T CELLS – UNDER-APPRECIATED IN COVID-19 RECOGNITION FROM ROUTINE & RESEARCH WITH IN VITRO ASSAYS
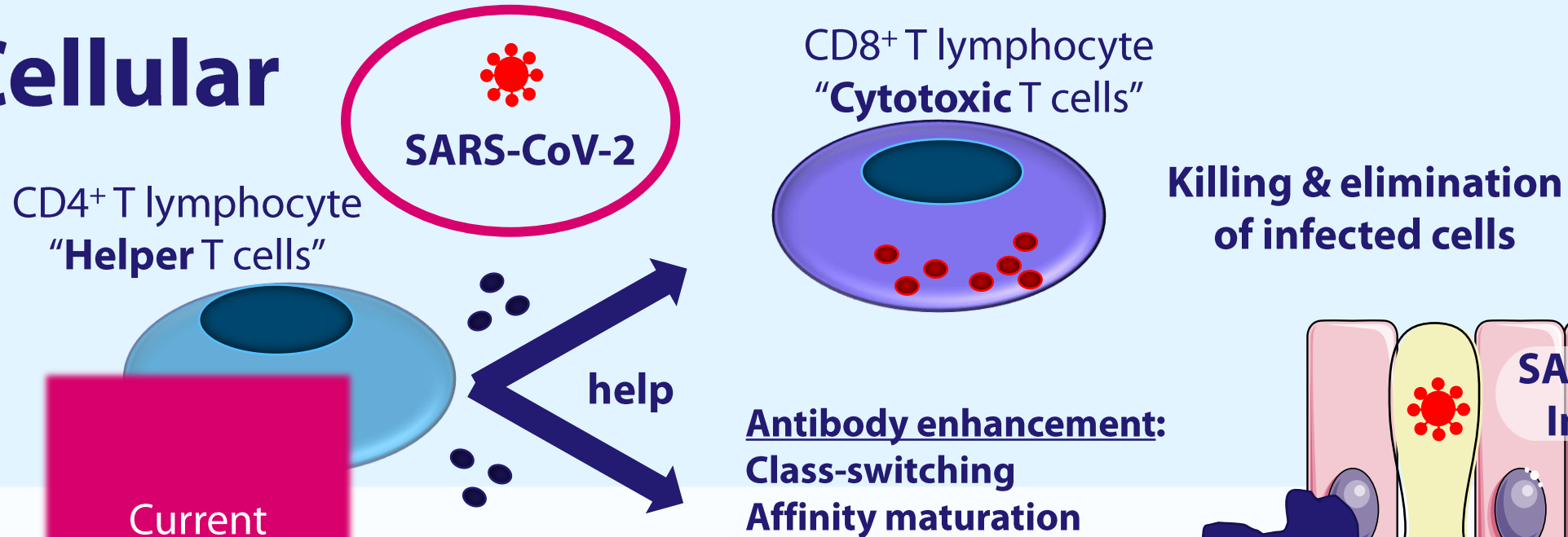
Dr. Lester Thoo

