CHAPTER 1

Introduction and Literature Review

1. Introduction and literature review

1.1. Introduction

1.1.1. Overview of cancer – demography and epidemiology

Cancer is one of the most dreaded 'non-infectious' diseases that has inflicted humans since time immemorial [1]. Cancer can affect any part of the human body and often metastasises to nearby organs becoming potentially life threatening [2]. The incidence of cancer has often been associated with a number of serious psychosocial and spiritual consequences for the patients and survivors, due to its pervasive, untreatable nature [3]. Most of these consequences can be described as distressing, debilitating and detrimental to the psyche of an individual (and their family) going through the rigours of oncological interventions.

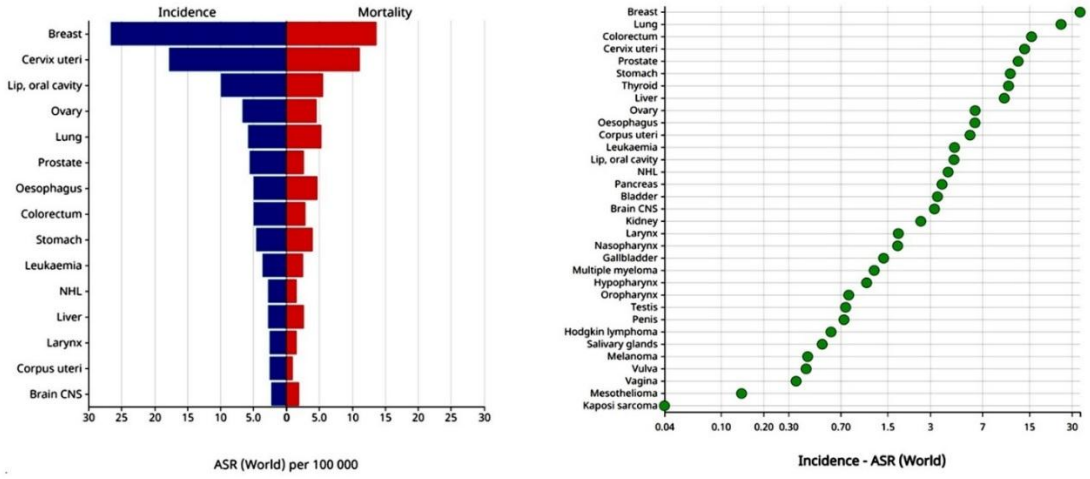


Figure 1.1. Age-standardized rate (ASR) of incidence and mortality of different cancers in India, as per the Global Cancer Observatory Report, GLOBOCAN 2024 published by the International Agency for Research on Cancer, World Health Organization (WHO).

Almost one-third of all diagnosed cancer patients have been seen to suffer from intense psychological trauma like shock, disbelief, denial, despair, anxiety, fear and suicidal ideation [4] often leading to co-morbid mental health issues aggravating their clinical conditions, undermining their social competence and executive abilities [5], inversely affecting the overall survival and quality of life and ultimately, requiring the intervention of a psycho-oncologist or a behavioural oncologist. It has been postulated through a number of community studies that elderly cancer patients (more than 65 years of age) are less prone to develop psychological symptoms than their younger counterparts; however, the presence of age-related underlying diseases often leads to complications in the elderly patients [6].

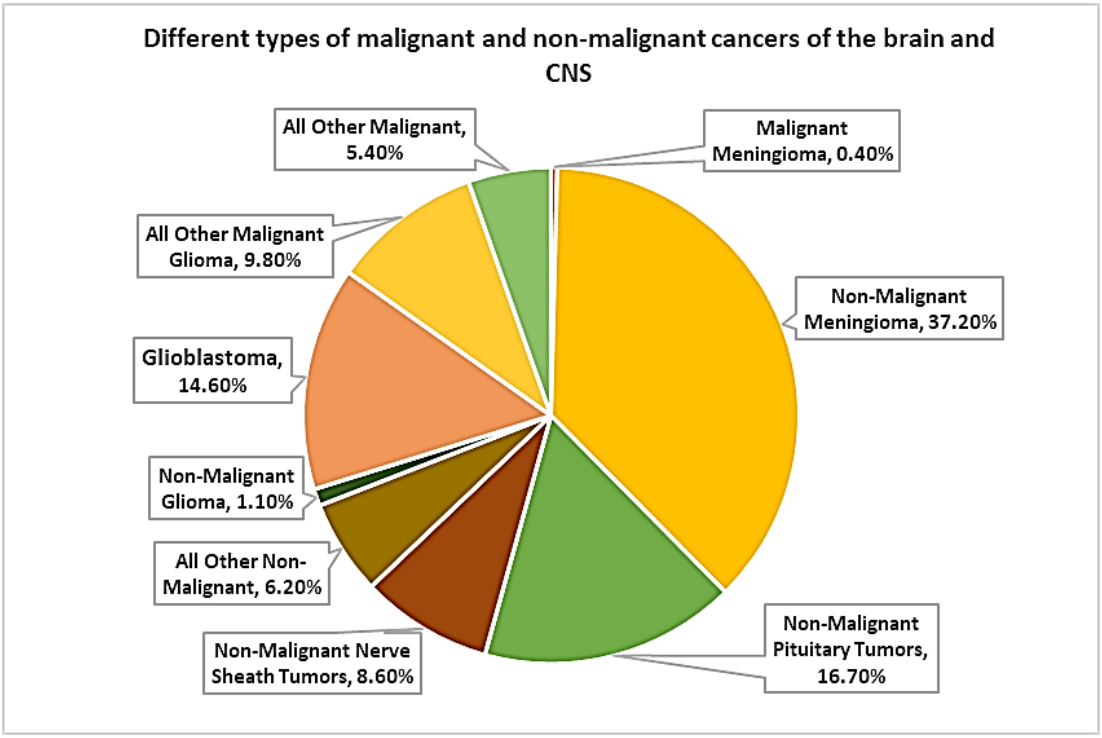


Figure 1.2. Different types of malignant and non-malignant cancers of the brain and CNS.

1.1.2. Overview of glioblastoma multiforme (GBM) and breast cancer

1.1.2.1. Glioblastoma multiforme (GBM)

Glioblastoma multiforme (GBM) is a carcinoma of the star shaped astrocyte cells of the brain and spinal cord and is a form of highly malignant glioma which is classified by the WHO as Grade IV astrocytoma [7, 8]. It is a form of solid tumor which is most aggressive and perfidious of all brain tumors and despite advanced interventional therapies, the prognosis of the disease is indeterminate and it remains extremely incurable. The mean survival rate (MSR) for this disease with treatment is only 14-16 months post-diagnosis with a 5-year relative survival rate of less than 7%. GBM has a positive correlation with patient's age and the incidence is highest in elderly adults aged above 75 years. Around 14.6% of all commonly occurring primary brain tumors (and 48.3% of all malignant tumors) comprises glioblastoma (**Figure 1.2**) [9]. The major symptoms of GBM are often non-specific and include a) blurred or double vision, b) headaches, c) loss of appetite, d) memory problems, e) mood or personality changes, f) muscle weakness or balance problems, g) nausea and vomiting, h) seizures.

The primary method of treatment for GBM is a) maximal surgical resection of the solid tumor (also known as cytoreductive surgery) [10] followed by b) post-operative external beam radiotherapy [11] and c) direct or adjuvant chemotherapy with standalone alkylating agents like temozolomide (TMZ) [12] or nitrosourea combinations (like the eponymous procarbazine, lomustine (CCNU), vincristine (PCV) regimen) [13]. Apart from these, some of the newer and emerging strategies to fight GBM include; a) molecular targeted drugs [14, 15], b) immunotherapy [16], c) viral vector-based gene therapy [17], d) alternating electric field therapy or tumor treating field (TTF) therapy [18], e) photodynamic therapy [19], f) tumor vaccines [20], g)

hybrid pharmacophores [21] and drug repurposing/repositioning [22] and h) strategies to increase tumor sensitivity to existing regimens of chemo and radiotherapy [23].

GBM often becomes highly resistant to conventional chemotherapeutic drugs like temozolomide leading to low response to therapy or shows tendency to relapse. This is due to a large number of factors [24, 25] like; a) the localization-delocalization factors i.e., presence of the blood-brain barrier (BBB), b) the DNA protecting or damage reversal factors (e.g., DNA damage repair by the enzymes O^6-methylguanine-DNA methyl-transferase (MGMT), poly-ADP-ribose-polymerase (PARP)), c) the epigenetic factors (e.g., gene mutation, signalling pathway deregulation, miRNAs), d) the chemical factors (e.g., hypoxia, acidic pH in tumor microenvironment), e) the neurotoxicity factor (e.g., resultant neurotoxicity of treatments directed at gliomas and astrocytoma), and f) the presence of a subpopulation of glioma stem-like cells (GSCs) (e.g., GSC initiators, GSC propagators).

Many molecular targets have been identified through genetic and epigenetic profiling of glioma cells [26] which play key roles in the development of GBM and have the potential to serve as adaptable targets for the development of newer anti-GBM drugs. All these functions have been proven to be responsible for full-fledged gliomagenesis. The most important ones include; a) the MAPK signalling pathway via RAS-RAF-ERK stem, b) the RAS-PI3K-Akt-mTOR signalling pathway, c) the JAK-STAT3 pathway, d) EGF and EGFR overexpression and mutation to EGFRvIII, e) presence of VEGF and VEGFR, f) the EGFR-Akt-PTEN pathway, g) mutations and suppression of the *p53* gene, h) mutations and suppression of the isocitrate dehydrogenase (*IDH*) gene and i) cyclin-dependent kinase inhibitor 2A (*CDKN2A*) gene.

All these pathways involve numerous mediators many of which have been designated as important biomarkers in the prognosis and molecular profiling of GBM. These mediators are proving to be potential targets for development of better drugs for GBM which are expected to circumvent the drawbacks faced by already approved drugs and have made it possible for a more pharmacogenomic approach towards anti-GBM therapy [14, 15, 27]. The drugs available for treatment of GBM, mainly temozolomide (TMZ) (discussed in brief in **Section 1.2.1.1**) suffer from extreme chemoresistance due to the presence of specialized glioma stem-like cells (GSCs) [28]. These GSCs cannot be killed by conventional chemotherapeutic agents; instead, they are enriched when the bulk of glioma cells are killed and due to their better oncogenic potential cause recurrence of even more refractory and malignant tumor.

1.1.2.2. Breast cancer

Breast cancer is the carcinoma of the mammary tissues and is one of the most-prevalent form of cancer affecting humans. As can be seen from **Figure 1.1**, breast cancer has the highest age-standardized incidence and mortality rate among all cancers, as per the GLOBOCAN report 2024. It is the most common type of cancer affecting women, with more than 22 lakh cases (22,96,840 cases) reported in the world in 2022 and more than 6.6 lakh cases of death from the disease ranking 4^{th} worldwide [29]. The disease is known to affect men too. In India, 1,92,020 breast cancer cases were reported in 2022 in women which is 26.6% of all types of cancer cases making it the most common type of cancer in India affecting women, of all ages (**Figure 1.3**). Breast cancer is caused due to various factors which includes age, genetic pre-disposition, environmental factors, hormonal factor and other factors like alcohol-induced increased blood levels

of oestrogen which interferes with folate metabolism and also, due to obesity, especially post-menopausal breast cancer [30].

