**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 working in the field of Immunology to understand the role of T cells in the inflammatory conditions like inflammatory bowel disease (IBD), viral and bacterial pathogenesis and cancer. The research at my laboratory divided into two broad categories namely basic and translational immunology research.

Under the basic immunology research, our focus is to understand the biology of inflammatory T cells such as Th9 cells for which I had secured prestigious India-Alliance Intermediate Fellowship. I contributed in understanding the biology of IL-9-producing Th9 in tissue inflammation of autoimmune diseases, allergic inflammation and extracellular infections. Our seminal work in discovering and identifications of transcriptional landscape of Th9 cells was recognized globally. In fact, we are among very few researchers who are working on Th9 cells and its transcriptional regulation in various inflammatory conditions. In last five years or so, we have published or research work on Th9 cells in high impact journals (**Nature Communications 2017**, **Nature Communication 2021, Frontiers in Immunology 2019, Journal of Leukocyte Biology, Scientific Reports, and Nature Communications 2023**).

In addition to understand the role of Th9 cells and inflammatory conditions, we have contributed to the understanding the role of micronutrients in anti-tumour immunity (**Science Advances 2021**). We significantly contributed to understand immune-phathology of COVID-19 (**ELife 2023**) induced by ancestral and its variants (**Communications Biology 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**). Since we established preclinical models for COVID-19, we also tested the role of Aayush herbal extract in mitigating the COVID-19 induced inflammation. This was an initiative of Aayush Ministry, Govt of India to identify Aayush herbal extract that can reduces SARS-CoV-2 induced inflammation and associated mortality. 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

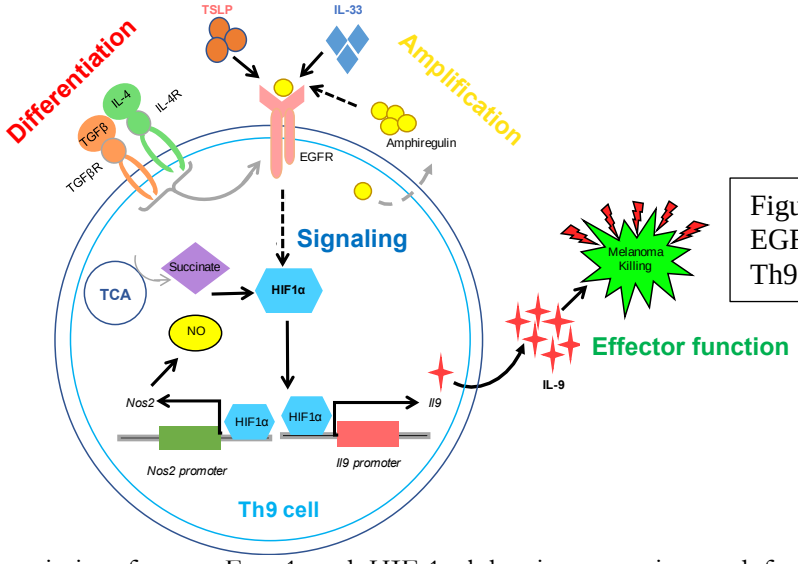


Figure 1: Schematic representation of EGFR-HIF1α signalling pathway in Th9 cells.

transcription factors, Foxo1 and HIF-1 alpha, in generation and function of Th9 cells. Briefly, we identified 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 further identified the role of IL-9-Foxo1 axis in the SARS-CoV2 infection, we found that IL-9 aggravates SARS-CoV2 infection and exacerbates SARS-CoV2-induced pulmonary pathologies[2].

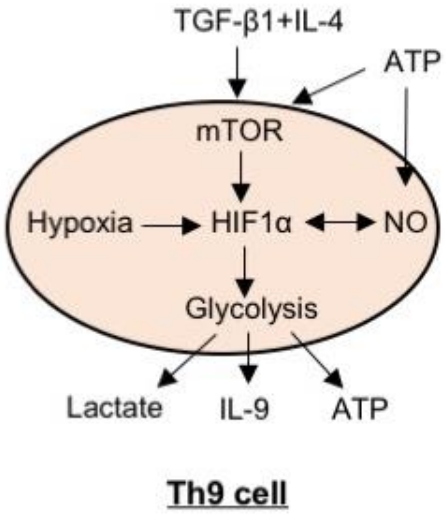


Figure 2: Schematic model of human Th9 cells differentiation

We further 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**)[3]. We further 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 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

