Lessons learned from monitoring T cell responses to SARS-CoV-2 infection and vaccination

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WCHH WOMEN'S COLLEGE HOSPITAL Health care for women REVOLUTIONIZED

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SARS-CoV-2 Convalescent Study

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COVID-19 IMMUNITY TASK FORCE
GROUPE DE TRAVAIL SUR L'IMMUNITÉ FACE À LA COVID-19

Immunity to SARS-CoV-2

What do T cells tell us about immunity following SARS-CoV-2 infection or vaccination?

STUDY 1

What does the T cell response to SARS-CoV-2 look like?

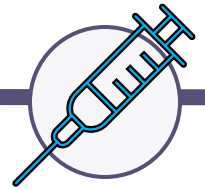
Law et al. Systematic examination of T cell responses to SARS-CoV-2 versus influenza virus reveals distinct inflammatory profile. **Journal of Immunology 2021 206, 37-50.** (N=13, 46% hospitalized 64% male)

Whole Spike and N stimulation

STUDY 2

Does the T cell response persist? What does it look like?

Law et al. Persistence of T cell and antibody responses to SARS-CoV-2 up to 9 months after symptom onset. **Journal of Immunology. 2022. 208, 429-443.** (N=24, mostly community infections)
S1,S2,N, M, E, peptide pools



Late 2020

STUDY 3

Immunogenicity of COVID-19 vaccines in immunocompromised individuals

Dayam, Law et al. Accelerated waning of immunity to SARS-CoV-2 mRNA vaccines in patients with immune mediated inflammatory diseases.
JCI insight. 2022. 7 (11).

Cheung et al. Third dose corrects waning immunity to SARS-CoV-2 mRNA vaccines in immunocompromised patients with immune-mediated inflammatory diseases
RMD Open 2022;8:e002622
161 patients

Cheung, Dayam, Shapiro et al. in prep.

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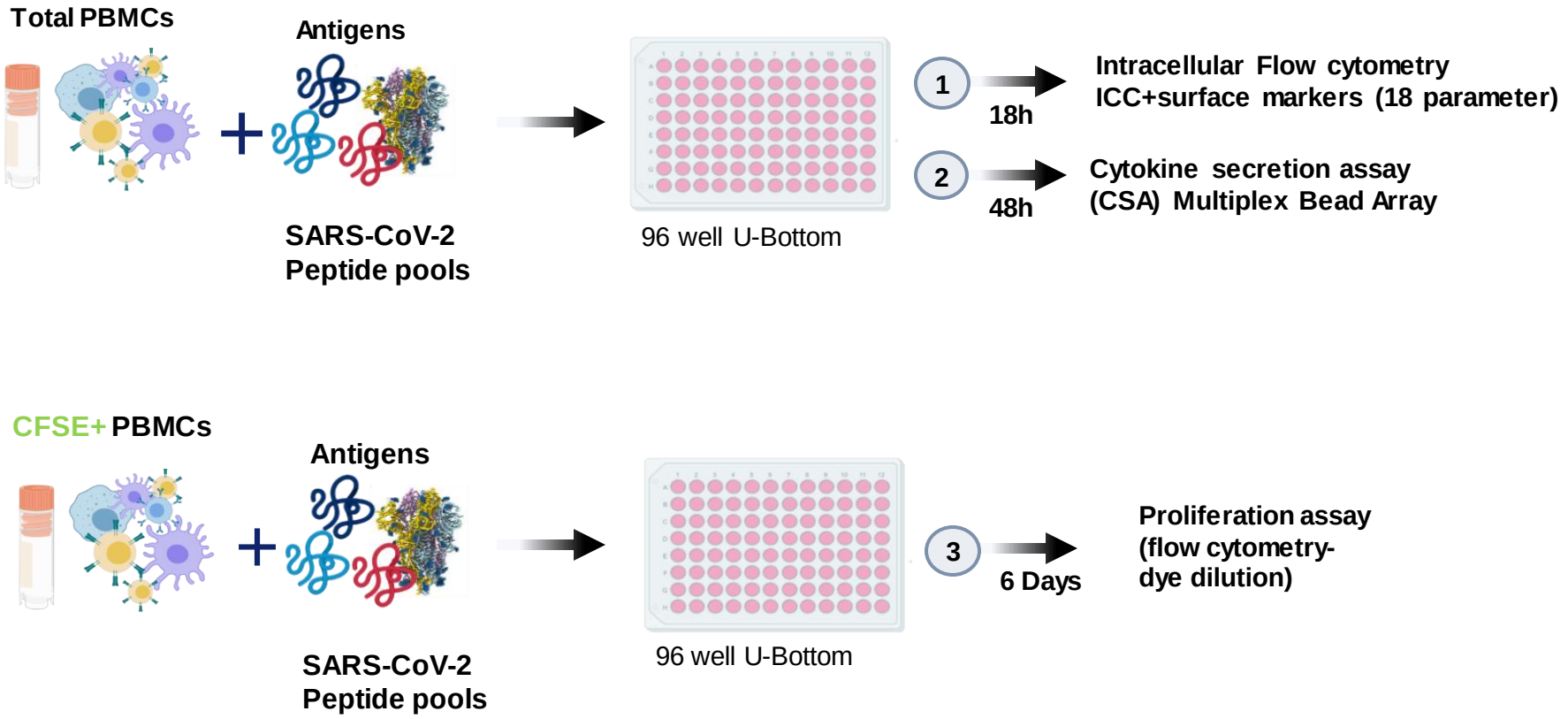
Assays we used to assess memory T cell responses to SARS-CoV-2 after infection