ADR-AC GmbH, Bern

Adaptive immunity in Covid-19
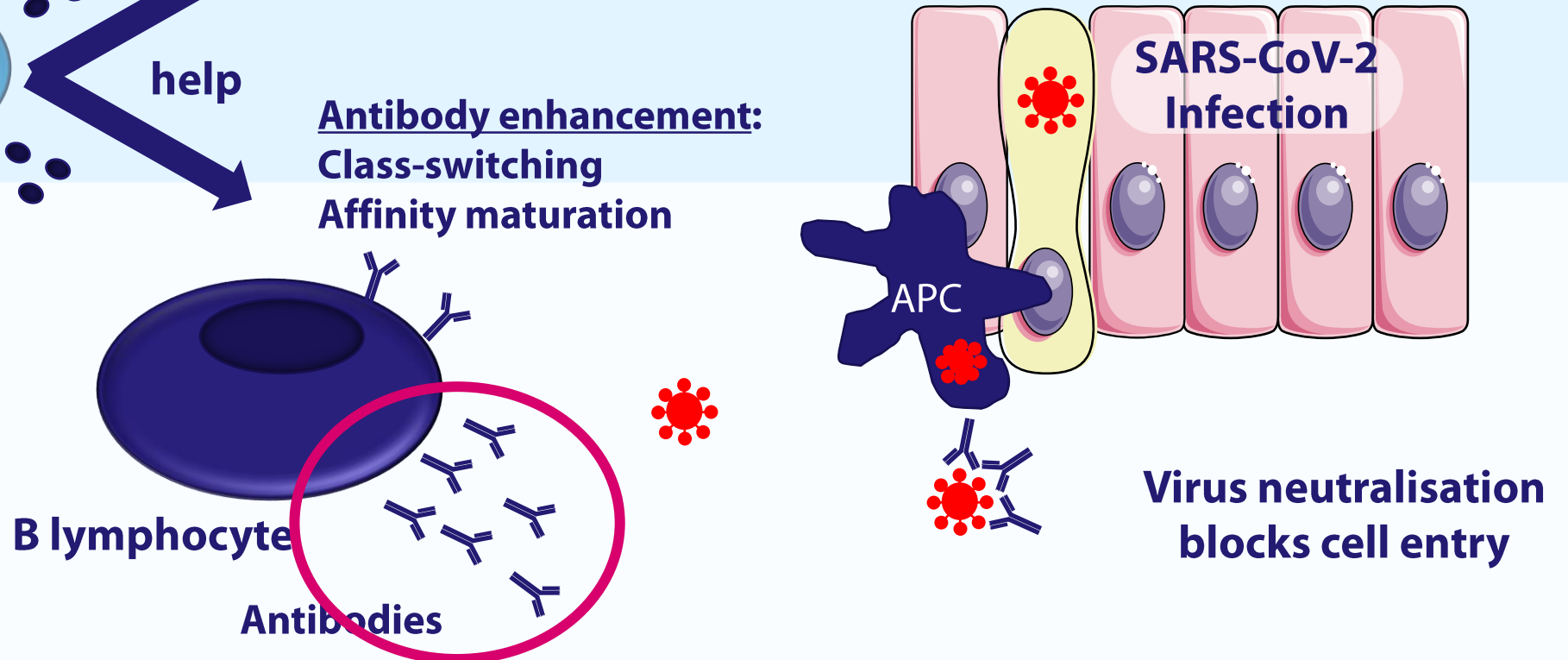
Cellular

Humoral

SARS-CoV-2
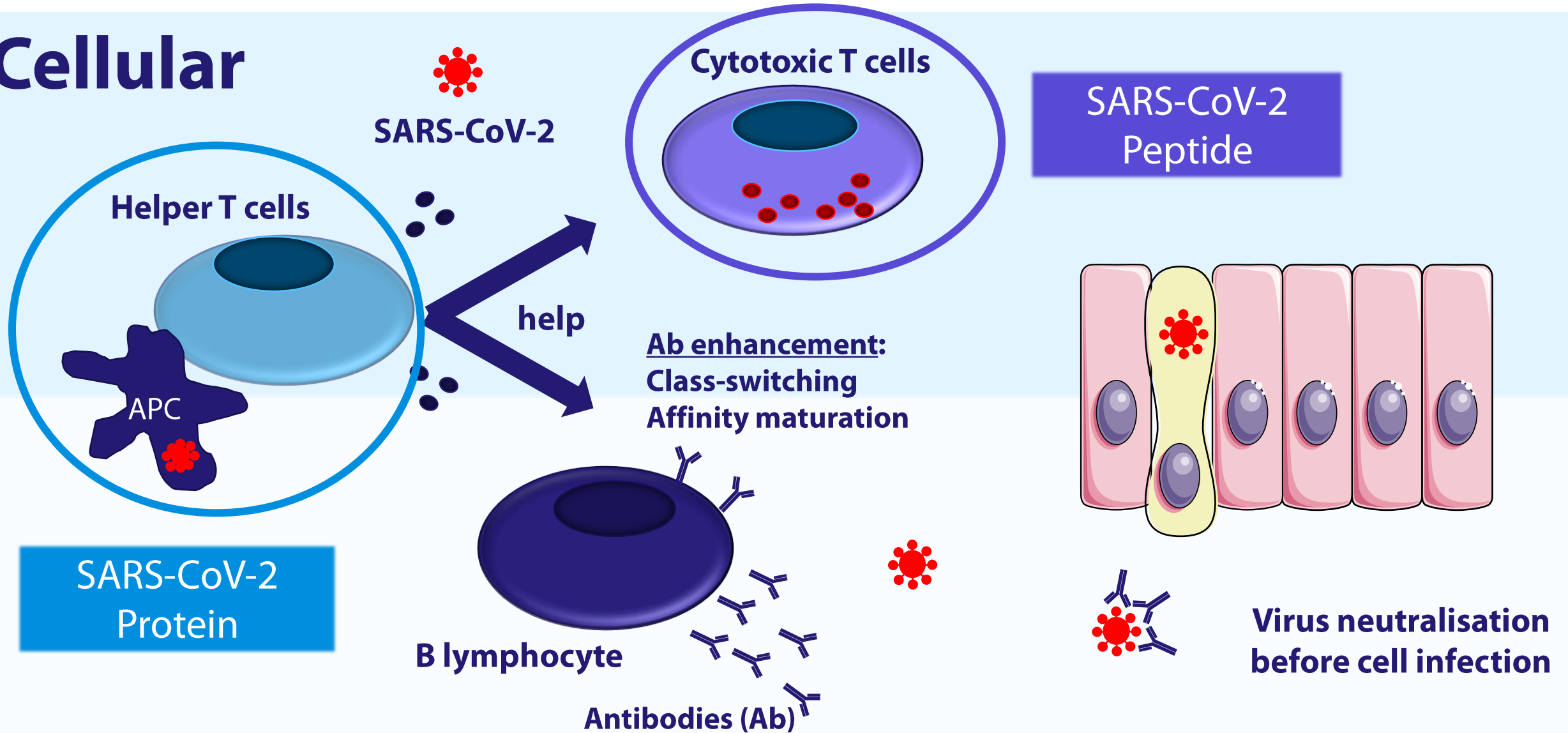
CD4$^+$ T lymphocyte "**Helper** T cells"

CD8$^+$ T lymphocyte "**Cytotoxic** T cells"

Killing & elimination of infected cells

Current diagnostic tests

help

Antibody enhancement:
Class-switching
Affinity maturation

SARS-CoV-2 Infection

APC

B lymphocyte

Antibodies

Virus neutralisation blocks cell entry

Cellular immunity test

Cellular

SARS-CoV-2

Helper T cells

APC

Cytotoxic T cells

SARS-CoV-2
Peptide

help

Ab enhancement:
Class-switching
Affinity maturation

SARS-CoV-2
Protein

B lymphocyte

Antibodies (Ab)

**Virus neutralisation
before cell infection**

SARS-CoV-2 structure
not just Spike!

