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Chapter IV

Targeted Therapy for Brain Tumors

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Abstract

Therapeutic agents affecting specific targets in cancer cells have begun to occupy an important position in cancer treatment. These treatments, known as targeted therapies, exert their effect via two main mechanisms. One group of these therapeutic agents is monoclonal antibodies which block receptors on the cell surface, deliver anti-neoplastic agents or facilitate the immune response. A second group of agents are small molecules which enter the cell and interrupt signaling pathways necessary for cancerous growth. Several drugs which affect single targets are used successfully for malignancies outside the brain in oncological practice. Imatinib mesylate, gefitinib, erlotinib, bevacizumab, inhibitors of farnesyltransferase and multitargeted agents such as antineoplastons (ANP) are also being used in extensive clinical studies in neuro-oncology. Imatinib, gefitinib and erlotinib do not appear to have antitumor activity in high-grade gliomas as single agents, but may produce a synergistic effect with chemotherapy. ANP shows promising results in phase II studies of high and low grade gliomas, brain stem gliomas and a number of responses in preliminary reports in primitive neuroectodermal tumors, atypical teratoid/rhabdoid tumors and multicentric gliomas. The efficacy of these agents has not yet been confirmed in phase III studies. This chapter reviews the most important molecular targets, mechanisms of action and results of clinical trials of new generation of antineoplastic drugs that selectively destroy neoplastic cells in brain tumors.

Introduction

The idea of developing drugs which are oriented against specific targets is not new and can be traced back to Paul Ehrlich whose 150th birthday anniversary was celebrated in the fall of 2004 [1,2]. The science of medicinal chemistry, which was founded by Ehrlich at the beginning of the 20th century, relied on libraries of synthetic and natural products that were screened in selected types of bioassays. The idea originated from Paul Ehrlich's research on synthetic dyes, which specifically stained targets in microorganisms. In collaboration with Hoechst Farbwerke, Ehrlich's team developed a targeted library of hundreds of compounds. In the fall of 1909, compound No. 606 from his library, named salvarsan, dramatically proved the validity of his idea [1]. A new generation of drugs that recognized specific targets are opening new perspectives for cancer treatment. The drugs which were initially approved for oncological indications were oriented against single specific targets. They raised tremendous hopes and excitement as well as disappointment [3]. The treatment of chronic myelogenous leukemia with imatinib resulted in dramatic responses and durable results in many patients. This is easy to understand since there is a clearly identifiable molecular target for imatinib in Philadelphia chromosome. The drugs which were developed to interrupt signal transduction in the pathways involving the members of the family of human epidermal growth factor receptor (HER) were less successful. The gene for HER2 (*ERBB2*) is amplified in approximately 1/3 of breast cancers. Trastuzumab, which is anti-HER2 antibody, may produce responses up to 35% of patients with marked over-expression of *ERBB2* in cancer cells [4]. Epidermal growth factor receptor (EGFR/ERBB1) inhibitor gefinitib was not as successful. It was approved by the FDA as a third line therapy for non-small-cell lung cancer based on phase II trials [5]. The results of phase III trial of gefitinib, in addition to chemotherapy, were disappointing and did not confirm prior clinical observations [6, 7]. To the contrary, the addition of chemotherapy enhanced responses to trastuzumab and resulted in extension of patients' survival [8].

Primary brain tumors are among the most disappointing diagnoses, both for patients and their physicians. With the exception of a small percentage of patients who have successful total surgery, in the majority of cases the disease will progress and lead to death, despite radiation therapy and chemotherapy [9]. The addition of temozolomide (TMZ) improved short-term results but did not change the final outcome [10, 11]. Further research confirmed that the benefits of TMZ were limited to the minority of patients with methylation of the promoter of 0_6-methylguanine-DNA methyltransferase (MGMT) [12]. Increased angiogenesis in brain tumors raised hopes that antiangiogenic therapy will provide better results. The first antiangiogenic drug approved by FDA-thalidomide was used in phase II trials of glioblastoma multiforme (GBM) and anaplastic astrocytoma (AA). Four different clinical studies using thalidomide as a single agent, and in combination with TMZ, carboplatin and radiation therapy did not provide the proof of efficacy and contributed to serious thromboembolic events [13-17]. It is the expert's opinion that the advancement in treatment of patients with brain tumors has been negligible and a completely new approach is needed for a significant change [9, 18]. The present chapter is trying to answer the question if targeted therapy may improve dismal results of the treatment of brain tumors? The author reviews the most

important strategies which resulted in introduction of the agents which can be useful in the treatment of brain tumors. The results of phase II trials are discussed and summarized.

Targeted Therapeutic Agents Used for Treatment of Primary Brain Tumors

The emphasis of research on pharmaceutical agents is centered on drugs effecting pre-selected targets. The last 10 years have seen dynamic expansion of the interest in this area. From approximately 500 molecular targets in 1996, the current therapeutic target database contains over 1200 targets [19]. In the area of neoplastic diseases, the current list includes 392 targets [19]. The therapeutic targets are extensively described in the literature. In this chapter, the attention is concentrated on the agents used in phase II clinical trials for primary brain tumors. Most of these agents affect single targets, including growth factor receptors, signal transduction pathways, G1-S transition, apoptosis, membrane transport or epigenetic changes. (Figure 1) Table 1 lists currently used drugs and their targets.

Figure 1 – See end of the section for colour presentation

The agents developed by our team, antineoplastons (ANP) are multitargeted therapeutics [20]. Four of these agents A10I (Atengenal), AS2-1I (Astugenal), A10 (Cengenal) and AS2-1 (Fengenal) are the subject of extensive clinical trials which are described in this chapter.

Pharmacological Targets for Currently Used Agents

Formation of brain tumor originates from dysfunction of many different regulatory pathways which consists of increased function of oncogenes and loss of the activity of tumor suppressor genes. Genetic and epigenetic mechanisms produce amplification, or over-expression of growth factors and their receptors, including epidermal growth factor (EGF), platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and transforming growth factor β1 (TGFB1). Beyond the membrane receptors, a number of oncogenes and tumor suppressor genes are involved in intracellular signal transduction and protection of neoplastic cells from apoptosis. The most important genes for the design of targeted therapy are oncogenes *RAS, AKT, mTOR, MYCC* and *MDM2*, and tumor suppressors *TP53, NF1, p21*^{C1p1WAF1} *(p21), PTEN,* and *INI1.* Finally, exploring differences in cellular and nuclear transport between normal and neoplastic cell may indicate additional targets for new agents.

Table 1. Targeted Therapeutic Agents in Clinical Use for Primary Brain Tumors

Agent	Brand Name	Company	Target	Type	Status Brain Tumors	Indication Approved
Generic Name						
Imatinib	Gleevec	Novartis	BCR-ABL	TKI	Phase II	Chronic myelogenous leukemia
Gefitinib	Iressa	Astra Zeneca	EGFR	TKI	Phase II	Advanced non-small cell lung cancer
Erlotinib	Tarceva	Genentech/OSI	EGFR	TKI	Phase II	Non-small cell lung cancer, pancreatic cancer
Lonafarnib	Sarasar	Shering-Plough	RAS	Peptidomimetic	Phase II	
Bevacizumab	Avastin	Genentech	VEGF	Monoclonal antibody	Phase II	Colorectal cancer in combination with 5-fluorouracil
Enzastaurin		Eli Lilly	PKC-β2 (VEGF)	PKC-β2 inhibitor	Phase II	
Temsirolimus (CCI-779)		Wyeth	mTOR	mTOR inhibitor	Phase II	
ANP - A10I	Atengenal	BRI	Multitargeted	DMM	Phase II	
ANP - AS2-1I	Astugenal	BRI	Multitargeted	DMM	Phase II	
ANP - A10	Cengenal	BRI	Multitargeted	DMM	Phase II	
ANP - AS2-1	Fengenal	BRI	Multitargeted	DMM	Phase II	

ANP - antineoplaston, BCR-ABL - BCR-ABL tyrosine kinase, DMM - DNA methylation modifying agent, EGFR - epidermal growth factor receptor, m-TOR - mammalian target of rapamycin, PKC-β2 - protein kinase C-β2, RAS - *RAS* oncogene, TKI - tyrosine-kinase inhibitors, VEGF - vascular endothelial growth factor.

Inhibition of Growth Factor Signaling

Interruption of signal transduction originating from activation of EGFR, PDGF receptor (PDGFR), VEGF receptor (VEGFR) and TGFBI receptor (TGFBR1) served as the basis for the design of a number of target-oriented agents.

EGFR

EGFR/ERBB1 is a member of a subclass 1 family of the receptor tyrosine kinase (RTK), which comprises of three additional members: ERBB2, ERBB3 and ERBB4 [21]. The receptors consist of three major parts: an extracellular region which binds ligands, a membrane-spanning region and RTK domain in the cytoplasm. The ligands which bind EGFR include EGF, transforming growth factor-α (TGFA), amphiregulin, betacellulin, epiregulin, and heparin-binding EGF. After ligand binding, EGFR forms homo- and heterodimers which leads to activation of RTK, and triggers intracellular signaling.