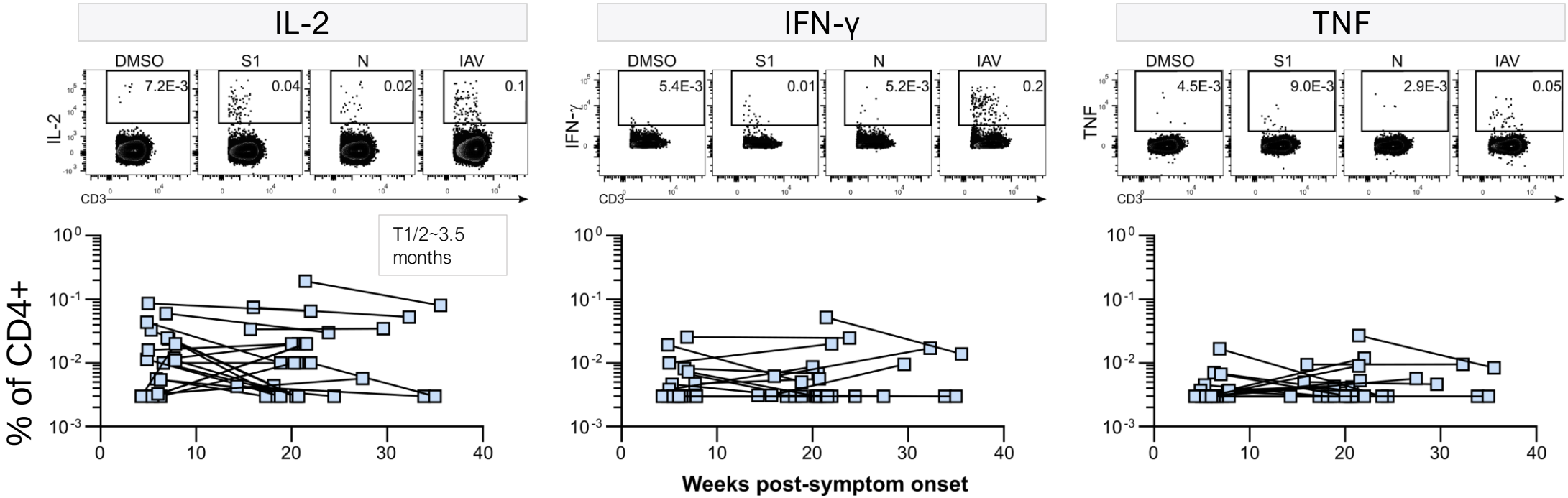
Experimental workflow
3 assays: ICC, Secreted cytokines, Proliferation



Jaclyn Law

S-specific response dominated by IL-2+ CD4s and persists up to 9 months

Response to spike peptide pools: T1 median 59 days (30-154) , T2v 160 days (55-249)