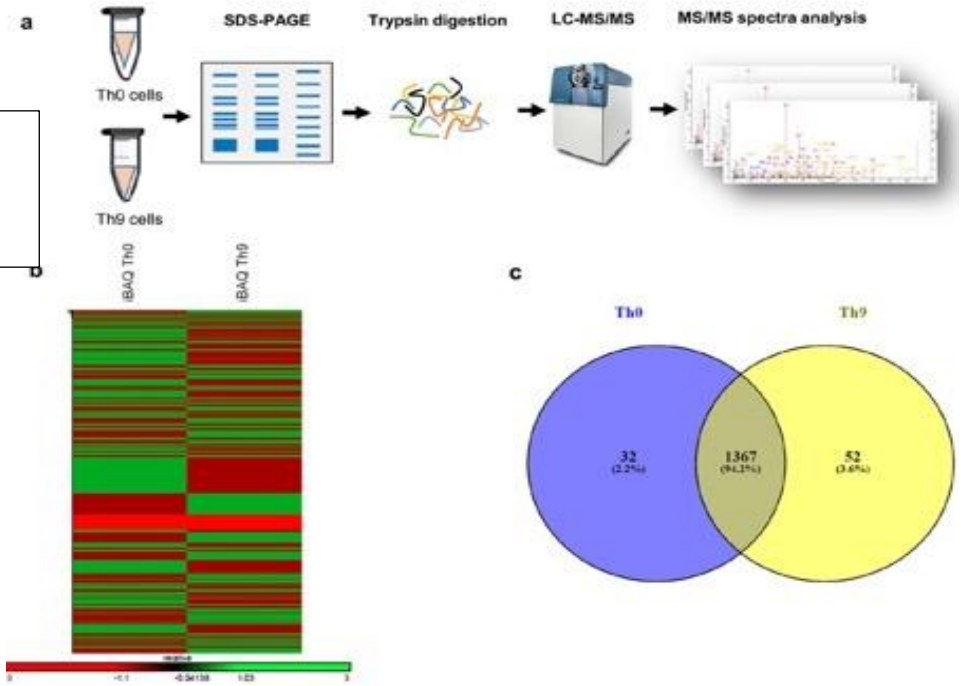


Figure 3: Schematic representation of proteomic analysis of Th9 cells.

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].

In addition, his group works on to understand the role of micronutrient and T cells in tissue inflammation in autoimmune diseases and cancer[5]. 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 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

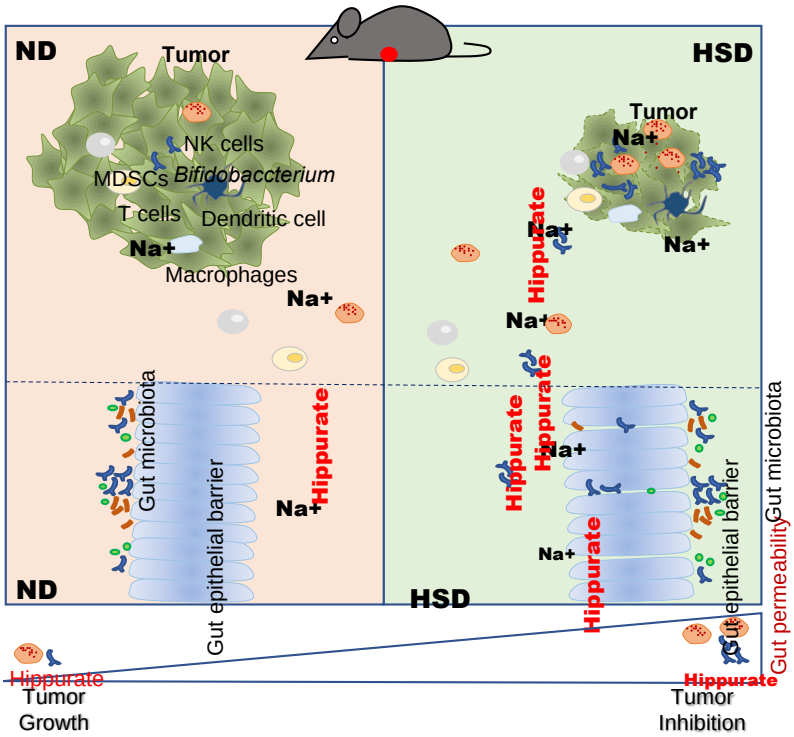


Figure 4: Schematic representation Salt induced anti-tumour functions through the modulation of gut microbiota

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**)[6].

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, Amit has shown, for the first time, that SarsCov2 infection causes cardiovascular dysfunction using hamster model[7].

Using the animal model, we studied the disease pathogenesis induced by SARS-CoV2 infection. We