In gliomas, EGFR is activated by mutation, amplification, and paracrine or autocrine biosynthesis of EGF and other ligands [22]. The most common change of EGFR is amplification which occurs in approximately 50% of primary GBM [23]. Such alteration is less common in low grade glioma. EGFR amplification is frequently associated with mutation in the extracellular domain, known as EGFRvIII variant [24]. This mutation which consists of a 5' deletion of codons 6-273, and allows activation without ligand binding, is found in 67% of EGFR positive tumors [25]. As much as 1/3 of GBM have multiple secondary changes which are associated with amplification and mutation of the *EGFR* [22]. Abnormalities of EGFR increase tumor invasiveness through up-regulation of matrix metalloproteinases (MMPs) and decrease of apoptosis through activation of BCL-X_L [26, 27].

A group of agents which target EGFR is subject of phase II trials in brain tumors. Small molecules, gefitinib and erlotinib, are competitive specific and reversible inhibitors of the ATP binding to the catalytic site of RTK of the intracellular domain of EGFR. Gefinitib was initially approved in Japan in 2002 and in the U.S. in May 2003 for the treatment of advanced non-small cell lung cancer after failure of other therapies [28]. Gefinitib inhibits receptor autophosphorylation and interrupts intracellular signal transduction. In animal tests using athymic mice with overexpression of EGFR in brain tumors, gefitinib substantially reduced EGFR phosphorylation and increased median survival by approximately 90% [29]. Its action, however, is limited only to cells with EGFR amplification, without EGFRvIII mutation [30]. Erlotinib shares similar mechanisms of action with gefinitib. It has been approved in the U.S. in November 2004 for the treatment of advanced non-small cell lung cancer after failure of at least one prior chemotherapy regimen, and contributed to increase in survival in phase III trials [31]. Erlotinib underwent only limited preclinical testing in brain tumors. The results of phase II trials of gefitinib and erlotinib in high grade gliomas are described in this chapter.

PDGFR

PDGFR and its ligand PDGF play an important part in pathogenesis of gliomas. The PDGFR is a protein tyrosine kinase receptor. Chemically, it is a glycoprotein and contains extracellular, transmembrane and intracellular domains [32]. The external domain binds PDGF ligand which leads to dimerization and autophosphorylation of tyrosine residues in

intracellular domains. This creates the sites for the attachment of factors instrumental in signal transduction through important pathways, including RAS, AKT and STAT [33]. PDGFR exists in two forms, α and β, and PDGF in four forms: A, B, C and D. Both PDGFR and PDGF form dimers of various chains (homo- and heterodimers) [22, 32, 34]. PDGFR and PDGF play an important function in the development of the central nervous system, and they are necessary for normal gliogenesis and myelination [35]. Aberrant upregulation of the genes seems to be the main factor which increases expression of PDGFR and PDGF. Another important factor is autocrine stimulation which occurs early in formation of brain tumors and leads to PDGFR expression in most cases [32, 34, 36, 37]. PDGF-A and B are expressed in all gliomas. There is reduced expression of PDGF-A in low grade gliomas, but it is markedly increased in GBM. To the contrary, there is marked expression of PDGFR-α in low and high grade gliomas. PDGFR β is expressed at low levels in all grades.

Imatinib mesylate is the first RTK inhibitor which was successfully introduced for the treatment of chronic myelogenous leukemia and gastrointestinal stomal tumors (GISTs). By interfering with binding of ATP to RTK, imatinib interrupts signal transduction mediated by PDGFR, BCR-ABL, and c-kit [38]. Cell culture and animal tests confirmed activity of imatinib against GBM [39]. The rationale for the use of imatinib in the treatment of brain tumors is based on blocking the activity of not only mutant BCR-ABL and c-kit, but also PDGFR-α kinase [40]. The results of phase II trials are described in this chapter.

VEGFR

The growth of solid tumor is dependent on formation of new blood vessels [41]. VEGF which was isolated as a factor which stimulates endothelial cell division plays a pivotal role in angiogenesis of brain tumors [42]. VEGF family consists of ABCDF and E members, and VEGF-A has at least four isoforms [43]. The members of VEGF family are glycoproteins which form homodimers and bind three different receptors: fms-like tyrosine kinase (FLT1/VEGFR1), kinase insert domain-containing receptor (KDR/VEGFR2), and fetal liver kinase (murine KDR/FLK1) [42, 43]. The receptors are expressed on endothelial and some neoplastic cells. Other stimulators of angiogenesis include fibroblast growth factor (FGF), PDGF and hypoxia-inducible factor 1 (HIF-1). HIF-1 is αβ heterodimer. The HIF-1α transcription factor is stabilized in the absence of oxygen, but HIF-1β occurs at uniform levels in the nucleus. In hypoxic conditions, HIF-1α accumulates in the nucleus, forms heterodimers with a β subunit, and initiates transcription of genes instrumental in angiogenesis and metastasis; among them VEGF [44].

Bevacizumab is a humanized monoclonal antibody against VEGF, which was approved by the U.S. FDA in February 2004 as the first-line treatment of metastatic colorectal cancer, in combination with 5-fluorouracil [45]. Initially, it has been contraindicated in the treatment of brain tumors due to the possibility of intracerebral hemorrhage. Recently conducted phase II trial in high grade glioma is described in this chapter.

TGFBRI

The transforming growth factor β, may have both positive and negative effect on brain tumor progression. It inhibits early stages of neoplastic processes through apoptosis and arrest of G1-S cell cycle transition, but promotes the late stages through angiogenesis and metastasis

[46]. TGFB is a polypeptide which exists in three versions: TGF- β1, β2 and β3. TGFB belongs to a super family of more than 30 ligands which are produced by many different kinds of cells, and also include activins, inhibins and bone morphogenic proteins [47]. They are polypeptides which have a common cluster of conserved cysteine residues connected by intramolecular disulfide bonds. TGFB is a product of the gene located on chromosome 19q, and is proteolytically cleaved to 25 kd size. TGFB is bound by TGFB-binding protein and is released by thrombospondin-1, or plasmin [22]. TGFB binds to receptor complex consisting of type I and II (TGFBRI and TGFBRII). The receptors which are serine-threonine kinases are activated by TGFB binding to TGFBRII, which phosphorylates TGFBRI in a glycine-serine-rich region (GS box) [48]. Activated TGFBRI promotes signal transmission through a number of different signaling pathways [46]. They can be grossly divided into Smad and non-Smad pathways. Currently, there are over 10 different Smad proteins which are homologs of the *Sma* and *Mad* genes of *Drosophila melanogaster* and *Caenorhabditis elegans*. The complex consisting of TGFB, TGFBRI and TGFBRII binds to the complex of Smad2, Smad3 and Smad-anchor for receptor activation (SARA) and phosphorylates Smad2 and Smad3. The phosphorylation releases Smad2/3 from a cytoplasmic protein and allows binding to Smad4 and migration into the nucleus [48]. Inside the nucleus, Smad2-4 complex regulates the transcription of numerous genes [46]. Among others it induces *INK4b (p15)* and *p21*, and represses *MYCC* and *cyclin-A*. The transmission in TGFB pathway is affected not only by Smad proteins, but also through other pathways, including phosphoinositide 3-kinase (PI3K)/AKT and mitogen-activated protein kinase p38 (p38 MAPK) [49]. Smads play an important part in TGFBs growth inhibition and apoptosis, but p38 and PI3K/AKT regulate transcription and motility [50]. The abnormalities in TGFB/Smad signaling pathway promote neoplastic growth and can occur through mutations of *TGFBRI, TGFBRII, SMAD2 and SMAD4*, which leads to the loss of expression of TGFB1 receptors and Smads. The increased activity of TGFB1 makes neoplastic cells more aggressive, and increases angiogenesis. The role played by TGFB in pathogenesis of gliomas is not completely understood [51]. TGFB is released by high grade glioma cells which also express the receptors [52]. GBM cells are expressing TGFBRI/II and TGFB2 at a higher level than low grade gliomas [53]. On the other hand, Smad2-4 are expressed at a lower level in some high grade glioma [54]. TGFB also down-regulates expression of PTEN [55].

Pre-clinical tests confirm the validity of targeted therapy directed against TGFBI [51, 56]. PG, which is the main ingredient of A10I, decreases expression of TGFB1, and shows activity against brain tumors in phase II studies as described later in this chapter [57, 58]. (Figure 2)

Figure 2 – See end of the section for colour presentation

Inhibition of Signal Transduction Pathway Intermediates

The most important signaling pathways in the treatment of brain tumors are RAS and PI3K/AKT. These pathways can transmit signals from any of the ligand-receptor systems described before.

