Signed details of the excellence in research work for which the Sun Pharma Research Award is claimed, including references and illustrations. The candidate should duly sign on the details

My laboratory at THSTI is working in the field of Immunology to understand the role of T cells in the inflammatory conditions, bacterial and viral pathogenesis, and cancer.

One of the focus area of the laboratory is to understand the biology of IL-9-producing Th9 cells in inflammatory conditions by identifying of transcriptional landscape of Th9 cells. In last five years or so, we deciphered the role of Th9 cells in allergic inflammation (**Nature Communications 2017),** cancer (**Nature Communication 2021),** and recently shown that Th9 cell contributes to the immunepathologies of SARS-CoV2 infection (**Nature Communication 2021)**.

We further identify the proteome signature of Th9 cells (**Scientific Reports)** and the signalling pathways that are required for generation and functions human Th9 cells (**Journal of Leukocyte Biology, Frontiers in Immunology 2019**). Our lab also identified small molecules that inhibits inflammation in experimental colitis by upregulating anti-inflammatory T cells that produce IL-10 (**European Journal of Immunology 2024**). In addition, we contributed to the understanding the role of micronutrients in anti-tumour immunity where we show as to how Salt modulates anti-tumour function of NK cells through gut microbiota (**Science Advances 2021**).

We contributed to understand immune-pathology of COVID-19 induced by ancestral and its variants ((**ELife 2023**, **Communications Biology 2023, European Journal of Immunology 2024, European Journal of Medical research 2023**). To understand the role of T cells in long lasting immunity against COVID-19, we have shown as to how proinflammatory cytokines shape the protective T cells response against COVID19 (**Vaccines 2022**), and moreover these T cells are critical in reducing the severity of COVID-19 and decreased the risk of hospitalization (**Lancet Infectious Diseases 2021**). In another study we addressed the longevity, durability, magnitude, and breadth of SARS-CoV-2-specific T cell responses in vaccinated individuals following the widespread circulation of omicron and its sub-lineages (**Journal of Medical Microbiology 2024**).

Using preclinical models of COVID-19, we tested Aayush herbal extract in mitigating the COVID-19 immunepathologies under the initiative of Aayush Ministry, Govt of India. We found that prophylactic use anu oil reduced viral entry and inhibited the virus induced inflammation (**Frontiers Pharmacology 2021**). In addition, we also show that prophylactic treatment of Glycyrhhiza and Withania inhibit SARS-CoV2 infection and associated pathology in hamsters (**Frontiers Immunology 2022; Frontiers Immunology 2023**). On the COVID-19 therapy, we identified a broadly neutralising monoclonal antibody that is able to neutralize SARS-CoV2 ancestral and Variant of Concerns (**Plos Pathogens 2022 (a); Plos Pathogens 2022 (b)**). These are some key findings of my laboratory in past five years that I would like the committee members to consider these publications for The Sun Pharma Research foundation award.

We delineated the molecular pathways that are required for generations and functions of Th9 cells using a variety of techniques like transcriptomics, proteomics, metabolomics. We have unravelled the role of transcription factors, Foxo1 and HIF-1 alpha, in generation and function of Th9 cells. Briefly, we identified

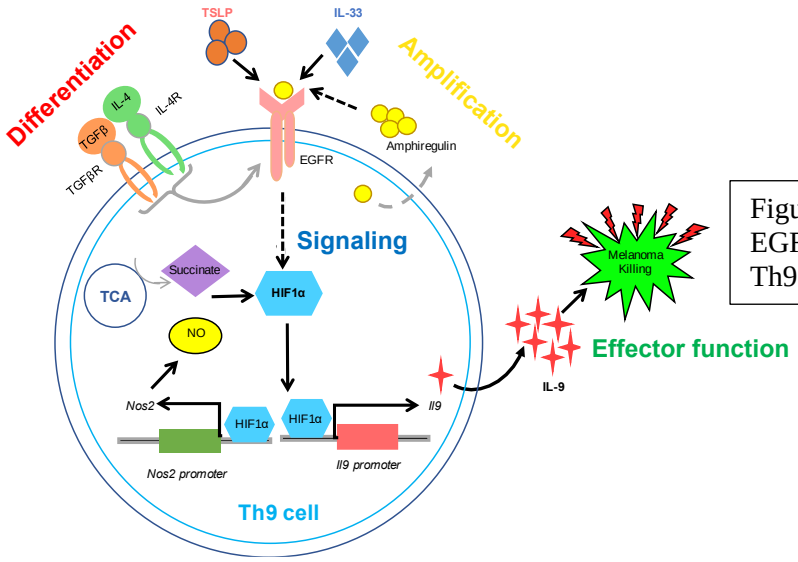

Figure 1: Schematic representation of EGFR-HIF1α signalling pathway in Th9 cells.

that Foxo1, a Fork head family transcription factor, is essential for the generation and function of Th9 cells. In fact, Foxo1 is essential for the induction and transactivation of IL-9 gene locus in other T helper cells such Th2, Th17 and Tregs[1].

