Despite recent advances in therapeutics, colorectal cancer remains the third deadliest cancer in the USA. This is mainly attributable to survival of a small population of cancer cells called stem cells (CSC) with the ability to self-renew, resist chemotherapy killing and metastasize (broadly speaking CSC phenotype). A therapeutic strategy gaining in strength is that selective targeting of CSC would be singularly effective in eradicating tumors. We hypothesize that paradigm-shifting opportunities are afforded by non-saccharide glycosaminoglycan mimetics (NSGMs) that promise highly selective modulation of interactions of glycosaminoglycans (GAGs) so as to interfere with CSC signaling, growth, self-renewal and differentiation.

One of us (Desai) is a recognized leader in the synthesis of NSGMs that are structurally unique, easy to synthesize and completely unlike any molecule reported in the literature to date. Preliminary studies with 53 NSGMs and two colon cancer cell lines (HT29 and HT116) have identified three highly promising agents (G2.2, G11.1 and G12.2) that selectively target CSCs by inhibiting self-renewal. Of these, a specific dimeric sulfated flavonoid called G2.2 is especially interesting because two closely related analogs, G1.4 (a related monomer) and G4.1 (a trimer), completely failed to inhibit CSCs. Moreover, G2.2 demonstrated a steep dose-response profile for primary spheroid inhibition. Interestingly, G2.2 exerts anti-CSC properties through activation of p38 mitogen activated protein kinase (MAPK).

In the current proposal we aim to establish upstream regulator of p38 MAPK activation by G2.2 to determine its direct molecular target, which will greatly aid our efforts to develop more potent analog(s) of G2.2 to advance ‘in vivo’ models of CSC growth in the future. We will accomplish this objective by carrying out two specific aims below.

Aim 1: Identify a molecular mechanism(s) of inhibition of CSC growth by G2.2. Hypothesis: G2.2 affects one or more processes involved in cell cycle, apoptosis and differentiation utilizing specific growth factor/morphogen signaling pathways, which result in activation of p38 MAPK. Based on literature search and our preliminary data, the probable upstream signaling mediators include fibroblast growth factor receptor (FGFR) and transforming growth factor (TGF)-β superfamily while potential downstream mediators include ATF-2 and C-terminal binding protein. The primary target of G2.2 will be identified using a combination of biochemical, molecular biology and biophysical techniques including pharmacological as well as genetic modulation of candidate target expression, luciferase reporter assay, western-blot and Q-PCR to determine the role of a candidate pathway on p38 activation and signaling as well as capillary electrophoresis, affinity chromatography/LCMS sequencing for binding of G2.2 to the target protein/GAG.

Aim 2: Establish detailed structure-activity relationships (SAR) by developing analogs of G2.2, potentially aided by delineation of mechanism of action, to identify most promising NSGM(s) to advance in vivo pre-clinical models. Hypothesis: We will develop a focused library of additional NSGMs, each based around the G2 scaffold, and study their anti-CSC targeting abilities (growth of colonosphere and EMT transformed-CSC enriched cells) to identify clinically relevant candidate(s).

Overall, selective targeting of colon CSCs by NSGMs is a major paradigm with clinical implications for all cancers because of the possibility of targeting underlying fundamentally similar mechanisms. Successful outcome in the two aims will lead to a R01 application on advanced mechanistic, pre-clinical pharmacological and toxicological, as well as animal model studies with an IP protected group of novel NSGMs.