Large single-stranded
RNA virus (30 kb)

16 non-structural proteins

3 **abundant**
structural proteins

Coronavirus Structure and Protein Visualization

Human coronavirus spike protein

Spike (S)

Nucleocapsid (N)
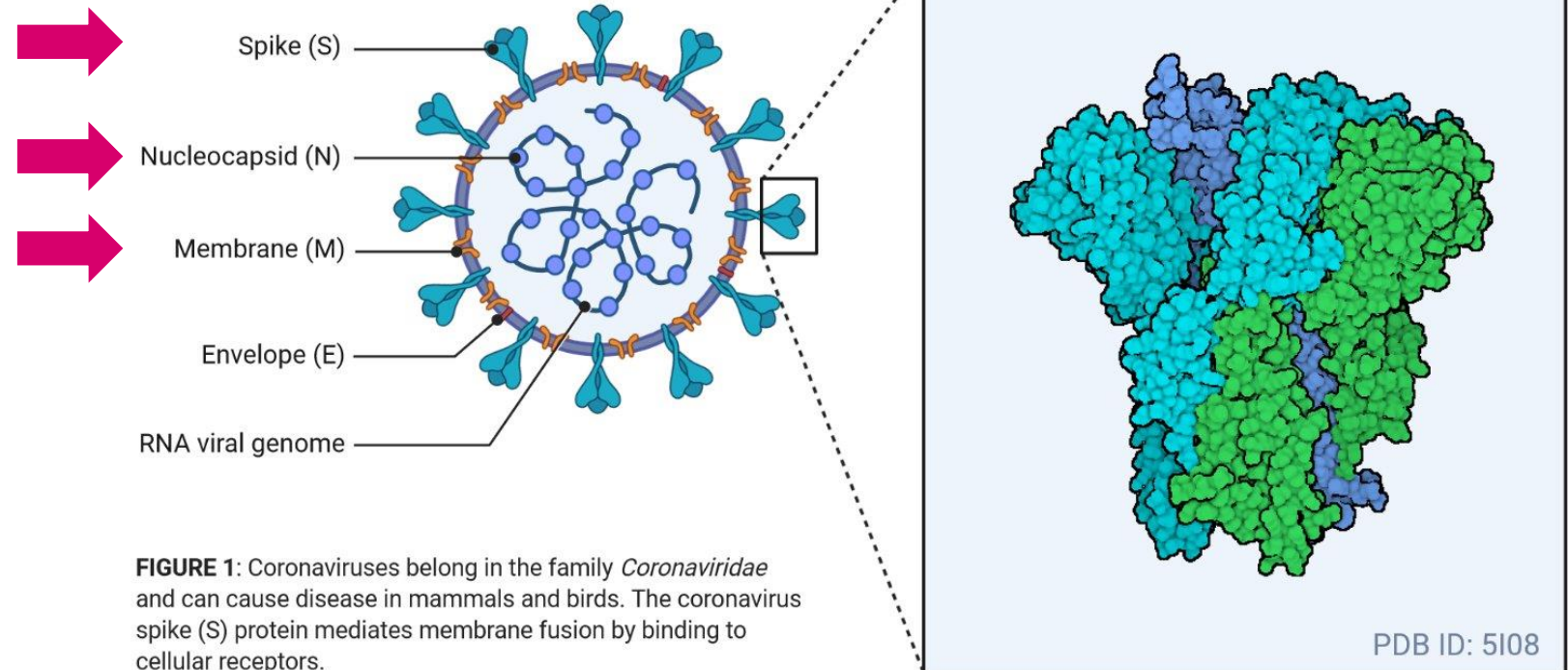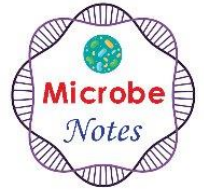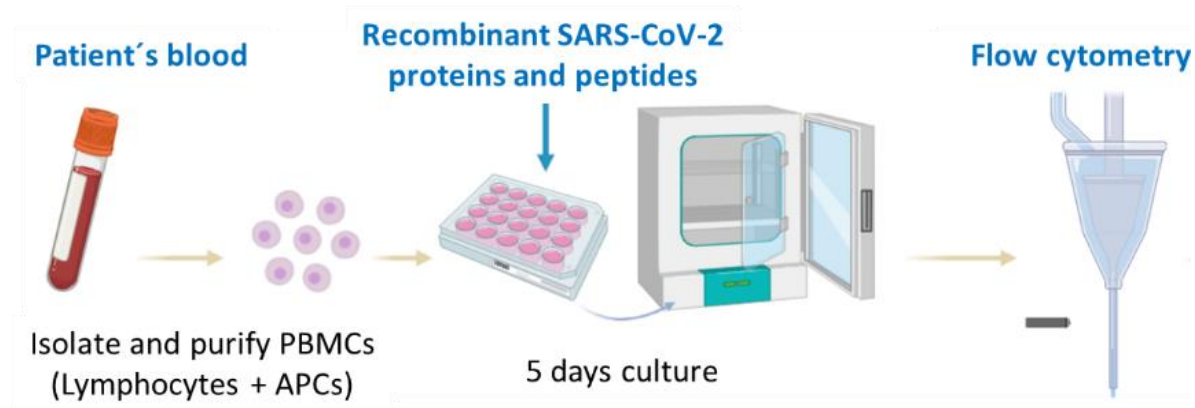
Membrane (M)

Envelope (E)

RNA viral genome

FIGURE 1: Coronaviruses belong in the family *Coronaviridae* and can cause disease in mammals and birds. The coronavirus spike (S) protein mediates membrane fusion by binding to cellular receptors.

