CHAPTER 2
REVIEW OF LITERATURE
2.0 Review of Literature

Hepatocellular carcinoma (HCC) is the predominant malignant tumor and 2\textsuperscript{nd} deadliest cancer worldwide, which is derived from well differentiated hepatocytes. It is a predominant malignant tumor seen across the globe and generally leads to death within 6-20 months (Giunchi et al., 2017). Several underlying conditions, such as cirrhosis, hepatitis virus B/C infections worsen the prognosis and make its management more challenging (Colombo et al., 2017). The incidence of HCC has been continuously enhancing over the past decade in western countries, and the overall incidence of HCC worldwide is expected to continue enhanced in near future (Torre et al., 2016). Symptomatically liver cancer can be classified into six main types from which HCC is most common liver cancer (Ahmed et al., 2009):

- **Hepatocellular carcinoma** (HCC): It is the most common type of liver cancer. The probability of HCC is increased after Hepatitis infection or cirrhosis.

- **Lymphoma of liver**: It is a type of lymphoma formed as a result of diffuse infiltrations to the liver and can also form liver masses.

- **Angiosarcoma and Hemiangiosarcoma**: They are a rare type of tumors generally originating in vessels. They are very fast growing and fatal. Most patients have life expectancy of only one year.

- **Cholangiocarcinoma**: It is a type of adenocarcinoma (forms glands) that originates in bile ducts which carries bile from the liver to the intestine. It accounts for 1 out of every 10 cases of liver cancer.

- **Hepatoblastoma**: It is a rare malignant tumor mostly formed in the right lobe.

- **Hemangioendotheliomas**: Cancer arising from the cells in the blood vessel in the liver.
2.1 Epidemiology

According to the WHO, HCC ranked 5th in men and 8th in women as most common cancer in globally. It has been estimated that every year globally almost 782,000 new cases diagnosed with HCC due to cirrhosis, HBV and HCV infections (Colombo and Maisonneuve, 2017). High hepatitis prevalent regions such as Asian and sub-Saharan African area have incidences of HCC as 120 cases per 100,000. Vaccination programs of hepatitis B for children are being carried out in some of Asian nations, which decrease risk of hepatocellular carcinoma among Asians countries (Siegel et al., 2016).

2.1.1 Mortality in HCC

The mortality verses incidence ratio in case of HCC is almost equal, due to the less possibility of survival after treatment. Only 5% patients survive after resection surgery and aggregate survival from time of diagnosis is just 6 months. Days of endurance mostly depend on the magnitude of cirrhosis in the liver. Most of cirrhosis affected people have shorter life due to less therapeutic interventions. The major complications of hepatocellular carcinoma that leads to death are liver failure, weight loss, abnormally dilated vessel bleeding (Torre et al., 2016). A study carried out by Bertuccio and colleague, reported International mortality rate for HCC from 1990 to 2014, and forecasted trends for numerous countries to 2020. They observed that Northern/Central Europe, North/Latin America have increased incidences of HCC. Eastern Asia demonstrated significant improvement in mortality rate; however these areas were 2 to 5 fold higher incidences than European countries as well as America. They have also predicted that till 2020 East Asian regions might have steady decline and European countries and USA might have increased incidences of HCC (Fig. 2.1) (Bertuccio et al., 2017).
Fig. 2.1: Epidemiological distribution of liver cancer (Source: GLOBOCAN (International Agency for Research on Cancer))
2.2 Risk Factors
There are various underlying factors responsible for HCC progression. Although, around one fourth of HCC cases analyzed in the United States don't have any known risk factor. However, major risk factors for HCC are liver cirrhosis, viral infection (hepatitis B and hepatitis C), cytotoxic compounds (alcohol, carcinogens present in food chain and aflatoxins), metabolic disorders (diabetes and non-alcoholic liver disease, inherited haemochromatosis) and immunological disorders (biliary cirrhosis and immune dysfunction in hepatitis) (Fig. 2.2) (Fares et al., 2013)

2.2.1 Hepatitis Infection
Hepatitis B and C virus encodes oncogenic viral proteins that contribute to induce hepatocarcinogenesis. The oncogenic proteins cause chronic inflammation in liver which leads to fibrosis and uncontrolled hepatocyte proliferation (Mittal et al., 2013).