RAS

RAS proteins and MAPK form one of the most important signaling systems for cell proliferation. It receives signals from growth factors receptors and transmits them to cell nucleus [59]. The main position in the pathway is occupied by RAS protein which is guanosine triphosphatase (GTPase) RAS. In mammals, there are four RAS proteins HRAS, NRAS and KRAS 4A and KRAS4B, which are the products of 3 genes [60]. *KRAS* gene is most frequently mutated (approximately 80-85% of total *RAS* mutation). *NRAS* and *HRAS* are mutated in lesser percentage of cases (correspondingly, 15% and 1%) [61]. The proteins which are products of oncogenes located on chromosomes 11p, 12p and 1p respectively encode very similar molecules but with different carboxyl termini. RAS proteins require modification through farnesylation, proteolytic cleavage, carboxymethylation and palmitoylation. NRAS and KRAS4A are acylated with one palmitoyl residue, HRAS with two and KRAS4B contains C-terminal polybasic amino acids instead of palmitoyl moiety [62]. The most important step in activation of RAS is farnesylation facilitated by farnesyltransferase (FTase), which attaches 15 carbon isoprenoid fragment. RAS functions at the plasma membrane and in Golgi complex [63]. Palmitoylated proteins are present in the Golgi and at the membrane; whereas, KRAS4B is attached only to the membrane. In this respect, there are two signaling platforms, membrane and the Golgi, but it is not known at present how these pathways are segregated [64]. Since RAS is involved in fundamentally different processes, proliferation and differentiation, its distribution to two cellular compartments may explain diverse aspects of the activity. The advantage of the Golgi location is the proximity of the nucleus which is at the receiving end of the signal [62]. At the membrane, RAS GDP is rapidly converted to RAS GTP as the result of binding ligands to growth factor receptors described earlier in this chapter. Adapter protein GRB2 binds SH2 domains of autophosphorylated receptor and conduct the signal to RAS guanine-nucleotide exchange factors (GEFs). After binding SH2 domains of GRB2, GEF activates RAS through the exchange of GDP to GTP. RAS GTP conducts signals to numerous effectors which are responsible for the variety of biological activities: transcription (RAF/MEK/ERK), cell cycle progression (PI3K/AKT) translation (PI3K/PDK1) apoptosis (RASSF-1), cell-cell junctions (AF6), endocytosis (RIN1), membrane (RAL) and nuclear transport (PLCε) [59]. In addition to various GEFs (the best known are SOS1/2), there are numerous naturally occurring inhibitors of RAS activation. RTK is inhibited by SPROUTY and ACK, which bind GRB2, and a number of GTPase-activaging proteins (GAPs). Among them is neurofibromin, the product of neurofibromatosis 1 gene (*NF1*). In this respect, inactivation of *NF1* activates RAS [65].

RAS gene is frequently mutated in human cancers (in approximately 30% of all cancers). Mutated *RAS* is losing sensitivity to hydrolysis catalyzed by GAP, which results in increased signal transmission. The mutations are rare in gliomas, but increased activity of RASGTP was found in GBM, and high grade astrocytomas [22, 66, 67]. It is generally accepted that the activation of RAS signaling in brain tumors is due to increased activity of receptors RTKs.

Agents which inhibit farnesylation of RAS have shown efficacy and low toxicity in pre-clinical and clinical studies [68]. The beneficial results, however, were limited to hematological malignancies [69]. Such agents fell short of expectations in the treatment of solid tumors [70]. The inhibitor of farnesyltransferase, lonafarnib has been used in phase II

studies of GBM, and the results are described in this chapter. One of the reasons for failure of these agents is "alternative prenylation" which permits modification of KRAS by geranylgeranyltransferase-1 (GGTase-1) [71, 72]. The alternative inhibition of farnesylation of RAS can be obtained through depletion of isoprenoids in cells [70]. This can be accomplished by inhibition of 5-pyrophosphomevalonate decarboxylase by PN (main ingredient of ANP AS2-1I) [73]. Interruption of signal transduction downstream of RAS protein creates interesting targets for anticancer agents. Based on the studies in breast cancer cell line, PG and isoPG (ingredients of A10I) inhibit phosphorylation of ERK [74]. The results of phase II trials with ANP are described in this chapter. (Figure 3)

Figure 3 – See end of the section for colour presentation

PI3K/AKT

PI3K/AKT pathway is of primary importance in pathogenesis of brain tumors [66]. The signal orginates from activation of PI3K which can be accomplished by numerous growth factor receptors and RAS pathway. The most important include insulin-like growth factor 1 receptor (IGF-1R), EGFR, PDGFR and VEGFR [75]. Second messengers generated by PI3K, phosphatidylinositols (PtdIns): PtdIns (4,5) P_2 (PIP_2) and PtdIns (3,4,5) P_3 (PIP_3) recruit AKT (which is serine-threonine kinase)to the cell membrane. AKT family includes three members: AKT1, AKT2 and AKT3, which are phosphorylated by 3-phosphoinositide-dependent protein kinase – 1 (PDK1) and PDK2. PIP_3 is inactivated through conversion to PIP_2 by phosphatase and tensine homolog (PTEN). This mechanism resembles inactivation of RASGTP by GAPs. AKT activates and inhibits numerous targets. Through activation of mTOR it promotes cell cycle progression, initiation of translation and survival. Inhibition of BAD and FOXO factors, and activation of NFκB by AKT blocks apoptosis. This is also accomplished through increased activity of MDM2 which inhibits p53 [76]. High grade gliomas show increased activity of PI3K/AKT pathway but amplification of AKT has not yet been described in human astrocytomas [77, 78]. The main mechanism is inactivation of PTEN, which is deleted in the majority of high grade gliomas, and increased activity of EGFR, PDGFR and RAS signaling [22, 78]. Increased activity of AKT is mediated in substantial degree by mTOR, especially when there is a loss of PTEN [79]. The impact of mTOR pathway is substantial in maintaining the transformed phenotype of cancer cells, but mTOR mutations, or overexpression, have not yet been reported in human cancers [76]. mTOR inhibitor temsirolimus has been used in phase II trials of recurrent GBM. PG, which is the main ingredient of ANP A10I, decreases expression of AKT2 and activates PTEN (through inhibition of TGFB1) [57, 58]. The results of phase II studies of temsirolimus and ANP are described in this chapter. (Figure 4)

Figure 4 – See end of the section for colour presentation

Inhibition of G1-S Transition

Progression of neoplastic process requires continuous cell divisions which occur in mitosis (M) phase of cell cycle. Before mitosis occurs, the cell must replicate its DNA in synthesis (S) phase. Two additional phases separate S and M. In G1 phase between the end of

mitosis and beginning of DNA synthesis, the decision is made within the cell to replicate its DNA or rest. In G2 phase (between S and M phases) the cell activates the mechanisms which prevent mitosis in case of DNA damage and delay cell division until the damage is repaired. At G1-S checkpoint, the cell is under influence of numerous signaling pathways. Among these pathways RAS and PI3K/AKT are the most important. Transition from G1 to S and initiation of DNA synthesis would not occur unless there is activation of cyclin-dependent kinases 2 and 4 (CDK2/4). In order to accomplish their tasks, CDKs form active catalytic complexes with cyclins; CDK2 with cyclins E1, E2 and A, and CDK4 and, CDK6 with cyclins of D type. At the beginning of G1, *MYCC* oncogene through MYC-MAX dimers activates genes *CDK4* and *CCND2*, which express CDK4 and cyclin D2 [80, 81]. *MYC* family of oncogenes (*MYCC, MYCL* and *MYCN)* play an extremely important part in regulation of G1-S transition [82, 83]. These oncogenes are over-expressed in over 50% of all malignancies, usually through epigenetic mechanisms and interact directly and indirectly with close to a thousand targets. MYC proteins are stabilized and activated by phosphorylation of serine 62 (Ser 62). Degradation (ubiquitination) of MYC is facilitated by phosphorylation of threonine 58 (Thr58) [84]. These processes are regulated by RAS and AKT signaling. In RAS pathway, the cascade of events (RAF1/extracellular signal-regulated kinase-ERK) affects phosphorylation (activation) of MYC at Ser 62 [85]. AKT prevents MYC degradation through inhibition of glycogen synthase3- β (GSK3-β), and phosphorylation of Thr58 [83]. Complex cyclin D/CDK4 binds and commit to ubiquitination an inhibitor of CDK2-KIP1 (p27) [86]. KIP1 serves also as an inhibitor of G1-S transition, and its inactivation is promoted by two genes, *CUL-1* and *CKS*, targeted by MYC [87]. In this respect, inhibition of MYC prevents formation of cyclin D2/CDK4 complex and inactivation of KIP1. Such inhibition can be accomplished through interruption of RAS and AKT signaling and MAD family proteins [81]. Neutralization of KIP1 activity eliminates inhibition of cyclin E/CDK2 complex. Elevated concentrations of cyclin E are necessary for entering S phase [88]. The expression of cyclin E is regulated by E2F factors [89]. At the beginning of G1 these factors are sequestrated by the retinoblastoma protein (Rb), and its related proteins p107 and p130. E2F1, E2F2 and E2F3 are inactivated, whereas, E2F4 and E2F5 become repressors [89]. Rb is inactivated through phosphorylation by complexes cyclin D/CDK4 and cyclin D/CDK6 [90]. Phosphorylation releases E2F from Rb binding and allows its function as a transcription factor for cyclin E, DNA polymerase α and other proteins necessary for DNA replication. Additional inhibitors of cyclin/CDK complexes are INK4A (p16), p15 and p21. p15 and p21 inhibit cyclin E/CDK2 and arrest cell cycle progression [91]. MYC-MAX together with MYC-interacting zinc finger protein 1 (MIZ1) blocks activation of *CDKN1A* and *CDKN2B* which are genes encoding p21 and p15 correspondingly. Cyclin D1/CDK4 is inhibited by p16 which is activated by INI1 tumor suppressor gene protein. On the other hand, AKT2 phosphorylation of GSK3-β prevents inactivation of cyclin D (phosphorylation of cyclin D by GSK3- β causes its destabilization) [92].

