Proposal Template

Name of the Institute: Indian Institute of Technology Roorkee

Incubator: TIDES Incubator/ BioNEST scheme at IIT-Roorkee

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Objective: Post-vaccination study to evaluate Covid-19 vaccine efficacy and immune correlates of protection

Type of Intervention: (Choose one)

1) Proposal on Vaccination drive community engagement
2) Proposal on Cold storages and Cold chains battery or solar operated for last mile connection
3) Last stage Vaccination development

√ 4) Post Vaccination studies. (**)

**** For this type intervention, please send a separate 1-2 pager proposal that will include abstract of the planned/proposed work, methodology, tentative budget, and estimated timeline.** (Attached)

Details of intervention:

(Should cover details about the product/ technology, methodology, milestones, timeline, Line-item wise financials along with tentative cost of transportation, annual maintenance etc.) (Attached, same as above)

Do you have State Government connection, or will you require support from CSR –
Yes, support from CSR required for coordinating regarding State Government connections
Few collaborations from AIIMS Rishikesh and Jolly Grant Hospital in Dehradun, through MoUs

States that you can provide technology to – Uttarakhand, Delhi, Haryana

Please answer following questions depending on the intervention you choose and if applicable to you:

Can you do the Community engagement yourselves or will need help by CSR- **Help needed from CSR**

If you have a Market ready technology available

- How do you plan to deploy: Not Applicable
- Number of unites available: Not Applicable

Do u wish to partner with an NGO? If yes, name the NGO and provide details on how u will partner? (item wise costing should include cost to NGO for their scope of work): Not Applicable

Title: Post-vaccination study to evaluate Covid-19 vaccine efficacy and immune correlates of protection

Abstract:

With two vaccines recently been approved for emergency use in India, we face new challenges for Covid-19 vaccine studies. India is among the most affected nations with SARS-CoV2 pandemic, with sparse data on asymptomatic infections and antibody persistence. The aim of this proposal is to conduct a post-vaccination study wherein human subjects will be recruited in collaboration with state government health care officials and vaccine efficacy will be assessed. Appropriate prospective case-control studies will be designed to assess efficacy of (i) Covishield, (ii) Covaxin and (iii) placebo, both after first and booster dose. From peripheral blood obtained from recruited individuals, serum and PBMCs samples will be isolated and characterized. Serological studies will involve testing for SARS-Cov-2 specific antibody titers and measuring pro- and anti-inflammatory cytokine levels using ELISA. Since both very-high and very-low titers of antibodies have been negatively associated with protection, generation of neutralizing antibody titers will be evaluated using virus-neutralization assays. Cellular analysis will involve enumeration of lymphocyte subpopulations (Total lymphocytes, B-cells, CD4T-, CD8T-cells, memory cells, and antibody-secreting plasma cells) through flow-cytometry. Genomic DNA will be isolated from human serum for testing presence of candidate SNP polymorphisms as biomarkers of risk for Covid-19. Further, immune repertoire studies will be undertaken to identify association between genetic diversity and risk/protection for Covid-19 in Indian population. In summary, the proposed study aims to investigate induction of both humoral and cellular immune responses, and investigate which of these may provide beneficial outcomes in vaccine recipients in order to identify immune correlates of protection. The study would also assess vaccine related side-effects and adverse events if any.

Methodology

1.1 Recruitment for Case-Control studies: In order to carry out the proposed research objectives mentioned in this study, human volunteers administered with one or both doses of (i) Covishield, (ii) Covaxin and (iii) placebo vaccines will be recruited through clinical collaboration with State Government facility along with age-matched healthy controls. Inclusion criteria for cases and controls: between 18-50 years of age, availability for clinical follow-up, willingness for completing informed consent and blood donation, HIV-negative, beta-HCG negative, and in general good health as per CBC test. For cases, the inclusion criteria would include: having no prior diagnosed Covid-19 infection (confirmed through RT-PCR or serology). Exclusion criteria for cases and controls: patients having recent Covid-19 infection, presence of underlying health issues and other immunocompromising conditions, corticosteroid use, and bleeding disorders.

The study design is detailed as follows: Total number of individuals to be recruited will be between 25 to 50 for each group. The sample size (n=25 to 50) allows the possibility of having minimum 10 patients following up for subsequent follow-up visits to the health care center for additional inputs if needed.

1.2 Isolation of Serum and PBMCs From each patient, peripheral blood obtained will be used to isolate serum and PBMCs using standard protocols. PBMCs obtained at different time-points will be frozen in aliquots for further downstream studies.

2.1 Measurement of Antibody titers: Serum samples will be screened for presence of cross-protective and broadly neutralizing antibodies by means of ELISA and various *in vitro* cell based assays. Virus neutralization assays will be performed and quantified using both plaque reduction (PRNT) and foci reduction (FRNT) techniques. ELISPOT assays will be performed to screen PBMCs for presence of SARS-CoV-2 specific antibody secreting plasma cells.

2.2 Measurement of Cytokine levels: Serum samples will be screened for presence of both Th1 (pro-inflammatory) and Th2 (anti-inflammatory) cytokines by means of ELISA and multiplex platforms like Luminex. Additionally, intracellular multiplex cytokine staining will also be performed using CBA assay (by means of flow cytometry) if needed.

