Chapter 27
Prerequisite Genetic Traits for Metastasis

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The genetic and epigenetic abnormalities in tumors influence the metastatic traits of disseminated cells by activation of oncogenes and inactivation of tumor-suppressor genes. Tumor-suppressor genes affect genome stability, cancer-cell survival and growth while also being involved in the response and repair of DNA. They are a part of the prerequisites for metastasis and determine initiation and continuous development of the oncogenic process resulting in unrestricted proliferation and resistance to cell death signals. Inactivation of tumor suppressor genes can occur through various mechanisms such as loss of heterozygosity and chromosomal damage as well as by genetic mutations and epigenetic mechanisms such as promoter hypermethylation (Nguyen and Massague 2007; Eccles 2005). The amplification and mutation of oncogenes in primary tumors, together with the selective pressures of the tumor microenvironment play a key role in the formation of metastasis (Bernards and Weinberg 2002).

27.1 Tumor Suppressor Genes

27.1.1 Retinoblastoma Pathway

The p16Ink4a–CyclinD1-CDK4-RB pathway regulates the cell cycle at the G1/S transition. Absent or mutated components of the RB pathway lead to the subsequent loss of the G1/S checkpoint in multiple cancers, thus promoting aberrant

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proliferation (Sherr and McCormick 2002). The unphosphorylated state of RB is maintained by p16INK4a which competes with the activity of cyclin D- dependent kinases (CDK), thus blocking the entry into S phase and the E2F (E2 transcription factor) transcriptional program (Knudsen and Wang 2010). Mutations in this pathway occur frequently in many cancers and the RB protein is functionally inhibited in 25 % of primary tumors. Once RB is hyperphosphorylated by the CyclinD1/CDK4 complex, it results in E2F-regulated gene expression, stimulating G1 to S transition. Persistent mitogenic stimulation could lead to overexpression of either CDK4 or Cyclin D1 resulting in inhibition of the RB pathway function by blocking the growth-suppressing activity of p16INK4a (Ortega et al. 2002). Transcription of the *cyclin D1* gene, its synthesis and assembly with CDK4, is regulated by ras (reticular activating system) and phosphatidylinositol 3-kinase (PI3-K) signaling (Kim and Diehl 2009).

Therapeutic Options Several therapeutic strategies are employed against defects in the RB-pathway and encouraging results are emerging in preclinical studies inducing the expression of p16Ink4a by means of adenoviral vectors containing human p16 cDNA (Craig et al. 1998). Additionally, positive results for reactivation of the RB-pathway are reported in studies using inhibitors of DNA methylation or histone deacetylases, which lead to the activation of epigenetically silenced p16Ink4a. The authors reported that DNA methyltransferase inhibitor 5-aza-2′-deoxycytidine (5–7aza-CdR) and the histone deacetylase inhibitor 4-phenylbutyric acid (PBA) caused cell cycle arrest, apoptosis and induced p16 (CDKN2A/INK4) and p21 (CIP1/SDI1/WAF1) in bladder carcinoma cells (Egger et al. 2007). Together, these studies suggest that RB-pathway activation could be used therapeutically.

Inhibition of Cdk4/6 kinase activity is another therapeutic option which was evaluated with second-generation CDK4/6 inhibitors in pre-clinical studies. Oral administration of these compounds induced tumour regression in xenograft animal models of human colon carcinoma causing elimination of phospho-RB. This therapeutic strategy of activating RB is currently under investigation in phase I-II trials (Knudsen and Wang 2010; Fry et al. 2004).

Flavopiridol is a semisynthetic flavone CDK inhibitor that interferes with CDK9-cyclin complex binding resulting in apoptosis. Phase I studies have revealed favorable responses in metastatic breast cancer carcinoma in combination with doxetacel (Freyer et al. 2003). Flavopiridol was also shown to have a synergistic effect with Herceptin, a drug active against Her2/neu (human epidermal growth factor receptor 2) in breast cancer cell lines (Nahta et al. 2002).

The cross talk between p53 status and levels of E2F activity influences the overall response to therapy. Therapeutic approaches that target p53 include stimulation of E2F-activity and restoration of the pro-apoptotic activity of p53 (Polager and Ginsberg 2009). It has been previously shown that RB-deficient tumor lines or those exhibiting deregulated E2F activity can be good targets for compounds that have the capacity to activate p53 (Kitagawa et al. 2008). Gene therapy using virus-activated E2F-regulated gene expression (pESM6), was shown to induce tumor reduction in

preclinical studies. These studies affirm the potential of pESM6 as a viable agent for gene therapy of DNA tumor virus-associated cancers (Lim et al. 2006). Also, gene transfer of a truncated variant of the retinoblastoma gene, (RB94), has been proven to inhibit proliferation of several human tumor cell types including pancreatic cancer (Roig et al. 2004).

