Defining the molecular mechanisms related to immune-modulation leading to faster HIV disease progression- a journey of a decade and a half:

The acquired immune-deficiency syndrome (AIDS), which results from an infection with the human immunodeficiency virus (HIV), is a significant contributor to mortality and has substantial burden on public health system of a country with adverse economic consequences. The viral RNA replication and retro-transcription processes are associated with high mutation rates, leading to spontaneous emergence of a pool of mutant viruses. With the discovery of highly efficacious anti-retroviral treatment, although the disease can be controlled to some extent with reduction in the transmission rates especially in the new born children to HIV+ mothers, yet the pandemic is still ongoing in many parts of the word. This may be due to many reasons including emergence of drug-resistance mutant variants of the virus leading to drug-failure in some individuals. Among these mutants, some may exhibit low-level to very high-level of drug resistance (DR). Keeping in mind the increasing demand of improving the drug efficacy, it was pertinent that there was an urge to identify these mutations and know how they affect the susceptibility to a particular drug. In the year 2005, our lab screened for mutations in the *pol* gene of HIV-1 associated with resistance to zidovudine and lamivudine (two of the drugs making 1st line regimen of ART) in HIV-infected treatment-naive patients from North India and found that a high proportion of mutant variants harboured mutations in the *pol* gene at codons- 70 and 184 coexisting with wild-type HIV-1 even in treatment-naïve situation (1). In the protease region, a major drug resistance (DR) mutation at M46I as well as at positions F53L and T74P likely to cause minor level of DR were observed. This was a significant observation at that point of time when the ART was just starting in India because in the RT gene, mutations in the hinge (M36I, R41K, H69K) and -helix (L89M) regions of the C-virus protease have been linked to increased catalytic activity (2).

In 2009, the National AIDS Control Organization (NACO, Government of India) introduced second-line therapy as a response to the emergence of treatment failure for first-line therapy, which typically involves a combination of Nucleoside Reverse Transcriptase Inhibitors (NRTIs) and Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTIs). This approach involves triple drug combination therapy (also known as highly active anti-retroviral treatment or HAART) that includes one protease inhibitor (PI) and two NRTIs. We also predicted drug-resistance in HIV-1 subtype C based on PR and RT sequences from ART-naive as well as first-line treatment failures in North India using genotypic analysis using Stanford DR database (which is based on subtype-B sequences) in addition to a self-proposed HIV-1 subtype-C specific docking model (3). The most common NRTI resistance mutations were in positions 118, 184, 210 and 215 in the RT gene, indicating the possibility of high-level resistance to Lamivudine (3TC) and Emtricitabine (FTC) in 92.3% of isolates. Common NNRTI resistance mutations were identified at position 98, 101, 103, 181 and 190 indicating a high-level resistance to Nevirapine (NVP) in 100% of first line therapy failures, while affecting the susceptibility to EFV in 76.92% (3,4). In addition, in a genotype study of subtype-C infected individuals from North India, we showed that high producer haplotype, CAG of TNFα gene associates with enhanced apoptosis of lymphocytes in HIV-1 infected individuals and may be a possible mechanism for faster progression to AIDS (8). Furthermore, genotyping results

revealed significantly higher frequencies of low producer AA genotype at +874T/A in IFNγ gene and 592C/A position in IL-10 gene and low producing haplotype 'ATA' at -1082, -819 and -592 loci in IL-10 gene were significantly over-represented in fast-progressors of HIV disease as compared to slow progressors and these individuals showed poor response to therapy in terms of CD4 count gains after one year of ART, compared to high producing haplotype (GCC) carriers (9,11).

We have also studied novel inter-relationships of co-receptor expression and their regulatory genes in therapy naïve individuals and how the virus manipulates the host machinery to its advantage and reported that Rac-1 regulates not only the functional conformation of CXCR4, but also the expression of both FoxP3 and NF-κB genes, thereby affecting the HIV disease progression (6). In addition, we found statistically significant differences in expression levels of twelve genes IL-1α, IL-1β, IL-7R, TNF-α, FoxP3, PDCD5, COX7B, SOCS1, SOCS3, RPL9, RPL23, and LRRN3 respectively among immunological non-responders compared to therapy responders, confirming their intimate relationship with immunological responsiveness to therapy (14).