2.2.2 Alfa-toxin (AFB1) and Chemical Carcinogen
Alfa-toxins are produced by fungal species Aspergillus, which mainly occurred in Asian and African continent. It has been seen AFB1 cause specific mutation in p53
tumor suppressor gene which leads to primary liver cancer. Diethylnitrosamine (DEN) considerably recognized to cause hepatocarcinogenesis is present in various food products, agricultural products, and pharmaceutical chemicals (Mittal and El-Serag, 2013). It induces single strand breaks in DNA of liver cells and also cause changes in several enzymes which are involved in DNA repair. Similarly N-acetyl-2-aminofluorene (2-AAF) has ability to form a covalent bond with DNA and develop DNA adduct of purine base such as N-(deoxyguanosin-B-yl)-2-AAF and 3-(deoxyguanosin-N2-y1)-AAF. The metabolism of these chemical carcinogens leads to ultimate formation of hepatoma cells (Sell et al., 1982; Malik et al., 2013). DEN and 2-AAF are widely used chemicals for induction of liver cancer in experimental animals like rat, mice, zebra-fish etc (Heindryckx et al., 2009).

2.2.3 Alcohol
Alcohol consumption leads to liver damage through inflammation, oxidative stress, and endotoxins. There are several stages such as steatosis; steatohepatitis and alcoholic hepatitis followed by fibrosis which culminates into HCC (Testino et al., 2014). Endotoxin mediated liver damage triggers release range of reactive oxygen species and cytokines, which increase vascular permeability in hepatic sinusoids cells later leads to metastasis of tumorigenic hepatocytes (Testino, 2013).

2.2.4 Cirrhosis
Cirrhosis in liver tissue may be caused due to chronic liver infection, hepatitis C/B virus (HCV, HBV), alcohol abuse (ALD) etc. Most of the time more than one symptom related to cirrhosis leads to HCC. The prevalence of cirrhosis has been increased almost two-fold in the last decade, which leads to higher incidence of HCC worldwide (Ioannou et al., 2013).
2.3 Molecular Basis of HCC

HCC involve many genetic alterations, that eventually, leads to malignant transformation of the hepatocytes. Tumor growth and survival depends upon microenvironment of the cell and interaction of multiple growth factors. However latest report on cancer stem cell (CSC) suggested that impaired signaling pathways and its receptors such as transforming growth factor β (TGF-β), different notch receptors (NOTCH1, NOTCH2, NOTCH3, and NOTCH4), VEGF/ FGF, Wnt MAPK, PI3k/mTOR and Hedgehog pathways are associated with hepatocarcinogenesis (Wu et al., 2012; Song et al., 2013). These signaling molecules and receptors can be considered as key for determination of HCC (Fig. 2.3) (Lachenmayer et al., 2012). According to study, alteration in Wnt pathway is majorly affected by Wnt-related molecules such as CTNNB1 and Wnt-TGFβ which stimulate hepatocarcinogenesis (Vilchez et al., 2016). Some studies on HCC indicate that CXCL12-CXCR4 (Chemokine) signaling cascade plays a major role in the process of invasion and metastasis that cause more than 80% of deaths in hepatocellular carcinoma patients (Manu et al., 2013). Gedaly and associates reported that advanced stage of HCC is activated due to increased signaling of Ras/Raf/MAPK and PI3K/AKT/mTOR pathways, which directly up-regulate growth factors like epidermal growth factor (EGF), Hepatocyte growth factor (HGF) and insulin –like growth factor (Gedaly et al., 2012). HCC is a highly vascular tumor; hence anti-angiogenesis strategies have become an important therapeutic modality for solid tumors (Fritsche-Guenther et al., 2016)
Chapter 2  
Review of Literature

2.4 Challenges with HCC

Multitude of researchers have been working to decipher molecular pathogenesis leading to advances in management of HCC, yet screening and early diagnosis of HCC remains a major concern. Major challenges associated with HCC are screening of disease, treatment, management of advance stage of HCC and palliative care.