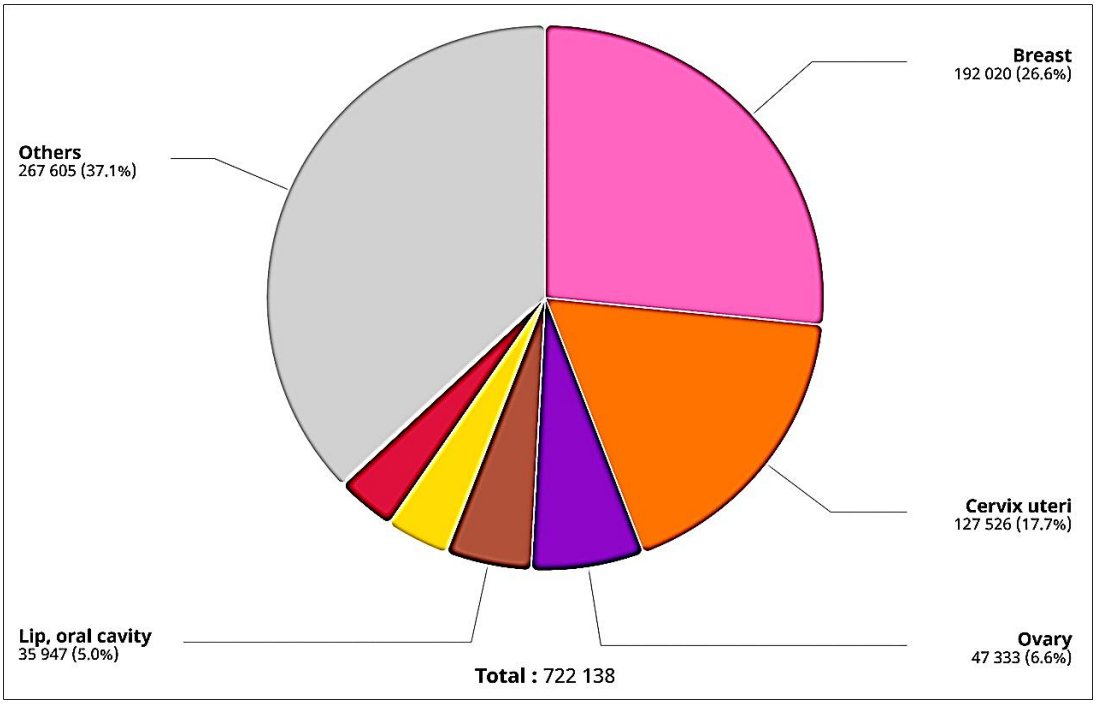


Figure 1.3. Pie-chart showing number of new cancer cases in 2022, in India for females of all ages. Breast cancer is the most-prevalent singular cancer affecting women in India.

Breast cancer can be classified according to the site of disease into the following types: i) non-invasive breast cancer, in which the cells get constrained to the ducts and do not breach the surrounding fatty and connective tissues of the breast. Ductal carcinoma *in situ* and lobular carcinoma *in situ* are the two forms of non-invasive breast cancer; ii) invasive breast cancer in which the cells burst through the duct and lobular wall and breach the surrounding fatty and connective tissues of the breast. Infiltrating lobular carcinoma and infiltrating ductal carcinoma are the two forms of this type of breast cancer [31]. Other rare forms of invasive breast cancer include medullary carcinoma, mucinous carcinoma, tubular carcinoma, inflammatory breast cancer and Paget's disease of the nipples.

Breast cancer can also be classified according to the hormonal status of the disease which is referred as molecular subtypes of breast cancer [32]. According to this classification, breast cancer is of the following subtypes; i) luminal A which contains tumor that are oestrogen receptor (ER) and progesterone receptor (PR) positive but HER2 negative and can be treated by hormonal therapy and chemotherapy, ii) luminal B which includes tumors that are ER and HER2 positive but PR negative and can be treated by chemotherapy and hormone therapy and HER2 targeted therapy, iii) HER2-positive which includes tumor that are ER and PR negative but HER2 positive and is treated with chemotherapy and HER2 targeted therapy and iv) triple negative breast cancer (TNBC) which is also called basal-like breast cancer which includes tumors that are ER, PR and HER2 negative. This type of breast cancer solely gets treated by chemotherapy.

Current treatments for breast cancer include surgery viz. breast conserving therapy (BCS) or lumpectomy, partial mastectomy or quadrantectomy and total mastectomy, radiotherapy, chemotherapy like adjuvant or neo-adjuvant chemotherapy, hormonal and targeted therapies. Targeted therapies (also called biological therapies) are a comparatively new access to breast cancer treatment and target definite biological processes on the molecular level that are responsible for tumor growth. Targeted therapy is either given just after chemotherapy as maintenance or in conjugation with other therapies e.g. chemotherapies or hormonal therapies at various stages of advanced disease in accordance with their approved label. Drugs used in targeted therapy are trastuzumab, lapatinib, neratinib, abemaciclib, everolimus, olaparib etc. [33-35].

Several signal transduction pathways have been identified, in which, modifications promote tumor cell proliferation, progression, and survival in breast cancer [36]. These

include; i) PI3K/Akt/mTOR pathway, ii) Notch signalling pathway, iii) Wnt signalling pathway, iv) hormone receptor signalling pathway. Other signalling pathways that are altered in breast cancer involve one or more of the following molecular mediators like p53, ERK, PTEN etc. Modifications in these cell signalling pathways promote breast tumor cell proliferation, progression, and survival and accordingly, they serve as highly adaptable and druggable targets for the therapeutic management of breast cancer.

1.1.2.3. Cell lines for GBM and breast cancer

Cancer cell lines are important for oncological experimentations. There are several dedicated cell lines for glioblastoma and breast cancer which are well established and commercially available. Cell lines have proved essential to understand the biology/biochemistry behind tumorigenesis and pathogenesis of GBM and breast cancer, to run molecular diagnostic studies in order to identify key signalling pathways and mediators, to evaluate newer therapeutic strategies and develop new anti-cancer drugs and, to develop new vectors for gene therapy.

The American Type Culture Collection (ATCC) enlists an array of cell lines for different tumors of the brain and breast [37] which were originally derived from different carcinoma patients and are commercially available. Some of the prominent cell lines derived from human and rat brains and useful for GBM are U87MG, A172 (both human-derived) and C6 (rat-derived) [38], Interestingly, the ATCC provides a dedicated Glioma Tumor Cell Panel named ATCC® TCP-1018™ which consists of '5 glioma tumor cell lines with varying degrees of genetic complexity'. The 5 components of the panel are as follows: A172, SW1088, H4, U118MG, and U87MG [39]. Breast cancer cell lines are considered crude models of the disease and cannot capture all

features of the tumor. Nevertheless, there are well-established cell lines like MCF-7, an estrogen receptor (ER)-positive cell line that is often used in research on ER-positive breast cancer, MDA-MB-468 and MDA-MB-231, ER-negative cell lines that are epidermal growth factor receptor (EGFR) positive, 4T1, an aggressive murine cell line resembling human metastatic TNBC cells etc [40].

1.1.2.4. Current challenges in the treatment of GBM and breast cancer– problem statement-I

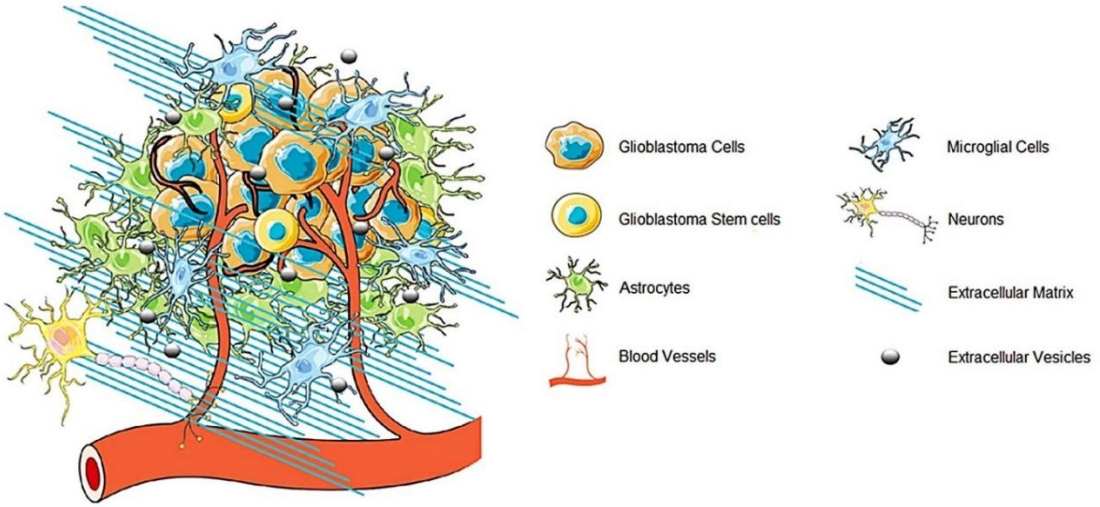


Figure 1.4. Cellular heterogeneity of the GBM microenvironment (Reproduced from reference 46)

As we have discussed in the previous sections, GBM is characterized by a very low prognosis and presents numerous challenges in successfully treating the disease to complete remission. The factors responsible for this have been described earlier where the most important factors are identified to be the localization of the tumors in the brain and the heterogeneity of the tumor microenvironment (TME) (**Figure 1.4**) [41]. GBM tumors are composed of a diverse variety of cellular components including the highly malignant and aggressive stem-like cells which are responsible for chemoresistance and relapse of the disease. In addition to that, the inability of most chemotherapy drugs to

cross the BBB poses an added challenge in the treatment of the disease as the drugs do not reach therapeutic concentrations within the target site. Other specialized treatment options like immunotherapy have failed due to the intrinsic and adaptive resistances presented by the disease resulting in strong immunosuppression in the intra- and intertumoral spaces [42]. Targeted therapies including combination therapy have seen some success of late due to the molecular level of tackling the disease though the abovementioned precluding factors often pose problems including chemoresistance [43].

Breast cancer presents less challenges than GBM in workable treatment options and success in managing the disease, yet there are some ubiquitous issues plaguing the global priority it deserves [44]. Recent information from the World Health Organization (WHO) indicates that although 70% of countries have established cancer guidelines and 62% report screening programs, at the same time, 40% report important management and treatment access restrictions and less than half have palliative care plans [29]. The main challenge is the development of resistance and deleterious side effects of chemotherapy. In addition, recurrence of the disease due to the fickle breast cancer stem cells (BCSC) poses an added challenge. Another issue is the progress of the disease to the metastatic stage which makes it difficult to accurately target the tumor cells which obviously makes an early detection extremely important [45].

The challenges in successfully managing GBM and breast cancer have rendered these as thrust areas for researchers across the globe and with their soaring impact on all populations, it is pertinent to address these treatment challenges to better the outcome. The unique challenges of GBM management and the extremely high prevalence rate of

breast cancer make these diseases relevant and suitable to be addressed with fundamental research which has been attempted in the current work.

1.1.3. Overview of SHP2 enzyme

1.1.3.1. Structure and functions of SHP2

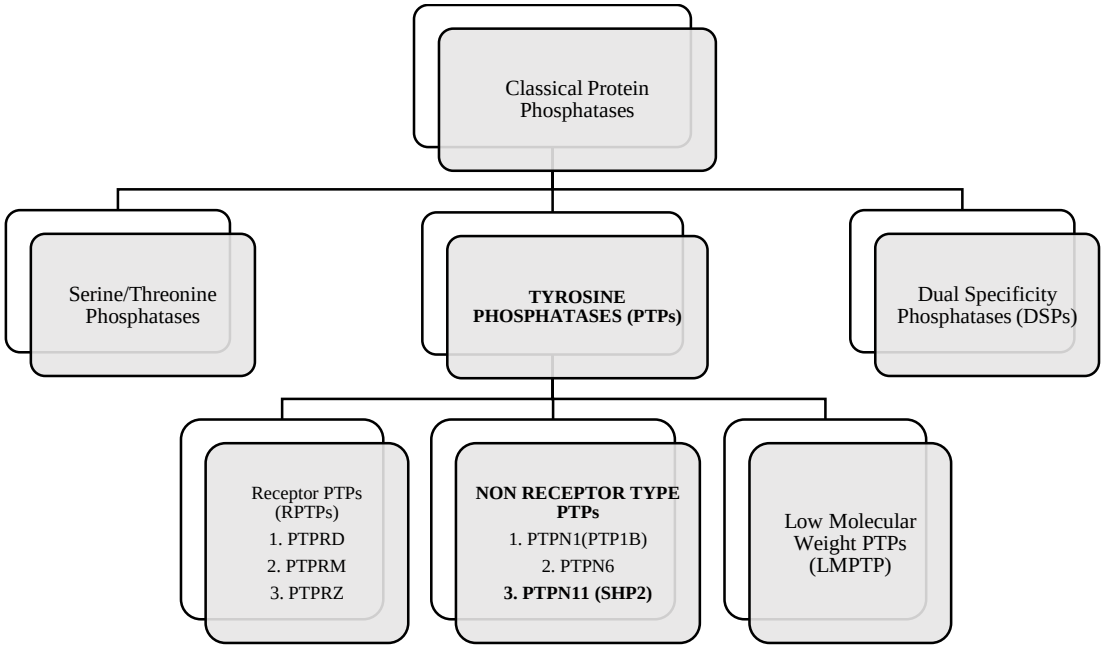


Figure 1.5. Protein tyrosine phosphatase (PTP) superfamily

The Src homology-2 (SH2) domain-containing phosphatase-2 (SHP2), also known as PTPN11, is a member of the subfamily of non-receptor bound protein tyrosine phosphatases (PTPs) (**Figure 1.5**) encoded by the *PTPN11* gene (NCBI accession ID: XP_054228694.1) [46].