Key Points Therapeutic strategies for reactivation of the retinoblastoma pathway

- Retinoblastoma gene transfer therapy
- Induction of the expression and activation of epigenetically silenced p16Ink4a
- Inhibition of Cdk4/6 kinase activity
- Stimulation of E2F-dependent apoptosis

27.1.2 The p53 Tumor Suppressor Gene

The p53 tumor suppressor gene maintains genomic integrity. Its transcription factor is induced in response to DNA damage, hypoxia, and oncogene activation. P53 regulates a number of downstream genes including p21, MDM2 (Mouse Double Minute 2), GADD45 (Growth Arrest and DNA Damage), BAX (Bcl2- associated X protein), as well as cell cycle (G1/S) and G2/M DNA check-points. This allows for cellular repair mechanisms or initiation of apoptosis through both extrinsic and intrinsic pathways (Sherr and McCormick 2002). The p53 tumor suppressor gene is the most frequently mutated gene in human tumors resulting in loss of its biological responses and inhibition of apoptotic mechanisms.

Therapeutic options Several strategies for restoration of wild-type p53 function and induction of apoptosis in tumors have been explored. These have included p53 gene–replacement therapy in which the E1 adenoviral region is replaced with the cDNA of the p53 gene, driven by a cytomegalovirus promoter (Ad-p53, ADVEXIN [Introgen Therapeutics, Inc.]) (Invitrogen 2007). Preclinical studies have shown encouraging results for this treatment modality with regard to antitumor activity and feasibility of gene therapy (Bianco et al. 2007). Evidence of clinical activity was also observed in clinical trials, where re-expression of wild type p53 by viral-mediated gene transfer induced tumor regression and stabilization in patients with NSCLC (non-small-cell lung cancer) and squamous cell carcinoma of the head and neck (HNCC) (Vecil and Lang 2003; Wiman 2007; Roth 1996; Nemunaitis et al. 2000; Clayman et al. 1999). However, no significant benefit was observed in patients with primary stage III ovarian cancer when treated with intraperitoneal delivery of a replication-deficient adenoviral vector expressing wild-type p53 (Zeimet and Marth 2003).

A different strategy employs a genetically modified adenovirus, (Onyx-015) which eliminates p53 by producing the early region protein E1B 55K. This protein binds p53 and targets it for destruction by inducing apoptosis in the cells expressing mutant p53. Evidence of clinical activity was reported after intra-tumor injection in clinical

trials in combination with chemotherapeutic agents in head and neck cancer as well as in pancreatic adenocarcinoma (Khuri et al. 2000; Hecht et al. 2003). Adenoviral vascular delivery for systemic metastases is also currently under investigation. Positive results have also emerged from additional therapeutic modalities involving small molecule therapy that functions through reactivation of mutant p53. In preclinical studies, p53 C-terminus derived semisynthetic peptides were shown to induce p53-dependent apoptosis in tumor cells (Haupt and Haupt 2004). Other methods exploit the p53- MDM2-mediated inhibition with drugs that interrupt the p53–MDM 2 interaction. For example, a synthetic class of cis-imidazoline analogs (Nutlins) interferes with the p53-MDM2 complex inhibiting tumor cell cycle and triggering apoptosis (Vassilev et al. 2004). Anti-sense mRNA therapy directed towards *MDM2* was shown to induce down regulation of the MDM2 and p53-mediated anti proliferative effects in human cancers cells, *in vitro* and *in vivo* (Wang et al. 2003). Other strategies include Hsp90 (heat shock protein-90) inhibitors where drug exposure was shown to induce destabilization of the mutant p53 protein in breast and prostate tumor cell lines (Blagosklonny et al. 1995).

Key Points Therapeutic strategies involving tumor suppressor p53

- Adenovirus-mediated p53 gene therapy
- Introduction of wild-type *p53* gene into tumor cells using viral vectors
- Interference with p53–MDM2 and down-regulation of MDM2 expression
- Targeting mutant p53 (Hsp90 inhibitors)
- Adenovirus-mediated inactivation of mutant p53
- Restoration of inactive or suppressed wild type p53
- Reactivation of mutant p53 with small molecule therapy

27.1.3 BRCA1 and BRCA2 Tumor Suppressor Genes

The tumor suppressor genes *BRCA1* (Breast Cancer 1) and *BRCA2* (Breast Cancer 2) are involved in DNA repair and have been identified in breast cancer and ovarian cancer. In 80 % of the cases mutations in the *BRCA1 and BRCA2* genes involve abnormal truncation of the BRCA protein (Sobol et al. 1996; Welcsh and King 2001). Individuals with mutations in these genes have a 15–20 fold increase in risk of breast cancer compared with the general population (Wooster et al. 1995). *BRCA2* mutation carriers are at an increased risk of developing breast cancer (in both males and females), as well as melanoma, ovarian, prostatic, pancreatic, and carcinoma of the gall bladder. *BRCA1* gene replacement therapies have shown anti-tumor responses in preclinical studies. Additionally, responses were seen in Phase I trials of patients with extensive metastatic breast cancer when treated with retroviral *LXSN-BRCA1sv* gene therapy. However, a phase II trial in ovarian patients showed no response, and no vector stability in response to *BRCA1*gene therapy (Tait et al. 1999)

