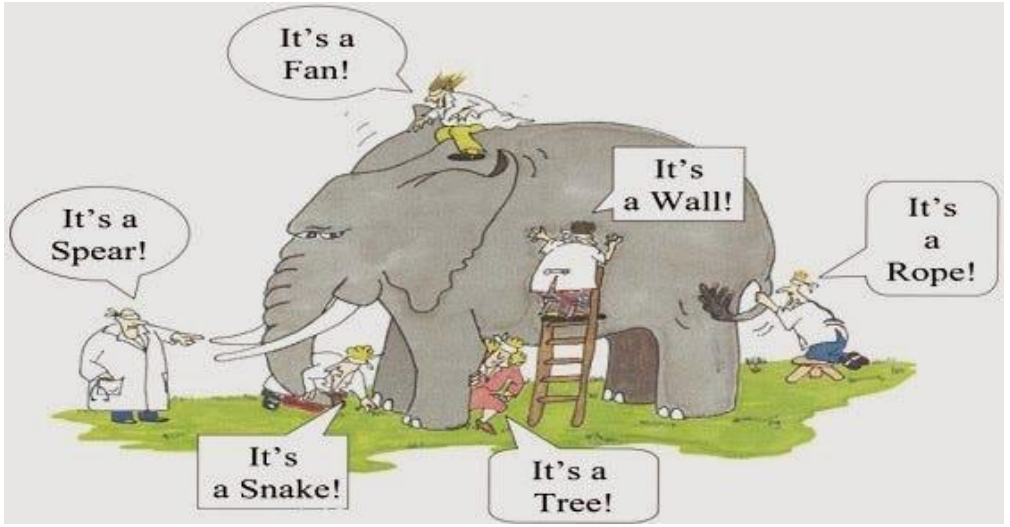
Conclusions



Need to integrate information from DNA, RNA, Protein, Metabolite, etc.

- Significant technological advances in multi 'omics (incl. bulk, single cell, spatial)
- Great potential for understanding dynamic processes
- Integration across "omic" types key to getting full picture
- Robust nature allows comparison across studies and contexts
- CXCL9 across studies pointing to importance of this pathway

Thank you

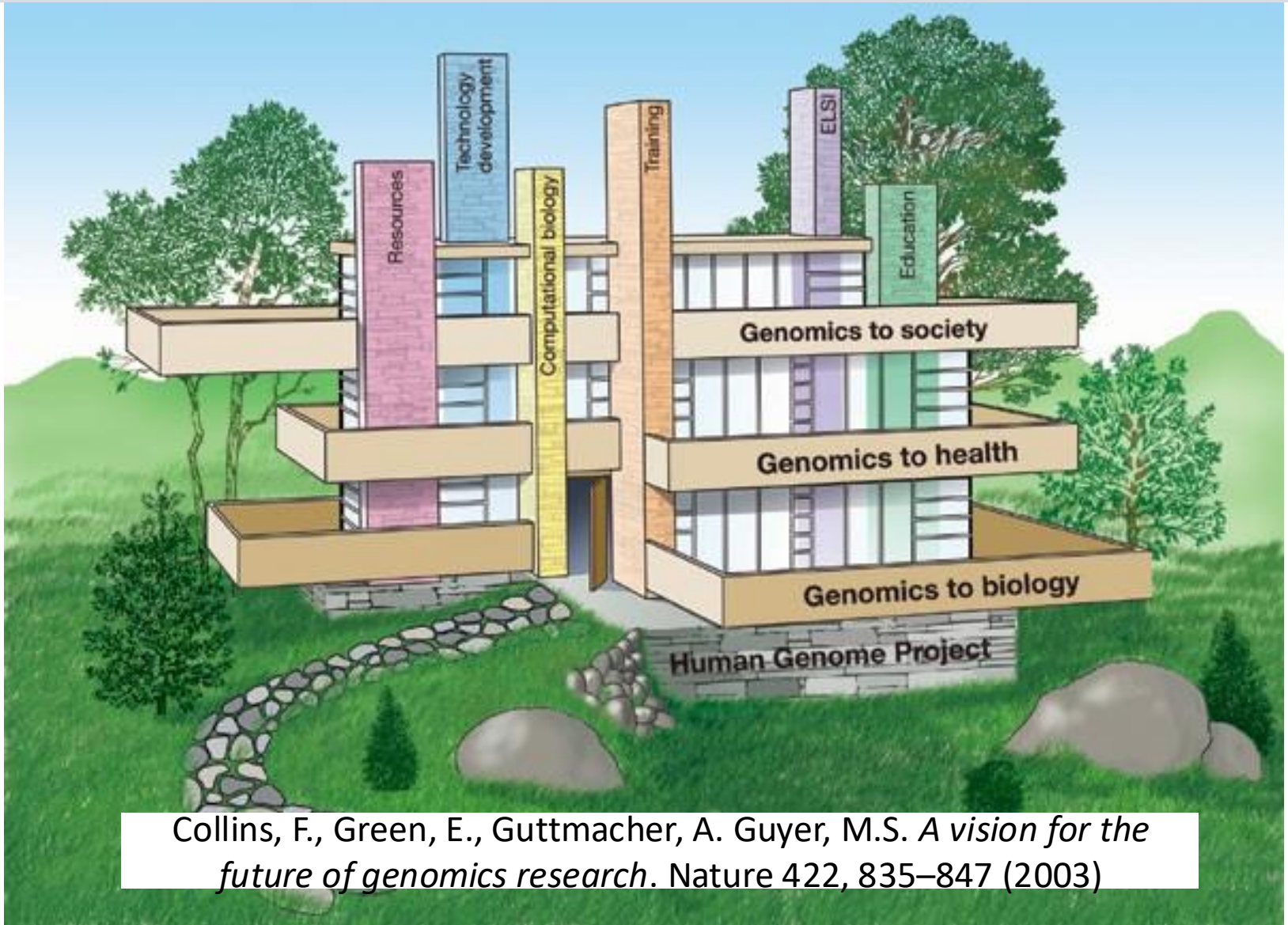
crohn's colitis

Genome Canada

GenomeQuébec

NIH National Institute of Diabetes and Digestive and Kidney Diseases

“The future of genomics rests on the foundation of the Human Genome Project; NHGRI 2003”