PDB ID: 5I08

Image: microbenotes.com

Assay Protocol

Flow cytometry
(cell activation markers)

–	1	RPMI
–	2	RPMI + DMSO
+	3	Phytohaemagglutinin (PHA)
T	4	Spike 1 protein
T	5	Spike 2 protein
T	6	Nucleocapsid protein
T	7	Membrane peptides mix
T	8	Spike peptides mix
T	9	Nucleocapsid peptides mix

Helper T cells

Cytotoxic T cells

Use in Routine Diagnostics

Highly specific and reliable in vitro diagnostic analysis of memory T and B lymphocytes in a Swiss cohort of COVID-19 patients

Lester Thoo[a], Pierre I. Gumowski[bc], Kevin Kammermann [a], Swelia Nussli[a], Benno Grabscheid[a], Oliver Hausmann[ade], Ulrika Axius[a], Werner J. Pichler[a], Daniel Yerly[a]

[a] ADR-AC GmbH, Adverse Drug Reactions – Analysis and Consulting, Bern, Switzerland
[b] INRAAIC, Meyrin, Geneva, Switzerland
[c] Clinical Immunology, Hôpital de la Tour, Meyrin, Switzerland
[d] Löwenpraxis Luzern, Lucerne, Switzerland
[e] Klinik St. Anna, Lucerne, Switzerland

	Antigen	Activation cut-off value (relative to negative control)	Convalescent (N=30)			Unexposed (N=10)			PPV	NPV
			+	-	Sensitivity	+	-	Specificity		
CD4+ T cells	S1	2	26	4	87%	1	9	90%	96%	69%
	S2							90%	96%	53%
	NC							100%	100%	63%
	Combined Statistics	2 in stim						90%	97%	82%
CD8+ T cells	Membrane Mix							70%	80%	28%
	Spike Mix							70%	83%	32%
	NC Mix							90%	94%	41%
	Combined Statistics	2 in stimuli condition	23	7	77%	4	6	60%	85%	46%

Combined Sensitivity 93% Specificity 90%

Analysis	Result
IMMUNOLOGY	
SARS-CoV-2 Lymphocytes Analysis	
T cells CD4	
Spike 1	▲ positive
Spike 2	▲ positive
Nucleocapsid	negative
T cells CD8	
Membrane mix	▲ positive
Spike mix	▲ positive
Nucleocapsid mix	▲ positive
B cells CD19	
Spike 1	negative
Spike 2	negative
Nucleocapsid	negative

Observation
- Significant activation of CD4+ T cells detected in response to Spike 1 and Spike 2 antigens.
- Significant activation of CD8+ T cells in response to the tested peptide mixes of Membrane, Spike and Nucleocapsid.
- No activation of B cells.

Conclusion
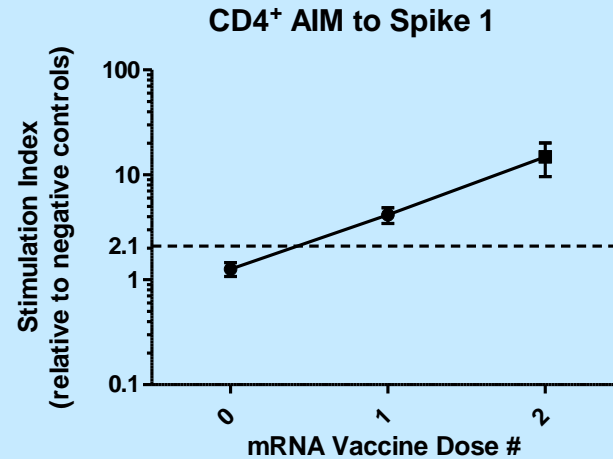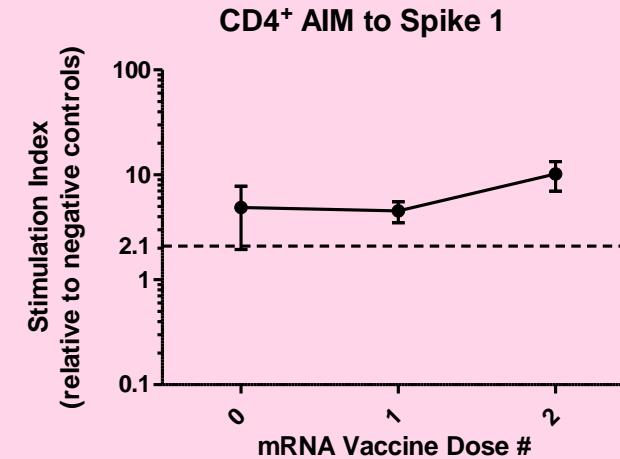Immunization to SARS-CoV-2 clearly detected.

Scan for details

Does vaccination induce T cell responses?

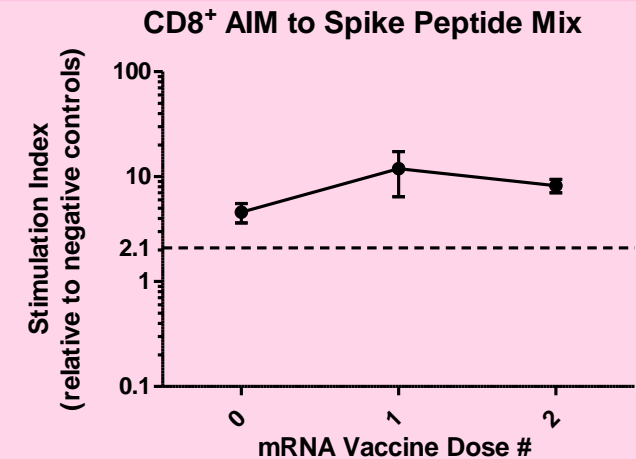**Vaccinated, N=9**
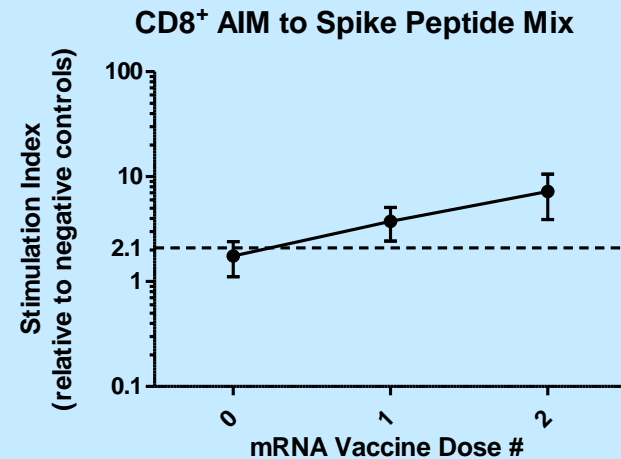
Convalescent, N=4