In the subsequent phase of our research, we explored the involvement of certain essential intrinsic factors that regulate cytokine signaling. Our objective was to identify the mechanisms underlying the functional deterioration of DCs in the context of HIV-1 infection. Our study indicated that the HIV-1 infected patients, particularly in the advanced stage of disease had an imbalanced expression of negative and positive regulators of cytokine signaling, leading to profound negative effect on JAK-STAT or TLR-NF-κB pathways exerting inhibitory effects on DC functions (10). Interestingly, the markedly increased expression of SOCS-1, SOCS-3, PIAS-1, and SHP-1 showed a positive correlation with PD-L1 expression in these DCs, revealing some of molecular mechanisms of DC dysfunctional state in the advanced stage of HIV disease. Our results imply that the elevated levels of negative regulatory factors observed during chronic HIV disease exert a significant down-modulatory effect on DC functions and contribute to the establishment of an overall exhausted state (12). Furthermore, we investigated the potential role of CD20 in T cell activation during HIV infection. Untreated HIV-1 patients exhibited a decrease in CD3+CD20+ T cells, which are restored to normal levels upon receiving HAART. The study indicated that CD20 and CD38 expression on T cells are regulated independently of each other. Unlike CD38, CD20 can replace ionophores for Ca2+ flux during early T-cell activation and significantly enhance cell stimulation in the presence of Ca2+ ionophores and can be a good marker of disease progression (5).

In our succeeding research phase, we evaluated a panel of lectins with fine specificity for distinct oligosaccharides and assessed their ability to inhibit infection of HIV-1 viruses known to have differing sensitivity to anti-HIV env antibodies. We concluded that the HIV-1 isolates display differential sensitivity to lectins specific for α1-3Man, α1-6Man, and α1-2Man binding lectins, in part due to the microheterogeneity of N-linked glycans expressed on the surface of the virus Env glycoprotein (13). Moreover, we investigated the significance of Env glycan composition and heterogeneity in conjunction with host membrane-associated carbohydrate-binding lectins, particularly DC-SIGN, and the fate of the virus upon uptake by cells expressing these c-type lectin receptors (CLRs) (17,19,20).

Of late, our laboratory has been actively involved in studying the molecular mechanisms underlying Mtb-mediated immune modulation in HIV-1 subtype-C infected individuals from North India. We reported a possibility of Mtb-mediated gene modulation in co-infected individuals, where there was significantly lower expression of HO-1 compared to patients with only HIV-infection, despite having comparable CD4 count. This observation correlated well with increased expression of CCR5 and CXCR4, as well as NF-κB and inflammatory cytokines IL-6 and TNF-α. Collectively, we proposed that these immunomodulatory changes might be contributing to enhanced viral replication and increased cell death in HIV-TB co-infected individuals (7). In order to further understand the related mechanisms, we have conducted studies on how HIV-1 evolves in hosts with Mtb co-infection or substance abuse. Our recent observations reveal that the Mtb co-infection leads to the emergence and accumulation of genetically diversified quasi-species with significantly higher frequencies of drug-resistance (DR) related mutations in the RT gene and additional NF-κB binding sites in the LTR region of the viral genome in the co-infected host as compared to HIV-only infected individuals, which likely contributed to increased replication competence and non-responsiveness to conventional anti-retroviral drugs (15,16). These findings suggest the differential genetic evolution of HIV in a co-infected host with emergence of more resistant viral species with higher replication competence causing faster disease progression.

Lately, we reported that the T-memory stem cells (Tscm), which are one of the principal reservoirs, that have been relatively under-studied due to their presence in lower quantities and in anatomical locations, that are difficult to access and found that HIV-1 infected patients who experience a break in ART, have a higher number of CD8+ memory stem cells, but these cells exhibit dysfunctional behaviour. In order to understand the molecular mechanisms, we conducted RNA-seq studies of these cells and observed a distinct transcriptional signature of CD4+ Tscm in patients who had interruption of ART during the course of disease compared to those who continued receiving ART (18). These findings have great clinical implications in the pursuit to achieve complete cure from this disease.

More recently, we are trying to understand the impact of Mtb on dysregulated expression of host innate intrinsic factors, such as APOBEC3G, TRIM5α, SAMHD1, and Tetherin, on one hand and modulation of the expression of immune-check-point molecules on T-cells in HIV-TB co-infected individuals. We have some very interesting observations suggesting that Mtb may modulate the expression of these factors in favour of HIV (unpublished personal data).

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