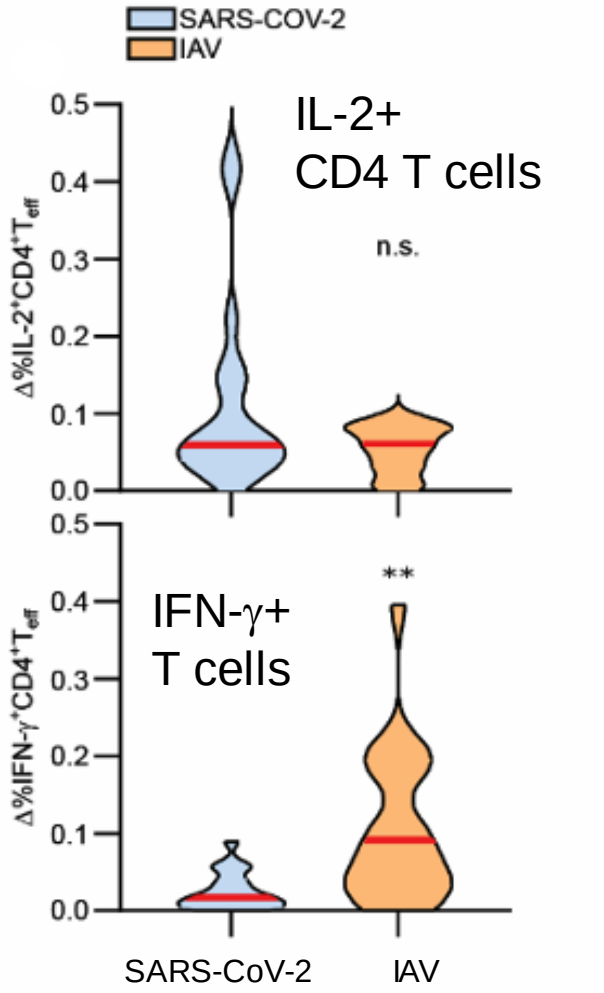
24 COVID-19 convalescent (12 male, 12 female), mainly mild cases

Similar results with other peptide pools tested: NP, M, E

Law et al. Journal of Immunology. 2022. 208, 429-443.

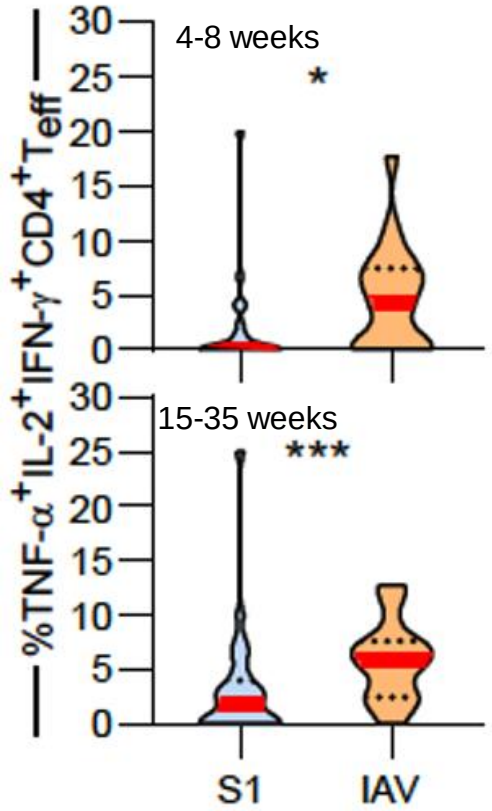
Dan et al. Science 2021 – 3 months
Cohen et al. Cell 2021 – 6 months