2.5 Early Detection

HCC patients may often remain unscreened due to lack of diagnoses, though they may be at advance stage of the disease. The main tools for screening HCC are AFP (Alfa-feto-protein, serum based biomarker) and ultrasonography. In cirrhotic patients both tools are recommended for diagnoses as well as follow-up in the patients having cancerous lesions <1 cm in (Terzi et al., 2016). However, the sensitivity and
specificity of AFP is very low due to their over expression in several liver disease, embryonic and some gastrointestinal tumors. Additionally, in small hepatic tumors AFP concentration is reduced, which makes it less sensitive in detection (Zhao et al., 2013). Therefore, early detection of HCC is a challenging task. The need of hour is an early surveillance of HCC and complete protein profiling of HCC patients for early detection.

2.5.1 Protein Based Biomarkers for Early Detection of HCC

Despite significant research in the area of diagnostic cancer imaging technology, surgical and resection and chemo-therapeutic, cancer remains one of the deadliest disease worldwide. Majority of carcinomas, in the absence of exclusive biomarkers hinder the attempt to meliorate early diagnosis and therapeutic interventions (Rhea et al., 2011). In recent times, substantial technologies have emerged such as MicroRNA and “gene fingerprinting” in field of cancer genetics (Hayes et al., 2014). Expression proteomics is more significant and specific than genomics. Unlike genomics, obtaining data for proteomic study is challenging task, due to lack of standardized method, sensitivity and duplicability. However, these hurdles can be managed high throughput proteomic techniques (Prieto et al., 2014). There are several types of proteins based biomarker for cancer diagnosis, prognosis and therapeutic interventions (Fig. 2.5).
In case of HCC, the major research in biomarker discovery is the identification and characterization of signature proteins for early detection. Currently used biomarker of HCC such as alpha-fetoprotein is of limited specificity and sensitivity and most of the time gives false positive results (Tsai et al., 2015). The diagnosis of HCC is usually brought up at later stages, when curative options are critically limited. Some significant markers for HCC at different stages for HCC are given in Table 2.1.
Chapter 2

Table 2.1: List of proteomic biomarker for diagnosis of HCC

<table>
<thead>
<tr>
<th>Name of Protein</th>
<th>Clinical benefit</th>
<th>Detected from</th>
<th>Sensitivity and Specificity</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFP (α-Fetoprotein)</td>
<td>Early diagnosis</td>
<td>Serological</td>
<td>43% sensitivity/ 78% specificity</td>
<td>(Wong et al., 2014)</td>
</tr>
<tr>
<td>(DCP)Des-γ-carboxy prothrombin</td>
<td>Early diagnosis</td>
<td>Serological</td>
<td>44% sensitivity/ 60% specificity</td>
<td>(Zhang et al., 2014)</td>
</tr>
<tr>
<td>α-L-Flucosidase</td>
<td>Early diagnosis</td>
<td>Serological</td>
<td>Sensitivity and specificity limited due to expression in non-cancerous and extra hepatic diseases</td>
<td>(Zhu et al., 2013)</td>
</tr>
<tr>
<td>Glypican-3</td>
<td>Diagnosis</td>
<td>Tissue and Serological</td>
<td>77% sensitivity/ 96%specificity</td>
<td>(Filmus et al., 2013)</td>
</tr>
<tr>
<td>Transforming growth factor β1 (TGF β1)</td>
<td>Metastasis</td>
<td>Serological</td>
<td>70% sensitivity/ 77%specificity</td>
<td>(Watanabe et al., 2016)</td>
</tr>
<tr>
<td>Epidermal growth factor receptor</td>
<td>Recurrence and progression</td>
<td>Tissue specific</td>
<td>Less sensitivity/Less specificity</td>
<td>(Wu et al., 2009)</td>
</tr>
<tr>
<td>Hepatocyte Growth factor (HGF)</td>
<td>Prognosis, invasion and recurrence</td>
<td>Serological</td>
<td>Less sensitivity/Less specificity</td>
<td>(Karabulut et al., 2014)</td>
</tr>
<tr>
<td>Golgi protein 73</td>
<td>Early Diagnosis</td>
<td>Serological</td>
<td>83% sensitivity/77% specificity</td>
<td>(Dai et al., 2015)</td>
</tr>
<tr>
<td>Squamous cell carcinoma antigen</td>
<td>Progression</td>
<td>Serological</td>
<td>high sensitivity/ low specificity</td>
<td>(Biasiolo et al., 2016)</td>
</tr>
<tr>
<td>Complement C3</td>
<td>Disease Progression</td>
<td>Serological</td>
<td>Low sensitive/</td>
<td>(Malik et al., 2013)</td>
</tr>
</tbody>
</table>