We identified that Epidermal Growth Factor Receptor (EGFR) is essential for IL-9 induction in Th cells. Moreover, amphiregulin (Areg), an EGFR ligand, is critical for the amplification of Th9 cells induced by TGF-β1 and IL-4. Furthermore, our data show that AREG-EGFR signalling induces HIF1α, which binds and transactivates IL-9 and NOS2 promoters in Th9 cells. Loss of EGFR or HIF1α abrogates Th9 cell differentiation and suppresses their anti-tumour functions. Moreover, in line with its reliance on HIF1α expression, metabolomics profiling of Th9 cells revealed that Succinate, a TCA cycle metabolite, promotes Th9 cell differentiation and Th9 cell-mediated tumour regression (**Figure 1**)[2]. We extended our work on human Th9 cells and identified that extracellular ATP (eATP) promote human Th9 cell differentiation through the production of nitric oxide (NO), which creates a feed forward loop in the differentiation of human Th9 cells. Inhibition of purinergic receptor signalling suppressed the generation of human Th9 cells while exogenous NO supplementation reversed the generation of Th9 cells even in the absence of purinergic receptor signalling. Moreover, we identified that ATP-induces transcription factors, mTOR and HIF-1a, which are essential for the induction of human Th9 cells. In this study, we identified that the ATP-NO-mTOR-HIF1a axis is essential for the generation of

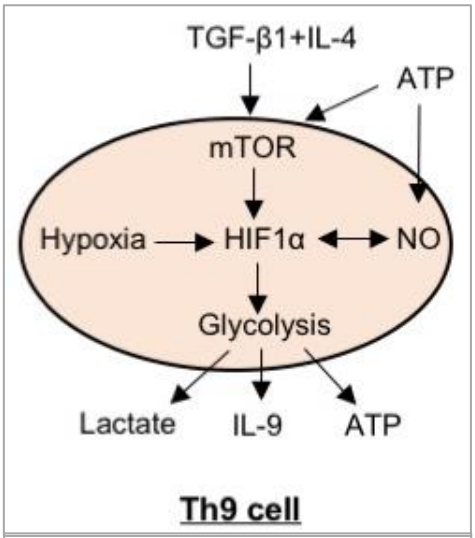

Figure 2: Schematic model of human Th9 cells differentiation

human Th9 cells and modulation of this axis may lead to therapeutic intervention of Th9-associated disease conditions **(Figure 3)**[4].

Above studies identify factors that are essential for Th9 cells development and function based on the transcriptomics analysis. One of the drawbacks of transcriptomics that it fails to identify proteins that are