ANP affects multiple targets at G1-S transition. The mechanisms involving RAS and AKT signaling pathways were described previously [20, 57, 58, 73]. In addition, PN, PG and isoPG activate p21and PG increases expression of MAD through MADS box transcription enhancer factor (MEFD2D) [57, 58, 74, 81, 93]. PG and isoPG increase the expression of p16 [74]. (Figure 5)

Figure 5 – See end of the section for colour presentation

Induction of Apoptosis

Induction of neoplastic cell death is the final goal of any targeted therapy, but some regimens used in treatment of brain tumors, explore also direct apoptotic mechanisms. Apoptosis is a highly complex process and it is the subject of numerous excellent reviews [82, 94-98]. It proceeds through two main pathways. Extrinsic pathway leads through CD95 receptor, tumor necrosis factor (TNF) and TNF-related apoptosis-inducing ligand (TRAIL) [99-101]. The main event in the second (intrinsic) pathway is the release of cytochrome c from mitochondria [102]. The extrinsic and intrinsic pathways are united in the common cascade of activation of proteolytic enzymes, caspases, which together with cytochrome c and apoptotic protease-activating factor 1 (APAF1) form apoptosome (wheel of death) and degrade proteins. Proteins of BCL-2 family play an important part in regulation of apoptosis. Anti-apoptotic proteins, BCL-2 and BCL-X_L inhibit pro-apoptotic BAX. Proteins BAX and BAD promote mitochondrial-outer-membrane permeabilization (MOMP) and release of cytochrome c. BAD is inhibited by RAS and AKT, and BAX is activated by p53 [82]. p53 protein plays a central role in this process, and its loss leads to inhibition of apoptosis [94, 103, 104]. Through transactivation of p21, p53 protein causes arrest of the cell cycle at G1-S checkpoint, and is also instrumental in transcription of many apoptosis-associated genes including *BAX* [105-107].

ANP are among agents used in phase II studies of brain tumors, which directly target apoptotic machinery. PN increases expression of p53 and p21, and inhibits BCL-2 [108-110]. PG inhibits BCL-X_L [57, 58]. Indirectly, PN and PG promote apoptosis of neoplastic cells through interruption of signal transmission in RAS and AKT pathways [20, 57, 58, 73]. (Figure 6)

Figure 6 – See end of the section for colour presentation

Inhibition of Membrane Transport

Cellular Membrane Transport

Active cancer growth requires effective transport of molecules through the cell membrane and also continuous shuttling of signaling proteins between nucleus and cytoplasm. Differences in membrane transport may create targets for anti-cancer drugs and they are utilized in ANP therapy of brain tumors. PG inhibits the uptake of growth-critical amino acids, such as L-glutamine and L-leucine in glioma cells [20, 57]. PG enters cells via the stereo-specific amino acid transporters for L-glutamine, decreases intracellular concentrations of L-glutamine and L-leucine, which contributes to anti-neoplastic activity.

Nuclear Transport

Important signaling molecules move back and forth between the cytoplasm and nucleus. The nuclear localization of the products of important tumor suppressor genes *TP53* and *INI1*

is necessary for their function. The mutation of INI1 gene plays a crucial part in development of atypical teratoid/rhabdoid tumor (AT/RT), and some cases of GBM [111, 112]. INI1 represses transcription of cyclin D1 and induces p16, blocking G1-S cell-cycle transition. Mutation of the *INI1* gene at 22q11 effects nuclear export signal and causes INI protein to migrate to the cytoplasm where it is not active [113]. INI1 transport from the nucleus to the cytoplasm also depends on RanGTP/RanGDP gradient, and Ran binding protein 1 (RanBP1), which causes increased concentration of RanGDP in the cytoplasm. PG decreases expression of RanBP1 and prevents escape of INI1 from the nucleus [57, 58, 114].

Modification of Epigenetic Changes

Aberrant methylation of promoters of tumor suppressors and decreased global methylation are the most common epigenetic changes occurring in primary brain tumors [12, 115-119]. Increased methylation of promoters causes silencing of numerous genes, including p15 and p16. It is important to note that silencing of p16 occurs in approximately 25% of gliomas and of the tissue inhibitor of metalloproteinase-3 (TIMP-3) in up to 80% [118, 119]. Global hypomethylation is very common and was found in both low and high grade tumors. It is causing genomic instability, amplification of oncogenes, multi-drug resistance, and is more pronounced in high grade tumors [119-121]. Mutation of *INI1*, which is part of SWI/SNF complex contributes to hypomethylation. Methylation is associated with chromatin remodeling and histone modification [122, 123]. Acetylation of lysine in histone tails loosens compact chromatin structures and allows gene expression. The reverse process – deacetylation catalyzed by histone deacetylase (HDAC) blocks gene expression. Pharmacological agents which modify abberant DNA methylation and histone acetylation create attractive possibilities for the treatment of brain tumors. Unfortunately, most of currently used agents lack specificity. The first drug in this group, azacitidine, an inhibitor of DNA methylation, was approved by U.S. F.D.A. for the treatment of myelodysplastic syndromes in May, 2004 [124].

ANP normalizes aberrant DNA methylation. PG affects global hypomethylation and PN decreases methylation of the promoters of tumor suppressor genes and inhibits HDAC [2, 58, 115].

Phase II Clinical Studies of Targeted Therapy in Brain Tumors

Treatment of High Grade Glioma

High grade gliomas (HGGs) represent approximately 60% of primary brain tumors and include GBM, AA, anaplastic oligodendroglioma and anaplastic oligoastrocytoma. GBM is clinically most important and affects approximately 12,500 patients in the U.S. [125]. With traditional treatment, median survival is 9-10 months with most patients dying within 2 years [126]. Standard treatment for newly diagnosed GBM consists of surgical resection followed

by radiation therapy. Adjuvant chemotherapy with nitrosourea did not produce a survival benefit according to randomized phase III trial of 674 patients [127]. The prognosis is even poorer for recurrent high grade glioma with median survival of approximately 7 months for chemotherapy [128]. Introduction of TMZ raised hopes for more successful treatment [129, 130]. Phase II study of TMZ versus procarbazine in patients with GBM at first relapse confirmed improved overall survival (OS), progression-free survival (PFS), as well as percentage of objective response in patients treated with TMZ [131]. Phase III trial involving 573 patients with newly diagnosed GBM compared overall survival of patients treated with radiation therapy alone versus radiotherapy plus concomitant and adjuvant TMZ[10]. The addition of TMZ to radiotherapy for newly diagnosed GBM produced statistically better survival with low toxicity. The results of recent trials are summarized in Table 2.

Imatinib successfully introduced for the treatment of chronic myeloid leukemia has been tested in a number of different phase II studies in recurrent GBM and AA as a single agent or in addition to hydroxyurea [132-135] . In studies with imatanib as a single agent, there was no significant benefit measured as PFS and objective response. Combination treatment with hydroxyurea provided better results with 3% of complete response (CR), 13% of partial response (PR), 37% of stable disease (SD) and 32% of PFS at 6 months [134]. Gefitinib was tested in recurrent GBM with no confirmed objective responses and PFS at 6 months from 12 to 14% [136, 137]. Additional 2 separate phase II studies with erlotinib in recurrent GBM produced better results. Vogelbaum, et al. reported 21% PR in 24 patients and Cloughesy et al., 2% of CR and 6% of PR in 48 patients [138, 139]. Bevacizumab in combination with irinotecan was administered to 21 patients with relapsed high grade glioma in recently reported phase II study [140]. The results consisting of 5% CR and 38% PR were encouraging. Farnesyltransferase inhibitor, lonafarnib, was administered in combination with TMZ to 23 patients with recurrent GBM, and contributed to 13% of PR and 44% of SD. PFS at 6 months was 13% which was higher in procarbazine but lower than TMZ study [141]. mTor inhibitor, temsirolimus and PKC-β2 inhibitor, enzastaurin did not produce significant responses in phase II studies in recurrent GBM and HGG [142, 143]

ANP therapy was studied in phase II trials of 22 patients with relapsed GBM, and contributed to 9% of CR and PR, 54.5% of SD and PFS at 6 months of 50% [144]. The analysis of the results of treatment of 173 evaluable patients with recurrent and progressed GBM in phase II trials with ANP have shown significant percentage of response rates and long-term survival (LTS) [145]. Seventy-nine of these patients were treated in the study protocols and an additional 94 patients were treated under special exception (SE) due to low Karnofsky Performance Status (KPS) below 60. 98% of patients failed prior surgery, radiation therapy and/or chemotherapy [145]. LTS was defined as survival more than 3 years after the initial diagnosis of GBM. For patients in the study group (SP), the LTS was 15.5% and in SE group was 7.1%. The maximum survival in SP was more than 13 years and in SE was more than 10 years. The results of targeted therapy in GBM and HGG, including additional data on ANP, are summarized in Table 3.

