Poster number
00241

Temporal associations of B and T cell immunity in a 16-week interval BNT162B2 regimen

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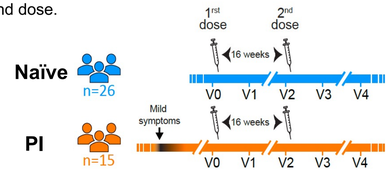
INTRODUCTION : Spacing of the BNT162b2 mRNA doses beyond the standard 3-week interval raised concerns about vaccine efficacy. We longitudinally analyzed B cell, T cell and humoral responses to two BNT162b2 mRNA doses administered 16 weeks apart in **43 SARS-CoV-2 naïve** and **previously-infected** (PI) donors. We examined blood samples at five time points from baseline to 4 months post second dose.



	Naïve	PI
Age	51 (41-56)	47 (43-56)
Male	11 (42%)	10 (66%)
Female	15 (58%)	5 (34%)
Days between days of symptom onset and first vaccine dose	NA	274 (258-307)
V0, days before first dose	1 (0-5)	24 (6-43)
V1, days after first dose	21 (19-26)	20 (19-21)
V2, days after first dose	83 (82-84)	89 (86-93)
V3, days after first dose	133 (130-139)	138 (132-142)
V4, days after first dose	112 (110-119)	113 (110-17)

METHODS : We used high-parameter flow cytometry to quantify and study: i) receptor binding domain (RBD)-specific B cells; ii) Spike (S)-specific CD4 and CD8 T cells by a 16h activation-induced marker (AIM) assay; iii) S-specific CD4 and CD8 T cells by a 6h intracellular staining (ICS) assay. To identify antigen-specific T cell responses we used an AND/OR Boolean combination gating strategy detected by

AIM assay:
- CD69$^+$CD40L$^+$
- CD69$^+$OX40$^+$
- CD69$^+$4-1BB$^+$

ICS assay:
- CD69$^+$TNFa$^+$
- CD69$^+$CD107a$^+$
- CD69$^+$IL-10$^+$

- CD69$^+$IFNg$^+$
- CD69$^+$IL-2$^+$
- CD69$^+$IL-17a$^+$

We did supervised and unsupervised (using Phenograph and FlowSOM) analyses of B and T cell subsets, and temporal association analyses.

RESULTS:

Marked differences of SARS-CoV-2-specific B and T cells between the two cohorts with convergent features after boosting

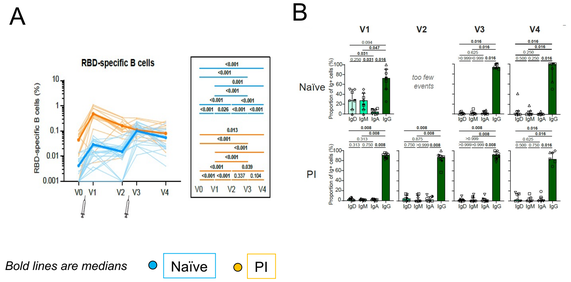


Figure 1. Comparisons between **(A)** Longitudinal RBD-specific B cell responses and **(B)** Dominant class-switched IgG$^+$ in RBD-specific B cells in naïve and PI donors.

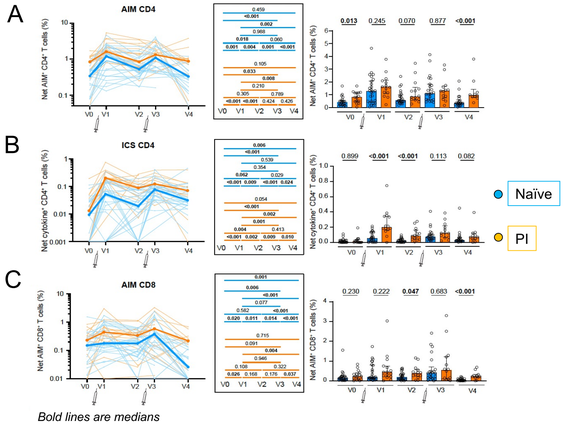


Figure 2. Longitudinal analysis of robust **(A)** S-specific AIM$^+$CD4$^+$, **(B)** Cytokine+CD4$^+$ and **(C)** S-specific AIM$^+$CD8$^+$ T cell responses in naïve and PI participants.