27.1.4 PTEN Tumor Suppressor Gene

PTEN (phosphatase and tensin homolog) functions as a tumor suppressor through its lipid phosphatase activity negatively influencing Akt through the dephosphorylation of phosphatidylinositol-3,4,5-trisphosphate (PIP3). Loss of PTEN is a common event in cancer and occurs through mutation, deletion, or epigenetic silencing inducing PI3K/Akt pathway hyperactivation. PTEN is mutated or deleted in about 30–40 % of tumors including brain, bladder, breast, prostate, and endometrial cancers. It correlates with poor prognosis and metastatic disease. Gene therapy with wild-type PTEN has been attempted in preclinical studies, however, clinical-translational therapeutic strategies focus in targeting PI3K-Akt-mTOR pathway in tumors with PTEN functional loss (Zhang and Yu 2010).

27.1.5 Other Tumor Suppressor Genes

The *FHIT* (fragile histidine triad) gene located on 3p14.2 is homozygously deleted and targeted by genomic alterations leading to a decrease or loss of gene and protein expression in many cancers (Joannes et al. 2009). Lack of FHIT expression correlates with tumor progression to metastasis as *FHIT* controls the invasive phenotype of lung tumor cells by regulating the expression of genes associated with epithelial–mesenchymal transition (Joannes et al. 2009). FHIT gene, re-expression by a recombinant adenoviral vector, resulted in apoptosis and reduced tumorigenicity in lung cancer (Ji et al. 1999). Additionally, gene therapy involving administration of the *FUS1* (cell fusion 1) tumor suppressor gene might have applicability in lung cancer (Ji and Roth 2008). Intravenous administration of nanoparticle encapsulated FUS1 expression plasmid had antitumor effects in human lung cancer xenograft models (Deng et al. 2008).This treatment was well tolerated in a Phase I clinical trial of FUS1-nanoparticles in patients with chemotherapy refractory stage IV lung cancer (Ji and Roth 2008; Lu et al. 2009).

27.1.6 Metastatic Suppressor Genes

Metastatic suppressor genes are differentially expressed between metastatic and non-metastatic cells and interfere with several signaling pathways involved in metastatic colonization. Examples include nm23 (non-metastatic gene 23) modulation of the ERK (extracellular signal-regulated kinase) pathway, BRMS1 (Breast cancer metastasis suppressor 1) alteration of phosphoinositide signaling, and MKK4 (mitogen-activated protein kinase kinase 4) activation of JNK (c-Jun NH(2)-terminal protein kinase) and p38 stress pathways (Rinker-Schaeffer et al. 2006). Silence inactivation or loss of heterozygozity of metastatic suppressor genes and low expression in tumors were associated with a higher risk of metastatic disease (Martins et al. 2008;

Bakalian et al. 2007). Therefore, re-expression of metastatic suppressor genes may have therapeutic effects on micrometastatic tumor cells (Steeg 2004). Several compounds that can elevate nm23 have been identified including indomethacin, gamma Linolenic Acid, trichostatin A, 5-aza-deoxycytidine, and medroxyprogesterone acetate. Results from in vivo models of lung metastasis demonstrated a reduction of the metastatic potential with administration of high doses of medroxyprogesterone acetate (Marshall et al. 2009). This therapeutic strategy is currently evaluated in a phase II clinical study investigating the effect of nm23 re-expression in breast cancer cells and subsequent metastatic colonization (Steeg et al. 2008).

27.2 Prerequisites for Metastasis: Oncogenes

Genetic instability in primary tumors increases the chance of further oncogenic mutational events and results in the induction of unrestricted proliferative capabilities and resistance to apoptotic signals. The amplification and mutation of oncogenes in primary tumors, together with the selective pressures of the tumor microenvironment play a key role in the formation of metastasis. This suggests that metastatic potential might be pre-programmed in tumors, whereas a selective population of cells might require additional alterations in tumor suppressor genes and oncogenes to initiate the metastatic cascade (Bernards and Weinberg 2002).