SHP2 is ubiquitously present in almost all animals and is an important regulator of signal transduction via appropriate tyrosyl dephosphorylations leading to a cascade of downstream events [47]. The tyrosyl dephosphorylations occur in the active site of the catalytic PTP domain with the explicit involvement of a cysteine-459 residue present in the phosphate binding loop (P-Loop) and stabilization of the thiophosphate

intermediate through other conserved amino acid residues like aspartate, arginine and a tyrosine moiety present in the WPD-loop (**Figure 1.6**) [48]. These events in turn, elicit a variety of cellular responses including growth, proliferation as well as cell senescence. Mutations and aberrant activation of human SHP2 have been implicated in conditions like Noonan Syndrome, LEOPARD Syndrome, *Helicobacter pylori* pathogenesis and most importantly, in cancers. In fact, SHP2 is the first member of the phosphatases to be classified as a proto-oncogene [48].

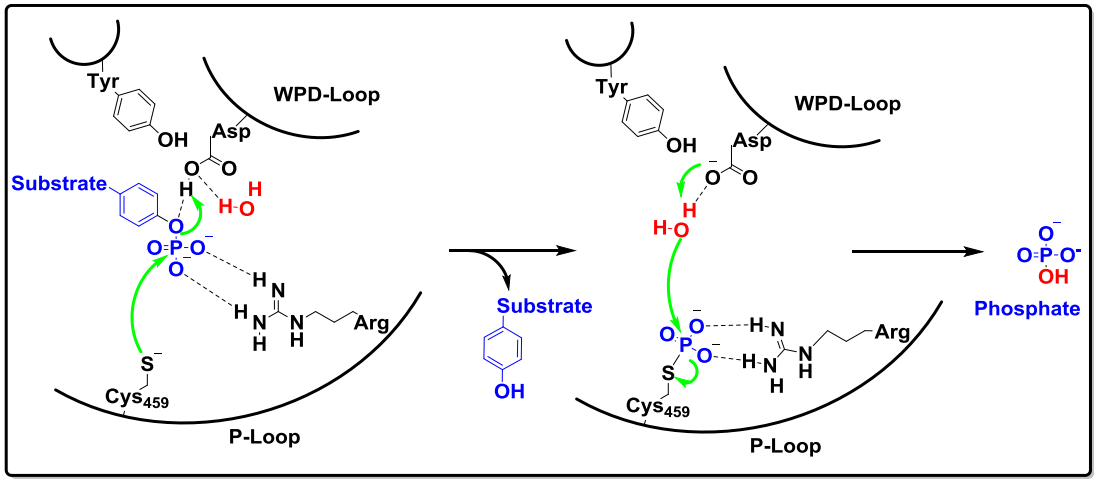


Figure 1.6. SHP2 molecular catalytic mechanism. WPD-Loop, tryptophan-proline-aspartate loop; P-Loop, phosphate binding loop. (Reproduced from reference 47 with modifications).

Several somatic mutations in SHP2 have been identified in carcinomas like juvenile myelo monocytic leukemia (JMML), breast cancer, prostate cancer, lung cancer, colorectal cancer, neuroblastoma, hepatocellular carcinoma and brain cancers [49-51].

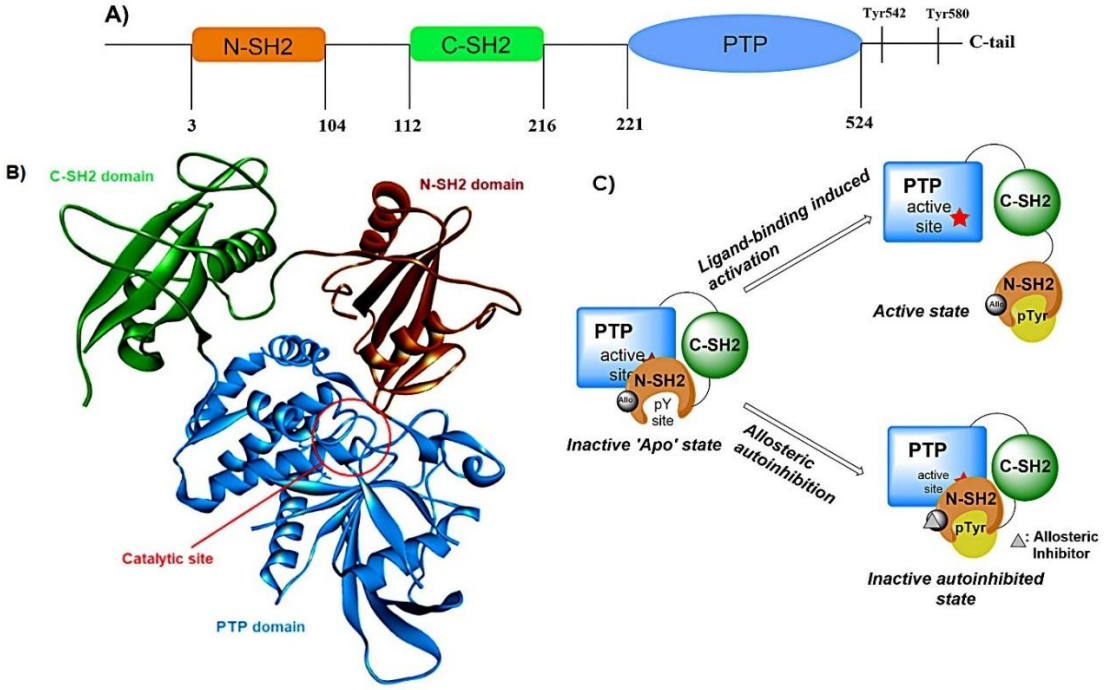


Figure 1.7. Structure of SHP2. A) Various domains of SHP2; B) 3D ribbon diagram of SHP2 in its auto-inhibited form ('apo' state); C) Schematic representation of SHP2 activation. (**Figure 1.7B** is reproduced from reference 52).

SHP2 is a non-transmembrane PTP which contains two tandem SH2 domains, the N-terminal SH2 domain and the C-terminal SH2 domain and a highly conserved and catalytically active PTP domain (**Figure 1.7A**). SHP2 remains in an auto-inhibited state when it is not bound by any ligands, wherein the N-terminal SH2 domain binds the PTP domain in a 'backside loop' and closes the active site of the enzyme making it inaccessible to any incoming potential ligand through a mutual allosteric inhibition (**Figure 1.7C**, left) [52]. The 3D structure of auto-inhibited SHP2 (**Figure 1.7B**) shows the interaction between the N-SH2 and the PTP domain (yellow and blue fragments, respectively) with the C-SH2 domain (in green) acting as a kind of intercessor [53]. Once the phosphotyrosine ligands of a receptor tyrosine kinase (RTK) or a Shp-binding protein (eg. Grb2) bind to the pTyr peptide-binding pocket of the unperturbed C-terminal SH2 domain followed by the N-SH2 domain, the auto-inhibition is removed

and the enzyme is opened and activated (**Figure 1.7C**, right upper panel) [54]. This activation leads to the recruitment of several molecular mediators in a so-called protein kinase cascade, ultimately stimulating the MAPK pathway via the RAS-RAF-ERK stem (**Figure 1.8**) [55, 56]. ERK phosphorylation has been shown to silence the p53 oncogene which suppresses apoptosis and cell senescence. Often, some ligand binding to the allosteric site (like, **SHP099**) will stabilize the enzyme in its auto-inhibited conformation thereby impeding its biological functions (**Figure 1.7C**, right lower panel) [57].

1.1.3.2. Role of SHP2 in gliomagenesis and in breast cancer

In a glioma and breast cancer cell, the above mentioned regulatory function of SHP2 has been well investigated and has been found to be an important factor in the determination of cellular phenotypes, along with some other important pathways which are, for all practical purposes, also controlled by SHP2 [58]. For instance, RAS which is stimulated by SHP2 in the ERK phosphorylation pathway, in turn up-regulates the PI3K-AKT-mTOR pathway by direct activation of PI3K [59]. Also, there is a direct and indirect stimulation of HIF-1/2α by SHP2 and by RAS respectively. Both these upregulations lead to increased translation and metabolism in the glioma cells leading to enhanced cell survival (**Figure 1.8**) [60]. SHP2 also negatively modulates the phosphorylation of EGFRvIII, a mutant form of EGFR mostly seen in GBM cells. [61]. On the other hand, SHP2 exerts inhibitory and anti-survival effects by negative regulation of STAT3 phosphorylation in GBM tumors, which is known as the 'STAT3 addiction phenotype'. This is achieved by dephosphorylation of the active phosphorylated-STAT3 (pSTAT) dimers and inactivation of the resultant ligand-receptor complexes which ultimately manifests as resistance of the GBM cells towards

apoptosis inducers in anti-GBM chemotherapy (**Figure 1.8**) [62]. These signal regulations by SHP2 through the RAS-ERK-MAPK and the JAK-STAT3 pathways, are eventually responsible for control of cellular phenotypes including proliferation, differentiation, angiogenesis, and ultimately, tumor progression as also resistive response to therapy and cell senescence [14].