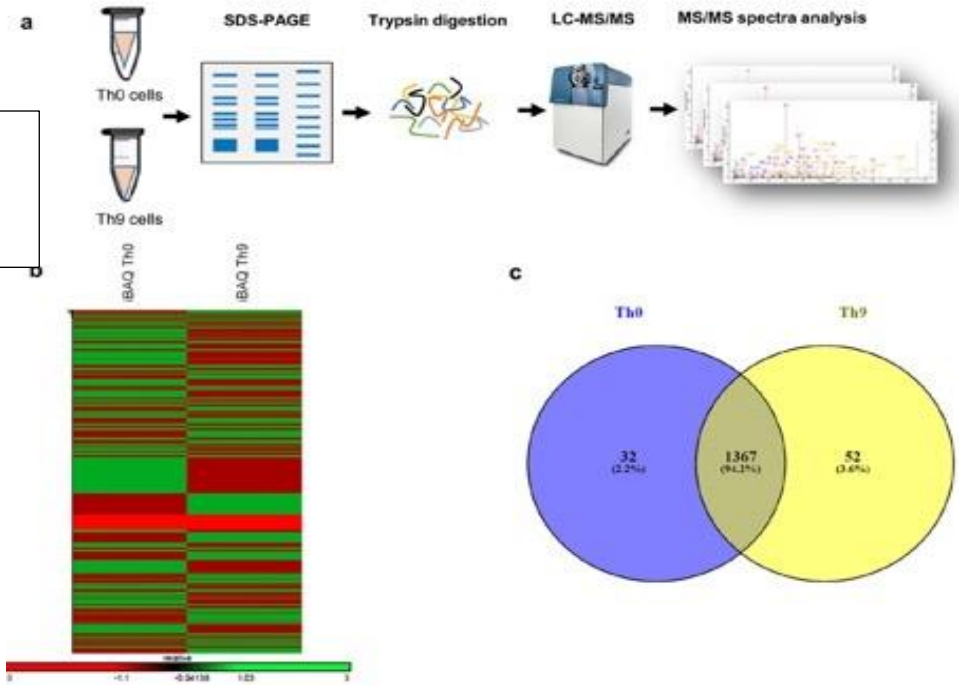

Figure 3: Schematic representation of proteomic analysis of Th9 cells.

regulated post-transcriptionally. Therefore, we performed proteome analysis of Th9 cells to understand the involvement of proteins that might be crucial for the anti-tumour functions of Th9 cells (**Figure 4**). In this study, we performed a comprehensive proteomic analysis of murine Th9 cells, and identified proteins that are enriched in Th9 cells. Pathway analysis identified an abundance of phosphoproteins in the proteome of Th9 cells as compared to controls. Among upregulated phosphoproteins, Ppp2ca (catalytic subunit of protein phosphatase, PP2A) was found to be highly enriched in Th9 cells. We further found that PP2A is required for the induction of Th9 cells, as PP2A inhibition leads to the suppression of IL-9 and expression of key transcription factors of Th9 cells. PP2A inhibition abrogates Th9 cell-mediated anti-tumour immune response in B16-OVA melanoma tumour model. Thus, we report that PP2A is essential for the differentiation and anti-tumour functions of Th9 cells[5].

We recently show, using K18-hACE2 transgenic (ACE2.Tg) mouse model, that IL-9 contributes to and exacerbates viral spread and airway inflammation caused by SARS-CoV-2 infection[6]. ACE2.Tg mice with $CD4^+$ T cell-specific deficiency of the transcription factor Forkhead Box Protein O1 (Foxo1) produce significantly less IL-9 upon SARS-CoV-2 infection than the wild type controls and they are resistant to the severe inflammatory disease that characterises the control mice[5]. Exogenous IL-9 increases airway inflammation in Foxo1-deficient mice, while IL-9 blockade reduces and suppresses airway inflammation in SARS-CoV-2 infection, providing further evidence for a Foxo1-Il-9 mediated Th cell-specific pathway playing a role in COVID-19[6].

Collectively, our study provides mechanistic insight into an important inflammatory pathway in SARS-CoV-2 infection, and thus represents proof of principle for the development of host-directed therapeutics to mitigate disease severity. We identified small molecules that inhibits inflammation in experimental colitis by upregulating anti-inflammatory T cells that produce IL-10[7].

In addition, his group works on to understand the role of micronutrient and T cells in tissue inflammation in autoimmune diseases and cancer[8]. Dietary salt (NaCl) is one of the key micronutrients that is essential for physiology. The role of dietary salt, however, is not known in modulating antitumor functions of the host. Tumour microenvironment is suppressive, which suppressed immune cells withing the tumour and