Treatment of Brain Stem Glioma

Brain stem gliomas (BSGs) are among the most difficult to treat brain tumors. They are relatively uncommon and constitute less than 10% of all primary tumors of the central nervous system [146, 147]. Approximately 80% of these tumors occur in children and 20% in adult patients. Based on MRI appearance they are usually classified in four different types: focal, dorsal exophytic, cervicomedullary and diffuse intrinsic (DBSG) [148]. DBSGs, which occur in 85% of cases, are the most difficult to treat. This is, however, not a homogenous group. These tumors have different histopathology and different clinical course, depending on the age of the patient. In infants, DBSG is more aggressive, but on the other hand it has as slower clinical course in patients between the ages of 22 to 39, and when it is associated with neurofibromatosis type I [147]. Patients over the age of 40 have worse prognosis and higher percentage of GBM (over 30%). Based on autopsy results, the majority of patients have AA. There is a lower incidence of a low-grade astrocytoma and GBM. Close to 50% of young adults less than 40 years of age have low-grade glioma [147]. It should be noted that only approximately 25% of BSG patients have a biopsy and that histopathology diagnosis is not accurate due to difficulty in securing sufficient size of sample in a strategic area of brain. It is also possible that a specimen taken from one area of the tumor does not represent histopathology of the area which was not biopsied. It is a general consensus of neuro-oncologists that all DBSG should be treated as high-grade gliomas. On the other hand, the remaining three types of BSG usually have histopathological diagnosis and are treated the same way as tumors in other parts of the brain. The aggressive behaviour of DBSG is manifested also by dissemination through cerebrospinal fluid in approximately 20% of cases. The data on genetic abnormalities in DBSG are quite limited, but they indicate that in more than 30% of these patients, EGFR protein is detected and half of these patients have *TP53* mutated [149]. *EGFR* gene is amplified only in some patients, and in half of the cases there is loss of *PTEN* [150, 151].

DBSG are non-resectable. Radiation therapy is the main treatment for newly diagnosed tumors [152]. A number of studies confirmed that standard radiation therapy is preferable over hyperfractionation and combination with chemotherapy [152, 153]. The prognosis is poor with approximately 7% survival at 2 years and no survival over 5 years in most of the studies [147, 152, 153]. Numerous chemotherapy regimens including TMZ given before, concomitant and after radiation therapy and high-dose treatment with autologous bone marrow transplantation did not improve the results and contributed to toxicity [154-162]. The prognosis is especially poor for recurrent DBSG with no standard chemotherapy available, including TMZ [11]. Patients with recurrent DBSG usually do not survive longer than 6 months, despite the treatment given.

Table 2. Results of Radiation Therapy in Combination with Chemotherapy for Glioblastoma Multiforme

Author	Study Type		Patients N	Treatment		Efficacy								
				Radiation Therapy	Additional Chemotherapy	OS			MST	PFS 6 M	CR	PR	SD	PD
				Gy		1 yr %	2 yrs %	5 yrs %	M	%	%	%	%	%
Yung et al., 2000 (131)	Phase II	Arm 1	112	None	Temozolomide	25	NA	NA	NA	21	0	5	40	NA
	RGBM	Arm 2	113	None	Procarbazine	15	NA	NA	NA	8	0	5	27	NA
Stupp et al., 2005 (10)	Phase III	Arm 1	286	60	None	50.6	21.2	NA	12.1	36.4	NA	NA	NA	NA
	NGBM	Arm 2	287	60	Temozolomide	61.1	26.5	NA	14.6	53.9	NA	NA	NA	NA

CR - complete response, M - months, MST - median survival time, N - number, NA - not available, - NGBM - newly diagnosed GBM, OS - overall survival, PD - progressive disease, PFS - progression-free survival, PR - partial response, RGBM - recurrent GBM, SD - stable disease

Table 3. Results of Targeted Therapy for Glioblastoma Multiforme and High Grade Glioma

Author	Study Type	Tumor Type	Patients N	Treatment		Efficacy								
				Targeted Therapy	Additional Treatment	OS			MST	PFS 6M	CR	PR	SD	PD
						1 yr %	2 yrs %	5 yrs %	M	%	%	%	%	%
Wen et al. (2004)(132)	Phase II	Total	48	Imatinib	None									
		RGBM	29	Imatinib	None	NA	NA	NA	NA	3	0	3	21	NA
		RAA	19	Imatinib	None	NA	NA	NA	NA	11	0	0	10	NA
van den Bent et al. (2004)(133)	Phase II	RGBM	51	Imatinib	None	NA	NA	NA	NA	NA	0	6	18	NA
Dresemann et al. (2005)(134)	Phase II	RGBM	30	Imatinib	Hydroxyurea	NA	13	NA	NA	32	3	13	37	47
Reardon, et al. (2005)(135)	Phase II	Total	64	Imatinib	Hydroxyurea									
		RGBM	32	Imatinib	Hydroxyurea	NA	NA	NA	NA	26	NA	9	35	NA
		RAA	32	Imatinib	Hydroxyurea	NA	NA	NA	NA	NA	NA	NA	NA	NA
Rich et al. (2004)(136)	Phase II	RGBM*	57	Gefitinib	None	NA	NA	NA	10*	NA	0	0	NA	NA

Table 3. Results of Targeted Therapy for Glioblastoma Multiforme and High Grade Glioma (Continued)

Author	Study Type	Tumor Type	Patients N	Treatment		Efficacy								
						OS			MST	PFS 6M	CR	PR	SD	PD
				Targeted Therapy	Additional Treatment	1 yr %	2 yrs %	5 yrs %	M	%	%	%	%	%
Franceschi et al. (2005)(137)	Phase II	Total	28	Gefitinib	None	NA	NA	NA	NA	14	0	4[†]	18	NA
		RGBM	16	Gefitinib	None	14	6	NA	NA	12	NA	NA	NA	NA
		RAG	12	Gefitinib	None	NA	NA	NA	NA	NA	NA	NA	NA	NA
Vogelbaum et al. (2004)(138)	Phase II	RGBM	24	Erlotinib	None	NA	NA	NA	NA	NA	0	21	21	42
Cloughesy et al. (2005)(139)	Phase II	RGBM	48	Erlotinib	None	NA	NA	NA	10	17	2	6	37	NA
Stark-Vance (2005)(140)	Phase II	RHG	21	Bevacizumab	Irinotecan	NA	NA	NA	NA	NA	5	38	52	NA
Gilbert et al. (2005)(141)	Phase II	RGBM	23	Lonafarnib	Temozolomide	NA	NA	NA	9	13	0	13	44	NA
Galanis et al. (2005)(142)	Phase II	RGBM	52	Temsirolimus	None	NA	NA	NA	NA	10	0	0	NA[‡]	NA
Fine et al. (2005)(143)	Phase II	RHG	85	Enzastaurin	None	NA	NA	NA	NA	NA	1[§]	16[§]	NA	NA
Weaver et al. (2004)(144)	Phase II	RGBM	22	ANP	None	82	27	5	16.4	50	4.5	4.5	54.5	36.5
Weaver et al. (2005)(145)	Phase II	Total	173	ANP	None	58	19	5	13.5	44	6	8	35	51
Study	Phase II	RGBM	79	ANP	None	65	27	8	15.5	39	9	10	29	52
Special Exception	Phase II	RGBM	94	ANP	None	52	12	3	12.7	48	3	6.5	40.5	50

ANP - antineoplastons A10I and AS2-1I, CR - complete response, M - months, MST - median survival time, N - number, NA - not available, OS - overall survival, PD - progressive disease, PFS - progression-free survival, PR - partial response, RAA - recurrent anaplastic astrocytoma, RAG - recurrent anaplastic glioma, RGBM - recurrent glioblastoma multiforme, RHG - recurrent high grade glioma, SD - stable disease.

[*] 79% of patients did not have baseline measurable disease

[†] unconfirmed

[‡] 16 patients had evidence of decrease in T2, signal abnormality on MRI,

[§] based on 79 evaluable patients

Table 4. Treatment of Recurrent and Progressed Intrinsic Brain Stem Glioma with Temozolomide and ANP

Author	Study Type	Patient N	Treatment	Efficacy						
				OS		MST	CR	PR	SD	PD
				2 yrs %	5 yrs %	M	%	%	%	%
Lashford (11)	Phase II		Temozolomide	NA	NA	6.2	0	5.6	16.7	77.8
Burzynski et al. (165)	Phase II		ANP							
Total		65		38.5	21.5	15	20	14	37	29
Study		30		43	30	20	27	20	23	30
Special Exception		35		34	14	13	14	9	48	29

ANP - antineoplastons A10I and AS2-1I, CR - complete response, MST- median time survival, N - number, OS - overall survival, PD - progressive disease, PR - partial response, SD - stable disease

Table 5. Treatment of High-Risk Pediatric Brain Tumor Patients with ANP

Author	Diagnosis	Study Type	Patients N	Efficacy						
				OS		MST	CR	PR	SD	PD
				2 yrs %	5 yrs %	M	% (N)	% (N)	% (N)	% (N)
Burzynski et al. (168)	PNET	Phase II	13	77	46	42	23 (3)	8 (1)	31 (4)	38 (5)
Burzynski et al. (177)	Multicentric Glioma	Phase II	11	100	64	82	36.5 (4)	27 (3)	36.5 (4)	0
Burzynski et al. (114)	AT/RT	Phase II	8	12.5	0	15	25 (2)	12.5 (1)	12.5 (1)	50 (4)

ANP - antineoplastons A10I and AS2-1I, AT/RT - atypical teratoid/rhabdoid tumor, CR - complete response,
PNET - primitive neuroectodermal tumor, M - months, MST - median survival time, M - months, MST - median survival time, N - number,
OS - overall survival, PD - progressive disease, PR - partial response, SD - stable disease.