2.5.2 Proteomic Approach for Identification of Protein Based Biomarkers

Proteomics is emerging approach for biological system, which comprise qualitative and quantitative analysis of proteins occurred in the cell. Overall proteomics can be denoted as analysis of protein expression, modifications, signalling pathway and
protein interaction at particular stage of cell cycle. This analysis provides complete
details such as extraction, identification, significance and characterization of proteins
at various cell cycle stages (Veenstra et al., 2006). Changes in expression profile of
proteins can be measured in a systematic fashion and followed by elucidation of mode
of action for particular proteins at normal, diseased as well as therapeutic conditions.
The expression proteomics is one of the oldest and common methodologies for
identification of proteins in diseased as well as treatment regimen. Over or down
expression of protein in cellular conditions can be used as tool for identification of
biomarkers (Fig. 2.6) (Kuramitsu et al., 2010).

Fig. 2.5: Scheme diagram of proteomic approach for identification of
pharmacodynamic biomarker

Numerous analytical techniques such as 1 and 2 dimensional (1&2D) electrophoresis,
high-performance liquid chromatography (HPLC), liquid chromatography and Gas
chromatography are the most common separation methods, whereas mass
spectrometry (MS) is best method for protein identification (Larance et al., 2015). Recent high-throughput analytical techniques such as protein chips, isotope-coded affinity tag (ICAT) protein profiling etc have been established as the current capabilities in this field, though allowing for the measurement of polypeptide chain size, surface affinity and protein interaction potential. These techniques have shown promise in the areas of target identification and assessment of efficiency (Gillet et al., 2016). Number of high throughput techniques such as mass spectroscopy, LCMS MALDI-TOF has potential to identify, characterize and validates target specific protein which can be used as PD biomarker (Prieto et al., 2014; Tsai et al., 2015).

Bioinformatics is an intact element of proteomic. It is a depositary which analyzes and manages all type of biological database. Bioinformatics is collaborative work analytic life sciences, computational methodology and biostatistics analysis. It is an essential component of proteomic research which includes data comparison, similarity search, taxonomical analysis, interaction profiling of identified proteins (Kumar et al., 2017). Several commercial tools for 2D gel analysis image analysis software such as ProteinMineTM (Scimagix), ImageMaster 2D Platinum (GE Healthcare), PDQuestTM (Bio-Rad Laboratories), Melanie 3, and the Z3 2D-Gel Analysis System (Compugen Limited) provide a significant help in analysis of differentially expressed proteins in control verses treatment groups (Vizcaíno et al., 2013).