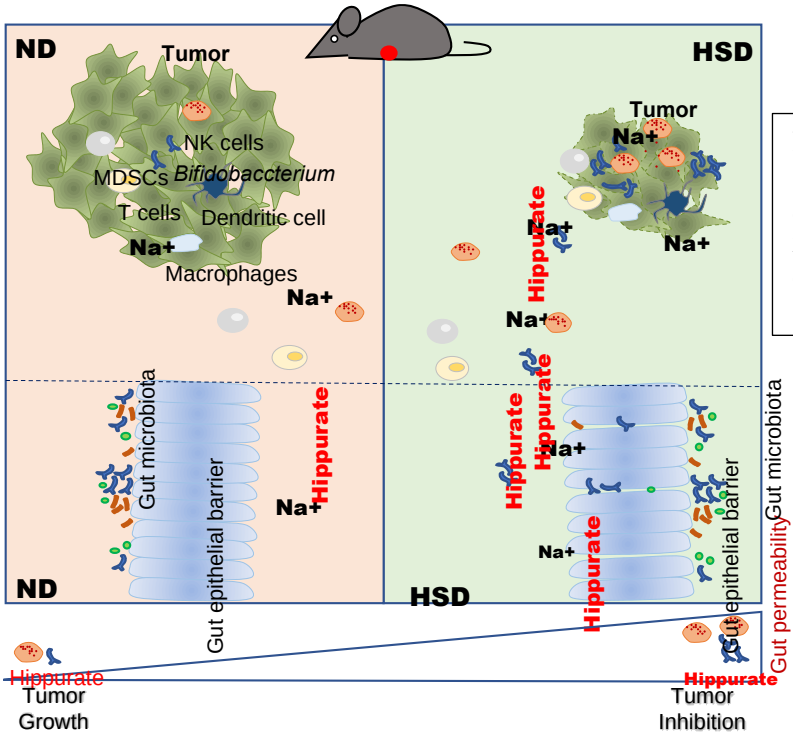

Figure 5: Schematic representation SARS-CoV2 induced pulmonary and extrapulmonary pathologies including cardiovascular complications.

thus failed to eliminate tumour. Within the immunosuppressive tumour microenvironment, high salt diet (HSD)-induced inflammatory response could enhance tumour immunity. However, HSD-induced antitumor immunity and its association with gut microbiota is yet to understood. In this study, we report that HSD-induced tumour immunity via enhancing IFN gamma production, NK cells functions and downregulation of key checkpoint inhibitor programmed cell death protein 1 (PD1). While depletion of NK cells abrogated HSD-induced anti-tumour functions, combination of salt with anti-PD1 antibody leads to a robust anti-tumour immune response. HSD modulates gut microbiota in mounting tumour immunity, as depletion of microbiota blunted HSD-induced tumour immunity. Faecal matter transfer (FMT) from HSD mice restored anti-tumour immunity associated with NK cell activity. Metagenomic profiling revealed that HSD enriches *Bifidobacterium* abundance, as colonizing *Bifidobacterium* resulted in NK cell functions in tumour regression. Our results, thus, indicate a potential use of *Bifidobacterium* to enhance cancer immunotherapy via modulation of gut microbiota-dependent NK cell activation (**Figure 4**)[8].

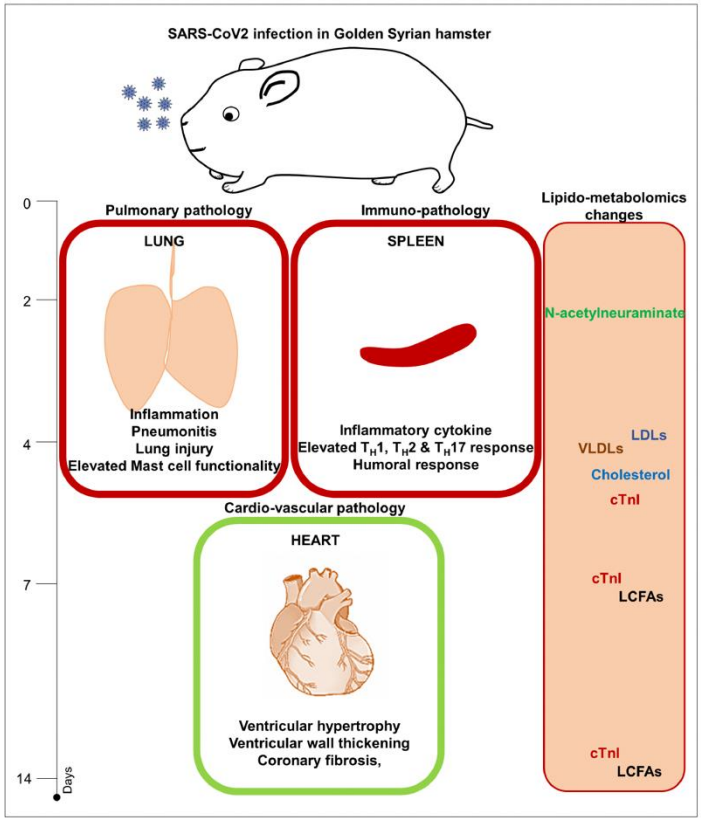

Figure 4: Schematic representation Salt induced anti-tumour functions through the modulation of gut microbiota

In addition, my lab significantly contributed to the Covid19 pandemic by establishing 'cellular assays' 'animal models' platforms to study the pathogenesis of SarsCov2 infection, and to support academic and industry partners to test their vaccine candidates and antiviral drugs. In fact, we have shown, for the first time, that SarsCov2 infection causes cardiovascular dysfunction using hamster model[9].