2.3 Enumeration of lymphocyte subpopulations: Lymphocyte populations will be characterized from PBMCs obtained from recruited patients by flow cytometry. An aliquot of PBMC sample will be stained with B cell markers and characterized by flow cytometry to identify presence of SARS-CoV-2 antibody secreting B cells (ASCs) and memory cells. Multicolor flow cytometry will be performed to simultaneously stain and assess CD4 T and CD8 T cell populations, memory cells and plasma cells.

3.1 Biomarker SNP Analysis: Serum genomic DNA will be utilized for SNP analysis to identify host genetic risk factors, namely few identified and published candidate genes associated with Covid-19. SNP association studies will be performed using PCR sequencing/or Genome-wide human SNP array 6.0 (Affymetrix).

3.2 Immune repertoire analysis: Heavy and Light chain antibody genes of the sorted single B cells would be sequenced, using nested single cell Ig PCR amplification approach. Molecular characteristics will be detailed. Pooled cDNA RT primers (for heavy chain and light chain isotypes) will be used for single cell template. The sequences at the heavy and light chain genes will be aligned with germline databases to study family/gene usage, VDJ rearrangement, heavy/light pairings, CDR3 length and codon analysis, SHM, N/P nucleotides, Reading Frames, clonal expansion and selection.

Budget Details

HEAD	YEAR 1	YEAR 2	YEAR 3	TOTAL
(A) Non- Recurring Equipment	Rs 90,00,000/-	0/-	0/-	Rs 90,00,000/-
(B) Recurring				
Manpower (one RA)	Rs 5,64,000/-	Rs, 5,64,000/-	Rs 5,64,000/-	Rs 16,92,000/-
Consumables	Rs 8,36,000/-	Rs 8,36,000/-	Rs 8,36,000/-	Rs 25,08,000/-
Contingency	Rs 50,000/-	Rs 50,000/-	Rs 50,000/-	Rs 1,50,000/-
Travel	Rs 50,000/-	Rs 50,000/-	Rs 50,000/-	Rs 1,50,000/-
(6) Overhead charges	Rs 5,00,000/-	Rs 5,00,000/-	Rs 5,00,000/-	Rs 15,00,000/-
Grand Total (A+B)	Rs 1,10,00,000/-	Rs 20,00,000/-	Rs 20,00,000/-	Rs 1,50,00,000/-

Expected Outcome

Evaluation of vaccines for prophylaxis and treatment of Covid-19 is currently the need of hour. The proposed study and experiments outlined herewith will provide crucial information for analyzing vaccine efficacy outcome (comparing the two different vaccine formulations approved as of now in India, with a placebo group) in healthy individuals and serve to identify those at major risk of severe COVID-19. The most

important results of the proposed study would be detailed analysis of the humoral and cellular immune responses against Covishield and Covaxin vaccines, in an independent cohort from Uttarakhand. Although the importance of antibody-mediated protection against Covid-19 is well established, extremely high levels of antibodies have been reported to be detrimental. Similarly, a balanced cytokine production has been advantageous as opposed to unchecked cytokine secretion leading to cytokine storm. The proposed study aims to identify protective immune correlates induced upon vaccination which may provide protective benefits to recipients on infection, against severe disease and mortality. Identifying serological (SARS-CoV-2 specific antibody titers and cytokine levels) and cellular correlates (lymphocyte subpopulations) which are induced and/or upregulated/downregulated upon vaccination, and can be associated with disease free status in followed-up recipients will help in future vaccination drives and understanding parameters for long-term protection. Genetic SNP association studies will lead to identifications of host Biomarkers predicting risk for Covid-19 infection. This would enable early detection of high-risk individuals and enable preventive therapy. Repertoire analysis of Indian patients who are administered either of the two Covid-19 vaccines and are followed-up over a period of time is expected to provide insights regarding crucial features of protective immune response and will aid in designing more efficient vaccines. This project will help to better understand overall response to vaccination and widely reported variety of side effects. This will not only improve the understanding of response but also may lead to better vaccine candidates. As mutants of the virus are being reported, designing improved vaccine candidates by understanding current vaccine responses is essential. To summarize, results from this study will have a significant impact on COVID-19 vaccination drive in India.

Timeline

Milestones	Year 1	Year 2	Year 3
Objective 1.1	Recruitment of Covid-19 vaccine recipients and controls (collaborators: AIIMS Rishikesh; Jolly Grant Hospital Dehradun and Institute Hospital IIT Roorkee)		
Objective 1.2	Isolation of Serum and PBMCs from peripheral blood samples		
Objective 2.1		Measurement of Antibody titers by ELISA and ELISPOT assays (PRNT)	
Objective 2.2		Measurement of Th1 and Th2 cytokines by ELISA and Luminex assays or Flow cytometry	
Objective 2.3		Enumeration of lymphocyte subpopulations using Multicolor Flow cytometry	
Objective 3.1			Biomarker SNP Analysis
Objective 3.2			Immune Repertoire Analysis