2.5.3 Pharmacodynamic Biomarkers
Pharmacodynamic (PD) biomarkers are indicator of treatment on the target organ in particular organism. PD biomarker can be employed to analyze connection between drug target interaction and biological response against tumor treatment. In the
literature several imaging techniques such as dynamic contrast enhanced magnetic resonance imaging (DEC-MRI), ultrasonography, computed tomography have been used as pharmacodynamic biomarker or treatment specific scanning (Hayano et al., 2015; O’Neill et al., 2016). According to the National Cancer Institute various imaging techniques and clinical assay method have been established by cancer treatment division for assessment of PD biomarkers (https://next.cancer.gov/developmentresources/pd_biomarker.htm). Identification of Protein based PD biomarkers are an emerging area of research. Although researches on cancer-genomics have been evolved in past few decades, it only gives us an over view of genetic alterations. The need is to assess what is actually occurring in cancer patient in diseased condition as well as after treatment. The need of today is to detect significant proteins that can give complete insight about biological processes with cancer development and therapeutic interventions (Topalian et al., 2016) Identification and characterization of changes in protein expression profile with disease and the successful therapy using cytotoxic drugs for treatment can provide a fundamental idea of pharmacodynamic biomarker. these biomarkers have the important role in diagnosis and screening of HCC and can be used as complementary of histopathological analysis (Panis, 2015). Onco-protein mediated molecular therapy can target only cancer-specific protein network and exhibit real-time assessment for efficacy and toxicity evaluation of various therapeutic interventions. Besides, oncoproteomics is also used for the discovery of new therapeutic receptors for drugs and also analyze their effects (Zhou et al., 2015). The Major research for the screening and therapy of cancer patients lies in the profound understanding of molecular basis of disease initiation followed by progression and later effective treatment on the basis of biomarker discovery (Kalia, 2015). Cytotoxic or curative effect of any therapy can be monitored to measure the potency of a drug using protein expression profile.
Chapter 2

The magnitude of alterations in specific protein not only provides a potential biomarker, but also exhibit complete insights of the diseased condition (Nagaraj et al., 2011). These proteins are used as target for drugs and can be emerging tool for pharmacodynamic biomarker. A few putative drug targets have been identified with respect to treatment (Patel et al., 2015). For example expression of several plasma proteins related to angiogenesis are PD biomarker for response of several antiangiogenic drugs like sorafenib, lenvatinib and sunitinib etc (Koyama et al., 2014; Masuda et al., 2015).

2.6 Systemic Therapy

The major treatments methodology for HCC comprises radical liver resection, orthotropic liver transplantation, loco regional treatment (ultrasonography-guided tumor ablation), and systemic therapies. Most of the therapies are recommended at early stage of HCC, when single or multiple nodules are less than 3cm in diameter (Nathan et al., 2013). However, for unresectable HCC, systemic therapy is the only way-out, which includes molecular targeted therapy, chemoembolization, antiviral drugs, nutraceuticals and chemoprevention (Fig. 2.4) (Ge et al., 2015).
Majority of HCC patients have unresectable and metastatic HCC due to poor progression and systemic therapy can provide a substantial benefit at this stage. Although, there is chance of recurrence after surgical resection, systemic therapies are helpful to prevent these situations (Zhu, 2006). The efficient systemic therapies for HCC have been difficult task due to poor chemo sensitivity, which is mostly associated with up-regulated expression of drug resistance proteins (P-glycoprotein and multidrug resistance protein) that enhance the cellular efflux of cytotoxic drugs. Another problem is the dysfunction of liver which leads to the obstruction of drug delivery. Cirrhotic liver deregulate drug metabolism as well as affects the formation of plasma-binding proteins which regulate active drug concentration in serum (Giglia et al., 2010). There are numerous types of systemic therapy such as, chemotherapy, hormonal therapy and molecular targeted therapy. The molecular target drug sorafenib has shown enhanced survival in advanced HCC patients (Villanueva et al., 2009).
We have retrieved some targets and its inhibitor drugs for the treatment of HCC (Table 2.2).