27.2.1 Myc

Oncogene amplifications affect distinct genetic programs leading to cell cycle progression, invasiveness and metastasis, for example downstream EGFR (epithelial growth factor receptor), C-ERbB2, Myc (myelocytomatosis viral oncogene) and ras signaling (Nguyen and Massague 2007). The *Myc* proto-oncogene family encodes nuclear products which are deregulated as a result of point mutations, gene amplification and translocation. *Myc* family genes are activated in a wide variety of human hematological malignancies and solid tumors as a consequence of activation of one or more signalling pathways. These include RAS-RAF-MAPK, PI3K, WNT- β catenin pathways and STAT (signal transducer and activator of transcription) pathways (Pelengaris et al. 2002). *Myc* is a key regulator for many biological activities including cell-cycle progression, apoptosis, tumor growth, angiogenesis, cell adhesion and motility. It is associated with poor prognosis and metastasis (Nesbit et al. 1999).

Strategies currently employed for targeting *Myc* include antisense oligonucleotides resulting in tumor cell growth arrest and induction of apoptosis in a variety of tumor cell lines. Experiments in xenograft models of breast carcinoma, melanoma and neuroblastoma resulted in prevention of tumor formation (Vita and Henriksson 2006).

Phosphorodiamidate morpholino oligomers (PMOs) are DNA antisense oligonucleotides that inhibit *Myc* gene expression by preventing its mRNA translation. This agents inhibited tumor growth and induced apoptosis in prostate cancer xenografts. Further clinical studies evaluated them in adenocarcinoma of prostate and breast tumor tissues (Devi et al. 2005) and assessed its safety in human trials (Iversen et al. 2003).

Other agents which interfere with *Myc* promoter and transcription are DNA analogs. These compounds specifically hybridize to DNA and/or RNA in a complementary manner thus inhibiting transcription and translation of the *Myc* target gene (Pession et al. 2004).

Cationic porphyrin (TMPyP4), which inhibits *Myc* transcription by blocking G quadruplexes, inhibited the *in vitro* transcription of *Myc* and decreased tumor growth rates in xenograft models (Grand et al. 2002)

The regulatory effect on gene transcription of *Myc* is dependent on dimerization and complex formation with a b-HLH-LZ protein Max. Targeting Myc–Max complex with small molecules is another therapeutic option (Berg et al. 2002). Small interfering RNA (siRNA) against *Myc* resulted in apoptotic effects and tumor growth reduction in xenograft models (Shen et al. 2005). Therefore targeting expression or function of Myc shows interesting promise and development of agents with improved delivery and efficacy is further anticipated in clinical settings (Ponzielli et al. 2005).

27.2.2 HER-2

The human epidermal growth factor receptor (HER, ERB) family consists of EGFR (HER1 or ERBB1), HER2 (EGFR2 or ERBB2/NEU), HER3 (EGFR3 or ERBB3), and HER4 (EGFR4 or ERBB4) (Rowinsky 2004). The *HER-2* (human epithelial receptor 2, also known as *HER-2/neu* or *ER*B-2) gene is located on chromosome 17q and encodes a 185-kDa trans-membrane receptor tyrosine kinase with a key role in normal cell growth and differentiation. The amplification and over-expression of the *HER-2* gene results in malignant transformation of cells and affects up to 30 % of patients with metastatic breast cancer correlating with increased metastatic potential in ovarian, breast cancer and in NSCLC (Yarden and Sliwkowski 2001; Slamon et al. 1989).

Trastuzumab (Herceptin®; Genentech, Inc.; South San Francisco, CA), is the first approved humanized monoclonal antibody designed to block the receptor extracellular domain of human epidermal growth factor receptor-2 (HER2) that is over expressed in metastatic breast cancer and affects intracellular signaling and tumor cell growth. Trastuzumab therapy alone or in combination with taxanes chemotherapy provided the proof of principle that targeting HER-2 receptors results in cytotoxic and cytostatic effects. This combination demonstrated clinical benefit in terms of response rate and survival for patients with HER-2-positive disease and represents the first-line therapy for these patients (Cobleigh et al. 1999; Slamon et al. 2001; Vogel et al. 2002). Other combinations of trastuzumab with chemotherapy are also

Table 27.1 Monoclonal antibody therapies targeting EGFR (ERB-1 and ERB-2)

Trastuzumab (Herceptin, Genentech Inc)	Breast cancer
Cetuximab (Erbitux, ImClone Systems)	Colorectal, NSCLC, pancreatic, breast cancer and HNSCC
Panitumumab (Vectibix, Amgen)	Colorectal cancer
ABX-EGF (Amgen)	NSCLC, colorectal, prostate, renal, HNSCC
Matuzumab (EMD 72000, Pharma)	NSCLC, colorectal, ovarian cancer, HNSCC, pancreas
Pertuzumab (Omnitarg;)	Prostate, ovarian, breast and NSCLC
Nimotuzumab (hR3)	Squamous cell carcinoma of head and neck

currently under investigation. Clinical data indicate that the therapy with trastuzumab may induce a decrease in ejection fraction, cardiac dysfunction in about 1–4 % of patients treated with trastuzumab and this side effect may be augmented in combination with chemotherapy (Perez and Rodeheffer 2004).

