

TCR repertoire sharing between CD4⁺ T follicular helper cells in human peripheral blood and tonsil

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Introduction and Objectives

T follicular helper cells (Tfh) are specialized CD4⁺ T cells primarily localized to germinal centers (GC) in secondary lymphoid organs that provide help to B cells. Recently different subsets of circulating Tfh (cTfh) cells in peripheral blood have been studied^{1,2,3,4}, but how the specificity of cTfh compares to germinal center Tfh (Tfh-GC) in lymphoid tissues is unknown^{4,5}. Also, the identity of memory cTfh cells in comparison to follicular memory Tfh cells is still unclear and it is not known whether cTfh and effector Tfh-GC cells originate from rest of memory (ROM) cells or whether they represent a well-defined separate population.

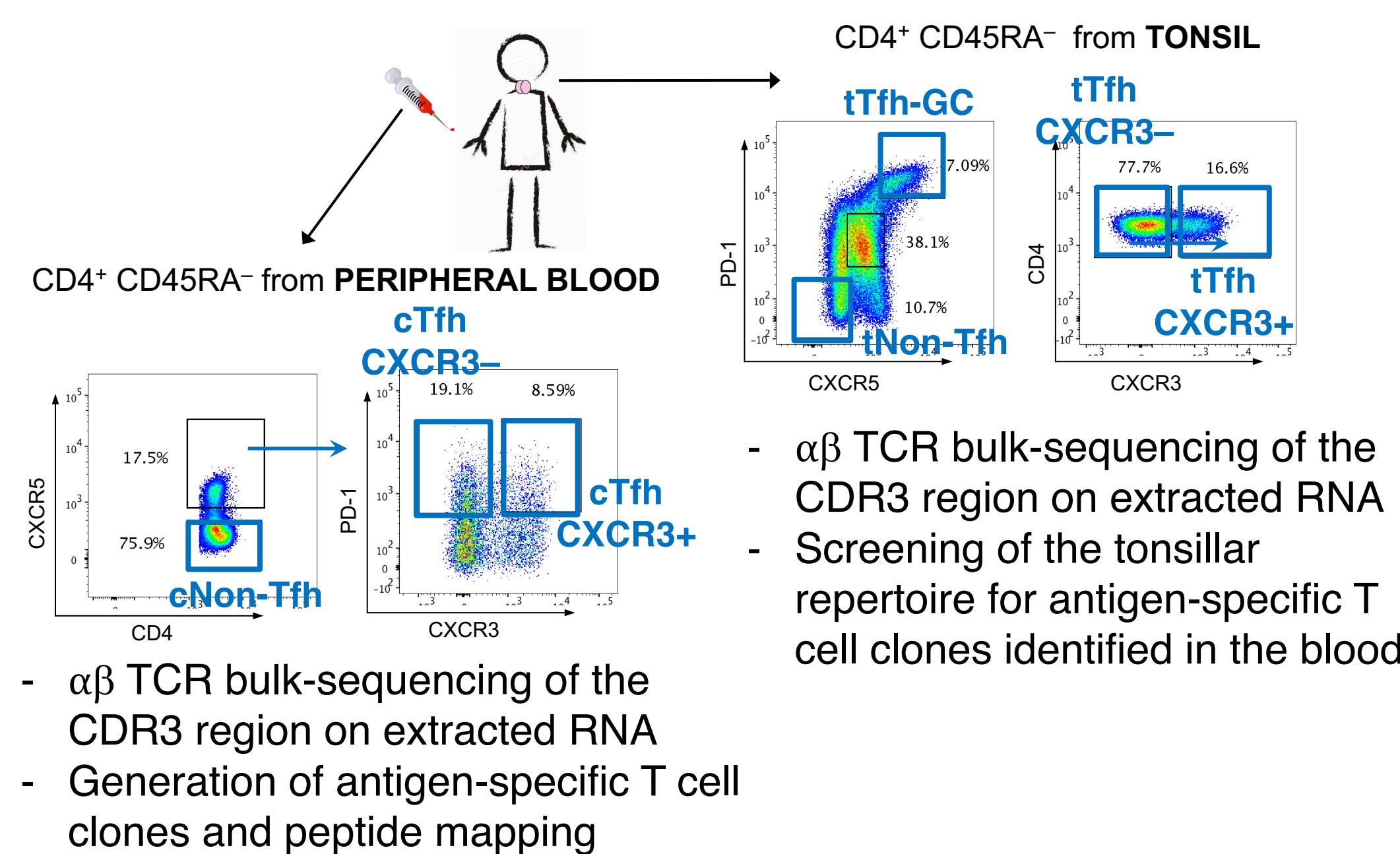
AIMS:

- compare the T cell receptor (TCR) repertoire of tonsillar and circulating Tfh cells
- to evaluate how distinct Tfh specificity is from that of the rest of memory CD4⁺ cells
- to gain insight into the TCR repertoire of Tfh and non-Tfh cells responding to a specific antigen, using influenza virus haemagglutinin.

