An assessment of NK and T cell functional immunity in response to vaccination whilst taking acalabrutinib or ibrutinib monotherapy

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INTRODUCTION

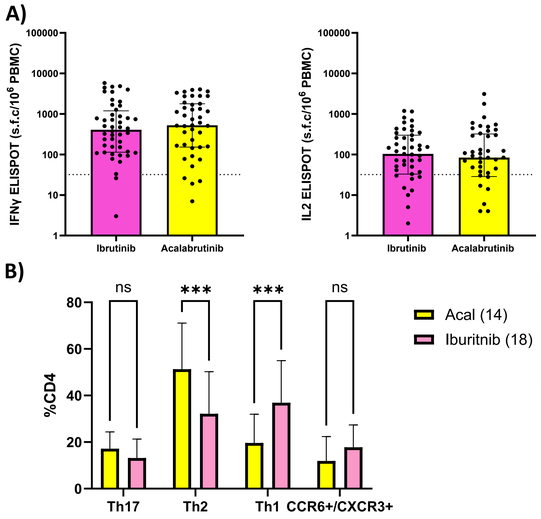
- Humoral immunity to vaccination is known to be impaired with continuous BTKi therapy, but less is known regarding antigen specific cellular responses to vaccination.
- Cellular immunity has been reported to improve during covalent BTKi therapy, evidenced through enhanced antigen specific responses to latent herpes viruses[1] and the improved generation of CAR-T cells amongst patients previously treated with BTKi[2].
- The contribution of NK and T cell antigen-specific immunity and a comparison of functional responses elicited by the different covalent BTKi drugs following vaccination, remains unknown.

METHODS

- Fresh whole blood samples from 96/99 participants of the IMPROVE trial (ISRCTN 14197181)[3] were obtained 3 weeks following vaccination, stimulated with SARS-COV-2 peptides overnight and IFNγ production assessed using QuantiFERON ELISA. Matched frozen PBMC samples were subsequently defrosted, rested and stimulated for 4 hours with 2 different peptide pools consisting of 315 peptides (15-mers with 11amino acid overlap) covering the spike glycoprotein) (S1 and S2). IFNγ production measured by ELISPOT. Using correlation analysis of these 2 functional assays, the top and bottom 17% of functional responders were identified.
- Using 10 colour flow cytometry panels, the surface phenotype and transcription factor profiles of CD3+ T cells and NK cells were assessed and compared amongst participants exhibiting the greatest and poorest cellular responses. To phenotype antigen-specific responses, PBMC samples were next rested overnight and subsequently stimulated with either S1 or S2 peptide pools for 4 hours before measuring IFNγ, TNFα and IL2 by flow cytometry alongside NK and T cell phenotypic markers.

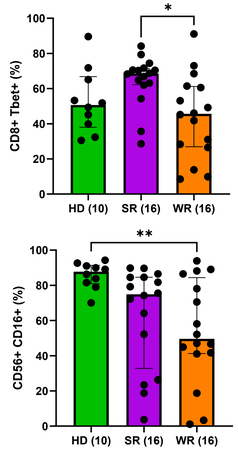
RESULTS

Comparable cellular responses to vaccination were observed in acalabrutinib and ibrutinib treated patients, despite a greater % of Th1 CD4+ T cells amongst ibrutinib treated.



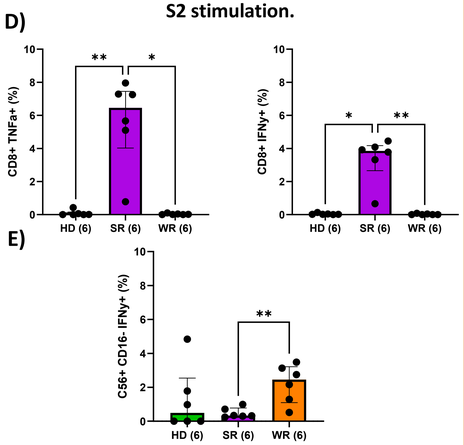
A) Comparison of IFN (left) and IL-2 (right) production following COVID-19 vaccination between patients receiving Ibrutinib (n= 49) and Acalabrutinib (n=43) therapy.
B) Comparison of the proportions of CD4 Th memory subsets[4] between patients receiving Acalabrutinib (n=14) and Ibrutinib (n=18)