Clinical trials evaluating the response to trastuzumab and other cytotoxic agents such as vinorelbine (Burstein et al. 2003), gemcitabine (Loesch et al. 2008), and capecitabine (Tevaarwerk and Kolesar 2009) have shown positive response rates and increased overall survival times in patients with metastatic breast cancer. Tanespimycin a new 17-AAG analog has demonstrated promising antitumor activity and tolerability in a Phase II clinical trial in patients with HER 2-neu positive metastatic breast cancer. These results were reported for a combination of 17-AAG with trastuzumab in patients previously nonresponsive to Herceptin alone (Modi et al. 2007).

Other humanized anti-EGFR (ERB-1 and ERB-2) monoclonal antibodies cetuximab and panitumumab bind to the extracellular domain of EGFR, thus leading to inhibition of its downstream signaling. These agents are currently being investigated in phase II and III clinical trials in NSCLC (Jatoi et al. 2010). Cetuximab and panitumumab have shown evidence of activity in combination with cytotoxic chemotherapy and radiotherapy in the treatment of metastatic colorectal cancer or as monotherapy for the treatment of metastatic head and neck squamous cell carcinoma (HNSCC). It is indicated for the treatment of KRAS wild-type metastatic colorectal cancer in combination with chemotherapy or as a single agent in patients refractory to chemotherapy (Cutsem et al. 2009; Bokemeyer et al. 2009). The presence of activating K-ras mutations has been identified as a potent predictor of resistance to cetuximab or panitumumab therapy (Tol and Punt 2010; Keating 2010). Cetuximab monotherapy is currently the only approved molecular target therapy in patients with recurrent or metastatic HNSCC, and has been shown as a radiation-sensitizing agent in primary radiation therapy of this disease (Jackisch 2006; Cripps et al. 2010). Other monoclonal antibodies targeting HER-2 include humanized antibodies matuzumab (EMD72000), nimotuzumab (hR3), and pertuzumab (Genentech),which are currently in preclinical or phase I and II clinical studies in low HER-2-expressing breast cancers, NSCLC, colorectal, ovarian cancer, pancreas, prostate and ovarian cancer (Bianco et al. 2007). Examples of monoclonal antibody agents are shown in Table 27.1.

The HER, ERB family of trans-membrane receptors forms dimers upon ligand binding, resulting in activation of the intracellular tyrosine kinase domain, and

Table 27.2 EGFR tyrosine kinase inhibitors that are currently under investigation for various malignancies

Inhibitor	Specificity	Selected tumor types
Gefitinib (Iressa®; AstraZeneca)	ErbB-1 tyrosine kinase inhibitor	Metastatic NSCLC, head and neck squamous cell carcinoma, breast, ovarian, prostate, glioma, pancreatic, colorectal cancer
Erlotinib (Tarceva™; Genentech)	ErbB-1 tyrosine kinase inhibitor	NSCLC, metastatic pancreatic cancer, HNSCC, breast, ovarian, prostate, colorectal, glioma
Lapatinib (GlaxoSmithKline)	Dual effect ErbB-1 and ErbB-2	Colorectal cancer and HNSCC

triggering of the downstream effector pathways involved in cellular proliferation, angiogenesis, and metastasis. Mutations in the EGFR tyrosine kinase receptor family of receptors have been associated with poor prognosis in breast cancer, ovarian and NSCLC (Paez et al. 2004; Lassus et al. 2006; Generali et al. 2007)**.** Tyrosine kinase inhibitors bind to the intracellular ATP-binding site on the receptor and inhibit cell proliferation by blocking intracellular signals that stimulate gene expression. The mechanisms of action include inhibition of cancer cell proliferation via G_0/G_1 cell cycle arrest, anti-angiogenic effects and inhibition of invasion and metastasis (Olayioye et al. 2000). These agents are reported to be able to cross into the CNS and have excellent oral bioavailability (Roy and Perez 2009; Gril et al. 2008).

Novel treatment regimens under investigation for patients with advanced breast cancer and NSCLC include HER tyrosine kinase inhibitors, gefitinib (Iressa®; AstraZeneca Pharmaceuticals) and erlotinib, (Tarceva™; Genentech) which are specific for EGFR and lapatinib (Tykerb, GlaxoSmithKline) a dual EGFR and HER-2 inhibitor (Table 27.2). In a phase III clinical trial that led to FDA approval for erlotinib, 731 patients with NSCLC previously treated with one or two chemotherapy regimens were randomized to receive erlotinib or placebo. Erlotinib treatment was shown to be superior to placebo in survival, quality of life, and related symptoms in advanced and metastatic NSCLC patients (Shepherd et al. 2005). However, the combination of erlotinb with first-line chemotherapy such as carboplatin and paclitaxel has failed to show additional benefit when compared with chemotherapy alone (Herbst et al. 2005; Gridelli et al. 2007). Also, the results of a phase III clinical study with combination therapy between erlotinib and gemcitabine in pancreatic cancer patients showed a modest improvement in the median overall survival (Moore et al. 2007).