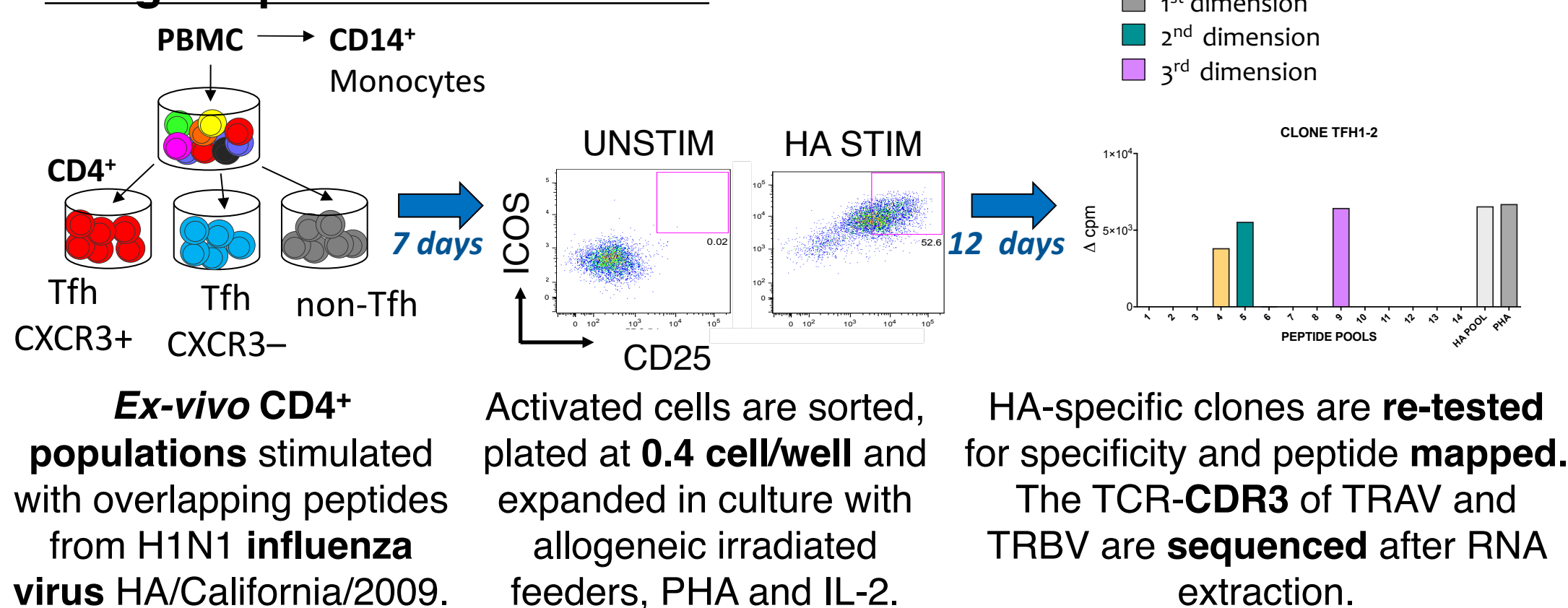
Methods

Tfh subsets from tonsil and blood:

Samples were collected from adult donors undergoing **tonsillectomy** who consent to donate **80-100mL of blood**.