Using the animal model, we studied the disease pathogenesis induced by SARS-CoV2 infection. We identified, for the first time, that SARS-CoV2 infection leads to cardiovascular dysfunction and ventricular hypertrophy and presented that Golden Syrian hamster could potentially be a good model to study cardiovascular dysfunctions[9]. SARS-CoV-2 infection in the Golden Syrian hamster causes lung pathology that resembles human coronavirus disease (COVID-19). However, extrapulmonary pathologies associated with SARS-CoV-2 infection and post-COVID sequelae remain to be understood. In this study, we show, using a hamster model, that the early phase of SARS-CoV-2 infection leads to an acute inflammatory response and lung pathologies, while the late phase of infection causes cardiovascular complications (CVCs) characterized by ventricular wall thickening associated with increased ventricular mass/body mass ratio and interstitial coronary fibrosis[8]. Molecular profiling further substantiated our findings of CVC as SARS-CoV-2-infected hamsters showed elevated levels of serum cardiac troponin I, cholesterol, low-density lipoprotein, and long-chain fatty acid triglycerides. Serum metabolomics profiling of SARS-CoV-2-infected hamsters identified N-acetylneuraminate, a functional metabolite found to be associated with CVC, as a metabolic marker was found to be common between SARS-CoV-2-infected hamsters and COVID-19 patients. Together, we propose hamsters as a suitable animal model to study post-COVID sequelae associated with CVC, which could be extended to therapeutic interventions **(Figure 5)**[9]. Among

extrapulmonary pathologies, we found SARS-CoV2 infection leads to thymic atrophy which ultimately affect T cell repertoire and peripheral T cells dysfunctions[10]. Using the animal model we identified broadly