Small molecule therapy with lapatinib, a dual oral inhibitor for EGFR and HER2 showed antitumor activity in preclinical studies (Rusnak et al. 2007). Lapatinib combined with capecitabine (Xeloda; Roche) demonstrated significant improvements in the time to progression and response rate when compared with capecitabine alone in breast cancer patients and this combination is currently approved for treatment of HER-2-overexpressing chemorefractory breast cancer patients (Tevaarwerk and Kolesar 2009; Jackisch 2006; Higa and Abraham 2007). Lapatinib was proven to have manageable side effects including diarrhea and skin rash.

A phase III, randomized, open-label study comparing the efficacy of gefitinib for first line therapy with carboplatin–paclitaxel demonstrated an increase in objective

response rates, significantly longer progression-free survival times and improved quality of life among EGFR mutation–positive patients who received gefitinib alone (Jiang 2009). Positive results are also emerging from other phase III clinical trials that investigated the clinical efficacy of gefitinib as monotherapy and in combination with chemotherapy for the treatment of NSCLC. These trials have revealed the comparable efficacy of gefitinib compared with docetaxel, (Douillard et al. 2010; Kim et al. 2008).

HER-2 inhibitors have been proven in clinical trials as beneficial therapeutic strategies for metastatic disease. Insights into future development of drugs that target this biochemical pathway will determine optimal sequence of administration as well as markers for the group of patients most likely to respond.

27.3 Growth Factor Receptors and Their Effector Pathways

Identification of oncogenic kinases has paved the way for further development of anticancer agents. Specifically, inhibitors of receptor tyrosine kinases (RTKs), such as BCR-ABL, c-KIT, PDGFR, EGFR, IGF1R, Met and Src, may have a role in the treatment of cancer.

27.3.1 KIT

KIT (c-KIT receptor) gene encodes a trans-membrane receptor tyrosine kinase which activates downstream signaling pathways involved in cellular proliferation and survival. Mutation and activation of *KIT* oncogene have been described in a variety of malignancies, such as gastrointestinal stromal tumors (GIST), acute myelogenous leukemia (AML) and result in aberrant signaling, increased proliferation and antiapoptotic effects. Imatinib (imatinib mesylate, Gleevec) targets the c-KIT tyrosine kinase, the Bcr–Abl tyrosine kinase and PDGFR (platelet-derived growth factor receptor) (Druker 2008). Clinical studies in patients with advanced GIST, where mutations in *KIT* have been reported in 75–80 % of tumors, demonstrated the efficacy and safety of imatinib mesylate treatment, leading to its approval for targeted therapy (Demetri et al. 2002). However, phase II clinical studies of imatinib mesylate in patients with metastatic melanoma and an activating *KIT* mutation, showed insufficient therapeutic effect. (Wyman et al. 2006). Also, although the drug was generally well tolerated, it had minimal activity in recurrent or persistent uterine carcinoma (Huh et al. 2010), recurrent ovarian cancer (Alberts et al. 2007) or primary peritoneal carcinoma (Schilder et al. 2008).

Other small molecule tyrosine kinase inhibitors that affect c-KIT are in various stages of clinical development. Examples include sorafenib, and sunitinib which have potent KIT inhibitory effect while also inhibiting other tyrosine kinases involved in oncogenic growth and progression, such as vascular endothelial growth factor receptors (VEGFR 1, 2, and 3), and PDGFR. Sunitinib was FDA approved for second-line therapy in GIST and RCC (renal cell carcinoma) (Faivre et al. 2007)

whereas Sorafenib demonstrated potent effects in RCC and hepatocellular carcinoma (HCC) (Hahn and Stadler 2006).

Recently, a small-molecule multikinase inhibitor Dasatinib, (BMS 354825), an orally available therapeutic agent was shown to inhibit Bcr-Abl and Src-family kinases, but also c-KITand PDGFR. This drug demonstrated potent effects and was approved for the treatment of patients with Bcr-Abl-positive chronic myeloid leukemia (CML) as well as acute lymphoblastic leukemia (ALL) resistant or intolerant to imatinib. Given its activity against c-KIT, PDGFR and Src kinases, this drug was evaluated and demonstrated favorable effects on several human solid tumor lines (González et al. 2006; Buettner et al. 2008; Coluccia et al. 2006). It is currently being investigated in clinical trials in patients with metastatic breast cancer to the bone (Rose and Siegel 2010) and in patients with metastatic prostate cancer (Yu et al. 2009).