Strong cellular responders following vaccination have increased proportions of Tbet-producing CD8+ T cells and CD16+ CD56+ NK cells compared to weaker cellular responders.



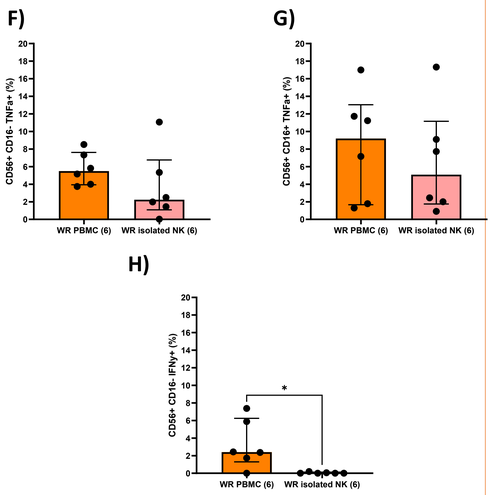
C) Comparison of proportion of CD8+ Tbet+ T cells (above) and CD56+ CD16+ NK cells (below) between Healthy Donors (HD =10), Strong Responders (SR, n=16) and Weak Responders (WR, n= 16) to COVID-19 vaccination.

Strong responders to vaccination have a greater proportion of IFNγ and TNFα- producing CD8+ T cells following pooled Spike 1 peptide stimulation (S1), but weak responders have a greater proportion of IFNγ – producing NK cells following S2 stimulation.



D) Proportional comparison of CD8+ T cells producing TNFα (left) and IFNγ (right) between Healthy Donors (HD), Strong Responders (SR) and Weak Responders (WR) following S1 stimulation.
E) Proportional comparison of IFNγ-producing CD56+ CD16- NK cells between HD, SR and WR following S2 stimulation (n=6 in all groups).

Isolated NK cells can produce TNFα, but not IFNγ following spike peptide pool stimulation, without T cell help



F) Direct comparison of TNFa production in CD56+ CD16- NK cells in Weak Responder whole PBMC vs isolated NK cells
G) Direct comparison of TNFa production in CD56+ CD16+ NK cells in Weak Responder whole PBMC vs isolated NK cells
H) Direct comparison of IFNγ production in CD56+ CD16- NK cells in Weak Responder whole PBMC vs isolated NK cells
(n=6 for both groups in all results).

CONCLUSION

- 97% of participants demonstrated either a cellular or humoral response following a median of 5 doses of COVID-19 vaccination.
- Regardless of which covalent BTKi is taken, an improved cellular response to SARS-CoV-2 is observed following vaccination and will provide important protection for patients, particularly in the 1/3 of participants who are lacking an antibody response.
- CD8+ T cell responses to S1 peptide pool provide the strongest cellular responses and are associated with increased Tbet expression.
- CD56+ CD16- NK cells in weaker responders produce IFNγ following S2 stimulation, suggesting a compensatory mechanism where Tbet+ CD8+ T cells are lacking.
- Following isolation, NK cells can produce TNFα following COVID peptide stimulation, suggesting they can recognize peptide independent of T cells.
- However, isolated NK cells appear incapable of producing IFNγ in the absence of T cell support.

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1. https://doi.org/10.3389/fimmu.2019.02832. 2. https://doi.org/10.1182/blood.2023021306. 3 doi: 10.1016/S2352-3026(25)00008-0
4. https://doi.org/10.1182/blood.2023020554