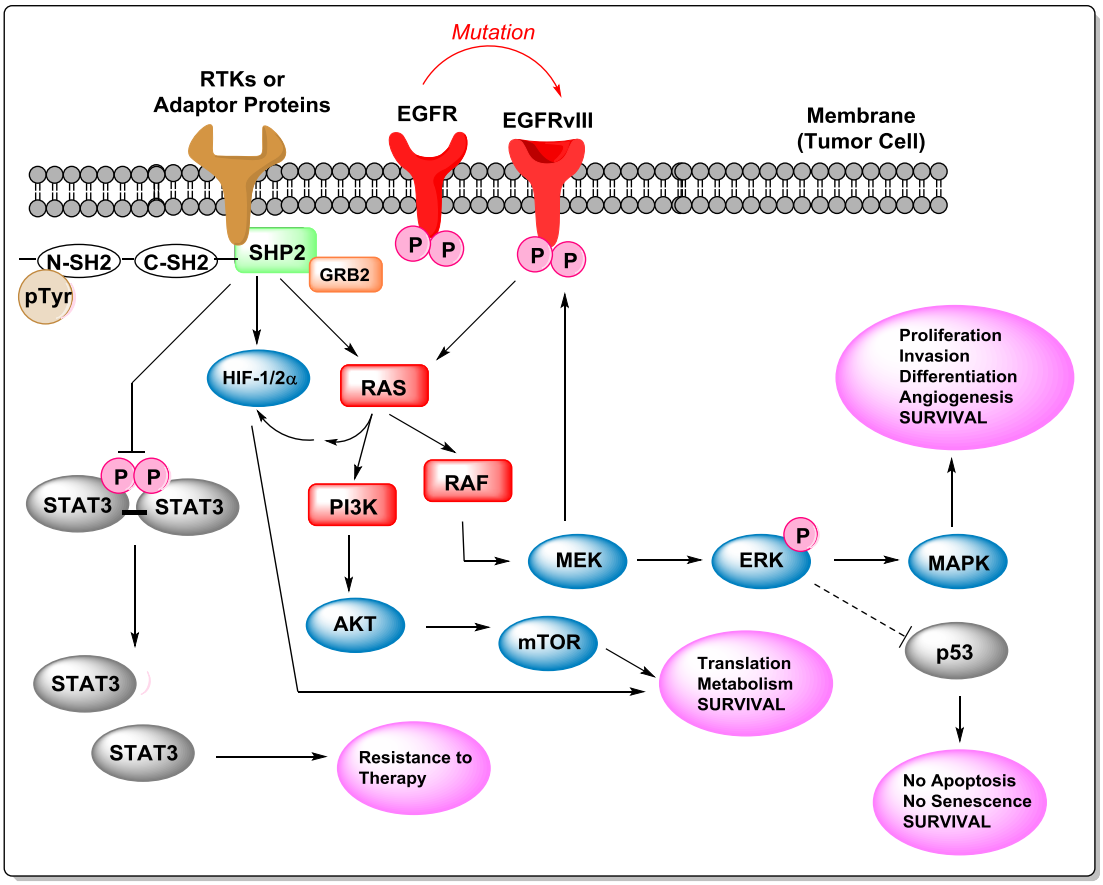


Figure 1.8. Role of SHP2 as a molecular mediator in GBM and breast cancer. Mediators which undergo mutation or are overexpressed in GBM are shown in red whereas those which are inhibited are shown in black. Key mediators of the 'kinase cascade' are shown in blue. SHP2 has been shown in its open activated form only.

As mentioned above, SHP2 is the first tyrosine phosphatase to be classified as a proto-oncogene. Several gain-of-function (GOF) mutations occur in SHP2 at the PTP or N-SH2 domain residues in its auto-inhibited apo state. However, when it comes to solid tumors like GBM, lung cancer and breast cancer, GOF mutations are much less

frequent and have often been found to play dual specific roles of activation or suppression although an overall pathogenic role has been attributed to the GOF-mutant SHP2 (e.g., $SHP2^{E76A}$) towards the occurrence, progression, and metastasis of these carcinomas. Thus, *PTPN11* is a mutational cancer driver in GBM and breast cancer [63].

SHP2 is required for the proliferation of breast cancer cells *in vitro* and tumor growth *in vivo* through regulation of cyclin D1 abundance, thereby accelerating cell cycle progression. Notably, SHP2 modulates the ubiquitin–proteasome-dependent degradation of cyclin D1 via the PI3K/AKT/GSK3β signaling pathway [64].

1.1.3.3. Role of SHP2 in glioblastoma and breast cancer stem cells

SHP2 function is also essential for glioma stem-like cells (GSCs) and breast cancer stem cells (BCSC) proliferation and tumorigenesis [58, 65]. The GSCs are responsible for resistance to chemotherapy and cannot be killed by conventional chemotherapeutic agents; instead, they are enriched when the bulk of glioma cells are killed and due to their better oncogenic potential cause recurrence of even more refractory and malignant tumor. The catalytic PTPase domain of SHP2 has been shown to essentially induce oncogenic transformation of EGFRvIII GBMs. In BCSCs, knockdown of SHP2 eradicated breast tumor-initiating cells in xenograft models, and SHP2 depletion also prevented invasion in three-dimensional cultures and in a transductal invasion assay *in vivo* [65]. Notably, SHP2 knockdown in established breast tumors blocked their growth and reduced metastasis. Thus, classical SHP2 inhibitors maybe potential candidates for targeting the GSC and BCSC compartment along with the regular cancer cells for bettering the response to therapy [58].

1.2. Literature review

1.2.1. Natural and synthetic small molecules in GBM and breast cancer management

1.2.1.1. Natural and synthetic small-molecule drugs for GBM

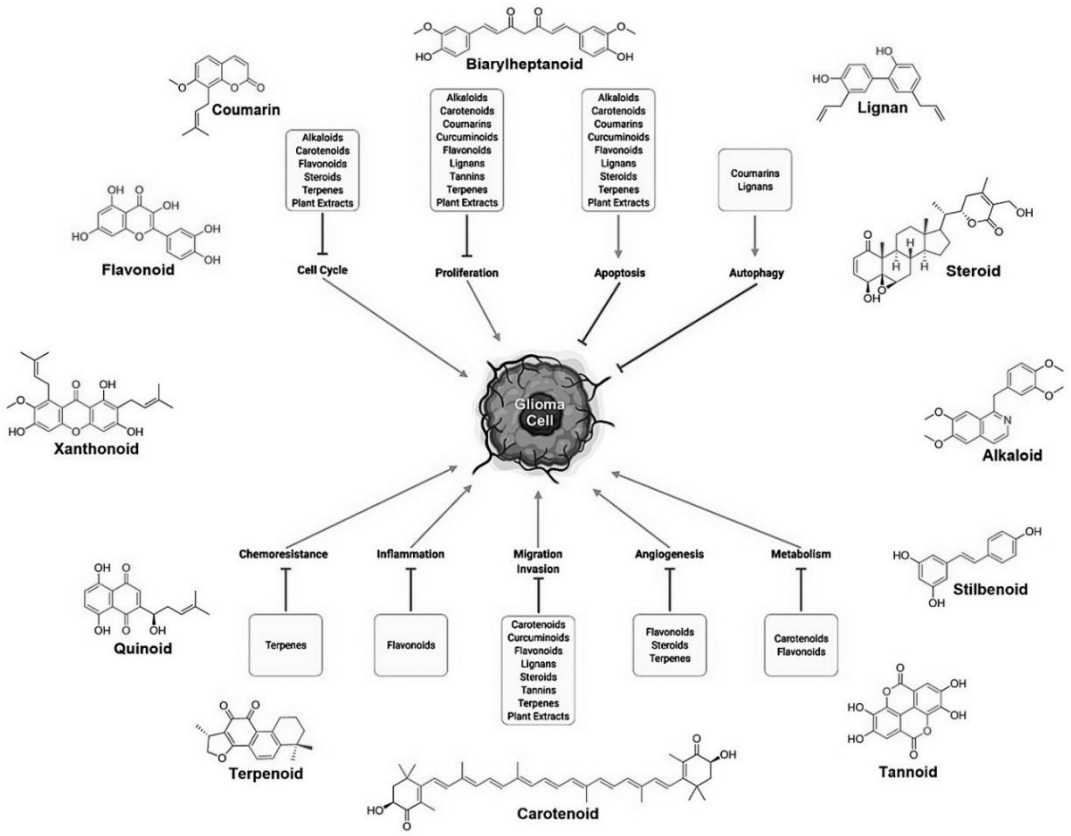


Figure 1.9. Major pathways modulated by natural substances (shown alongside) in GBM

Many natural products (NPs) have been identified that modulate various pathways associated with GBM. As can be seen in **Figure 1.9**, these diverse classes of NPs may have multiple targets within the gliomagenetic pathway. These compounds with established biological benefits exert oncological effects on GBM cells in vitro and in vivo. The most potent NPs include alkaloids, flavonoids, terpenoids, xanthonoids, quinoids, coumarins, lignans, stilbenoids, carotenoids and biarylheptanoids.

Development of synthetic small-molecules as anti-GBM drugs has been challenging so far, as has been discussed previously. The impeding factors of BBB, chemoresistance

and TME heterogeneity make anti-GBM drugs discovery difficult and as such, there is a dearth of clinically approved drugs for the disease. As a matter of fact, till date only ten synthetic small-molecule drugs have received USFDA approval for the treatment of all forms of brain cancer (viz, glioma, high-grade glioma, neuroblastoma and GBM) (**Table 1.1**). Of these, TMZ and the nitrogen mustards, carmustine and lomustine are alkylating agents (**Figure 1.10**);

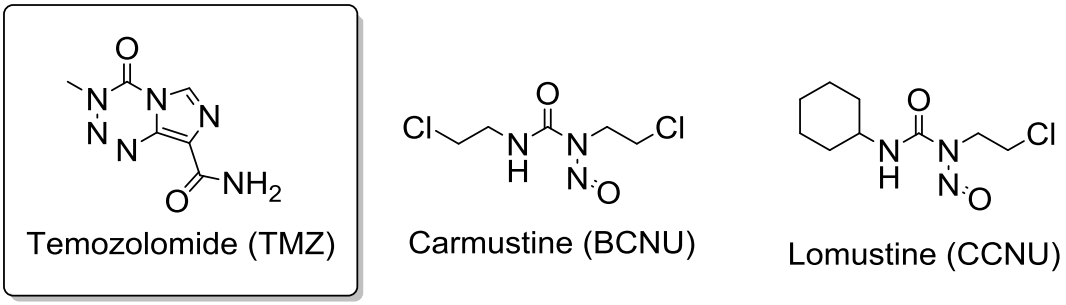


Figure 1.10. Alkylating agents approved for GBM therapy

The other class of drugs is the targeted enzyme inhibitors that target specific enzymes whose overexpression or mutations are responsible for gliomagenesis. inhibitors of several key molecular mediators of the glioma cell signal transduction pathway like HIFα, the kinases like MEK, ERK, mTOR etc. have been developed (**Figure 1.11**) [66].

Table 1.1. FDA approved chemotherapeutic drugs for different gliomas

Category	Examples	Mechanism of action/function
Alkylating agents	Temozolomide (TMZ), Carmustine, Lomustine	Damage DNA and result in death of growing cells.
Enzyme inhibitors	Belzutifan (HIF α Inhibitor)	Targets specific enzymes whose overexpression or mutations are responsible for gliomagenesis
	Dabrafenib (B-Raf Inhibitor)	
	Trametinib (MEK Inhibitor)	
	Tovorafenib (B-Raf Inhibitor)	
	Vorasidenib (IDH1/2 Inhibitor)	
	Everolimus (mTOR Inhibitor)	
Tubulin blockers	Vincristine	Bind to the tubulin protein, stopping the tubulin dimers from polymerizing to form microtubules

Few small molecules have also been developed to target the DNA Damage Reversal (DDR) factors in an attempt to better the chemotherapeutic outcome. Potent PARP inhibitors like olaparib and talazoparib [67] were developed to improve progression-free survival in GBM and to sensitize chemotherapeutic response of TMZ when used as adjuvants (**Figure 1.11**) [68].

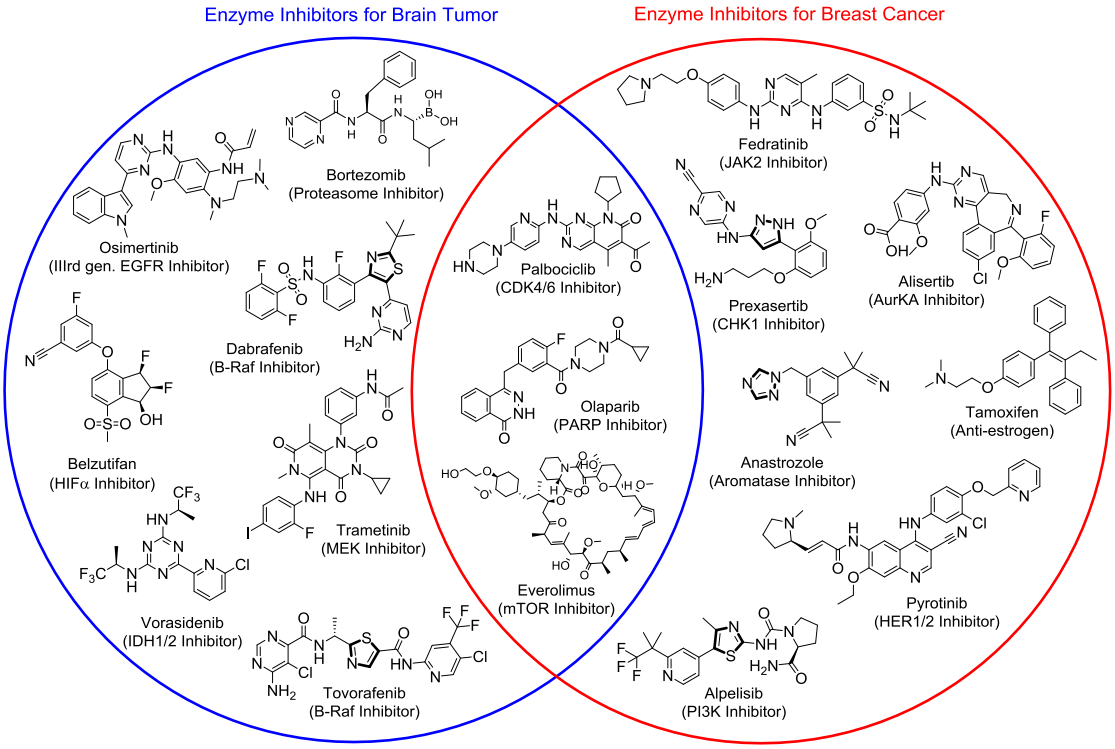


Figure 1.11. Targeted enzyme inhibitors for brain tumors and breast cancer, shown within blue and red circles respectively. Drugs that are clinically proven useful in both the cancers are shown in the intersecting segment.