Unsupervised and supervised analysis reveal a polyfunctional cluster of CD4$^+$ T cell stably maintained at the late memory timepoint.

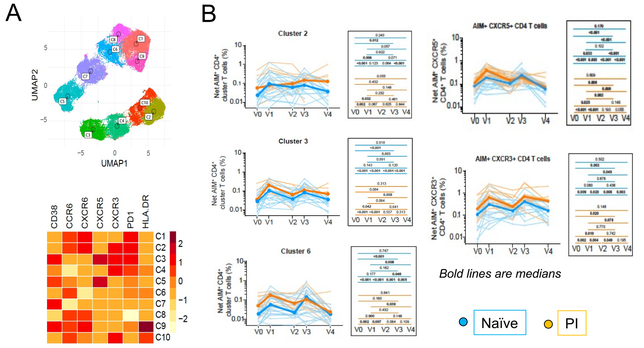


Figure 3. Multiparametric UMAP representation of **(A)** S-specific AIM$^+$ CD4$^+$ T cells and **(B)** Longitudinal frequencies of selected clusters and CXCR5 and CXCR3-expressing AIM$^+$CD4$^+$ T cells respectively analyzed by unsupervised and supervised analysis.

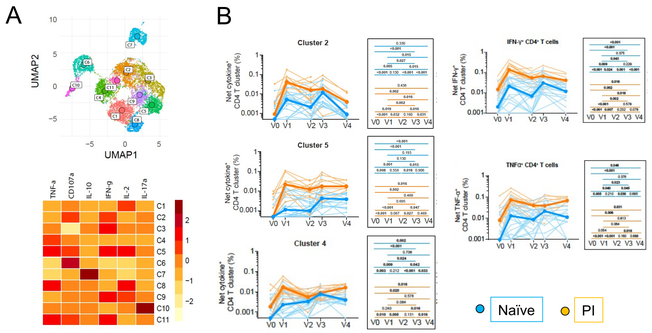


Figure 4. Multiparametric UMAP representation of **(A)** S-specific ICS$^+$ CD4$^+$ T cells and **(B)** Longitudinal frequencies of selected clusters and cytokines+ ICS$^+$CD4$^+$ T cells analyzed by unsupervised and supervised analysis.

Convergence and temporal associations between SARS-CoV-2-specific T and B cells in the two cohorts after two vaccine doses

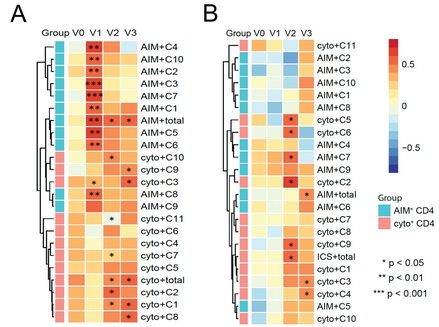


Figure 5. Temporal correlation between S-specific CD4$^+$ T cells at the V0, V1, V2 and V3 timepoints and **(A)** RBD-specific B cell frequencies measured at V3; **(B)** AIM$^+$ CD8$^+$ T cell frequencies measured at V3 showed immune component-specific associations over-time.

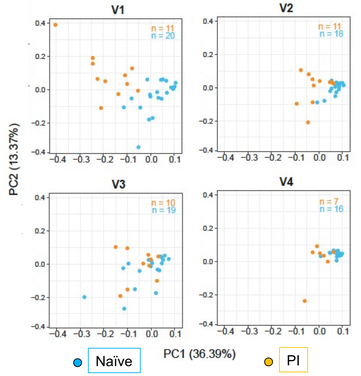


Figure 6. Integrated PCA analysis, combining various immune features, between naïve and PI vaccinees after two vaccine doses.

CONCLUSION : The 16-week interval schedule is associated with **robust**, **multifaceted recall cellular responses** after the second dose, consistent with highly functional immune memory. After the delayed second dose, immune profile kinetics shows only partial, and transient, **convergence** between both groups. The early induction of robust CD4 responses and their associations with longer-term B cell and humoral immunity support their central role in the **efficacy** of this vaccine regimen.

Acknowledgements : The authors are grateful to the study participants. We thank the CRCHUM BSL3 and Flow Cytometry Platforms for technical assistance and the financial supports.