Helper T cells CD4+

CD4+ AIM to Spike 1

CD4+ AIM to Spike 1

Cytotoxic T cells CD8+

CD8+ AIM to Spike Peptide Mix

CD8+ AIM to Spike Peptide Mix

✓ **Yes**: T cell responses are induced by vaccination

# Clinical applications for Lymphocyte Analysis

(1)
- Persons with **immunodeficiencies/suppression**
  - Immunosuppression **treatments**
    - e.g. Transplantation, Cancer therapy, Biological treatments
  - Primary immunodeficiency

(2)
- Do T cells induced from vaccination & past infections still recognise **mutant** variants?
  - Omicron (2 years after original strain identified in Wuhan)

T cell immunity in **immunosuppressed** individuals

T cell responses to Spike

	Helper T cell		Cytotoxic T cell	
Immunodeficient	**no**	**yes**	**no**	**yes**
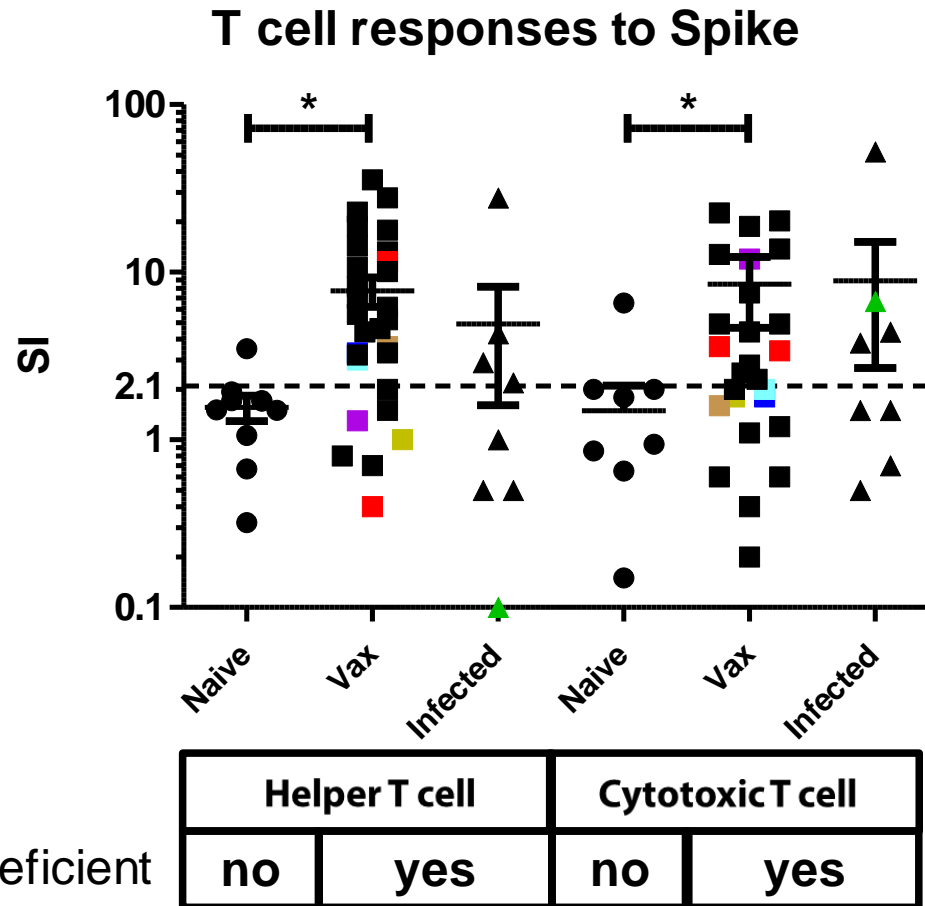
coloured points = antibody negative individuals

Healthy naïve controls   N=10

Immunosuppressed   (N=40)
- Vaccinated   N=32
- Infected   N=8

Even in immunosuppressed individuals:
✓ Vaccination induces both helper & cytotoxic T cell responses

✓ Past infection also has a trend towards positive helper and cytotoxic T cell responses