Several targeted inhibitor small-molecules are under investigation at various phases of clinical trial as chemotherapeutic agents for GBM. Most of the trials involving these drugs are concerned with combination therapies of the drug-of-choice temozolomide with an adjuvant investigational drug belonging to diverse pharmacological classes like HDAC inhibitors (e.g., vorinostat), kinase inhibitors (e.g., sunitinib, pazopanib), proteasome inhibitors (e.g., bortezomib) etc (**Table 1.2**) [69].

Table 1.2. Major classes of small-molecule enzyme inhibitors in clinical trial for GBM therapy

Category	Examples	Mechanism of action/function	Clinical trial phase	Clinical trial ID
HDAC inhibitors	Vorinostat	Inhibit epigenetic target HDAC to arrest cancer cell differentiation	Phase 1	NCT00268385
PARP inhibitors	Fluzoparil	Inhibit PARP to reduce chemoresistance	Phase 2	NCT04552977
Kinase inhibitors	Sunitinib (HIF α inhibitor)	Target different players of the kinase cascade	Phase 2	NCT02928575
	Pazopanib (Pan-kinase inhibitor)		Phase 2	NCT02331498
	Anlotinib (Tyrosine Kinase inhibitor)		Phase 2	NCT04157478
	Ibrutinib (BTK inhibitor)		Phase 1	NCT03535350
	Peposertib (DNA-PK inhibitor)		Phase 1	NCT04555577
Proteasome inhibitors	Bortezomib	Inhibit proteasome to block ubiquitin-tagged protein degradation	Phase 2	NCT03643549
MDM2 inhibitors	Brigimadlin	Inhibit MDM2-p53 interaction to restore the transcriptional activity of p53	Phase 1	NCT05376800

1.2.1.2. Natural and synthetic small-molecule drugs for breast cancer

Several different classes of natural and synthetic small-molecule drugs have been developed for breast cancer which address diverse aspects of the disease (**Table 1.3**). Due to a hormonal component for the progression of the disease, a unique class of hormonal inhibitors were developed as drugs for breast cancer which block hormonal receptors, prevent joining of oestrogen to cancer cells hence stops cell from growing or block the effect of male hormones on breast cancer cells. However, these drugs are only useful in ER and PR-positive breast cancer like luminal A and B type and are not applicable for TNBC. Enzyme inhibitors that provide targeted therapy are also available for the management of breast cancer. These molecules target specific molecular

mediators (enzymes, polypeptides) whose dysregulation and mutations lead to carcinogenesis. Several combination therapies for breast cancer have been approved by the USFDA that are grouped based upon the mode of action or their intended target(s) [70] e.g., anthracycline based like doxorubicin + cyclophosphamide, taxane based like doxorubicin + paclitaxel or monoclonal antibody (mAB) based like doxorubicin + cetuximab.

Table 1.3. FDA approved chemotherapeutic drugs for breast cancer

Category	Examples	Mechanism of action/function
Alkylating agents	Cyclophosphamide, Cisplatin	Damage DNA and result in death of growing cells.
Antimetabolites	Methotrexate, 5-Fluorouracil (5-FU)	Interfere with intermediary metabolism of proliferating cells
Topoisomerase inhibitors	Topotecan, Irinitecan	Block the resealing of DNA strands leading to large amount of DNA fragment hence destabilizes the cell and cause cell death.
Mitosis inhibitors	Docetaxel, Paclitaxel, Vincristine	Target microtubules and associated proteins required in cell division
Hormonal inhibitors	Tamoxifen (Anti-estrogen)	Blocks hormonal receptors, prevents joining of oestrogen to cancer cells hence stops cell from growing or blocks the effect of male hormones on breast cancer cells
	Flutamide (Anti-androgen)	
	Goserelin (LHRH antigen)	
	Anastrozole (Anti-aromatase)	
Enzyme inhibitors	Fedratinib (JAK2 Inhibitor)	Targets specific enzymes whose overexpression or mutations are responsible for gliomagenesis
	Alisertib (AURKA Inhibitor)	
	Prexasertib (CHK1 Inhibitor)	
	Ribociclib (CDK4/6 Inhibitor)	
	Everolimus (mTOR Inhibitor)	

Several targeted inhibitor small-molecules are under investigation at various phases of clinical trial as chemotherapeutic agents for breast cancer (**Figure 1.11**). Many of these molecules belong to the enzyme inhibitor class targeting specific molecular mediators that have been identified as druggable targets for the disease. In preclinical and clinical investigations, several small-molecule kinase inhibitors have demonstrated

effectiveness in combination with other targeted medicines or chemotherapy. Improved outcomes for breast cancer patients may result from further research into combination therapy. Molecules like tucatinib, a small-molecule tyrosine kinase inhibitor (TKI) that is effective in blocking HER2 is useful in HER2-positive breast cancer. Some advantages of small-molecule kinase inhibitors in breast cancer include targeted therapy, oral administration, less invasive, and lower resistance. However, small-molecule kinase inhibitors also face challenges, including medication resistance, finding biomarkers, and optimizing dose and schedule [71].

Few approved enzyme inhibitor drugs as mentioned in **Table 1.1** and **1.3** are useful in both brain and breast cancers as they target enzymes that are responsible for the disease progression in both the cancers. Molecules like the kinase inhibitors and the mTOR inhibitors like everolimus are effective and indicated against paediatric low-grade glioma (pLGG) and HER2-negative breast cancer (HN-BC) (**Figure 1.11**) [72, 73]. SHP2 is one such enzyme that is implicated in both these diseases due to its central role and thus, can be used as a druggable target for effective management of these two diseases.

1.2.2. Natural small molecule inhibitors of SHP2

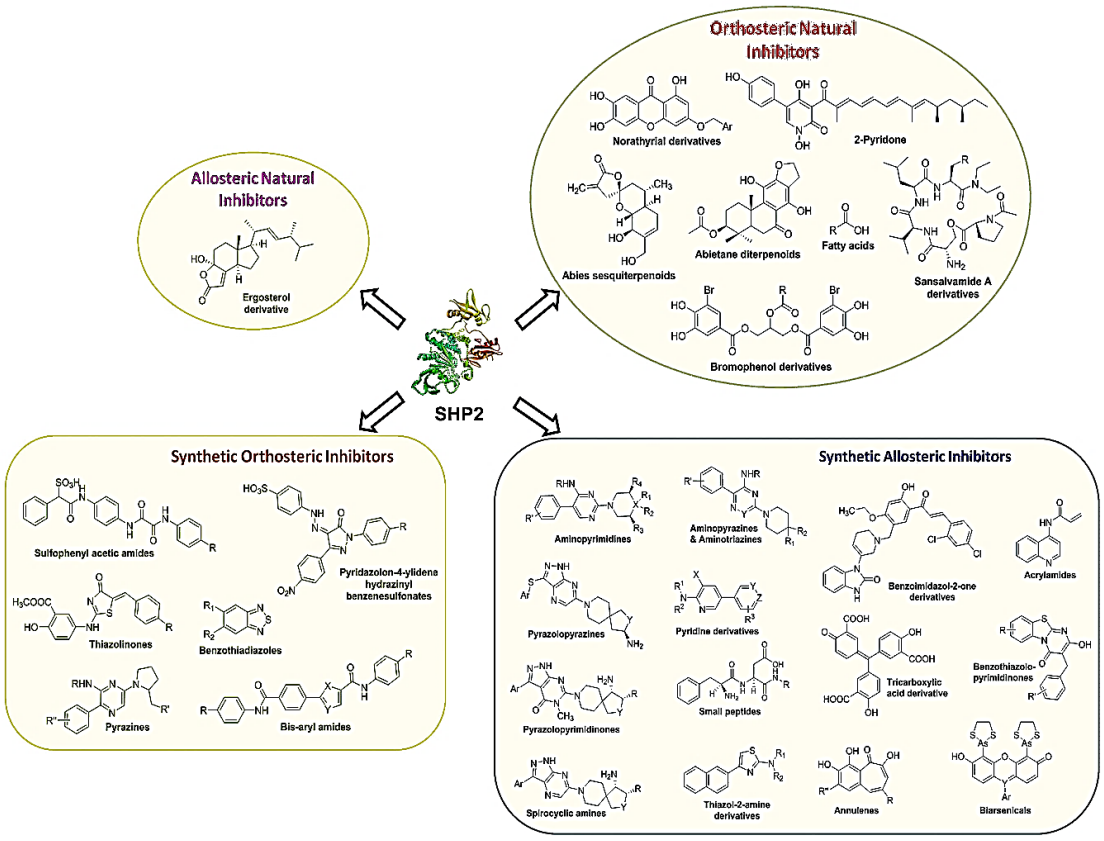


Figure 1.12. Different natural and synthetic small-molecule SHP2 inhibitory scaffolds. Image is reproduced from reference 74.

Several diverse natural molecules have been identified that show potent SHP2 modulating activity and as such can be useful for management of disease related to the enzyme. The natural SHP2 inhibitory scaffolds can also be classified into orthosteric and allosteric inhibitors, however, the number of orthosteric molecules are more with the natural scaffolds due to their enzyme substrate mimicking tendencies. **Figure 1.12** shows few important classes of natural orthosteric and allosteric inhibitors of SHP2 like the abietane diterpenoids, abies sesquiterpenoids, bromophenol derivatives, advanced fatty acid derivatives, 2-pyridone derivatives, norarythrial derivatives, sansalvamide analogues and the tanshinone analogues i.e., the quinoid diterpenes as orthosteric inhibitors and ergosterol derivatives as the unique natural allosteric SHP2 inhibitory

scaffold [74]. All these scaffolds displayed potent anti-SHP2 activity in the submicromolar range and a manifold selectivity over SHP1 and PTP1B.