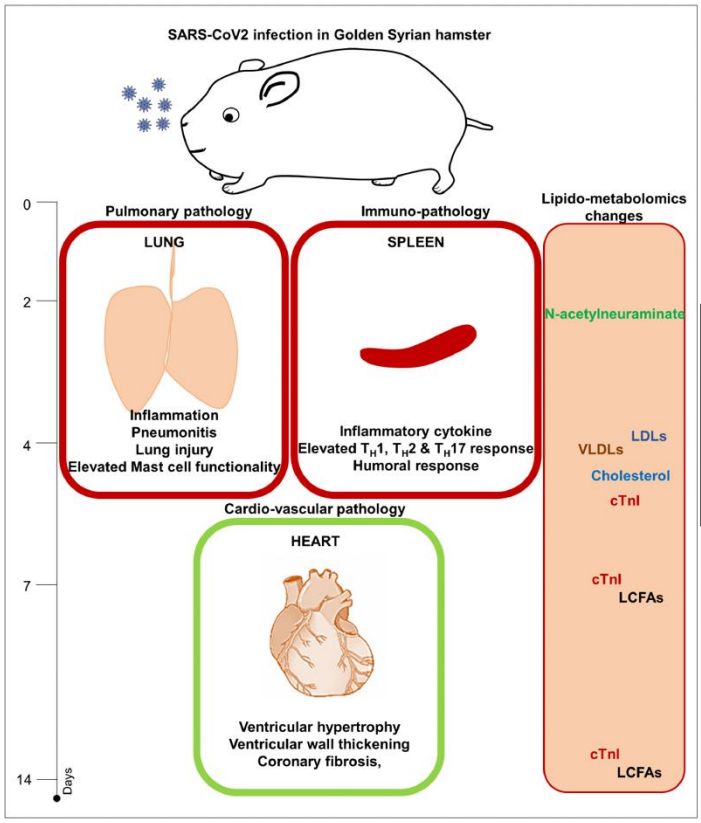


Figure 5: Schematic representation SARS-CoV2 induced pulmonary and extrapulmonary pathologies including cardiovascular complications.

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[7]. 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[7]. 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)** [7]. Using the animal model we identified broadly neutralising antibody that is able inhibits ancestral and other variants of SARS-CoV2[8]. 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[9].

Using the cellular assay platform I established at THSTI, we studied the vaccine effectiveness during Delta surge in 2021[10]. 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

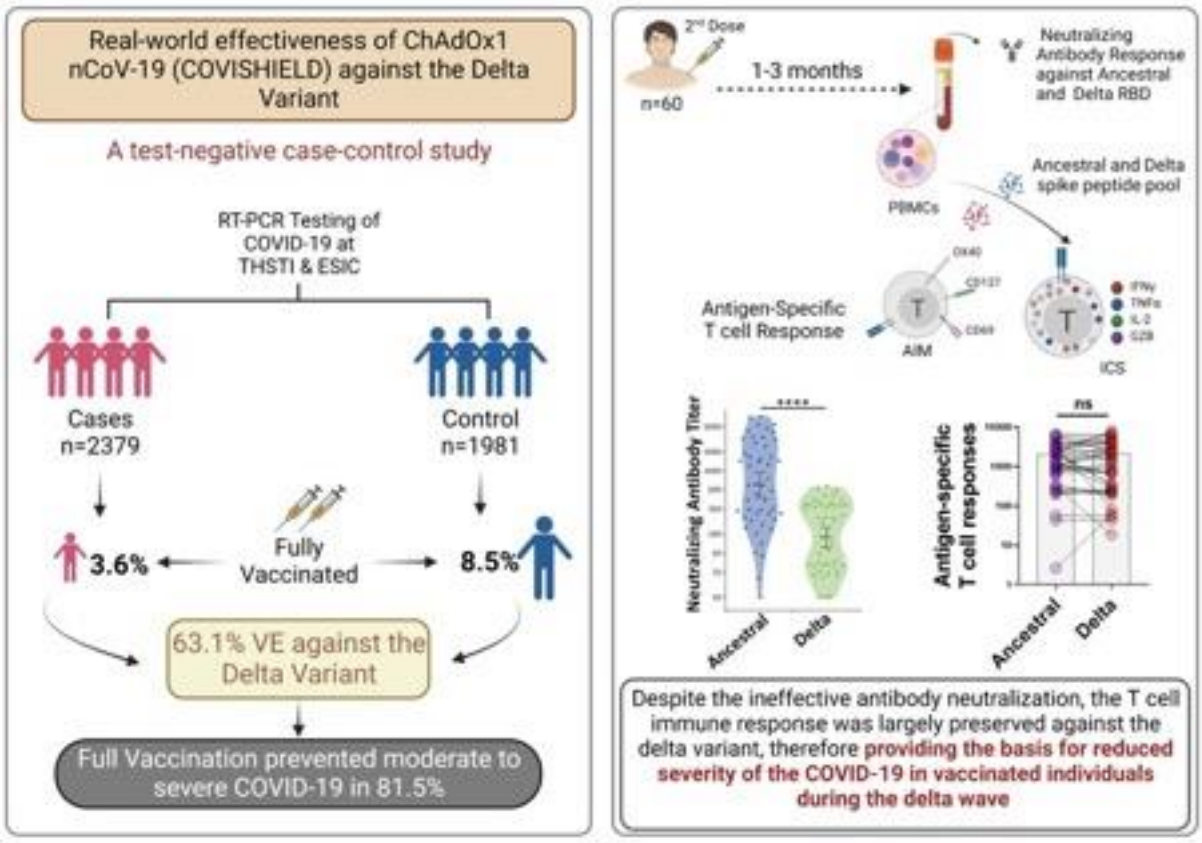


Figure 6: Schematic representation to summarise vaccine effectiveness study conducted during Delta variant surge in 2021.

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**)[10]. It was further 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[11].

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