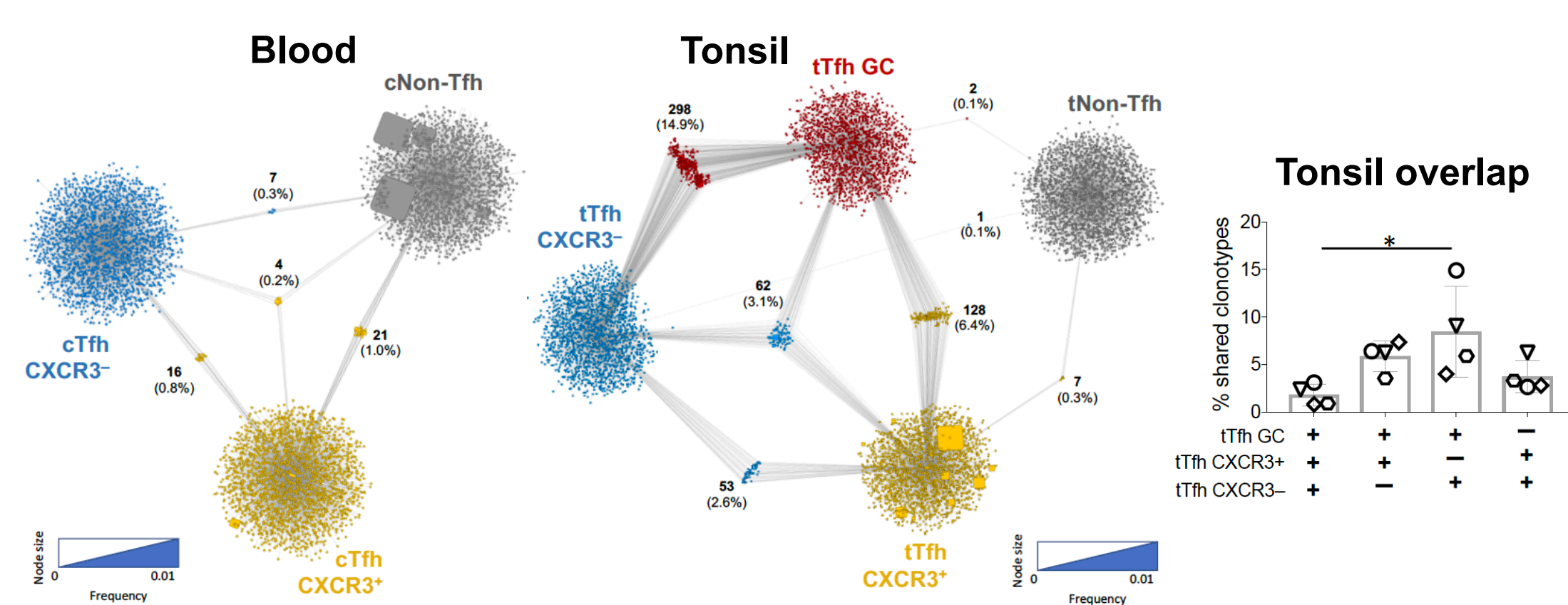


Antigen-specific T cell clones:



Tfh cell subsets are clonally distinct from non-Tfh cells

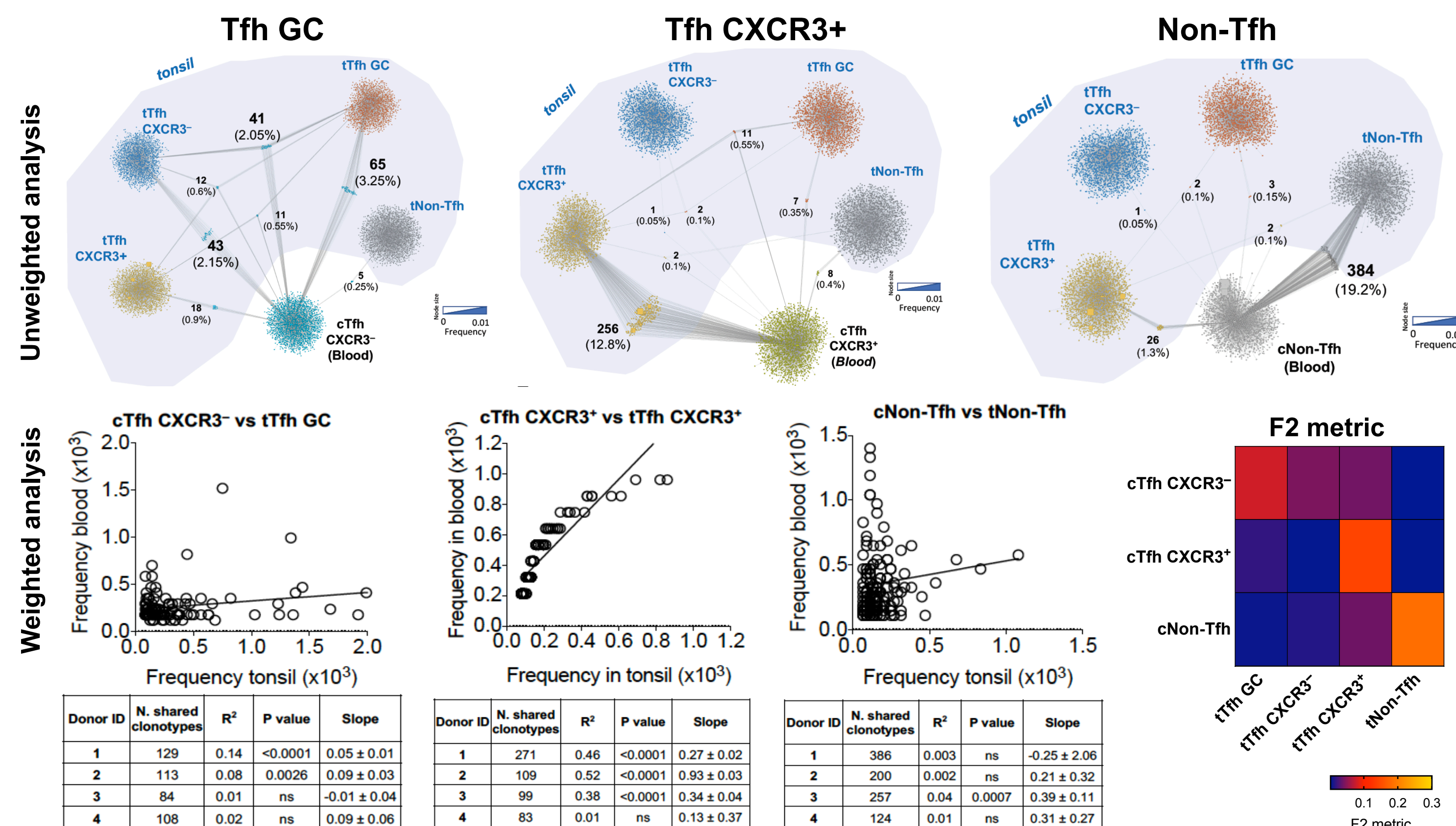
The TRBV CDR3 sequencing analysis showed that Tfh cells from both blood and tonsil tissues have a repertoire distinct from that of non-Tfh memory populations in the same tissue, although Tfh-GC and Tfh CXCR3⁻ in tonsils show considerable overlap.