✓ but…
Important to check at the **individual patient level** (***personalised*** healthcare)
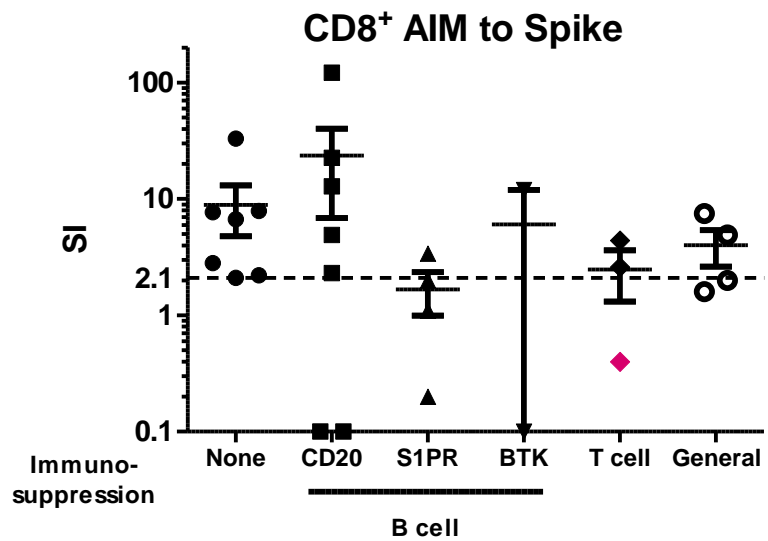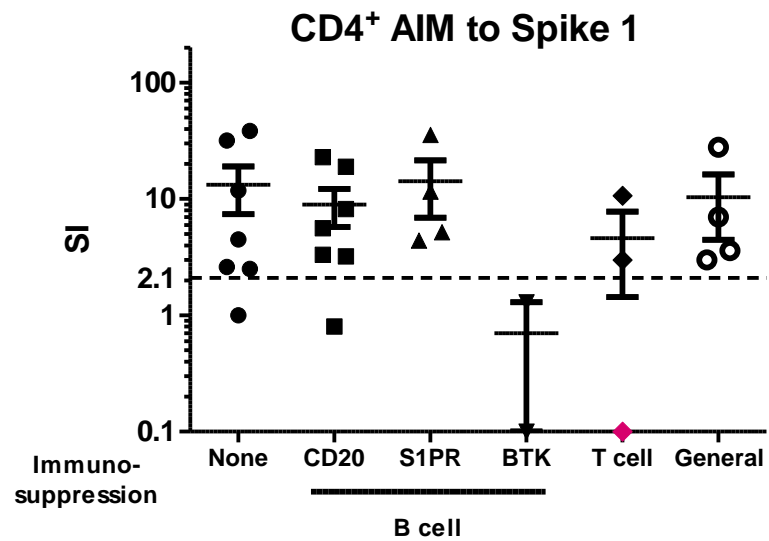
T cell immunity across **different immunosuppressants**
→ overall good vaccination-induced T cell responses

Secondary immunodeficiency			N=20
Target	**Cells affected**	**Substances**	**N**
CD20		Rituximab, Ocrevus, Obinutuzumab	7
Sphingosine 1-phosphate receptor (S1PR)	B cells	Fingolimod	4
Bruton's Tyrosine Kinase (BTK)		Ibrutinib	2
Calcineurin (→ IL-2)		FK506 (Tacrolimus)	1
CTLA-4	T cells	Belatacept	1
IL-12 & IL-23		Stelara (Ustekinumab)	1
General suppression	Lymphocytes	Imurek, Revlimid, Cellcept, Prednisolone	4

2° Immunodeficiency
N=20
after 2X vaccination

Healthy Donors
N=7
after 2X vaccination

CD4$^+$ AIM to Spike 1

CD8$^+$ AIM to Spike

Immuno-suppression: None, CD20, S1PR, BTK, T cell, General

B cell

Overall:
✓ Good T cell response
❖ but some poor responders

Recommendation:
→ test for T cells
→ extra caution / booster

Omicron – master of (antibody) evasion
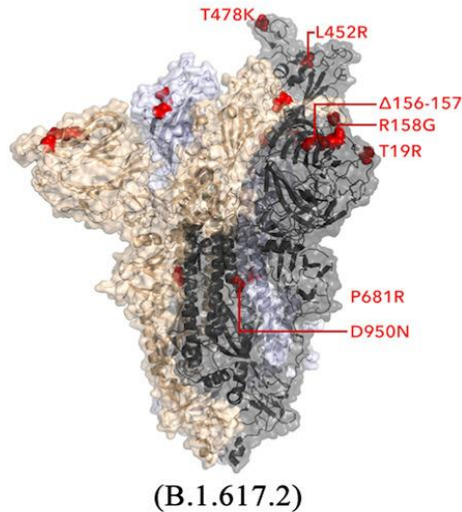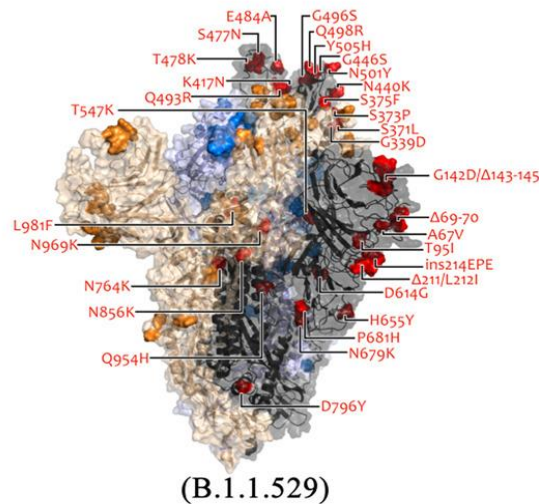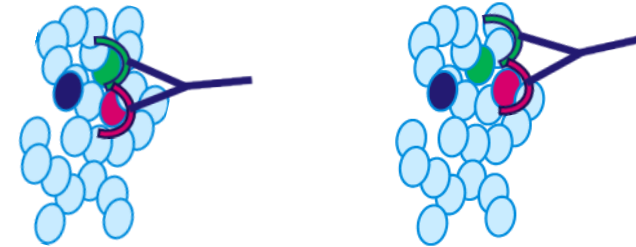
Spike protein

(A) Delta

T478K
L452R
Δ156-157
R158G
T19R
P681R
D950N

(B.1.617.2)