1.2.3. Synthetic small molecule inhibitors of SHP2

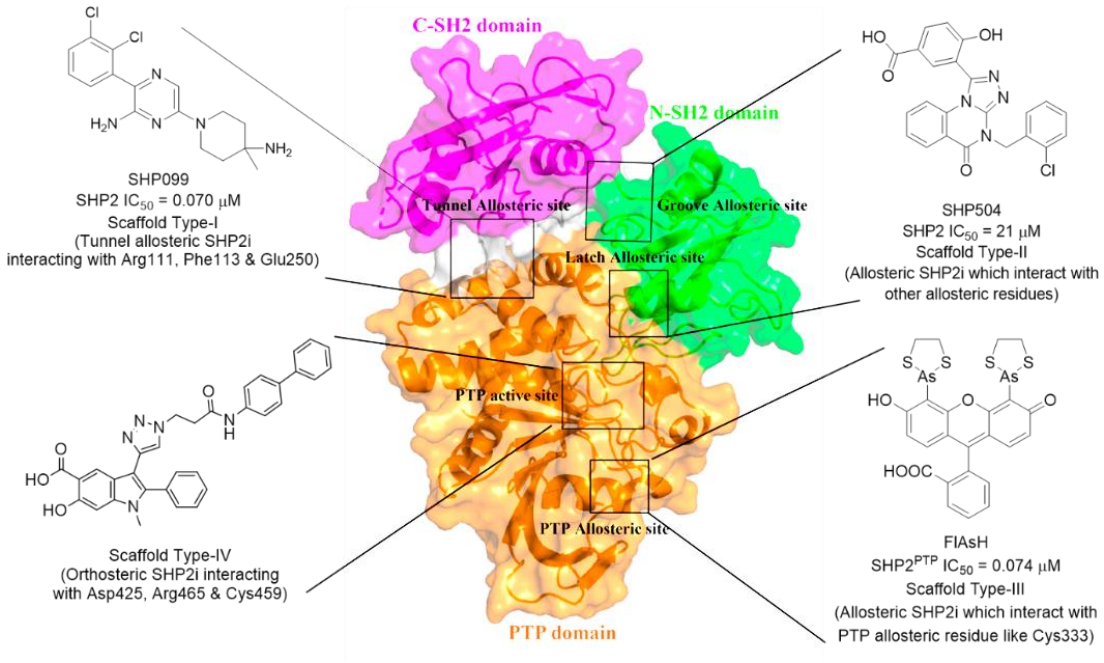


Figure 1.13. Different allosteric and orthosteric site of SHP2 and inhibitory scaffolds targeting them. The central image of SHP2 has been reproduced from reference 63 with necessary modifications.

Several allosteric sites have been identified in SHP2 (which are dispersed throughout its various domains) along with an orthosteric (active) site lodged in the PTP domain (**Figure 1.13**) [75]. Small molecule inhibitors targeting these sites have been developed and reported [74, 76, 77]. The allosteric inhibitors, the prototype being **SHP099** (IC_{50} = 0.070 μM, **Figure 1.13**), mostly bind to the interface formed by the N-SH2, C-SH2 and the PTP domains to stabilize SHP2 in its closed state and have demonstrated remarkable inhibitory potentials and favourable pharmacokinetic properties [74, 78]. These molecules have thus been termed as 'molecular glues' due to their so-called adhesive action on the autoinhibited state of SHP2 [79].

The following allosteric SHP2 inhibitory molecules have advanced into various stages of clinical trials mainly for solid tumors viz. RLY-1971 (Migoprotafib), TNO155 (Batoprotafib), RMC-4630 (Vociprotafib), JAB-3068, JAB-3312, BR-790, BBP-398, BPI-442096, ERAS-601, ICP-189, SH-3809, PF-07284892, ET-0038 and HBI-2376 (details of selected few are given in **Figure 1.14**) [80-85]. In recent years, many distinct strategies have been adopted to pharmacologically modulate the effect of SHP2 including targeted SHP2 degraders i.e., PROTACs [86, 87], dual inhibitors [88-90] and the macromolecular protein-protein interaction (PPI) inhibitors that target the interaction between various domains of SHP2 mainly, the two SH2 domains (N-SH2 & C-SH2) [91].

However, till date the best and most clinically successful strategy has been the development of allosteric inhibitors of SHP2; all clinical candidates mentioned above belong to the allosteric inhibitor class and share their pharmacological backbone with the prototypic allosteric SHP2 inhibitor molecule **SHP099** (**Figure 1.13**). As seen in **Figure 1.14**, most clinically advanced SHP2 inhibitors are analogues or modifications of this prototype structure of **SHP099** and contain the following "common" pharmacophoric features; (A) a mono/poly-substituted aromatic carbocycle (marked in blue, **Figure 1.14**), (B) a heteroaromatic ring core like pyrimidinone, pyrazine, indole etc. (marked in red, **Figure 1.14**), (C) an amino or alkylamino group or analogous fused azacycle on the heterocyclic core (marked in green, **Figure 1.14**) [92, 93]. These unvarying and necessary features, thus, carry the potential to be exploited judiciously and rationally to design and develop newer and better SHP2 inhibitory scaffolds in future.

It can be seen from the above discussion and **Figure 1.12** that quite a few SHP2 inhibitory scaffolds contain 5- or 6-membered heterocycles specifically azacycles either as a centrally placed core ring system like the thiazol-2-amines, oxadiazoles or the aminopyrazines & aminotriazines. The later molecules are some of the most successful classes of SHP2 inhibitors that has given rise to a potent group of advanced clinical molecules for different solid tumors as discussed (vide **Figure 1.14**).

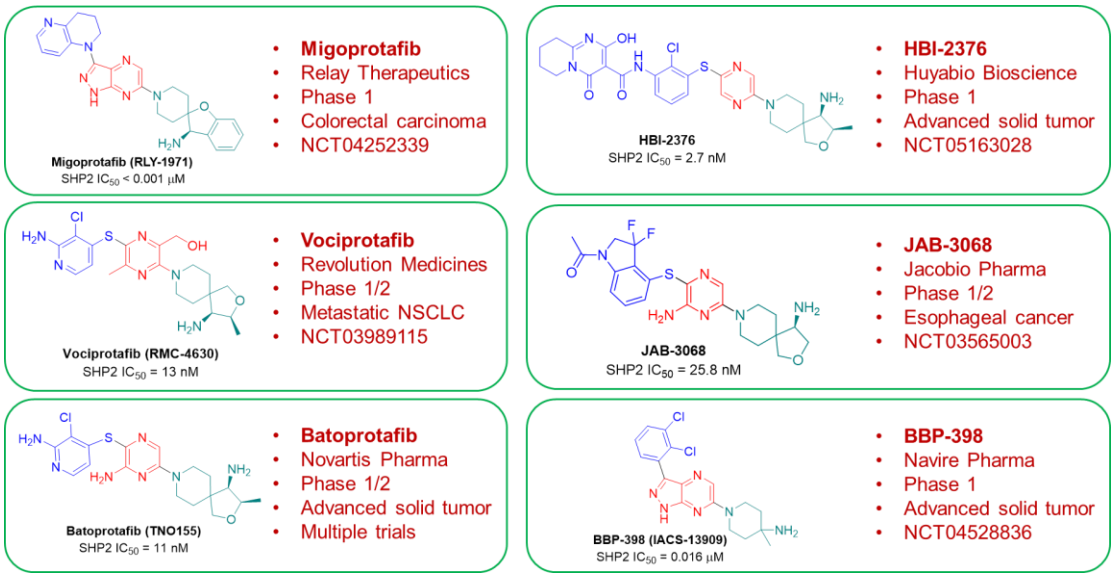


Figure 1.14. Selected synthetic small molecule SHP2 inhibitors in clinical trial in the last 10 years.

1.2.3.1. Development of small-molecule SHP2 inhibitors for GBM and breast cancer therapies

SHP099, a potent allosteric inhibitor of SHP2, was shown to have great promise in GBM therapy by rational analysis of both 2D and 3D structure activity correlations [94]. Sang and co-workers have comprehensively shown that **SHP099**, when administered alone or as an adjuvant with TMZ, proved to be an orally active inhibitor of tumor growth in GBM and GSC cell lines and patient derived xenografts which had activated PDGFRα signaling [95]. This in turn was correlated to the fact that **SHP099** by its allosteric binding to SHP2, downregulates PDGFRα signaling, inhibits ERK1/2

phosphorylation downstream and offers significant survival benefits in GBM xenograft models. It was further shown to penetrate the BBB in a very efficient way to accumulate in efficacious concentrations after oral administration [95, 96]. The immense potential of **SHP099** in SHP2 inhibition and resultant GBM suppression has led many researchers to utilize the various structural features of **SHP099** as templates for designing closely related scaffolds with **RMC-4550**, a structural analogue with minor modifications, showing immense potential (SHP2 IC_{50} = 0.0538 µM) and also serving as a prolific template. Many of these scaffolds (for e.g., methylpyrimidinones, thiazolyl-2-amines, dichlorophenyl-2-heteroarylamines) contain one or more of the key pharmacophoric fragments of **SHP099** to incorporate optimum pharmacological and PK/PD profiles in the final molecules (**Figure 1.15**).

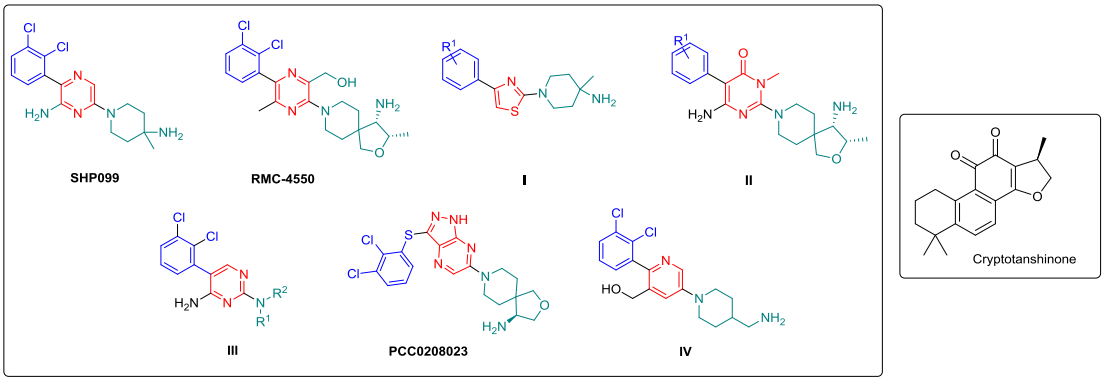


Figure 1.15. Various fragmental analogues of **SHP099** and cryptotanshinone.

Similarly, the natural molecule **cryptotanshinone**, a quinoid diterpene (**Figure 1.15**) was shown to be effective in suppressing gliomagenesis in vitro by inhibiting STAT3 activation and inhibition of intracranial tumor growth and in vivo glioma cell proliferation in human glioma cell line U87 and T98G xenografts. It also inhibited cell migration and invasion of U251 glioma cells in a dose-dependent manner as seen by a scratch wound assay and directly modulated tyrosine phosphatase activity of SHP2. As

such, in spite of its moderate potency against SHP2, cryptotanshinone can serve as a potential template for anti-GBM drug development in future through its effects on SHP2 activity [97, 98].

SHP2 is also being studied as a potential treatment for breast cancer because it is an oncogenic protein that is activated in many breast tumors, as discussed earlier. Genetic and pharmacological inhibition of SHP2 has been shown to block the growth of breast cancer tumors in mice and xenograft models. Additionally, SHP2 inhibitors suppress cancer cell phenotypes, metastasis and the spread of breast cancer tumors. Different chemical classes of small-molecule SHP2 inhibitors are being developed for the management of newly diagnosed breast cancer as well as for cancer that has metastasized to other vital organs. For this reason, SHP2 inhibitors are often combined and may be most effective with other therapies, such as FGFR-targeted kinase inhibitors or PI3K inhibitors. New active site-directed and specific SHP2 inhibitors are under investigation e.g., **CNBDA** and **'57774'** that show promising effects in inhibiting RTK-induced and SHP2-mediated signaling and in suppressing BC cell proliferation and transformation (**Figure 1.16**) [99, 100].

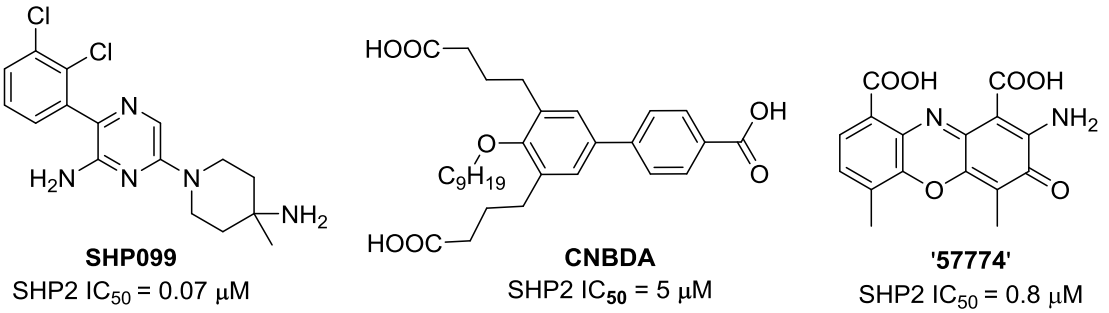


Figure 1.16. Few SHP2 inhibitors clinically proven effective in breast cancer.