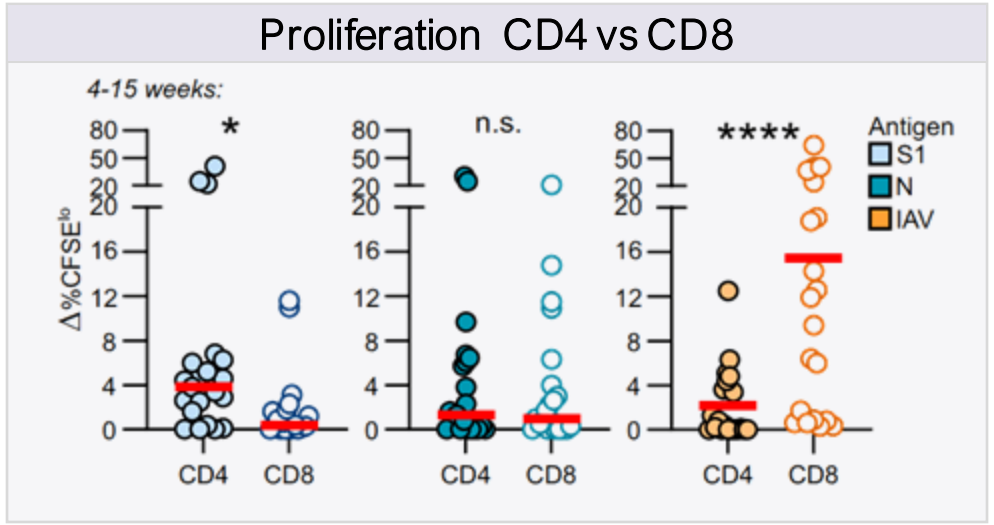
SARS-CoV-2: fewer IFN-γ+ T cells than IAV-specific responses, less multifunctional and CD4>CD8



4-8 weeks, CD4+ Responses:
Sum of all tested peptide pools
(S, NP, M, E) vs IAV

Multifunctional T cells





Law et al. Journal of Immunology. 2022. 208, 429-443.

Summary and Conclusions
T cell responses to SARS-CoV-2 infection

Findings:

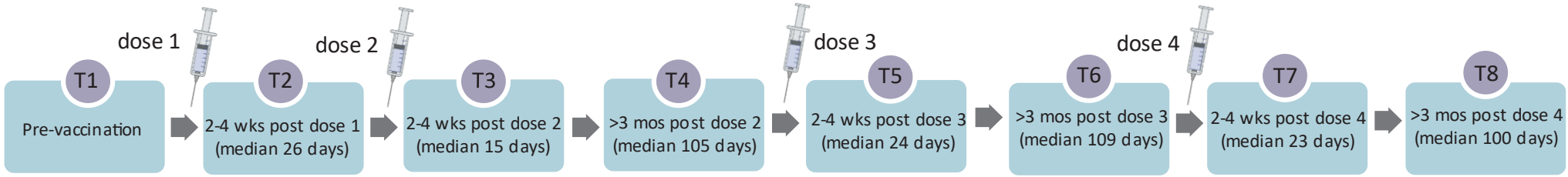
- IL-2 producing CD4 T cells were most frequent T cell subset to Spike, weak CD8 response
- Persistent phenotype up to 9 months PSO, moderate decline
- pTfh responses that correlate with plasma Abs
- More TNF response in the hospitalized cohort compared to the mild cohort (not shown)

Altogether, suggests SARS-CoV-2 T cell responses distinct from typical responses seen against influenza where CD8 IFNγ producing cells dominate

Lessons

- ICC - combined with other markers- wealth of info- but low frequency-limits accuracy, time consuming
- AIM assay- didn't capture all cytokine producing cells, didn't reveal cytokine profile
- Chose CSA for next study- higher throughput- similar cytokine conclusions revealed as ICC, albeit can't distinguish CD4 vs. CD8 responses

IMPACT Study: Immune response after COVID-19 vaccination during maintenance therapy in immune-mediated inflammatory diseases (IMID)



161 patients, >600 samples

Inclusions:
mRNA vaccines
healthy controls & IMID patients
inflammatory bowel disease *(Crohn's disease, UC)*
inflammatory joint disease
(rheumatoid or psoriatic arthritis, ankylosing spondylitis)
inflammatory skin disease
(psoriasis, hidradenitis suppurativa)

Exclusions: <18, COVID infections, steroids, B cell depletion

- Healthy controls
- IMID untreated
- methotrexate (MTX) / azathioprine (AZA)
- α-TNF+MTX/AZA
- α-TNF
- α-IL-17
- α-IL-23
- α-IL-12/23