27.3.2 Insulin-Like Growth Factor

The insulin-like growth factor (IGF) signaling axis is a prerequisite for oncogenic transformation and mediates tumor growth in a variety of human malignancies through its effects on proliferation and anti-apoptosis. The biological actions of the insulin-like growth factors, IGFI and IGFII, are mediated by activation of the IGFI receptor (IGFIR), a tyrosine kinase trans-membrane linked to the RAS-RAF-MAPK and PI3K-PKB/AKT signal transduction cascades. The IGF1R is over-expressed by tumors such as melanomas, colon cancer, pancreatic, prostate and renal cancer (Chitnis et al. 2008). This occurs as a result of loss-of-function and mutation of tumor suppressors such as wild-type p53, BRCA1 and VHL (von Hippel Lindau) resulting in transcriptional deregulation of the *IGFIR* gene (Werner and Roberts 2003). Stimulation of IGF1R-pathway results in activation of the RAS/RAF/MAPK pathway and induces differentiation and survival signals leading to tumor proliferation (Riedemann and Macaulay 2006). Anti-apoptotic effects are mediated through interaction of the IGF-1R with one of its major substrates, insulin receptor substrate 1 (IRS-1) which activates the PI3K-AKT pathway (Kulik et al. 1997).

IGF1R activation is linked to cancer progression and metastasis through multiple signaling intermediates. IGF1R-mediated signaling enhances ß-catenin transcriptional activity and interferes with E-cadherin expression, actin polymerization and focal adhesion complex formation, thus inducing loss of cellular adhesion (Morali et al. 2001; Playford et al. 2000). Another possible way in which IGF1 pathway induces the metastatic phenotype is interaction with integrin-mediated signaling pathways. These include $\alpha_v\beta_3$ (Shen et al. 2006) and IGF-induced secretion of matrix metalloproteinases (MMPs) as well as regulation of the urokinase plasminogen activator/plasmin (uPA) system of proteolysis resulting in degradation of the extracellular matrix (ECM) (Bahr and Groner 2005). These mechanisms were confirmed by several models which demonstrated that IGF1R over-expression confers anchorage-independent growth and promotes an invasive, metastatic phenotype (All-Ericsson et al. 2002; Economou et al. 2008; Lopez and Hanahan 2002; Chernicky et al. 2000).

Table 27.3 Examples of IGF1R tyrosine kinase inhibitors that are currently under investigation

Small molecule inhibitor	Specificity	Selected tumor types
OSI-906 (OSI Pharmaceuticals)	IGF1R	Phase I advanced solid tumors
	IR	Phase III Adrenocortical, Phase I ovarian
Insm-18 (NDGA) Insmed	IGF1R	Phase I advanced solid tumors
	HER2-Neu	
NVP-AEW54 1(Novartis)	IGF1R	Preclinical
NVP-ADW742	IGF1R	Preclinical
BMS-536924 (Bristol-Myers-Squibb)	IGF1R	Phase I
	IR	
AG1024 (Calbiochem-EMD Biosciences)	IGF1R	Preclinical
	IR	
Picropodophyllin PPP (Karolinska Institute/Biovitrum)		Uveal melanoma
PQIP (OSI Pharmaceuticals)	IGF1R	
XL 228	IGF1R	Phase I study of patients with solid malignancies
	SRC	

Altering IGF1R function might inhibit tumor cell growth and also has effects on anchorage-independent growth, survival, migration, invasion and colonization of tumor cells. Different strategies in blocking the IGF-1R signaling pathway include small molecule inhibitors, blocking antibodies, antisense oligonucleotides and plasmids, antisense and siRNA. In preclinical models as well as in early phase clinical trials down-regulation of IGF-1R revealed favorable results (Chitnis et al. 2008; Bahr and Groner 2005; Li et al. 2009).

A. IGF1R tyrosine kinase inhibitors The development of tyrosine kinase inhibitors downstream of the IGFI receptor has led to the development of compounds with a high degree of selectivity for IGF1R (Table 27.3). However, as there is a high degree of sequence homology between the insulin receptor (IR) and IGF1R, this type of inhibition could potentially result in metabolic changes (Pollak et al. 2004). Examples of small molecules that compete for the ATP binding pocket of IGF1R are NVP-ADW742 and NVP-AEW54 1(Novartis). Preclinical data for these compounds reported anti-proliferative activity in cancer cells by interfering with cell cycle progression (Martins et al. 2006) and anti-tumor effects in multiple myeloma xenografts (Mitsiades et al. 2004) and fibrosarcoma xenografts (Garcia-Echeverria et al. 2004).