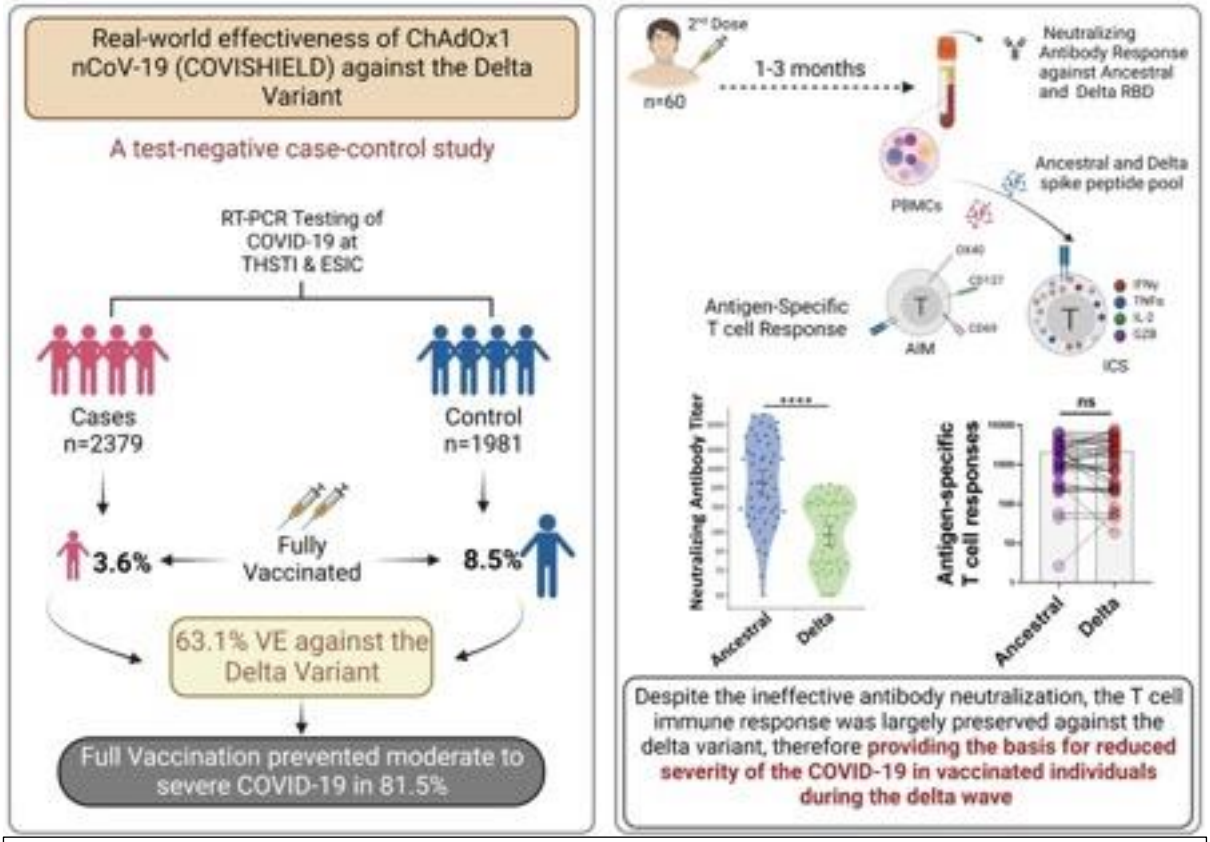

Figure 6: Schematic representation to summarise vaccine effectiveness study conducted during Delta variant surge in 2021.

neutralising antibody that is able inhibits ancestral and other variants of SARS-CoV2[11]. On the same line, we identified a Fangchinolone, a pan beta coronavirus inhibitor, which inhibit SARS-CoV1, SARS-CoV2 and its variants and MERS-CoV entry[12].

Using the cellular assay platform that my group established at THSTI, we studied the vaccine effectiveness during Delta surge in 2021[13]. In this study, we identified the role of T cells in preventing severe disease in ChAdOx1 nCoV-19 vaccinated individuals during Delta variant of SARS-CoV2 in India. Of the 2766 cases of confirmed SARS-CoV-2 infection, 3·1% were fully vaccinated compared with 7·1% of the 2377 controls giving an adjusted OR of 0·37 (95%CI 0·28, 0·48); this translated to 63·1% (95%CI 51·5, 72·1) vaccine effectiveness against SARS-CoV-2 B·1·617·2 variant, seen in 80% of the infected population. Full vaccination prevented moderate-severe COVID-19 in 81·5% (95%CI: 9·9, 99·0). The effectiveness of single-dose vaccine was 46·2% (95%CI: 31·6, 57·7) against infection but 79·2% (95%CI: 46·1, 94·0) in preventing moderate-severe COVID-19. Among healthy vaccinated persons, plasma live virus neutralisation was 2·5-6·8 fold lower against B·1·1·7, B·1·351, B·1·617·1 and B·1·617·2 being lowest against B·1·617·2 (Delta). However, both CD4+ and CD8+ T-cell responses were found to be preserved

against the virus spike protein of the Delta variant suggesting cell-mediated immune protection. ChAdOx1 nCoV-19 VE was 63.1% against B·1·617·2 infection and 81·5% in preventing severe disease. Spike-specific T-cells responses against virus variants were maintained (**Figure 6**)[13]. It was important to understand as to how these T cells, which provide long lasting protection, are generated. We show that early induction of innate proinflammatory cytokines like IL-23, IL-1beta and IL-18 shape the long-lasting T cells response against COVID-19[14].

In another study we addressed the longevity, durability, magnitude, and breadth of SARS-CoV-2-specific T cell responses in vaccinated individuals following the widespread circulation of omicron and its sub-lineages[15]. By conducting a cross-sectional study involving 321 participants from Delhi-NCR, we compared the T cell response- young vs elderly & anti-RBD seropositive vs seronegative. Interestingly, detectable T cell response was observed in >78% of seronegative participants. We observed a dramatically increased T cell response post-omicron surge, suggesting broadscale asymptomatic infection. T cell response post-omicron surge in two-dose vaccinated participants was comparable to that of participants with multiple exposures to SARS-CoV-2 antigen. The study also explores cross-reactivity against emerging variants, including BA2.86 with preserved frequencies of stem-cell-like memory T cells. Notably, in silico prediction suggests mutated epitopes of JN.1 and KP.2 retaining over 95.6% of their HLA binding capability. Implications of this study are far-reaching, particularly in the current global context of COVID-19. Our data indicate that asymptomatic exposures to omicron subvariants contribute to elevated & lasting T cell response, underscoring the potential for long-term protection.

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