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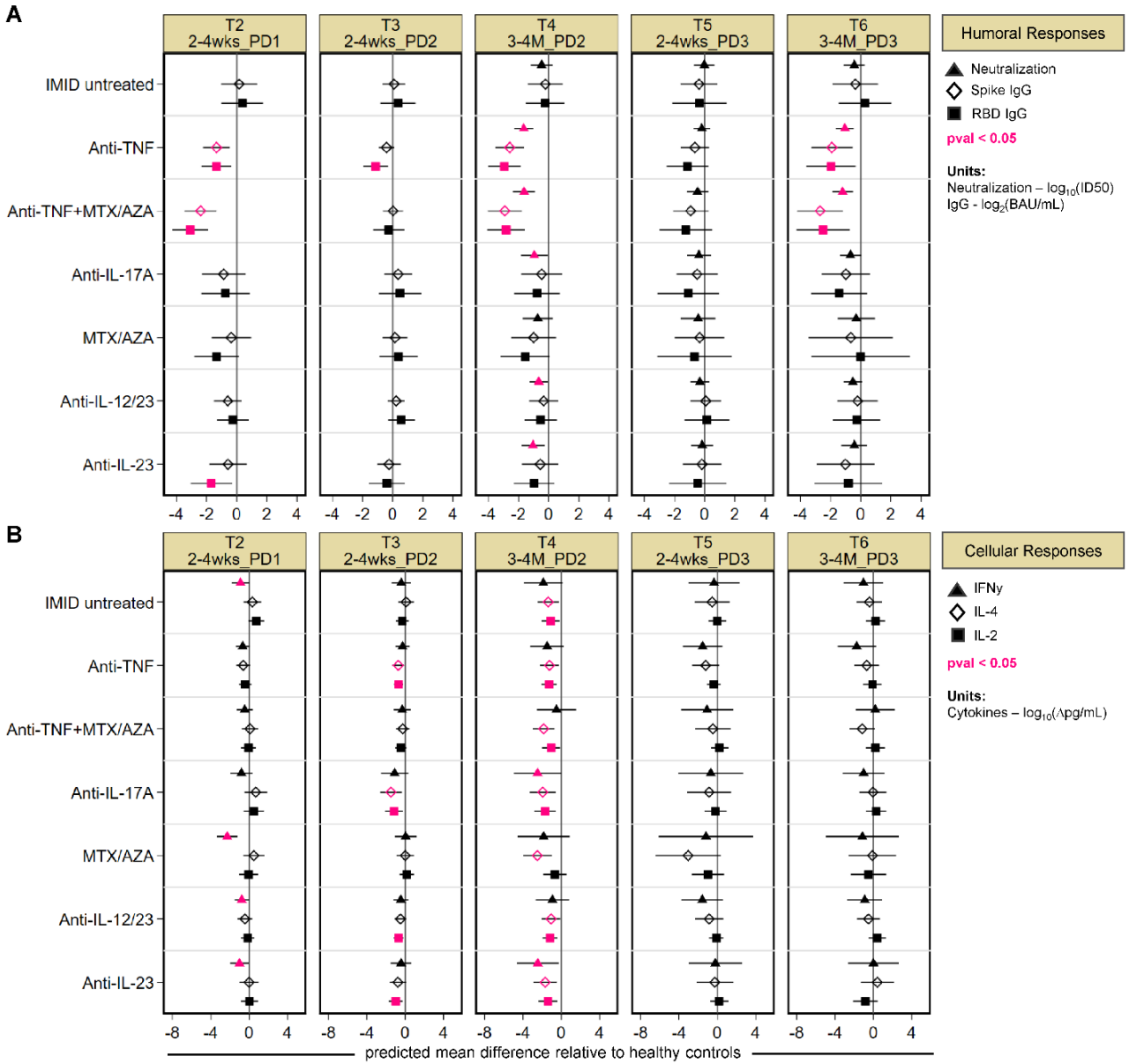
Antibody levels, neutralization, T cell stimulation- secretion of cytokines/cytotoxic molecules
Multivariate analysis controlled for age/sex/BMI/vaccine type

