# Evaluation of the PHH-1V COVID-19 Vaccine-Induced T-Cell Responses in Adolescents: HIPRA-HH-3 Study

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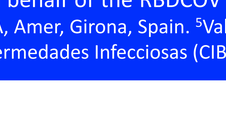
## BACKGROUND

PHH-1V (BIMERVAX®) is an adjuvanted recombinant protein vaccine based on a dimeric receptor-binding domain (RBD) of the Alpha/Beta SARS-CoV-2 variants (1,2). In March 2023, PHH-1V received full marketing authorization across the European Union, following the European Medicines Agency's (EMA) recommendation for use as a booster to prevent coronavirus disease 2019 (COVID-19) in individuals aged 16 and older. The HIPRA-HH-3 study (Phase IIb, open-label, single-arm, multi-center, non-inferiority; NCT06234956) aims to assess the safety and immunogenicity of PHH-1V in 300 healthy adolescents (pediatric population), aged 12 to 17 years, who had previously received primary vaccination with mRNA-based SARS-CoV-2 vaccines. Here, we evaluate the T-cell immune response in a group of 16 study participants.

## METHODS

The HIPRA-HH-3 study is a Phase IIb, open-label, single-arm, multi-center, non-inferiority study (NCT06234956). Participants included adolescents aged 12 to 17 years who had received **primary vaccination with mRNA-based SARS-CoV-2 vaccines.**

**SARS-CoV-2 specific T-cell responses** were analyzed in peripheral blood mononuclear cells at baseline (D0) and day 14 (D14) after PHH-1V booster.




Using 15-mer overlapping peptide pools covering the RBD region of B.1.351 (**Beta**; vaccine-matched) and **Omicron BA.1** SARS-CoV-2 variants.

Total T-cell responses were measured by **IFN-γ+ ELISpot.**

IFN-γ+ and IL-2+ cytokine-mediated CD4+ and CD8+ T-cell responses were measured by **intracellular staining.**

The average age of the participants was 15.2 years (n=16, range: 13-17), with 8 males (50%) and 8 females (50%). Cryopreserved peripheral blood mononuclear cells (PBMC) samples were obtained from participants on base line (D0) and day 14 (D14) after the PHH-1V booster. PBMCs were isolated and stimulated with 15-mer overlapping peptides pools covering the RBD region of the Spike protein from the Omicron BA.1 and Beta variants of the SARS-CoV-2 virus. T-cell responses were evaluated using ELISpot and intracellular cytokine staining (ICS) techniques.

**PHH-1V induces a strong Th1 T-cell response in individuals aged 12 to 17 years.**

**The IFN-γ-mediated T-cell response induced by PHH-1V appears to be greater in magnitude in adolescents than in healthy adults (2).**

## RESULTS

ELISpot assay (Figure 1) showed a significant increase in IFN-γ-producing T cells at D14 after vaccination with both RBD peptide pools tested (RBD Beta p<0.0001, RBD Omicron BA.1 p<0.0001). ICS assay (Figure 2) demonstrated that most of the IFN-γ-secreting cells have a CD4+ T-cell phenotype (RBD Beta p<0.0001, RBD Omicron BA.1 p=0.0001), although an increase of IL-4-secreting CD4+ T-cells was detected against peptides from the Beta variant (p=0.0381). No increases in IL-2-producing CD4+ and CD8+ T cells were observed.

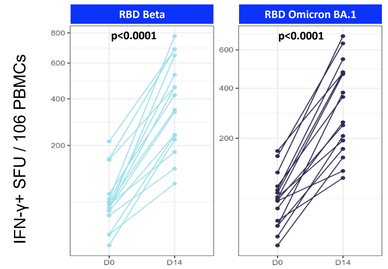



**Figure 1.** T-cell responses by IFN-γ+ ELISpot after stimulation of PBMCs with peptide pools encompassing the RBD region of Beta and Omicron BA.1 SARS-CoV-2 variants. Graphs show the number of IFN-γ+ SFU per $10^6$ PBMCs at D0 (n=16) and D14 (n=16). Statistical analyses were conducted using paired-samples t-tests. Statistical significance is peripheral blood mono nuclear cells; RBD, receptor-binding domain; SFU, spot-forming units.

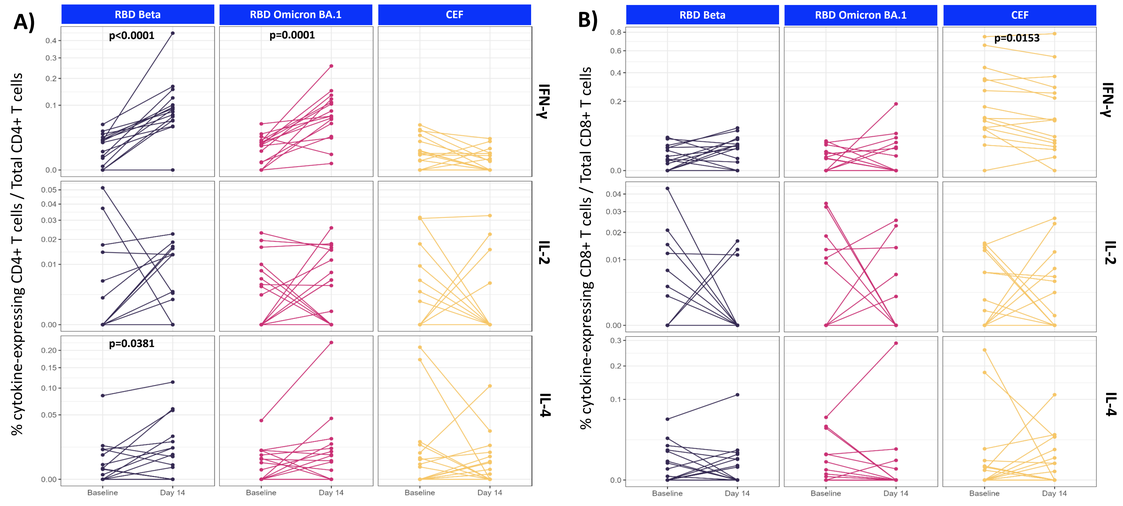



**Figure 2**. IFN-γ, IL-2, and IL-4-mediated responses in CD4+ (A) and CD8+ T cells (B) following stimulation of PBMCs with peptide pools covering the RBD region of the Beta and Omicron BA.1 SARS-CoV-2 variants and with the CEF stimulation control (n=16). The graphs display the percentage of CD4+ and CD8+ T cells secreting IFN-γ, IL-2, or IL-4 at baseline and day 14 post-vaccination with PHH-1V. Statistical analyses were performed using paired-samples t-tests. Statistically significant comparisons (p<0.05) are indicated by their corresponding p-value. CEF, cytomegalovirus, Epstein-Barr virus, and influenza virus; RBD, receptor-binding domain.

## CONCLUSIONS

PHH-1V vaccine elicits a robust IFN-γ T-cell response (Th1) in healthy pediatric adolescents when administered as a heterologous boost. Our findings are similar to those obtained in the heterologous boost study with healthy adults aged 18 to 65 years old (HIPRA-HH-2) (data not shown) (2). Noteworthy, the present study indicates a more pronounced IFN-γ T-cell immune response in adolescents than adults (data not shown) (2), potentially reflecting their maturating immune system. Additionally, adolescents presented high neutralizing titers against SARS-CoV-2 variants, as well as elevated binding antibody titers following PHH-1V vaccine boost (data not shown).

## REFERENCES

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