Clonotypic overlap between CD4⁺ Tfh cell populations from blood and tonsil

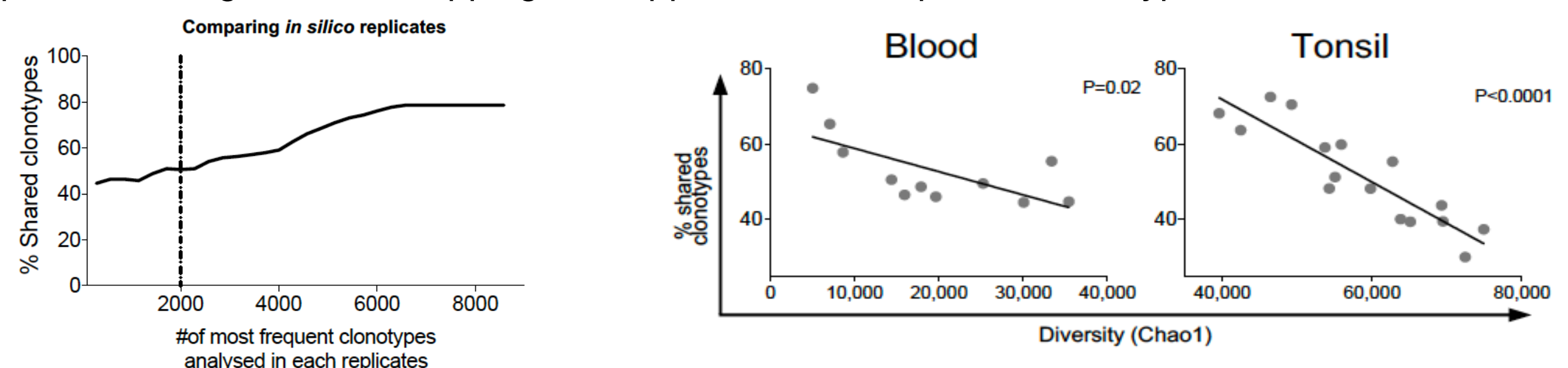
The TRBV CDR3 region sequencing analysis of the Tfh and non-Tfh populations from paired tonsils and blood samples (4 donors) showed consistent clonotypic overlap between:

- Tonsil Tfh-GC & Blood Tfh CXCR3⁻
- Tonsil Tfh CXCR3⁺ & Blood Tfh CXCR3⁺
- Tonsil non-Tfh & Blood non-Tfh



Repertoire diversity correlates with the degree of overlap from *in silico* analysis

To improve understanding of the magnitude of the overlap experimentally observed, an *in silico* replication using the bootstrapping was applied to re-sample the clonotypes in each subset.



