SUMMARY
Visceral leishmaniasis (VL) or kala-azar, a disseminated infection of the lymphoreticular system, is caused by the protozoan parasite(s) *Leishmania donovani* and/or *L. infantum/chagasi*. It still remains one of the most neglected tropical diseases worldwide, affecting largely the poorest of the poor residing mostly in developing countries. An estimate of 0.2-0.4 million global VL cases are reported each year with more than 90% of them occurring in India, Bangladesh, Sudan, South Sudan, Brazil and Ethiopia. It accounted a mortality rate (among cases from 2004-2008) of 2.4% globally (Alvar, Velez et al. 2012).

Active VL is characterized by suppression of cell mediated immunity (CMI), which is apparent from the unresponsiveness of patients to different delayed type hypersensitivity (DTH) tests (Leishmanin skin test or Montenegro test) as well as the defective lymphoproliferative response of the peripheral blood mononuclear cells (Ghalib, 1995 #455). Recovery from infection following an effective chemotherapy, on the other hand, is associated with a strong cell-mediated DTH response (Sachdeva, 2014). Thus, a favourable CMI in response to appropriate treatment marks the successful cure.

Regulatory T cells (Treg cells) are considered as a multitalented master of immune regulation that promotes bystander suppression of effector T cells and infectious tolerance through secretion of regulatory cytokines (Tang and Bluestone 2008). In infectious diseases, IL-10, TGFβ and other immunosuppressive cytokines are secreted from various regulatory cell populations. These cytokines not only suppress the protective immune response during the disease but are actively involved in differentiation of induced Treg cells. Furthermore, IL-12 driven IFN-γ and TNF-α dominated Th1 response promotes healing and parasite clearance. During VL, these cytokines are downregulated by elevated IL-10 and TGF-β (Adhikari, Gupta et al. 2012). The mechanism of immunosuppression during *Leishmania* infection is, however, still poorly understood. Moreover, IL-35, a newly discovered cytokine, which is a member of the IL-12 family and a heterodimer comprised of Ebi3 (IL-27β) and IL12a/p35 (IL-12β), is secreted by Treg cells and is required for maximal Treg function in vitro and in vivo. IL-35 was shown to inhibit the proliferation of mouse T
effector (Teff) cells in vitro (Collison et al. 2010). Nevertheless, its effects on leishmaniasis have not been investigated. Therefore, a comprehensive study of immune cells profile at the site of *Leishmania* infection, and their modulation is immensely important. In the present study, we have studied the profile of various immune cells and their cytokines and identified the major regulatory cell populations among them during progressive murine VL. We found CD4$^+$CD25$^+$FOXP3$^+$ and CD8$^+$CD25$^+$FOXP3$^+$ T cells were the most significant sources of immuno-suppressive cytokines, IL-4, IL-10, TGF-β and IL-35. Among these IL-35 and TGF-β secreted from Treg cells precede the other disease promoting cytokines and may be while acting synergistically make the advent of immunosuppressive milieu by downregulating immunoprotective responses resulting into exacerbation of the disease. Further, a combined neutralization of IL-35 and TGF-β led to induction of IFN-γ mediated protective response, which is pre-requisite for long time protection against VL.

Our work on Th17 cells and its cytokines indicate that Th17 cell may act as an additive to the Th1 response against progressive murine VL. We observed that even in the absence of a Th1 response, Th17 shows some protection against the disease. Moreover, we found that Treg mediated IL-35 along with TGF-β effectively suppress the Th17 response and neutralisation these cytokines results into upregulation of Th17 response.

In the absence of a successful vaccine, chemotherapy is the mainstay to combat VL. Amphotericin B is an excellent anti-leishmanial drug, especially when pentavalent antimonials, the first line treatment against leishmaniasis are globally challenged by the emergence of resistant strains. But AmB treatment is associated with nephrotoxicity, hepatotoxicity and hypokalemia. Due to this, liposomal formulations of AmB have been introduced which are however very costly. Thus, we evaluated a new ergosterol-rich liposomal amphotericin B formulation, KALSOME$^{TM}$10 for its toxicity, efficacy and immunomodulatory role. We found that treatment of *L. donovani* infected mice with KALSOME$^{TM}$10 resulted in almost complete parasite clearance from both liver and spleen without inflicting any liver and kidney toxicity.
The cure was well supported by significant fall in disease promoting cytokines, IL-10 and TGF-β with a subsequent increase in protective cytokines (IL-12 and IFN-γ). This drug is expected to reduce the treatment cost and chances for relapse with improved safety value.