Anti-TNF group shows impaired humoral responses to SARS-CoV-2, especially VOC



- Anti-TNF lower antibody responses compared to rest of IMID group and HC throughout, driven by IBD group

Not shown:

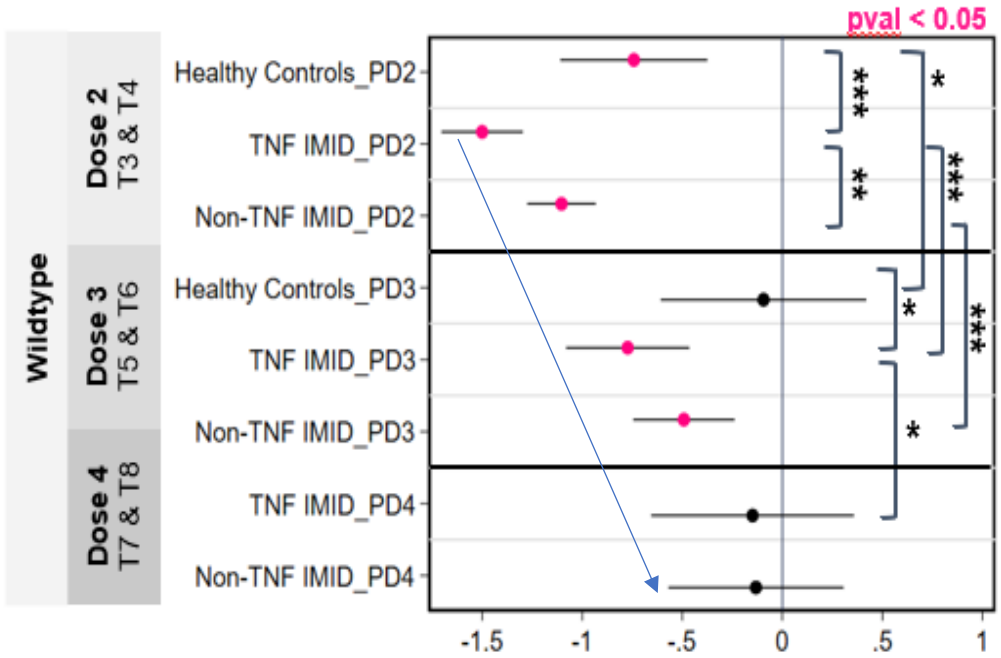
- 3 months after dose 2, anti-TNF treated IMID patients fail to neutralize VOCs (BA.1, BA.5): **3rd dose critical to broaden neutralization response to include VOC**

Least-squares linear regression models controlled for age, BMI, sex, and vaccine type.

Cheung, Dayam, Shapiro et al. submitted

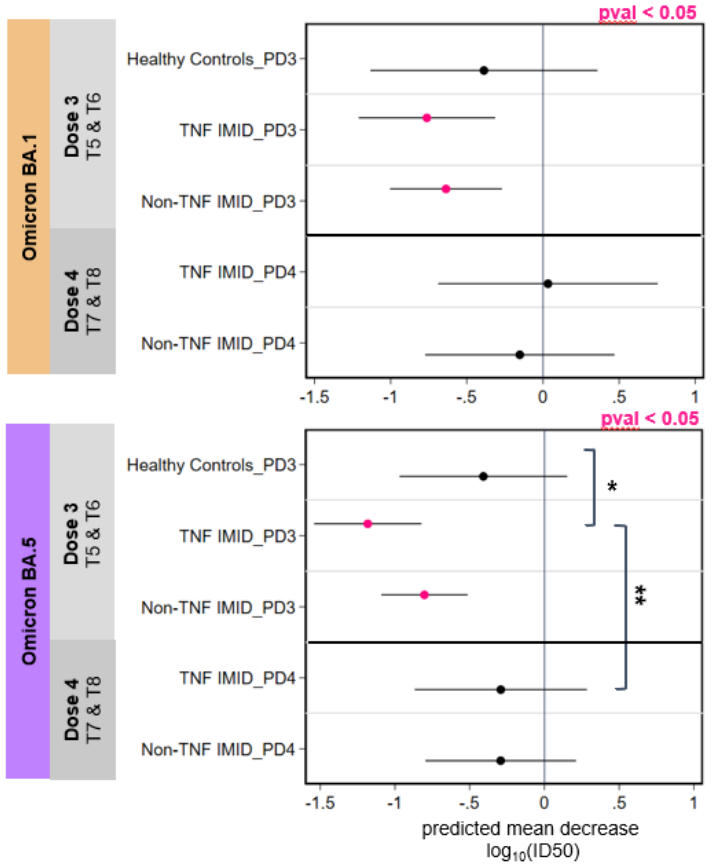
Reduced decay of neutralization after third and fourth vaccine doses