The repertoire overlap (%) changes depending on the diversity of the subset considered. This implies that the degree of overlap observed experimentally is an underestimate, because of the relatively small size, reinforcing the conclusion that there is substantial repertoire overlap between blood and tonsil Tfh cell populations.

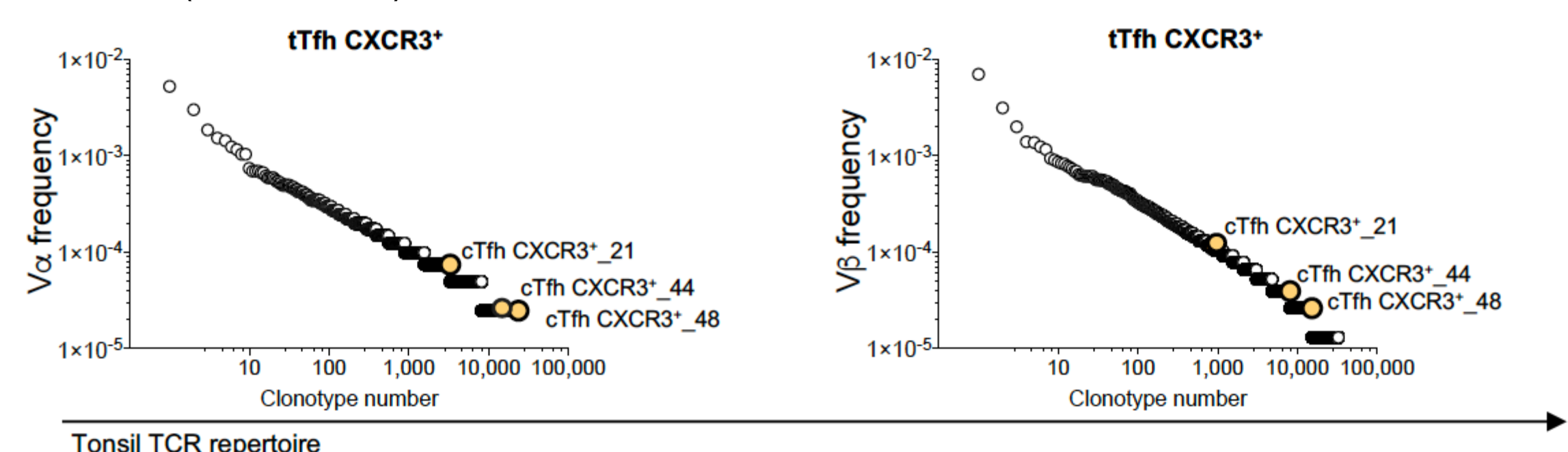
HA-specific Tfh CXCR3⁺ from blood can be detected in tonsil Tfh CXCR3⁺ population

Influenza virus HA-specific clones are predominantly found in Tfh CXCR3⁺ and non-Tfh populations from peripheral blood.

TCR sequencing of HA-specific clones showed no overlap between Tfh and non-Tfh clones that recognized the same peptide, supporting the hypothesis that Tfh cells are a distinct subset within the memory CD4⁺ T cell population.

CLONE ID	TRAV	TRAJ	TRAV_CDR3	TRBV_CDR3	TRBV	TRBJ
Tfh CXCR3 ⁺ _49	23/DV6	36	CAASARETGANNLF	CATSRRDRVGEKLF	15	1-4
Tfh CXCR3 ⁺ _01	23/DV6	26	CAASMRGEYQGNFV	CASSYSWGITEAF	6-5	1-1
Non-Tfh_41	39	44	CAVLEGTGTASKLT	CASLWGSAPSPEYQY	5-1	2-7

Blood-derived HA-specific Tfh CXCR3⁺ clonotypes can be found in the αβ TCR repertoire of both tonsil and blood (not shown) Tfh CXCR3⁺ subsets.



Conclusions

T cell receptor TRBV repertoire analysis identified a **consistent correlation** between **tonsil and blood Tfh subsets** and the degree of overlap was reinforced by the *in silico* and diversity analysis, as well as by the influenza-specific T cell clones generated from peripheral blood.

TCR Vβ deep-sequencing and αβTCR sequencing of antigen-specific T cell clones also suggest **no overlap between memory Tfh and non-Tfh subsets**.

These novel insights support the concept that circulating Tfh cells reflect the specificity of effector and memory follicular Tfh cells, indicating the utility of studying peripheral Tfh populations in infected individuals and human vaccine trial participants to gain insight into germinal centre responses.