Therapy, which affects single targets, was not yet studied in DBSG, but multitargeted therapy with ANP shows promising results [163-167]. Phase II studies of ANP are nearing completion. The results, which were recently reported, were based on the total of 65 evaluable patients [165]. The majority of the patients (72%) developed relapsed DBSG after standard therapy and 28% had progressive DBSG without prior treatment. The condition of 35 patients was not satisfactory for the admission to clinical studies (KPS from 40-50), which required SE from the FDA. Two thirds of these patients did not have a biopsy due to the dangerous location of the tumor, but in the remaining group of patients, 3 were diagnosed with GBM, 8 with AA, 5 with astrocytoma, 3 with pilocytic astrocytoma, 1 with oligodendroglioma, 1 with anaplastic mixed glioma and 1 with glioma. The results in comparison with TMZ chemotherapy are shown in Table 4.

Over 40% of the study patients survived over 2 years and 30% over 5 years. CR occurred in 27%, PR in 20%, SD in 23% and progressive disease (PD) in 30% in study patients. Median survival time (MST) for study patients was 20 months.

Another report described the efficacy of ANP in high-grade BSGs [166]. Sixteen patients with inoperable BSG were studied, including 4 with GBM and 12 with AA. In 9 cases, the tumor relapsed and in 1 case persisted after prior treatment and 6 patients did not have radiation therapy or chemotherapy. Thirty-one percent of patients survived over 2 years and 12.5% over 5 years. The maximum survival is over 6 years for recurrent GBM and over 18 years for recurrent AA. Complete and partial responses were obtained in 18.7%, SD in 14% and PD in 37.5%.

Another group of 10 evaluable patients with inoperable BSGs consisted of children less than 4 years' of age [167]. Three of these patients had a biopsy which confirmed 2 cases of AA and one pilocytic astrocytoma. The youngest patient was a 3-month-old infant. Three patients failed prior radiation therapy and chemotherapy. These patients have shown the best survival data; 60% at 2 years, 20% at 5 years and a maximum survival over 7 years. Complete responses were documented in 30%, SD in 40% and PD in 30%.

Treatment of High Risk Pediatric Patients with Primary Brain Tumors

Among pediatric neurooncology patients, there is a substantial incidence of high risk cases. Primitive neuroectodermal tumors (PNETs) are the most common malignancies of the central nervous system in children. They include medulloblastoma, pineoblastoma, supratentorial PNET, and ependymoblastoma [168]. PNET can be successfully treated with surgery, radiation therapy and chemotherapy [169]. Unfortunately, such treatment program is not successful after subtotal resection, dissemination of the disease, age younger than 4 years and certain histopathological and genetic characteristics, including large cell anaplastic variety, and overexpression of MYCC oncogene [170, 171]. Such difficult to treat and high risk patients were accrued to phase II studies with ANP [168]. The results are summarized in Table 5.

Recurrent, progressive multicentric gliomas (MCGs) have poor prognosis with no curative standard treatments available. Solitary low grade gliomas in children and adults were extensively studied and reviewed, but only a small number of reports were published on

MCG [172-175]. For a solitary tumor, the prognosis improves with the extent of surgical resection, which does not apply to MCG. There was no difference in overall survival of patients not treated after surgery versus patients who underwent radiation therapy [172]. The overall survival was also not different for patients after radiation therapy or after radiation and chemotherapy with lomustine. In one of the studies of MCG with 11 patients, 8 patients were treated with different chemotherapy agents, 1 with radiation therapy, 1 with chemotherapy and radiation therapy, and 1 was not treated [173]. Two patients obtained remission of unspecified type, 5 had stabilization and 4 patients died. The other reports describe single cases or a small series without statistical data [172-176]. ANP was used in MCG in phase II trials and the results of the treatment in the first 12 children were already published [177] (Table 5).

Atypical teratoid/rhabdoid tumors (AT/RTs) are highly malignant but rare pediatric neoplasms. Standard treatment includes surgical resection followed by radiation therapy and chemotherapy [178, 179]. The responses to the standard therapy have been described, but unfortunately they are short-lasting and are followed by progression and patient death [178-181]. Typical feature of AT/RT is mutation of INI1 gene [111, 112]. INI1 arrests the cells at the G1-S checkpoint through induction of p16 and repression of transcription of cyclinD1 [114, 182]. Mutated INI protein dislocates from the nucleus to the cytoplasm where it remains inactive. ANP inhibits complex cyclin D1/CDK4 and cyclin E/CDK2 and interrupts INI1 transport from nucleus to cytoplasm, which results in inhibition of AT/RT growth [57, 58, 183].

Preliminary results of phase II studies of ANP and AT/RT have already been reported [114]. The group of 11 children was treated under study protocol. All patients underwent prior surgery, 5 were followed with radiation therapy, 9 with chemotherapy and developed progression of the disease, except for one non-evaluable case. In the group of 8 evaluable patients, there were 2 cases of CR, 1 PR, 1 SD and 4 cases of PD. Maximum survival was approximately 2 years from the start of ANP.

The data of preliminary results of clinical studies in high-risk pediatric brain tumor patients are summarized in Table 5.

Concluding Remarks and Perspectives

Rapidly expanding understanding of molecular biology of cancer cells resulted in an increasing number of targeted therapies in clinical investigations. The initial excitement, centered around imatinib and trastuzumab, raised hopes that difficult to treat primary brain tumors will also respond to a new generation of highly specific treatments. The development of currently used agents began at the time when scientists were interested in individual genes and signaling pathways, and resulted in the design of drugs oriented toward single targets. The hopes that such drugs will control extremely complex system represented by neoplastic cells are unrealistic [184, 185]. Neoplastic cells form an extremely complex and robust system which is maintained in the presence of serious perturbations [184]. Most of neoplastic characteristics arise from complex interactions which form a number of interrelated networks; for instance, signaling, protein-protein, transcription and metabolic networks [186]. In this

respect, molecular interactions inside neoplastic cells can be compared to the internet. The networks are composed of nodes and links connecting them together. The most important nodes which regulate a large number of events form "hubs" [187]. Signaling network in human cells is extremely complex and consists of 1,543 types of receptors, 518 protein kinases, and close to 2,000 transcription factors [188-191]. The robustness of the neoplasm is assured by redundancy, feedback controls, modularity, structural stability, and gradual degradation of function in response to damage [184, 192]. These features help malignant cells escape from the control of the agents which are aimed at single targets. In this respect, imatinib was highly effective in the early stages of chronic myelogenous leukemia, but failed in the advanced stage [193]. Mutations in the *BCR-ABL* region are responsible for resistance to imatinib [194]. A higher success can be accomplished by designing the drugs targeting hubs of the network and multiple targets. Agents affecting multiple ERBB receptors (lapatinib, C1-1033, EKB-569) and ERBB and VEGFR (AEE788, EXEL 7647) may show higher clinical activity but their application may lead to unacceptable toxicity [22, 195]. On the other hand, a treatment using anti-ERBB agents in combination with mTOR may offer better results [196].

The ultimate success in controlling the neoplastic process would require elimination of neoplastic stem cells. Malignant stem cells have been identified in different human brain tumors [197-199]. The most promising targets in this area are Hh, MYC and Wnt pathways [200, 201]. Other important strategies include induction of apoptosis and cell cycle arrest through activation of *TP53* and *p21*. ANP explores multiple targets crucial in control of growth of brain tumors, including growth factor receptors (TGFBRI), signaling pathways (AKT/PTEN and RAS), cell cycle control (MYCC, p21, INI1) apoptosis, and cellular and nuclear transports, which gives this treatment a better chance for durable results.

Phase II studies of most of agents affecting single targets in primary brain tumors failed to show the results of clinical significance. The results of the treatment with erlotinib and imatinib with hydroxyurea are promising, but require confirmation by phase III studies [134, 135, 138, 139] . Combination therapy of bevacizumab and irinotecan contributed to a higher percentage of complete and partial responses, but was associated with significant toxicity. Clinical trials with multitargeted ANP in difficult to treat brain tumors, including recurrent and progressed GBM and DBSG resulted in favorable objective response rates, overall survival and progression free survival. The results of treatment of difficult to manage cases of PNET, multicentric glioma and AT/RT are encouraging, but will require confirmation in the larger population of patients. A low percentage of acute toxicity and no incidence of chronic toxicity allow administration of ANP for an extended period of time to assure proper control and prevention of tumor recurrence. Substantial data in this chapter are coming from meeting abstracts. Such data should be treated with caution until they pass the scrutiny of peer review. Dynamic expansion of the research on targeted therapies and identification of patients who are prospective responders promises the design and introduction of new agents of higher efficacy in the near future.