Table 2.2: Some signature receptors and their cytotoxic drugs for the treatment of HCC (Bertino et al., 2014)

<table>
<thead>
<tr>
<th>Target Protein</th>
<th>Drug</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>VEGFR-2 PDGFR-beta, Raf kinase</td>
<td>Sorafenib</td>
<td>Inhibition of angiogenesis Pathway</td>
</tr>
<tr>
<td>c-kit, Fit-3, and RET</td>
<td>Sunitinib</td>
<td>Inhibition of angiogenesis Pathway</td>
</tr>
<tr>
<td>PDGFR and VEGFR</td>
<td>Linifanib</td>
<td>Inhibition of angiogenesis and tumor cell proliferation</td>
</tr>
<tr>
<td>HGFR</td>
<td>Tivantinib</td>
<td>Inhibition of MAPK and PI3 K-AKT pathways</td>
</tr>
<tr>
<td>Humanized monoclonal antibody of VEGFR</td>
<td>Bevacizumab</td>
<td>Inhibition of angiogenesis through binding with VEGFR</td>
</tr>
<tr>
<td>FGF and VEGF</td>
<td>Brivanib</td>
<td>Inhibition through monoclonal antibody of VEGF and FGF</td>
</tr>
<tr>
<td>VEGFR-2</td>
<td>Ramucirumab</td>
<td>Inhibition through monoclonal antibody of VEGFR2</td>
</tr>
<tr>
<td>mTOR</td>
<td>Temsirolimus</td>
<td>Cell replication inhibition</td>
</tr>
<tr>
<td>mTOR</td>
<td>Everolimus</td>
<td>Cell replication inhibition</td>
</tr>
</tbody>
</table>

2.6.1 Sorafenib: A Systemic Drug

Sorafenib, a FDA (November 2007) approved drug (a bi-aryl urea, MW: 464.83) designed for treatment of primary liver cancer, is a Raf serine/threonine kinases inhibitor (Fig 2.7) (Gong et al., 2017). In spite of various researches over the years in the area of novel therapeutic agent discovery, no new medications have delivered positive outcomes to date. Among all the treatment available, sorafenib has ability to increase the overall survival. The vast majorities of the HCC patients are still
analyzed at their middle or advanced stages, where most of the curative methodologies are not feasible (Connock et al., 2010).

![Chemical structure of Sorafenib](image)

**Fig 2.7: Chemical structure of Sorafenib**

Sorafenib has the ability to interfere in cell proliferation and receptor tyrosine kinases which involved in generation of new blood vessels. In preclinical trials, Sorafenib is proven to be an inhibitor of several receptors including VEGFR13, PDGFR β, cKIT and FLT3 (Fig. 2.8) (Connock et al., 2010; Gholam, 2015). According to Pircher and colleagues, 400 mg dosage twice a day is the maximal tolerated dose (MTD) which confirmed the antitumor efficacy and tolerability (Pircher et al., 2011).

Sorafenib has shown a significant effect for second-line therapy, in case of minimal impact of initial treatment. According to a study, disease control rate (DCR) was significantly improved by 58.3% with sorafenib treatment after failure of initial chemotherapy using fluorouracil + cisplatin (Abdel-Rahman et al., 2015). Similarly a study conducted by the American Society of Clinical Oncology (2014) exhibited that sorafenib also acted as adjuvant therapy after surgical resection or radiation treatment. Additionally, this drug also provides a notable efficacy for prevention of HCC Recurrence (Bruix et al., 2014)
2.6.2 Challenges with Sorafenib

The adverse reaction of sorafenib therapy also exhibit common drug induce symptoms like diarrhea, skin rashes, hypertension, hand–foot skin disease, asthma, hair loss, vomiting, nausea, fatigue and esophagus inflammation (Sanoff et al., 2016). According to the SHARP (Sorafenib HCC Assessment Randomized Protocol) trials in 2008, HCC patient after treatment of Sorafenib reveled limited survival benefits with very low rates of tumor response.

