Conclusion
The present thesis hints that T11TS a novel molecule acts as an effective angiogenesis inhibitor in the glioma-induced rat model. We dealt with angiogenic protein expressions in ENU induced malignant glioma model that resulted in spontaneous development of angiogenesis and inflammation and also reversing the angiogenic and inflammatory mode with T11TS. The glioma angiogenic process involves several cell types of growth factors and mediators which interact to establish a specific microenvironment suitable for the formation of new capillaries from pre-existing vessels. Flowcytometry based CD31 & CD34 expression assays were the first convincing evidence of T11TS mediated glioma angiogenic perturbations and were the basis of entire study. This finding in this thesis established that the initiation of T11TS induced glioma angiogenic regression is preceded by the down regulation of CD31 and CD34 angiogenic phenotypic markers [Singh et al 2014]. These data indicate that glioma associated endothelial cells from rat brain represent highly angiogenic and vasculogenic cells (in situ slide). This study further revealed that after T11TS therapy the cell–cell contact and trans-homophilic binding efficacy of ECs is hindered. While in our histological findings we have been found that in the T11TS treated group the brain matrix was cleared up of the residual angiogenic vessels. Together, based on these observations, this study suggests that T11TS has the potential to stop and regress the angiogenic vessels in glioma bearing animals.

This thesis is also focused on matrix based cellular adhesion with major interest on matrix metalloproteinases (MMPs). It was interesting to observe that T11TS treatment causes downregulation of MMPs and upregulation of TIMPs in glioma bearing rats in a classical enzyme-inhibitor inhibition ratio that would have matched a 1:1 stoichiometry. It has also found that both MMP-2/TIMP-2 and MMP-9/TIMP-1 ratios follow a pattern which dictates disease severity. Moreover, our cell invasion matrigel assay showed that the invasive capacity of the ECs of T11TS group in glioma rats was dramatically decreased and suggests that T11TS inhibits the cell invasion hindering the metastasis in vivo. Further, T11TS administration causes the TIMP-1 and TIMP-2 upregulations in glioma bearing rats. TIMP-2 expression in T11TS treated animals prevents the interaction between integrin and matrix proteins and indirectly causes cessation of vessel development. Inhibition of cell migration in T11TS treated group is another valuable finding and suggests that T11TS stops the endothelial cell migration in vivo. This provides a broad hint to the mechanistic approach whereby T11TS inhibits angiogenesis which may be a key event leading to glioma regression in rat model. This study is one of the first to elucidate the upregulations of TIMP-1 and TIMP-2 modulating the expressions of MMP-2 & MMP-9 in glioma bearing rats [Singh et al 2014]. While, the causative Transforming growth factor TGF-β1, secreted from microglia cells have been effectively down regulated by T11TS administration, which can be well correlated with integrin -TGF-β cross-talk in glioma bearing rat. The present study in vivo
provides a good model for further exploration of the regulatory mechanism behind the balance between migration/invasion and proliferation.

Deprivation of growth factor such as MMPs as is evident from the study was responsible for the deactivation of other major glioma angiogenic factors VEGF and may be TNF-α also, since both proteins belong to the same category which finally induces changes in gene expression pattern. Previous workers have shown that TNF-α might stimulate the glioma angiogenesis by participating in tumor inflammation and endothelium destruction by direct cytotoxicity. Recently Bhattacharya et al.,2013 have shown that T11TS administration decreases VEGF expression in glioma bearing animals. In the present thesis it has been shown that T11TS also modulates the TNF-α expressions and inflammatory associated cytokine expressions in glioma angiogenic conditions. With respect to glioma-angiogenesis, various studies have analyzed isolated cytokines or panels of cytokines, and divergent results have been reported including alterations in macrophages/-microglia and lymphocytes behavior. The mechanisms whereby these processes are regulated are outlined, but not fully understood. In the present thesis it has been delineated that T11TS therapy modulates the cytokine expression patterns in microglia and endothelial cells. Aggressive tumor growth is associated with increased expression of pro-inflammatory factors. We observed increased expressions of several key markers of inflammation including TNF-α, NF-κB. It is shown here that T11TS induction causes the down regulation of pro-inflammatory cytokine TNF-α and also its receptor TNF-R on brain endothelial cells which further causes the inactivation of NF-κB regulatory gene. Simultaneously, in our experiment we have found that in glioma bearing rat expressions of IL-8 and IL-6 were high. IL-8, IL-6 are potential endothelial cell growth and survival factors interacting through their receptors expressed by endothelial cells. T11TS administration in glioma bearing rats decreases the secretion of IL-8 as well as IL-8R1 and IL-6 from endothelial cells thereby hindering the migration and survival of glioma associated cells. The next question was whether T11TS also modulate the expression level of anti-inflammatory cytokine. We observed IL-4 and IL-10 expressions and found that IL-4 & IL-10 expressions were decreased in ENU group and concomitantly increased after T11TS therapy in glioma bearing rats. In vivo, IL-10 most likely exerts its anti-inflammatory effects on the vascular system through inhibition of VECAM-1 levels through decrease in TNF-α levels and functionally blocks NF-kB activation through both the suppression of IkB kinase activity, preventing IkBa degradation. Induction of expression of anti-inflammatory cytokines after T11TS therapy showed that T11TS exerts its biological effects on microglia and endothelial cells by interacting with specific cell-surface (TNF-αR and IL-8R) receptors that restrict the tumor growth and angiogenesis through inhibition of pro-inflammatory cytokine expression.
The interaction of the tumor with its microenvironment determines the ability of the tumor to proliferate, migrate, and invade other organs. CD44 signaling involves homing and activation of leukocytes. Our present work showed that in the glioma microenvironment T11TS therapy decreases expression of CD44 in glioma associated endothelial cells. Simultaneously, our results demonstrated that T11TS regulates CD144/β-catenin activation pathway helping in glioma angiogenic regression. Through the clustering of CD144/β-catenin protein, ECs are able to sense the presence of other adjoining identical cells and react by limiting their growth and migration. This action may be exerted by VE-cadherin (CD144) recruiting growth factor receptors and modulating their activity. The mechanism by which such potentiation is achieved is multifactorial, involving the inhibition of cell proliferation, the stimulation of apoptosis, and the inhibition of angiogenesis. In conclusion, these findings unravel the molecular mechanism underlying the anti-angiogenic action as well as anti-inflammatory action of T11TS in rodent glioma model.

Overall through this study we have been able to concluded that the novel molecule T11TS acts in an multifactoral way to inhibit different steps of glioma angiogenesis through inhibition of endothelia cell migration and metastasis. This agent is unique mode of action when compared to other anti-invasive, anti-metastatic and anti-angiogenic agents which usually act on only one step or few other steps. So the therapeutic advantage of T11TS as an anti-neoplastic agent has an edge over other anti-neoplastic agents and has immense potential as a glioma therapeutic agent.