1.2.4. Opportunities and challenges in small-molecule SHP2 inhibitor discovery – problem statement–II

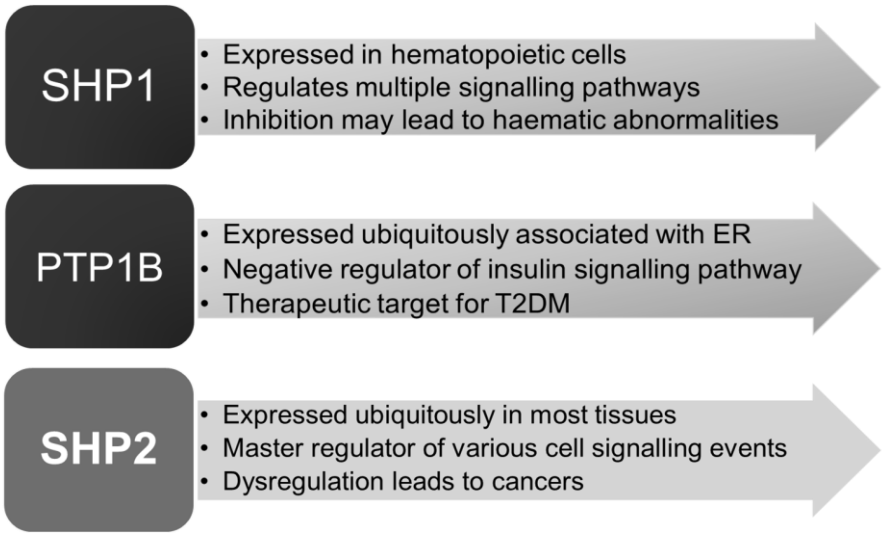


Figure 1.17. Comparison between the closely related paralogues of SHP2 belonging to the PTPN subfamily.

Though a diverse array of chemical classes has been developed as allosteric and orthosteric inhibitors of SHP2, nevertheless, there are no SHP2 inhibitory molecules that have been unequivocally translated into drugs against cancer. Direct orthosteric inhibition has proven challenging due to mainly two major issues [101]:

i) the polar nature of the catalytic PTP site (as can be seen in **Figure 1.7**). This is due to presence of the cationic amino acid residues and the nucleophilic environment required for the catalytic reaction to occur. This necessitates the orthosteric inhibitors to be essentially anionic species mimicking the native phosphotyrosine substrates (i.e., phosphate bioisosteres). It is a well-known fact that anionic molecules have poor bioavailability and pharmacokinetic profile thereby posing a problem in drug development.

ii) considerable sequence homology between the catalytic domains of different paralogues of the PTPN subfamily like SHP1 or PTP1B. This results in lack of selectivity in the orthosteric inhibitors leading to undesirable off-target effects due to the ubiquitous and homeostatic nature of the paralogous members (**Figure 1.17**).

Thus, there is a need to develop newer small-molecule SHP2 inhibitors that address these issues and are potentially effective in SHP2-mediated cancer therapies.

1.2.5. Overview of the 1,3,4-thiadiazole core scaffold

1.2.5.1. Chemistry of the 1.3.4-thiadiazole ring

The 1,3,4-thiadiazole ring system is a highly versatile and ubiquitous moiety that has seen extensive applications in medicinal chemistry as anticancer, antimicrobial, neuroprotective and other agents. The chemical nature of 1,3,4-thiadiazole is remarkable due to its weak basicity arising from the inductive effects of the heteroatoms and due to the reactivity of the nucleophilic centre localized on the ring nitrogen atoms & the electrophilic centre on the carbon of the C=N bond. Additionally, tautomerizable substitutions at C2 and C5 positions of the ring may give rise to enhanced reactivity towards electrophilic or nucleophilic attacks by various groups (**Figure 1.18**). The presence of the electronically rich and highly reactive heteroatoms as well as the rigid and stable structure and optimum ring size make such compounds lucrative for drug development. The tendency of the heteroatoms to form hydrogen bonds with the DNA residues and amino acid residues of the binding sites results in potent molecules having better anticancer profile. The 1,3,4-thiadiazole ring is favoured in for its stability and size factor and so it can be easily synthesized with readily available starting materials.

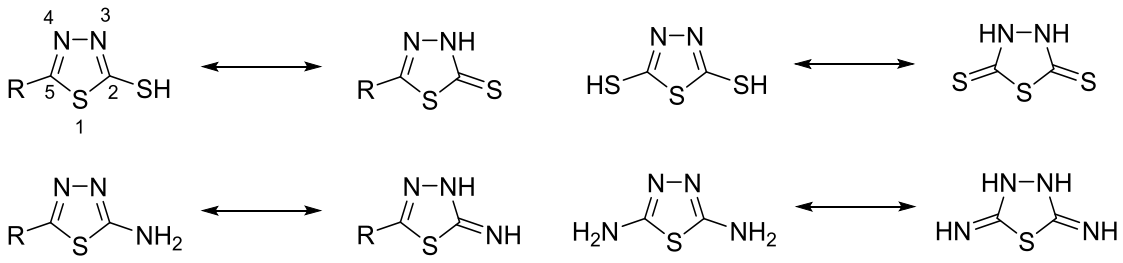
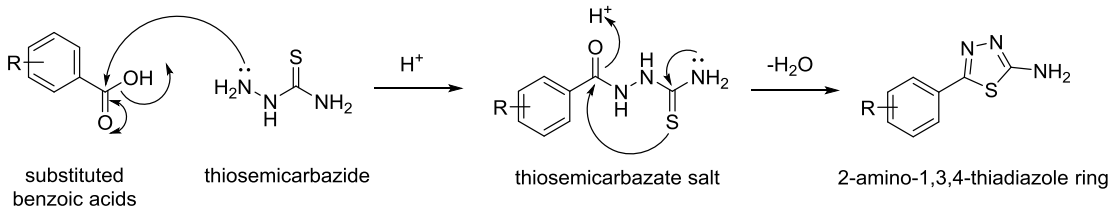


Figure 1.18. Canonical forms of 1,3,4-thiadiazole ring system

Diverse synthesis procedures are available in the art to prepare 2,5-substituted-1,3,4-thiadiazoles from starting materials like appropriately substituted benzoic acids etc. via the corresponding acylhydrazines or thiohydrazines or starting with the analogous 1,3,4-oxadiazole ring [102].

Commonly, 1,3,4-thiadiazoles are synthesized from cyclization of acylhydrazines including N,N′-diacylhydrazines and monoacylhydrazines or transformation from 1,3,4-oxadiazoles. 1,3,4-Thiadiazoles can also be obtained from thiohydrazines (convertibly obtained with the corresponding 1,3,4-oxadiazole by reacting with Lawesson's reagent) including thiosemicarbazide, thiocarbazides, dithiocarbazates, thioacylhydrazines, and bithioureas. Each derivative of thiohydrazines can introduce special kinds of substituents to the thiadiazole ring, which allows for 1,3,4-thiadiazoles with a broad spectrum of reactivity and bioactivity. Many syntheses of 1,3,4-thiadiazoles proceed from thiosemicarbazides, substituted thiosemicarbazides, or thiosemicarbazones. Cyclization of thiosemicarbazides or substituted thiosemicarbazides efficiently leads to 2-amino-1,3,4-thiadiazoles, which have been widely studied as crucial intermediates when preparing 1,3,4-thiadiazole derivatives. In fact, our current work is based on 2-amino-1,3,4-thiadiazole scaffold and its heterocyclic hybrids that were synthesized via the corresponding thiosemicarbazate salt

(vide **Scheme 2.2** and **Scheme 3.1**). In this reaction, acylation (**Scheme 1.1**) or Schiff base formation on the α-amino group initiates cyclization of thiosemicarbazate salt followed by reaction with a dehydrating agent such as TMSCl, PPh_3, $POCl_3$ etc. to obtain thiadiazoles.



Scheme 1.1. Mechanism of cyclization of thiosemicarbazate salt of benzoic acids to form 2-amino-1,3,4-thiadiazole ring system

1.2.5.2. Chemotherapeutic potential of 1,3,4-thiadiazole analogues

This scaffold displays a very broad-spectrum anticancer activity towards several carcinomas and solid tumors and has shown remarkable inhibitory efficiency against different molecular targets associated with the proliferation, survival and metastasis of cancer. Protein targets like protein tyrosine kinases (PTKs), Abl kinase, Akt/PKB, histone deacetylase (HDAC), carbonic anhydrase (CA), matrix metalloproteinases (MMPs), focal adhesion kinase (FAK) etc. have been effectively inhibited by compounds carrying this moiety (**Figure 1.19**) [102-104]. Due to its multitargeting potential against diverse cancer molecular targets, the 1,3,4-thiadiazole containing anticancer drugs exert their desired pharmacological and clinical effects by modulating multimodal pathways associated with cancer phenotypes like tumor invasion, angiogenesis, hypoxia factors, apoptosis and cell-cycle related events etc.

The enhanced level of pharmacological activity of 1,3,4-thiadiazole ring may be attributed to the tight binding between the ring and the interacting residues of the

intended biological target which in turn results from the electron-donating effect of the two nitrogen atoms of the ring that facilitate favourable H-bonds with the target binding sites. Also, substituents at C2 and C5 of the 1,3,4-thiadiazole nucleus (**Figure 1.18**) viz $-NH_2$, and -SH result in enhanced activity due to the active canonical forms of the system arising out of thione–thiol or amino–imino tautomerizations, respectively. These stable canonical forms participate in enhanced H-bond donation (shown in red, **Figure 1.18**) and H-bond acceptation (shown in blue, **Figure 1.18**) resulting in better interactions with appropriate residues of tumorigenic enzyme targets. One interesting fact specifically about the 2-amino-1,3,4-thiadiazole ring system is that the amine substituent at C2 (or, C5) is necessary for better antiproliferation activity as seen by consistent SAR observations [105].

Moreover, 1,3,4-thiadiazoles are bioisosteric to important biomolecules like the purine and pyrimidine bases of the nucleic acids. This makes them mimics of these molecules in the physiological system where they can exert their cytotoxic and cytostatic effects on tumor cells. 1,3,4-thiadiazole derivatives interfere with DNA synthesis and, consequently, inhibit replication of human tumor. This allows them to inhibit the multiplication of cancer cells. Thus, the 1,3,4-thiadiazole derivatives are promising candidates for new anticancer drugs. The molecule '**FPDT**' as seen in **Figure 1.19** is a 1,3,4-thiadiazole derivative that is effective in GBM cells with an IC_{50} ranging from 45 to 68 μM for different GBM cell lines [106].