The major issue with sorafenib treatment is the safety and efficacy for advanced HCC patients with defective, liver function. Interestingly, phase 3 trials of SHARP and ORIENTAL studies have not resolved the issue of Child-Pugh A (CPA) liver function. However, All data for sorafenib using retrospective studies showed only...
for liver function of Child-Pugh B (CPB,) even data for Child-Pugh C (CPC) liver function was limited (Chiu et al., 2012; Estfan et al., 2013). Another problem with Sorafenib treatment is efficacy and safety in elderly HCC patients. A retrospective analysis in the age group of 70 years or more also has less survival. Younger group showed 3.09 months survival, but older group exhibited 2.9 month survivals only. Therefore using this drug may not be good for older people (Wong et al., 2011).

A recent report on sorafenib efficacy suggested that over placebo survival rate of sorafenib is limited and has insufficient inhibition of morality rate in HCC patients which showed only 2.8 month survival as compared to placebo group. Almost 1,500 HCC patients (Child-Pugh B or C) were treated with initial sorafenib therapy and overall survival was 10.9 month as compared to placebo, where survival was 8.1 month only (Fig. 2.9). Several limiting factors were estimated such as age, immeasurable confounding and magnitude of cirrhosis in the liver cancer patients that leads to insufficient efficacy (Sanoff et al., 2016).

Fig 2.9: Clinical benefit of sorafenib: A pilot study by American Society of Clinical Oncology Source: (Sanoff et al., 2016)
2.7 Sorafenib Combination Therapies
Higher doses of sorafenib can also harm healthy hepatic cell, which are necessary for normal growth of liver cells (Azad et al., 2009). Sorafenib is the only standardized drug which has been tried in various combination of classical therapy such as TACE, liver resection chemotherapy and radio-ablation (Table 2.3) (Fig. 2.10). Additionally it has also been combined with other targeted molecules like bevacizumab, axitinib, doxorubicin and dalantercept (Gao et al., 2015). Therefore, sorafenib combination therapy can give better results than other individual chemotherapeutic medication (Abdel-Rahman et al., 2014).

Fig. 2.10: Schematic representation of different types of sorafenib combination therapy
### Table 2.3: List of sorafenib combination therapy for HCC treatment

<table>
<thead>
<tr>
<th>Combination of sorafenib with</th>
<th>Treatment Stage</th>
<th>Efficacy</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Everolimus</td>
<td>HCC recurrence treatment after liver transplant</td>
<td>Insufficient efficacy due to sorafenib complication</td>
<td>(De Simone et al., 2014)</td>
</tr>
<tr>
<td>TACE</td>
<td>Advanced HCC</td>
<td>Significantly enhanced time to progression (TTP) advanced stage HCC.</td>
<td>(Liu et al., 2014)</td>
</tr>
<tr>
<td>Radio-Ablation</td>
<td>(BCLC) Stage 0–B1</td>
<td>More effective than radio ablation alone</td>
<td>(Feng et al., 2014)</td>
</tr>
<tr>
<td>Dalantercept</td>
<td></td>
<td>Inhibition of angiogenesis in tumorigenic cells</td>
<td>(Bendell et al., 2014)</td>
</tr>
<tr>
<td>Axitinib</td>
<td>Second line treatment after sorafenib + bevacizumab</td>
<td>3.8% higher Response</td>
<td>(McNamara et al., 2015)</td>
</tr>
<tr>
<td>Refametinib</td>
<td>Advanced HCC</td>
<td>Enhanced Antitumorgenic activity</td>
<td>(Lim et al., 2014)</td>
</tr>
</tbody>
</table>

