SUMMARY
Characterization of the host immune response at the tumor microenvironment is essential for developing effective anti-tumor immunotherapy against most of the solid tumors. Although, the composition of the immune microenvironment varies among different tumors, however, majority of the solid tumors exhibit very high level of macrophage and T regulatory cell (Treg) infiltration. Moreover, it has been observed that both of the tumor associated macrophages (TAM) and Tregs are crucial for malignant progression of most of the solid tumors. Thus, it seems that effective immunomodulation of these two cell types may lead to the development of a promising anti-tumor immunotherapy against advanced stage solid tumors.

We first characterized the mechanism of defective activation of TAM from B16F10 melanoma tumor. We observed that the defective activation response of TAM was due to the alternative polarization of the TLR signaling pathway. Among the two different intracellular TLR signaling pathways, TAM selectively abrogated the MyD88 dependent signaling pathway. Moreover, TAM utilized the TRIF dependent TLR signaling pathway in an alternative pro-tumoral manner to enhance the expression of anti-inflammatory cytokines (IL-10 and TGF-β) as well as to block the MyD88 dependent signaling pathway by inducing the expression of a specific inhibitor of this pathway (IRAK-M). Furthermore, we observed that TRIF dependent ERK-1/2 MAP kinase activation was the key for generation of anti-inflammatory cytokines as well as enhanced IRAK-M expression in TAM.

Next, we investigated the role of Tregs in the initiation of cancer metastasis. Tregs are of two types- thymic or natural Tregs (nTregs) and adaptive or induced Tregs (iTregs). These two Treg subtypes are discriminated on the basis of neuropilin 1 (Nrp1) surface
expression and Nrp1\textsuperscript{hi} Tregs are known as nTregs whereas, iTregs exhibit Nrp1\textsuperscript{low} phenotype. We observed specific up-regulation of the Nrp1\textsuperscript{low} iTregs in the lung bearing visible B16F10 metastasis. Moreover, we found that the mediastinal lymph node (mln) macrophages of metastatic tumor bearing mice induced the differentiation of Nrp1\textsuperscript{low} iTregs predominantly in a B7-H4 dependent manner. Furthermore, inhibition of B7-H4 expression by IL-10 and TGF-\beta neutralizing antibody treatment significantly attenuated lung metastasis due to the down regulation of B7-H4 expression in the lung and mln of metastatic tumor bearing mice.

Based on our above findings, we hypothesized that modulation of the TAM and Tregs could be helpful for generation of an effective anti-tumor therapy against advanced stage B16F10 tumor. Accordingly, we used the immunomodulator, \textit{Mw}, for TAM repolarization and it induced reprogramming of TAM back towards its normal function \textit{in vitro} however, it failed to repolarize TAM \textit{in vivo}. Later, we observed that the presence of high number of Tregs at the tumor microenvironment quenched the immunomodulatory potential of \textit{Mw in vivo}. Thus, we utilized \textit{Mw} along with a Treg suppressing antibody, DTA-1, for our treatment purpose and the therapy was successful against advanced stage solid B16F10 tumor. The combination therapy was successful since; it was capable of TAM repolarization \textit{in vivo}, however, only DTA-1 treatment failed to do so and thereby could not provide host protective anti-tumor immunity.

Therefore, the present study for the first time describes a pathway for generation of a host protective anti-tumor immune response against advanced stage solid tumors by effective repolarization of the altered immune effector cells at the tumor microenvironment.