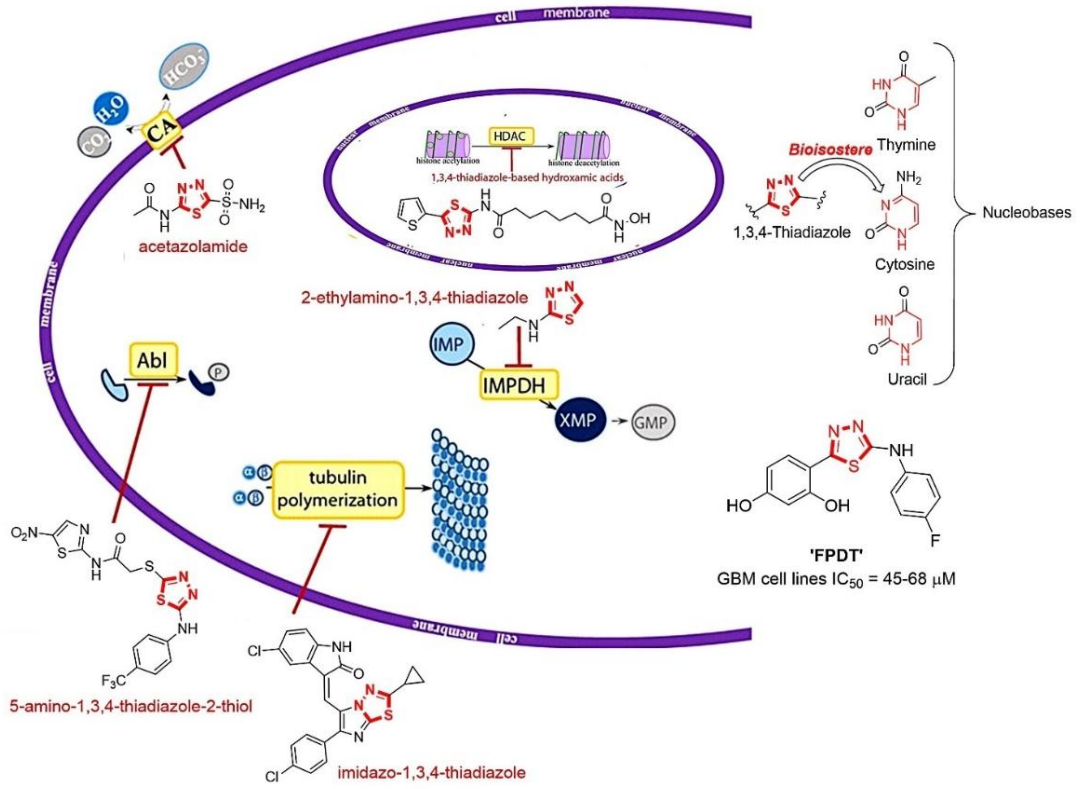


Figure 1.19. Anticancer mechanism of 1,3,4-thiadiazole derivatives

1.2.6. Summary

The review of available and current literature on GBM and breast cancer revealed the following salient points:

a) Glioblastoma multiforme (GBM) is a highly aggressive form of brain tumor characterised by poor prognosis, low survival rate and difficult clinical correlations, including high chemoresistance. Importantly, the presence of BBB impedes drug delivery to intended site of action in GBM.

b) Breast cancer is the most prevalent form of cancer affecting people worldwide with a high mortality rate and high chances of metastasis; recurrence of the disease due to the fickle breast cancer stem cells (BCSC) poses an added challenge.

c) SHP2, the first proto-oncogenic phosphatase, is a key mediator in gliomagenesis and breast cancer development which controls nearly all the signalling pathways through a kind of 'master control'. These signal regulations are responsible for control of cellular phenotypes including proliferation, differentiation, angiogenesis, tumor progression, resistive response to chemotherapy and cell senescence. It is a very appropriate target for the effective management of these two cancers.

d) There is considerable homology between the PTP domains of different members of the PTPN subfamily and the catalytic sites and the active intermediates binding to the orthosteric site are highly polar in nature.

e) Several small-molecule drugs have been developed to manage GBM and breast cancer and also, as SHP2 inhibitors with diverse clinical profiles. The orthosteric SHP2 inhibitor development is challenging due to paralogue sequence homology and polar substrate mimetics. Allosteric SHP2 inhibitors are a lucrative option as chemotherapeutic agents for GBM and breast cancer due to their selectivity, better PK profile and tolerance towards combination therapy.

f) The 1,3,4-thiadiazole scaffold core is a versatile ring system that appears in a multitude of clinical anticancer molecules due to its robust chemical properties and its bioisosteric nature towards the nucleobases.

Based on the above key findings, we envisaged to apply prevalent rational drug discovery approaches and relevant established protocols to discover new small-molecule SHP2 inhibitors and prove their applicability in GBM and breast cancer disease management.

1.3. Rationale, objectives and plan of work

1.3.1. Rationale and design strategy

Glioblastoma multiforme and breast cancer are some of the highly prevalent and highly malignant cancers that are difficult to cure. Synthetic small-molecule drugs for the treatment of GBM and breast cancer are still less in number and are fraught with challenges like low efficacy, chemoresistance and low bioavailability. It is thus imperative to address these challenges. Targeted therapy has seen some success in recent years by aiming for the various molecular mediators that participate in signal transduction mechanisms in carcinomas. Enzymatic targets are often adaptable and druggable enough to be utilized to design their small-molecule inhibitors that eventually act as disease modulators in GBM and breast cancer [41, 45]. SHP2 is the first identified proto-oncogenic phosphatase that has shown multimodal role in gliomagenesis and breast cancer progression and phenotype development. Due to its central role in these two cancers, it is needless to say that SHP2 is a lucrative and rational target for the management of these two cancers. Thus, our pharmacological rationale for the discovery of highly efficacious chemotherapeutic agents for GBM and breast cancer is to design and develop potent small-molecule SHP2 inhibitors [58, 64]. The 1,3,4-thiadiazole scaffold as discussed previously, is a multi-target binding, potent antiproliferative pharmacophore due to multiple chemical and structural factors [102]. Its presence in a potential anticancer molecule is justifiable due to its bioisosteric nature with the nucleobases; this additional feature may confer enhanced anticancer activity to our final designed scaffolds.

Virtual screening as a computational tool to discover new SHP2 inhibitors has been popular since the last decade. Many drug discovery research groups have resorted to

structure-based or ligand-based virtual screening protocols to identify structurally distinct and potent SHP2 inhibitors. Pharmacophore-based virtual screening (PBVS) is a pharmacoinformatics methodology that employs physicochemical knowhow of the chemical space into the dynamic environs of computational technology to extract virtual molecular hits that are precise and promising for a drug target. All these drug discovery techniques have proved to be important in generating virtual hits that have the potential to be modified via lead optimization approaches. The identified virtual hits act as templates for the subsequent judicious modifications of their chemical structure through functionalization of the various pharmacophoric groups and linkers and sometimes by simplification of the general structure where the computational outcome indicates a simpler molecule for better binding and inhibition [107-109].

Thus, our implicit strategy to obtain novel scaffolds as small-molecule SHP2 inhibitors was virtually screen relevant drug database(s) to identify virtual hits and utilise the same to design heterocyclic molecules by introducing the 1,3,4-thiadiazole moiety tethered via activated linkers to carbocycle or other heterocycles to get the added advantage of a hybrid structure. A pharmacophore-based virtual screening exercise was initially done and the identified & experimentally evaluated virtual hit was used as an inhouse template to design the novel pharmacophoric scaffolds by using ligand-guided lead modification and lead simplification wherever applicable. The 1,3,4-thiadiazole nucleus was retained in all the series of compounds thus designed due to its effective role as an antiproliferative. Detailed drug design strategy for individual series of compounds is given in the relevant chapter.

1.3.2. Objectives

Based on the above pharmacological and drug design rationale, we formulated the following overall objectives, accomplishment of which would eventually lead us to address the problem areas identified as mentioned earlier. These objectives are selected and planned by applying judicious methodological knowhows that are relevant and already present in the art of this research area and also, by envisaging a wholesome workflow that contains all or most of the following procedures. The objectives of the present work are;

- To utilize virtual screening approach to develop a pharmacophore model using established prototypical and potent allosteric SHP2 inhibitory scaffolds for identification of novel small molecule SHP2 inhibitory scaffolds and to experimentally evaluate identified virtual lead through *in vitro* biochemical and cellular assays
- To carry out in-house lead-guided design, synthesis and physicochemical & spectral characterization of different series of homologous compounds
- To perform *in vitro* biochemical screening of all compounds against fl-SHP2 enzyme by using established enzyme inhibition assay protocol to obtain the IC_{50} data and determine the mode of inhibition of lead compound(s) and to investigate the *in vitro* antioxidant and blood-brain barrier (BBB) permeability properties of selected compounds of each series
- To evaluate antiproliferative, anti-survival and antimigratory activity of the lead compound(s) in relevant GBM and breast cancer cell lines by employing suitable *in vitro* cell-based assays

- To perform mechanistic studies using flow cytometry protocol to evaluate the mechanism of the antiproliferative activity of the lead compounds in suitable cancer cell line(s)
- To evaluate the *in vivo* safety profile of the lead compounds in mice/rat to find the LD_{50} value by conducting an acute oral toxicity study in accordance with OECD Guidelines 423
- To carry out reverse phase-HPLC based *in vivo* pharmacokinetic profiling of selected lead compound(s) in rats/mice to establish the end-stage PK parameters of the molecule(s)
- To perform *in silico* evaluation of the different libraries of compounds by molecular docking and MD simulation to the allosteric site of 3D X-ray crystal structure of SHP2 to obtain any correlation between the *in vitro* and *in silico* enzyme activity
- To perform *in silico* prediction of physicochemical and ADMET properties of the synthesized molecules

1.3.3. Comprehensive plan of work

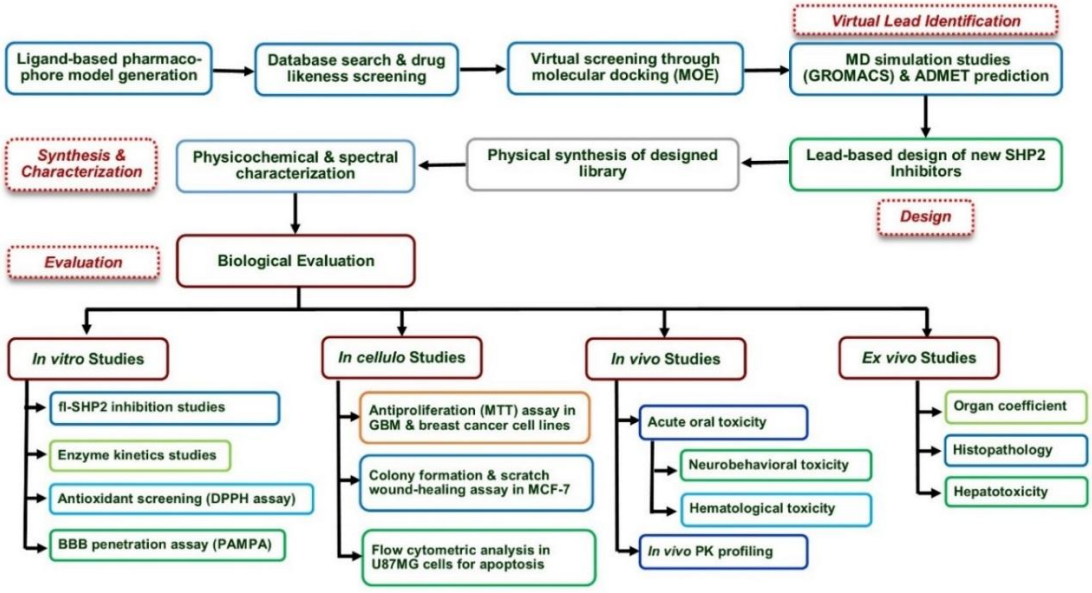


Figure 1.20. Schematic representation for the plan of work

In order to accomplish the abovementioned objectives of our research, an elaborate and systematic workplan was devised consisting of multifarious and hyphened techniques. A schematic representation of the proposed workflow is presented in **Figure 1.20** where we can see that the work is broadly divided into i) design of the pharmacophoric scaffolds through virtual screening and ligand modification methods, ii) synthesis & characterization of the designed scaffolds using conventional methodologies and iii) biological evaluation of the characterized molecules for their intended activity like SHP2 inhibition and cancer cell antiproliferation activity and for their toxicity profile. In the following chapters, a detailed discussion is presented on the design rationale, synthesis, characterization and biological evaluation of few novel 1,3,4-thiadiazole bearing pharmacophoric scaffolds as SHP2 inhibitors and their efficacy as anticancer agents.