SUMMARY AND CONCLUSIONS
In the present work, the primary objective is to elucidate the role of APase activity in the proliferative and differentiative phenomena of myeloma cells using levamisole as a tool. The mechanism of action of levamisole has also been analyzed in terms of the expression of CD138 and secretion of Interleukin-6 by the myeloma cells.

The salient observations and conclusions are:

1. Alkaline phosphatase activity was not detectable in resting and mitogen-stimulated normal human peripheral blood lymphocytes. When pokeweed mitogen was used, a significant proliferative response was observed with no increase in APase activity. This observation is in contrast to that observed in LPS stimulated murine splenic B lymphocytes wherein enhancement in APase activity occurs concomitant with proliferation.

2. APase activity could be measured in lymphocytes isolated from bone marrow of myeloma patients and in human myeloma cell lines - RPMI 8226 and U266 B1. This observation suggested that in case of human B lymphocytes, APase activity is not expressed in normal human lymphocytes, but only in malignant lymphocytes. Based on this observation, it is proposed that APase activity could be used as a therapeutic target in malignant B cells. Levamisole, an uncompetitive inhibitor of APase activity and an anti-cancer agent which is used in clinical medicine has been chosen for further studies.

3. Initial experiments were carried out using murine lymphocytes to analyse the effect of levamisole. The enhanced expression of APase activity and IgM secretion correlated well with proliferation and differentiation respectively of B cells in mitogen (LPS) stimulated murine splenic lymphocytes. Levamisole inhibited the LPS-induced proliferative response as well as secretion of immunoglobulin in murine splenic B lymphocytes.
4. Incubation of myeloma cell lines, RPMI 8226 and U266 B1 with increasing concentrations of levamisole (0.1-2.5 mM) in culture for a period of 48-72 hours showed a significant cytostatic effect at low concentrations (up to 0.5 mM) and a potent cytotoxic effect at high concentration (>1.0 mM). The proliferative response of both the myeloma cell lines was significantly inhibited as assessed by $^{3}$H-thymidine incorporation into DNA as well as by MTT assay.

5. In order to understand the specificity of levamisole effect on myeloma cells, the binding of $^{3}$H-levamisole to unstimulated and mitogen-stimulated murine B cells, myeloma cell lines as well as pokeweed mitogen stimulated human peripheral blood lymphocytes was studied. Binding of $^{3}$H-levamisole was significantly higher in LPS-stimulated splenic B lymphocytes and multiple myeloma cells (which express APase activity) when compared to PWM-stimulated normal B cells (no APase activity). The binding of $^{3}$H-levamisole was more in lysates than in intact cells which suggests that the binding site of the putative target, i.e. APase is less accessible in intact cells.

6. Effect of levamisole on IL-6 secretion and CD138 expression of myeloma cell lines was also analyzed. Levamisole treatment enhanced the secretion of IL-6 and increased the shedding of CD138. The expression of CD138 has been shown to be regulated by activation of at least two receptor sub classes (G-protein coupled and protein tyrosine kinase). It is possible that inhibition of APase probably leads to the activation of these receptors and subsequent shedding of CD138 (syndecan-1) from the cell membrane. On exposure to levamisole, myeloma cells showed an enhanced secretion of IL-6 into the supernatant. This increased secretion of IL-6 by myeloma cells could be an attempt to protect themselves from undergoing apoptosis.

7. In levamisole treated myeloma cells, DNA fragmentation, enhanced cytosolic Ctochrome C concentration and increase in Caspase 3 activity were observed as compared to untreated cells. These results suggested that the cytotoxic effect of levamisole could be via mitochondrial pathway of apoptosis.
8. The cytotoxic effect of levamisole on myeloma cells was observed to be irreversible.

9. Levamisole has been shown to be degraded in cell culture. HPLC analysis of cell culture supernatants of levamisole treated myeloma cells has shown that products of levamisole appeared with progressive culture period indicating a metabolic transformation. Hence, the concentration of intact levamisole available for interaction with cells could be lower than the concentration added initially. In order to examine the effect of serum, myeloma cells were cultured in the absence of serum and it was found that levamisole extracted from the culture supernatants with FCS is less than in those without FCS. This decrease could also be due to the degradation of levamisole in presence of serum.

10. The effect of levamisole as a cytotoxic adjuvant when used in combination with atorvastatin was analyzed using myeloma cells. It was found that the combination of levamisole with atorvastatin did not show any synergistic cytotoxic effect.

Although APase has been identified and characterized in many malignant conditions, its role in the malignancy per se has not been studied in detail. Presence of APase activity specifically in malignant human B lymphocytes, but not in normal human B cells provided a experimental model to analyse the role of APase in the proliferative and differentiative events of these cells. The results obtained in the present work strongly suggest that APase could be used as potential therapeutic target to control the proliferation of malignant B lymphocytes. The ability of levamisole to inhibit APase activity, growth of human myeloma cells and to induce apoptosis opens up possibilities to design, develop and characterize new compounds as anti-myeloma agents using APase as a molecular target.