Several phytochemical constituents also share Chemopreventive and angioprevention properties and should be tried with chemotherapeutic compounds to reduce the drug induced toxicity. Some plant based compounds such as silymarin, silibinin and Chalcones (flavonoid) are recognized as antioxidant activity, abundantly present in fruits, vegetables and various spices (Moon et al., 2006). Out of all plant based compounds silibinin follows similar antiangiogenic pathway as sorafenib and has other cytotoxic ability against tumorigenic cells. Silibinin is major constituent of silymarin complex extracted from milk thistle and has potential for adjunct chemotherapy. Silibinin has ability to enhance pro-apoptotic factors, cell cycle inhibitors and tumor suppressor gene as well as inhibits cell proliferating and pro-
angiogenic proteins (Fig. 2.11) (Wing et al., 2010). Silibinin has been combined with sorafenib and gefitinib for inhibition of HCC cell line growth (Gu et al., 2015). The novel combination of different plant based antioxidant with sorafenib can be better alternative for targeted therapy for HCC.

![Fig. 2.11: Summarized mode of action for silymarin and silibinin]

2.8 Polymer-Drug Conjugates Based Treatment
Since last two decades various strategies have been emerged to formulate new drugs that can significantly modulate carcinogenic process. However, modification in already existing drugs can be done for better efficacy on tumor site. Most of the molecular targeted drugs have low bioavailability and therapeutic index. Covalently joining synthetic drug with water soluble polymer is emerging trend that can enhance the bioavailability of FDA approved drug. In mid-1970s Dr Ringsdorf proposed a model of polymer attached chemotherapeutic agents that can modulate pharmacokinetics of drugs (Andruzzi et al., 1970; Feng et al., 2016). Prodrugs...
Polymer bounded chemotherapeutic compounds are broadly utilized as a part of the targeted therapy and can deliver cytotoxic agents to the diseased cells. To date, prodrugs have accomplished excellent feature in terms of target determination, therapeutic efficacy and physicochemical quality of the cytotoxic drugs. Modification in physicochemical characteristics of drugs (e.g., shielding of charges or protection of ionization groups) before reaching to their sites of action can enhance activity of drugs. Prodrugs have a long history of overcoming physiologic barriers such as the GI tract (Mohanty et al., 2015). Several clinical trial have shown advantage of drug conjugate over parent drugs, including lesser side effect, easy drug administration mode, enhanced patients comfort, increased drug permeability and its retention (EPR) and prolonged release (Pelegri-O’Day et al., 2014).

The major challenge of sorafenib is therapeutic index and bioavailability (Liu et al., 2014). During the past decades, various researches have been done for delivery and activation of prodrugs which have enhanced plasma blood concentration as well as molecular targeted delivery. PEGylation of small molecular makes drug more effective and specific delivery (Nathan et al., 2013). Pegylated liposomal delivery of doxorubicin has been done for better efficacy and targeted drug delivery and less toxicity (Rom et al., 2014). Individual sorafenib PEGylation has not been tried by researched, although sorafenib in combination with gadolinium co-loaded liposomes has been promising nano-carriers for treatment HCC (Xiao et al., 2016). Therefore, sorafenib conjugate could be an innovative method for treatment of HCC. Similarly, other polymers such as chitosan, pullulan and cyclodextrin are also well established coating material for sustained release of drugs to achieve prolonged therapeutic effect as well as steady drug concentration in blood (Carpenter, 2014; Boateng et al., 2015).
Pullulan is thermally stable, biodegradable and water soluble compound which consists of stable neutral linear polysaccharide chain of α-1; α-1,6- maltotriose monomer (Rekha et al., 2007). Pullulan has higher affinity to asialo-glycoprotein receptors of hepatic sinusoidal cells and is abundantly expressed in liver. This unique feature of pullulan has been utilized by researchers for targeted drug delivery to the hepatic cells (Prajapati et al., 2013; Singh et al., 2015). Pullulan based prodrugs can enhance the bioavailability of anticancer drugs and improve tumour targeting. A latest report on pullulan conjugated nanoparticle (such as pullulan-DOX conjugate nanoparticle) reaffirm the significance of this polysaccharide in targeted therapy of liver cancer (Pelegri-O’Day et al., 2014; Ahmed et al., 2015).