WT



Mixed-effects multivariate linear regression controlling for age, sex, BMI and vaccine type

VOC

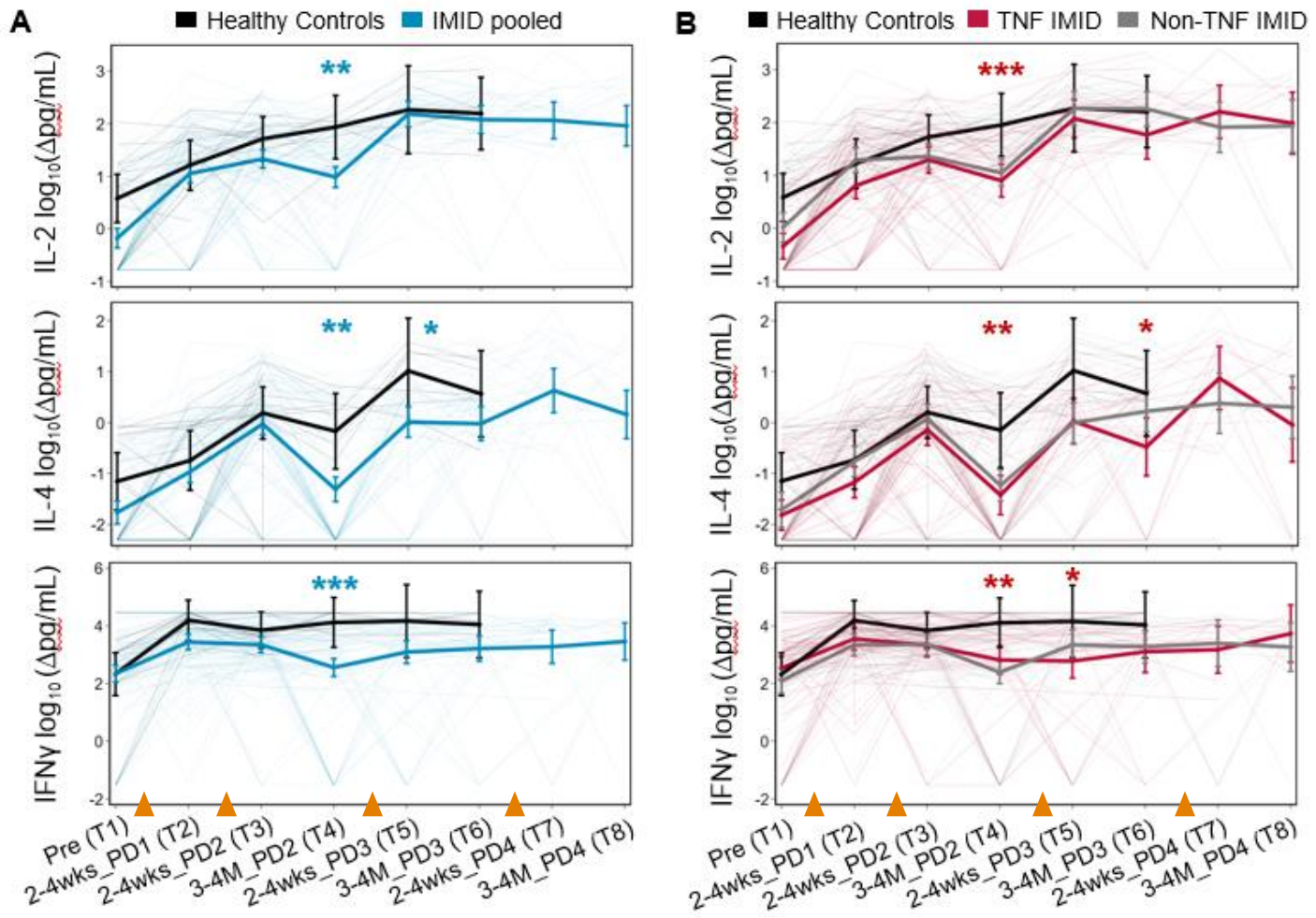


4th dose essential in IMID patients to prolong response against VOCs

3rd and 4th doses reduce the magnitude of waning of neutralization activity in IMID patients.