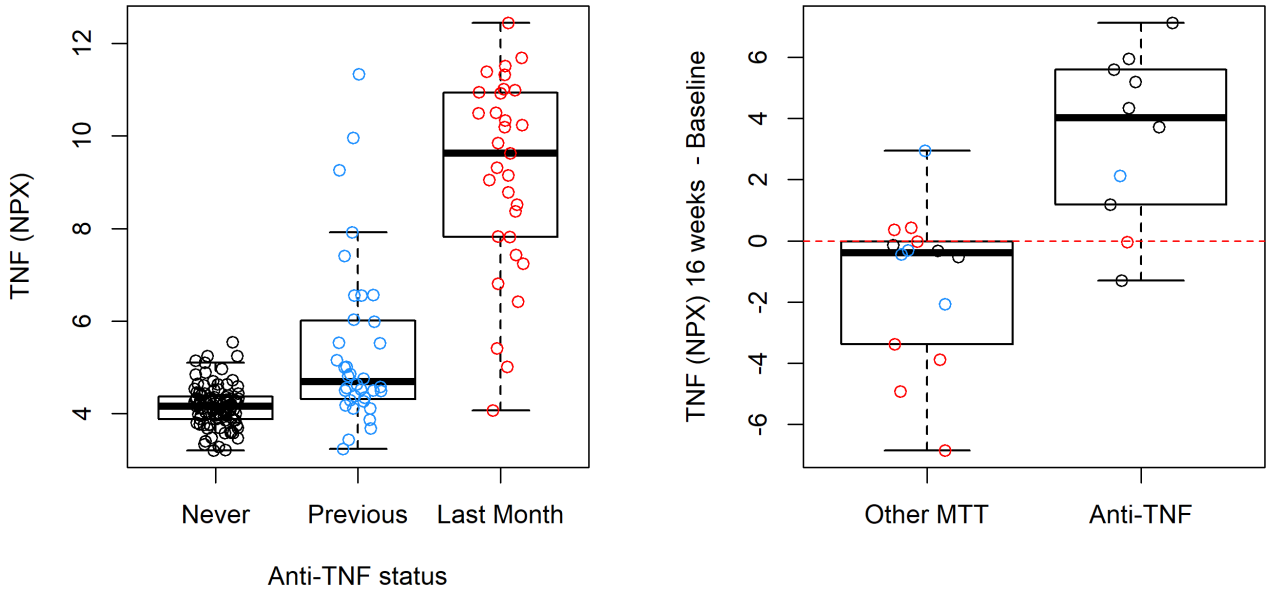


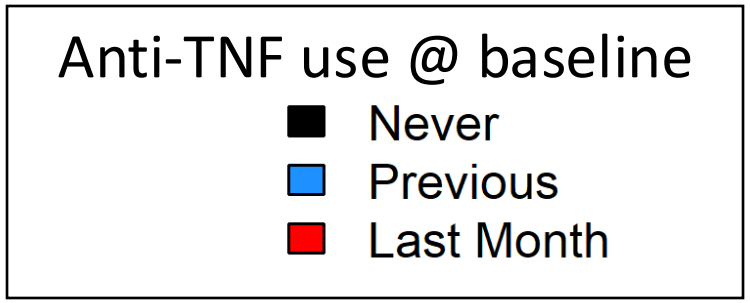
Collins, F., Green, E., Guttmacher, A. Guyer, M.S. *A vision for the future of genomics research*. Nature 422, 835–847 (2003)

Preliminary results – serum proteomics

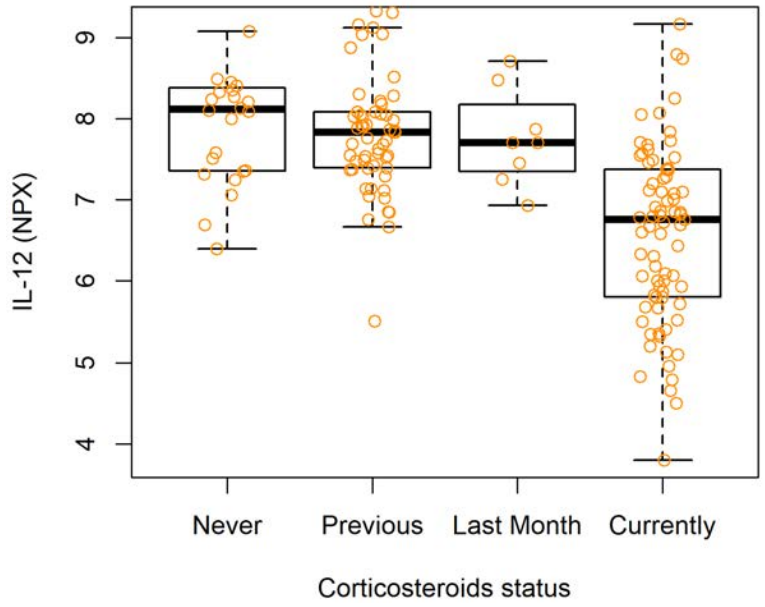
(128 analytes post QC)

Accumulation of complexed TNF





Likely effect of corticosteroid use on serum IL-12 levels



Rioux, unpublished