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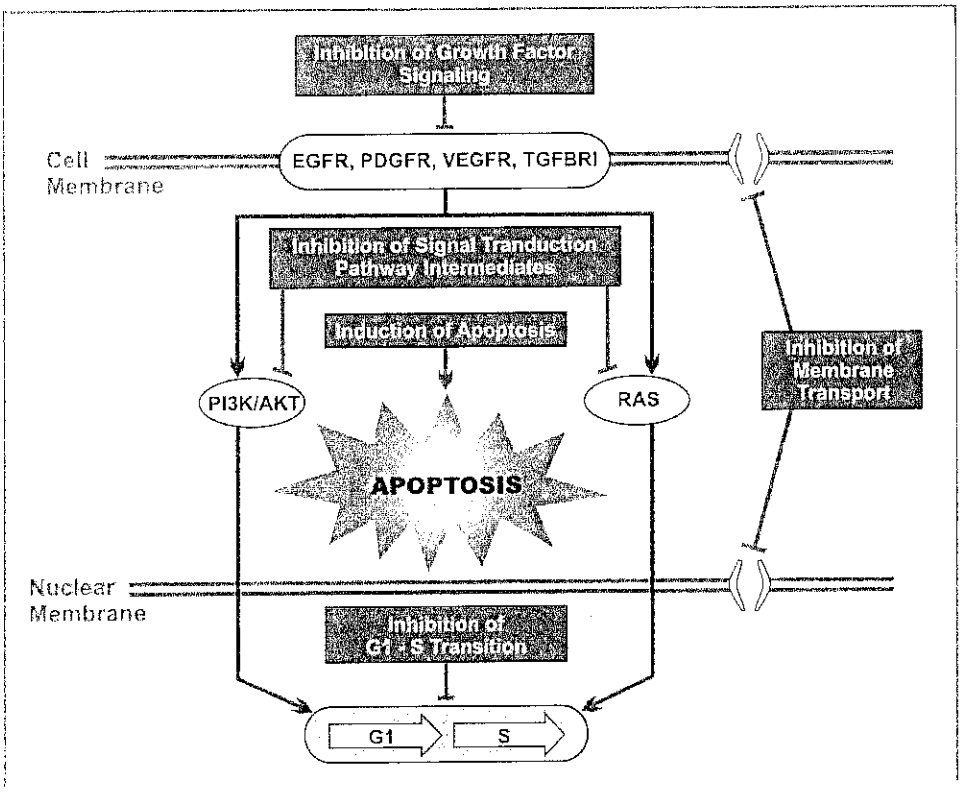

Figure 1. Targets for therapeutic agents used for treatment of primary brain tumors. EGFR, PDGFR, VEGFR, TGFBR1- growth factor receptors, PI3K/AKT, RAS - oncogene proteins, G1-S-phases of cell cycle.

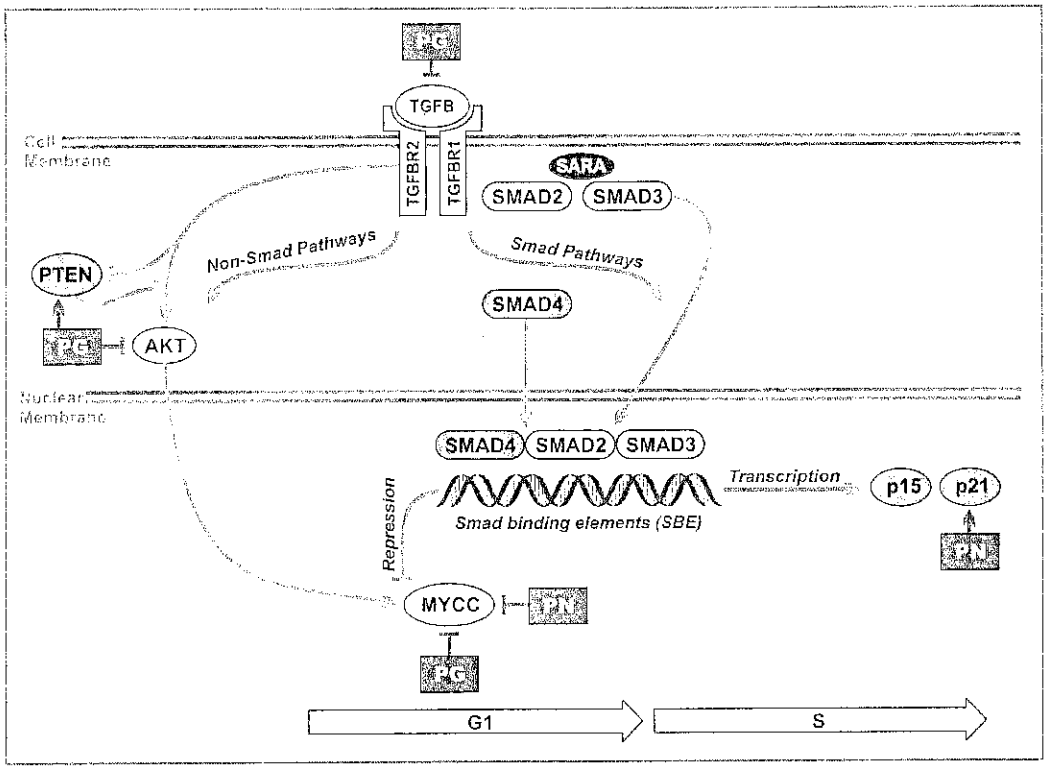

Figure 2. Pharmaceutical agents targeting transforming growth factor β signaling. TGFB, TGFBR1, TGFBR2 - transforming growth factor β, and its receptors, SMAD2, 3, 4 – Smad proteins, SARA – Smad – anchor for receptor activation, PTEN, p15, p21 - tumor suppressor gene proteins, AKT, MYCC – oncogene proteins, PG – phenylacetylglutaminate sodium, ingredient of ANP A10I, PN – phenylacetate sodium, main ingredient of ANP AS2-1I.

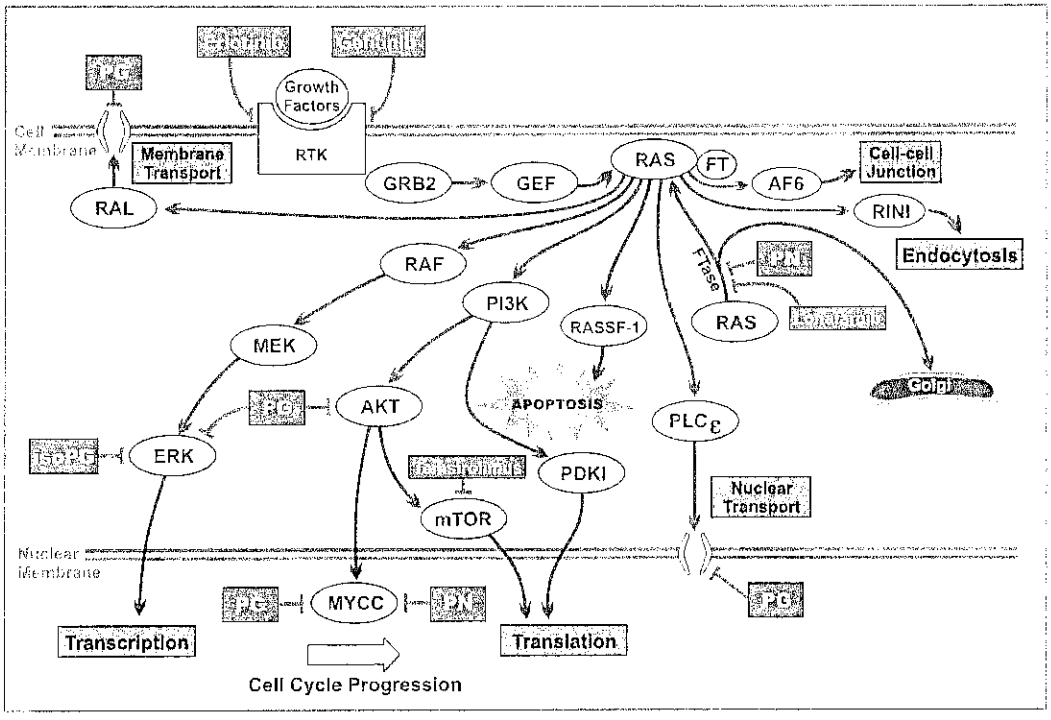

Figure 3. Pharmaceutical agents targeting RAS signaling pathway. RTK – receptor tyrosine kinase, GRB2 – adapter protein, GEF - guanine – nucleotide exchange factor, RAS – *RAS* oncogene protein, RASFT – farnesylated *RAS* protein, AF6, RIN1, RAL, RAF, RASSF-1, PLCε, MEK, ERK – RAS effectors, PI3K, AKT, mTOR, MYCC – oncogene proteins, PG – phenylacetylglutaminate sodium, ingredient of ANP A10I, isoPG – phenylacetyisoglutaminate sodium – ingredient of ANP A10I, PN – phenylacetate sodium, main ingredient of ANP AS2-1I.