(B) Omicron

E484A
S477N
T478K
K417N
Q493R
T547K

G496S
Q498R
Y505H
G446S
N501Y
N440K
S375F
S373P
S371L
G339D
G142D/Δ143-145
Δ69-70
A67V
T95I
ins214EPE
Δ211/L212I
D614G
H655Y
P681H
N679K

L981F
N969K
N764K
N856K
Q954H

D796Y

(B.1.1.529)

Image from: Kumar *et al.* (2022). *J. Med. Virol.* **94(4)**
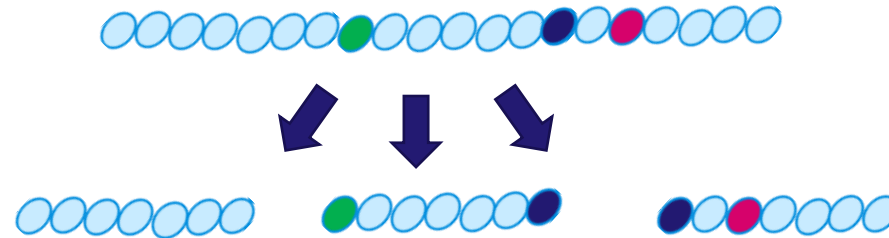
mutation leading to
<u>3D changes</u>

antibody binding
impaired
→ viral evasion of
antibodies

degradation into linear peptides

T cells recognise <u>linear peptides</u>
✓ less affected by conformational changes
(recognition sites are conserved)

❖ when T cell epitopes are mutated,
problematic only for <u>specific HLA-haplotypes</u>

Stimuli adaptation for Omicron
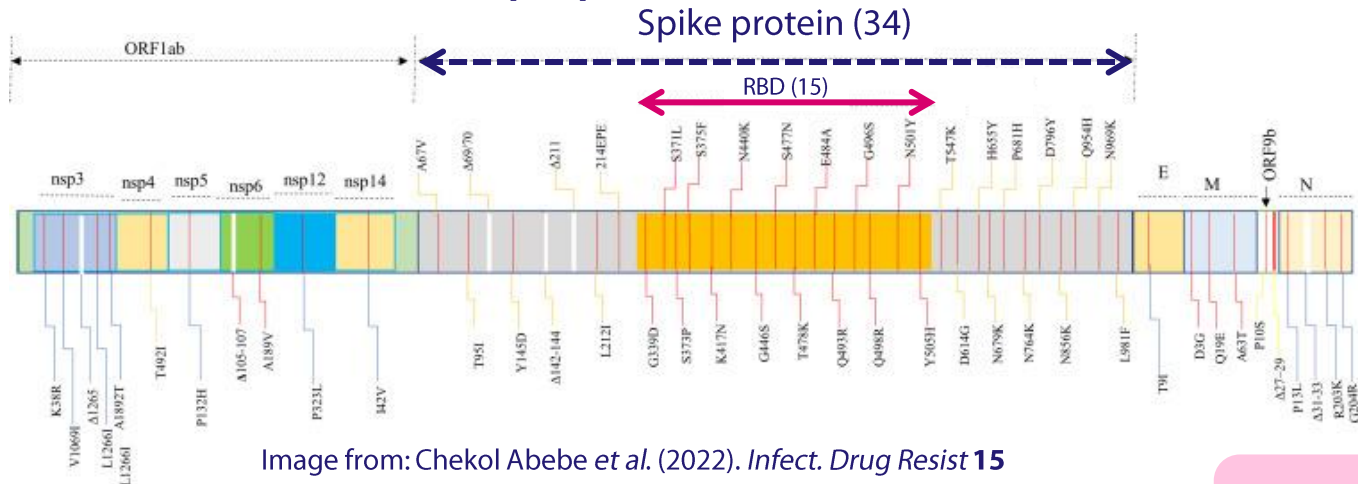
Omicron mutations (60)

Spike protein (34)

RBD (15)

Image from: Chekol Abebe *et al.* (2022). *Infect. Drug Resist* **15**

Wild-type Peptide sequence	Affected HLA alleles			Adapted for Omicron Peptide sequence
TEKSNIIRGW	B*44:02	B*44:03		**I**EKSNIIRGW
GEVFNATRF	B*40:01	B*44:02	B*44:03	**D**EVFNATRF
N**S**AS**FS**TFK	A*23:01			NL**A**P**F**FTFK
KIADYNYKL	B*07:02			**N**IADYNYKL
AE**H**VNNSY	B*44:03			AE**Y**VNNSY
S**P**RRARSVA	B*07:02			S**H**RRARSVA

→ only 6 / 28 tested spike peptides affected
→ most abundant HLA allele A*02 <u>not</u> affected

-	1	RPMI
-	2	RPMI + DMSO
+	3	Phytohaemagglutinin (PHA)
T	4	Spike 1 protein → Spike 1 RBD
T	5	Spike 2 protein
T	6	Nucleocapsid protein
T	7	Membrane peptides mix
T	8	Spike peptides mix
T	9	Nucleocapsid peptides mix

Helper T cells (rows 4–6)

Cytotoxic T cells (rows 7–9)

Is immunity still effective against Omicron?