Cheung, Dayam, Shapiro,Law et al. submitted

T cell cytokines increase with successive vaccinations



IL-2: HC and IMID- max PD3

IL-4: increases in the anti-TNF IMID even after 4 doses

IFN-γ response max PD1
IMID patients: achieve stability PD3

Anti-TNF treated and total IMID- similar deficits 3 months post-dose 2

See Cheung et al. Poster for Further details

Cheung, Dayam, Shapiro,Law et al. submitted

What did we learn?

3rd dose

- **Reduces** the magnitude of **waning** over next 3 months
- Critical for achieving neutralization of VOC in anti-TNF treated patients; T cell responses to VOC largely unaffected
- Important for maximal cytokine responses in IMID patients (IL-2, IFNγ)

4th dose

- Maintains antibody and neutralization responses as measured to 3 months post vaccination
- Increases T cell IL-4 production in anti-TNF/combo treatment group

3rd dose most critical to broaden and sustain responses; 4th dose has more subtle effects than 3rd dose

Lessons

- **Supports the view that this is a 3-dose vaccine, with additional effects of 4th dose in IMID group**
- **IMID patients show more rapid waning and need to be monitored for additional boosting**
- **Measuring more than one T cell cytokine is informative**

T cell cytokines differ after vaccination vs. infection

- **Vaccination: IL-4, no TNF**
- **Infection: TNF, but no IL-4; suggests more inflammatory, less help?**

Limitations: limited sample size; attrition of participants; heterogeneity of diseases and treatments

Lessons

- While antibody responses are easy to standardize, T cell responses are more difficult, multi-step and wide range of assays used
- Need to choose T cell assay depending on goal- in depth immunophenotype or survey of population
- General conclusions were similar across the literature, but we are not at the point we can give someone a T cell “score”
- Rapid funding mechanisms were crucial for the rapid response
- You need trained people in the lab to respond rapidly to pandemics-limited the number of studies we could take on- early grants 6mos or 1 year- difficult to recruit to in timely manner
- Broad funding for research across disciplines is the only way to ensure that we can respond to future threats

Table I How to Choose a T cell Assay During a Pandemic

Assay	ICC	AIM	Multiplexed CSA	Proliferation	ELISpot
Incubation time	18-24 h	18-24 h	48 h	6 days	24-48 h; variable
Advantages	• Single-cell resolution • Assess function based on cytokine production • Can distinguish T cell subsets • Can concurrently measure AIM expression • Short stimulation can estimate *ex vivo* responses	• Single-cell resolution • Measures total T cell responses • Simple protocol • Less sample manipulation • Short stimulation can estimate *ex vivo* responses	• Large panel of cytokines • Higher throughput • Simple protocol • Less sample manipulation • Quantitative	• Single-cell resolution • Expands low frequency responses	• Single-cell resolution • Quantitative • Assess function based on cytokine produced • More sensitive than ICC
Disadvantages	• Limited number of cytokines • Readouts are qualitative • Lower throughput	• Cannot assess function • Cannot distinguish T cell subsets	• Cannot distinguish source of cytokine in question	• May expand weakly cross-reactive T cells • Long incubation period may introduce culture artefacts	• Cannot distinguish source of cytokine • Cannot distinguish T cell subsets
When to use	• In depth analysis of a limited number of samples by flow cytometry	• Larger population studies • Sorting for further analysis such as single cell RNA-sequencing	• For complete cytokine profile • Small as well as larger cohorts	• To detect weak responses	• Larger population based studies • When a flow cytometer is not available

Jaclyn Law and Tania Watts, J. Immunol. Brief Review. Submitted.