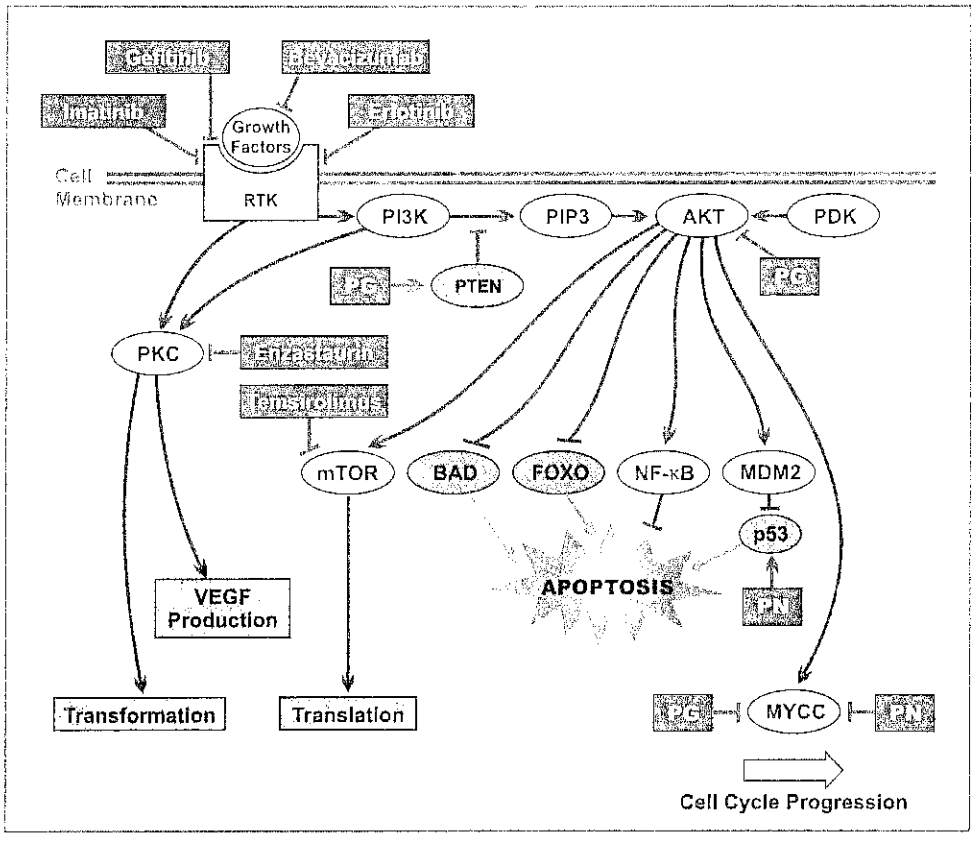

Figure 4. Pharmaceutical agents targeting PI3K/AKT signaling pathway. RTK- receptor tyrosine kinase, PI3K - phosphoinositide 3-kinase, PIP3 -phosphatidylinositol 3,4,5, PDK – phosphoinositide-dependent protein kinase, AKT – serine-threonine kinase, product of AKT oncogene, mTOR-mammalian target of rapamycin, BAD-apoptosis inducing protein, FOXO, NF-κB, MDM2, MYCC - oncogene proteins, p53 – p53 protein, PG – phenylacetylglutaminate sodium, ingredient of ANP A10I, PN – phenylacetate sodium, main ingredient of ANP AS2-1J, VEGF – vascular endothelial growth factor, PKC – protein kinase C-β2.

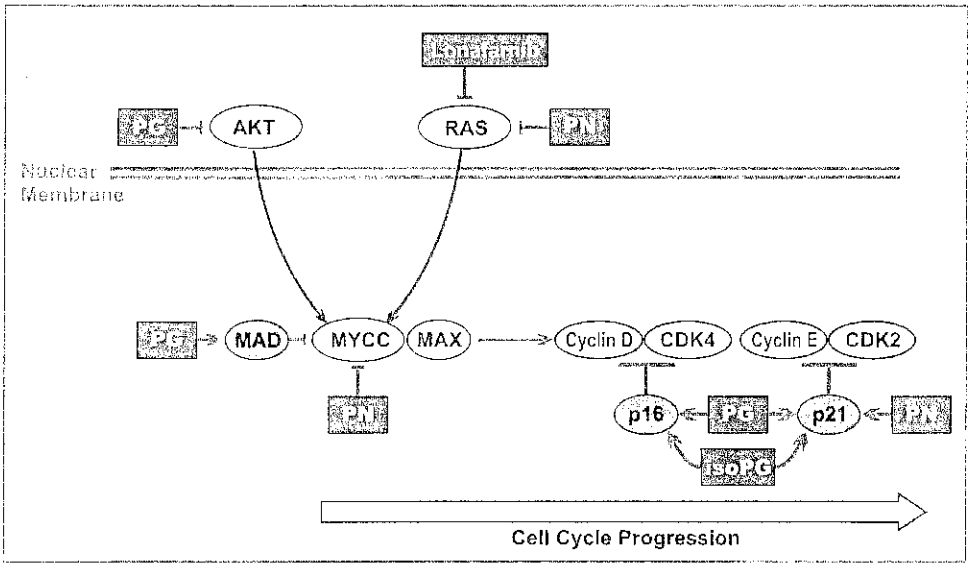

Figure 5. Pharmaceutical agents targeting G1-S transition. AKT, RAS, MYCC, MAX – oncogene proteins, CDK2 – cyclin-dependent kinase 2, MAD, p16, p21 – tumor suppressor gene proteins, PG – phenylacetylglutaminate sodium, ingredient of ANP A10I, isoPG - phenylacetyisoglutaminate sodium, ingredient of ANP A10I, PN – phenylacetate sodium, main ingredient of ANP AS2-1I.

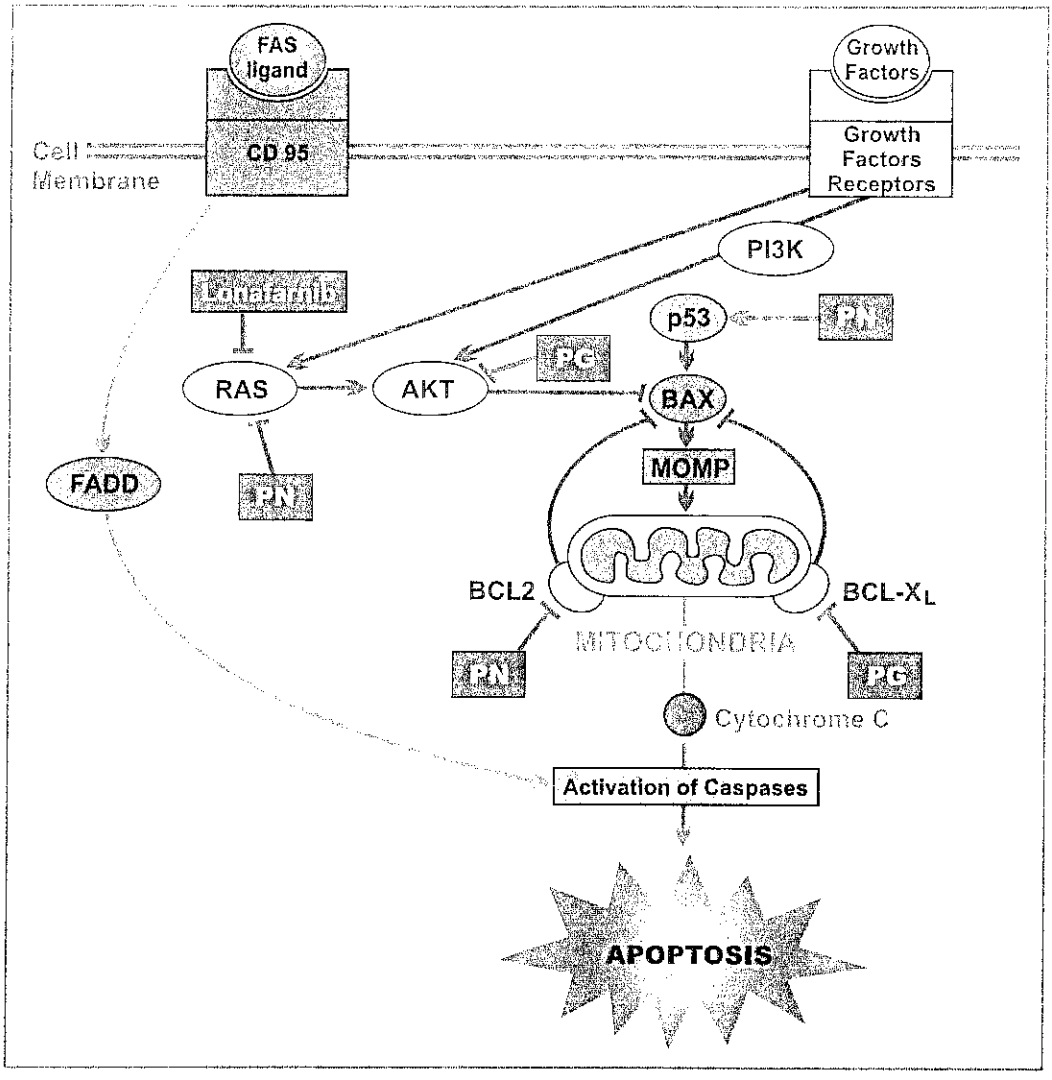

Figure 6. Induction of apoptosis by targeted pharmaceuticals. CD95 – CD95 receptor, PI3K, AKT, RAS – oncogene proteins, p53 – p53 protein, BAX – pro-apoptotic protein, FADD – intracellular adapter protein (FAS-associated death domain), MOMP – mitochondrial-outer-membrane permeabilization, BCL2, BCL-$_{XL}$ – anti-apoptotic proteins, PG - phenylacetylglutaminate sodium, ingredient of ANP A10l, PN - phenylacetate sodium, ingredient of ANP AS2-1I.