Cohort

Vaccinated;   N=4
Infected (**from January 2022**) ± Vax;   N=6   ← likely Omicron infection
(🇨🇭 ~80% Omicron)

Helper T cell

Cytotoxic T cell
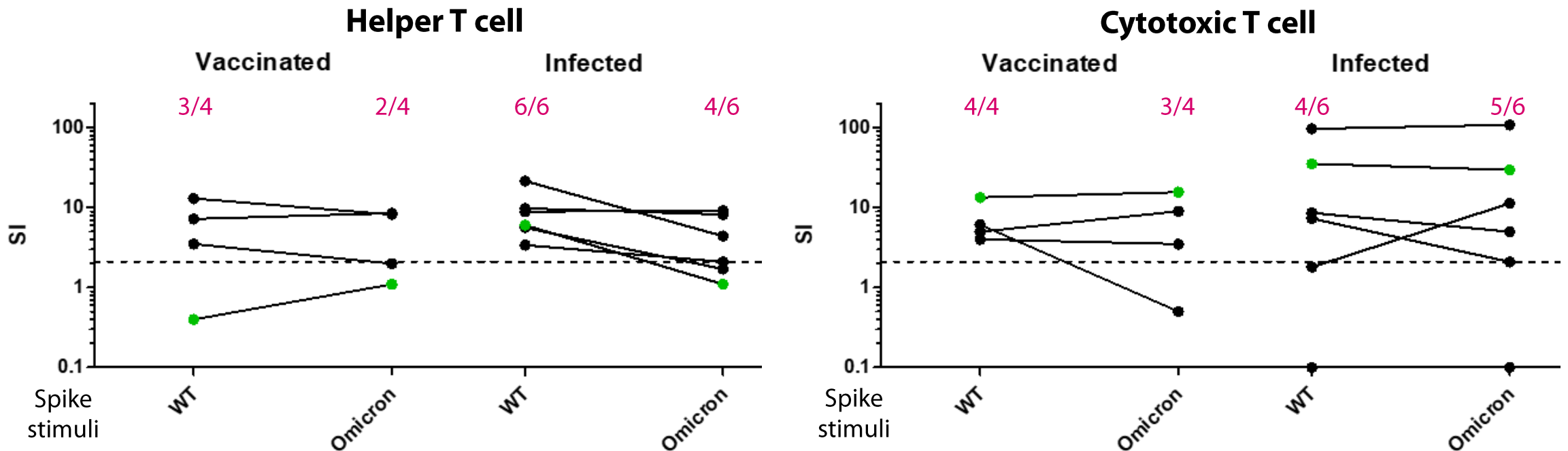
T cells induced by vaccination / infection **still recognise Omicron mutant Spike protein**
→ likely **explains the reduced Covid-19 severity** despite increased breakthrough infections

Key Messages

**Virus Clearance
Mild Disease**

**Virus Persistence
Disease**
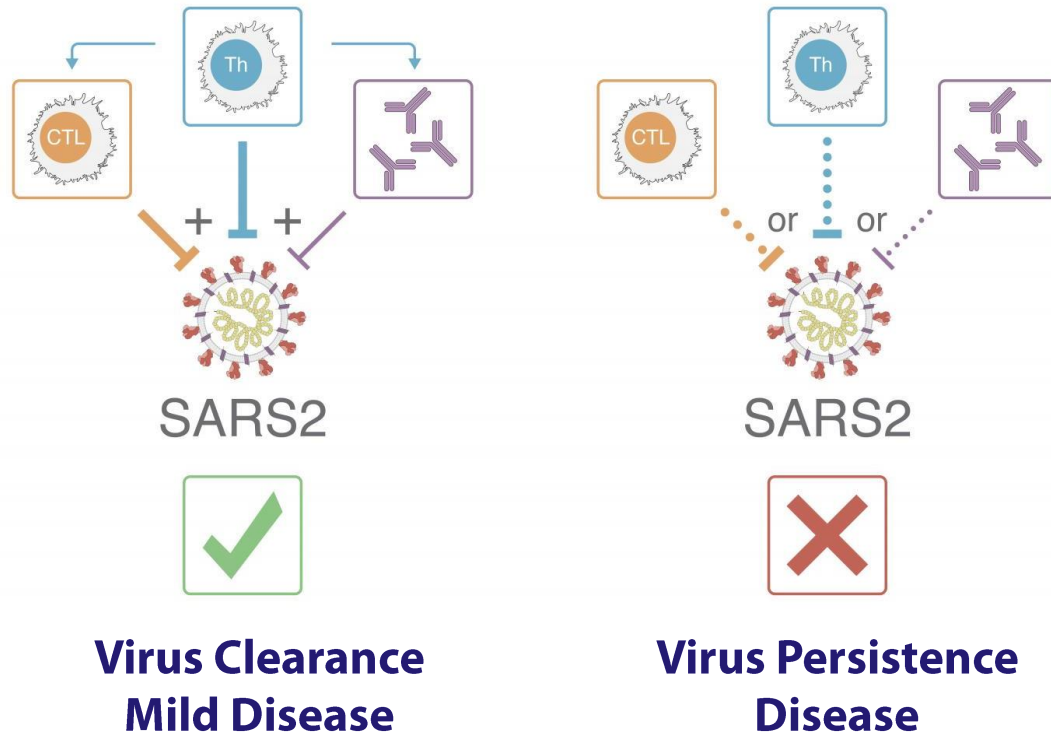
Immunity exists as a
coordinated response

Immunity against SARS-CoV-2

Antibodies

Cellular immunity

Lymphocyte analysis

Detection of SARS-CoV-2 specific T lymphocytes possible

Test **available** and **in-use** for routine diagnostics

Application in routine diagnostics

✓ **Personalised** healthcare
✓ Immunodeficient patients:
  Poor / No Antibody response –
  → **test for T cells**

Providing an unmet diagnostic tool

Confirmation of past-exposure / vaccination response
  → advice for caution when multiple immune parameters absent

  → preparedness for future mutants / other viruses

…any suggestions? Questions?
We are open to collaborate for further studies!

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