OSI-906 (OSI pharmaceutical) is a new small molecule, dual kinase inhibitor of both IGF-1R and IR. Data from a phase I clinical trial in patients with advanced solid tumors indicated that OSI-906 was well-tolerated and showed that at a low dosing schedule retained strong anti-tumor activity, with reduced incidence of IR-mediated side effects (Macaulay et al. 2010). This drug is currently evaluated in a Phase III clinical trial in adrenocortical carcinoma and in a Phase I/II clinical trial in ovarian cancer [1].

[1] NIH's ClinicalTrials.gov. Available from (http://www.clinicaltrials.gov/) and ClinicalTrialsFeeds.org (http://www.clinicaltrialsfeeds.org/) web sites.

Insm-18 (NDGA) (Nordihydroguaiaretic Acid) (Insmed), is an orally available small molecule IGF-1 tyrosine kinase inhibitor that has demonstrated anti-tumor activity in preclinical studies of breast, lung, pancreatic and prostate tumors (Chitnis et al. 2008; Hewish et al. 2009). This agent is currently evaluated in phase I clinical studies with non-metastatic recurrent prostate cancer (Harzstark et al. 2007).

Another small-molecule inhibitor, BMS-536924, (Bristol-Myers-Squibb) had an effect on insulin receptor kinase activity and reduced tumor cell proliferation of breast cancer cell lines *in vitro* (Litzenburger et al. 2009) and was also effective in reducing tumor xenograft size in vivo (Haluska et al. 2006)

Tyrphostin (AG1024), a substrate competitive, specific inhibitor of IGF-1R was proven to inhibit tumor cell growth in prostate, breast cancer and melanoma cell lines (Hewish et al. 2009).

Cyclolignans are selective inhibitors of tyrosine phosphorylation of the IGF-1R. Xenograft data has shown efficacy for one of these compounds, picropodophyllin (PPP), in Ewing's sarcoma cells, melanoma cells, and prostate carcinoma cells (Girnita et al. 2004).

PQIP (OSI pharmaceutical) is a 1,3-disubstituted-8-amino-imidazopyrazine derivative inhibitor of IGF-1R kinase. It has recently been reported to be particularly effective in breast cancer (Zeng et al. 2009), pancreatic cancer, and ovarian cell lines as well as in HNSCC and NSCLC. In xenograft models, this agent inhibited IGF-1R dependent tumor growth in colorectal cancer which correlated with the degree and duration of inhibition of IGF-IR phosphorylation (Hewish et al. 2009; Ji et al. 2007).

B. Monoclonal Antibodies IGF1R neutralizing monoclonal antibodies block the receptor–ligand interactions subsequently resulting in receptor internalization and degradation blocking intracellular signaling. The antibody-induced IGF1R down-regulation is selective against the IGF1R without interfering with IR and possibly induces less metabolic toxicity than that seen with the IGF1R small molecule inhibitors (Gualberto and Pollak 2009).

IMC-A12 (cixutumumab), Imclone, has the ability to induce IGF1R down-regulation and has shown promising activity in human tumor xenograft models of breast, lung, colon, and pancreatic cancers (Rowinsky et al. 2007). This agent was well tolerated evidence of stable disease were reported in a phase I clinical trial in patients with advanced solid tumors (Higano et al. 2007; Rothenberg et al. 2007). A similar study combining an IMC-A12 and a mTOR inhibitor (temsirolimus) in patients with solid tumors or lymphoma reported that the combination is well tolerated and demonstrated prolonged stable disease in two patients with metastatic prostate cancer and breast cancer (Naing et al. 2009). IMC-A12 is currently evaluated in patients with prostate cancer, metastatic colorectal cancer, Ewing's sarcoma and in a pediatric population with refractory solid tumors (Atzori et al. 2009).

CP-751871 (figitumumab, Pfizer) a fully human IgG2 monoclonal antibody, that blocks IGFI binding, and prevents activation of IGF1 causing down-regulation of IGF1R *in vitro* and in tumor xenografts of breast cancer, colon cancer, and multiple myeloma (Cohen et al. 2005). Phase I studies have suggested a favorable toxicity profile and signs of disease stabilization in patients with advanced solid tumors (Molife

et al. 2010). Clinical trials are ongoing and include prostate, breast, colorectal and melanoma patients. Preliminary data from a Phase II clinical trial in NSCLC evaluating CP-751871 in combination with paclitaxel and carboplatin (Karp et al. 2008) suggested promising results showing a 46 % response after addition of CP-751871 in comparison with a response rate of 32 % for patients treated with chemotherapy alone (Karp et al. 2009). However, results from a phase III study conducted to test the efficacy of the combination of paclitaxel, carboplatin, and CP-751871 reported that the addition of CP-751871 did not increase overall survival and resulted in adverse side effects resulting in discontinuation of this trial (Jassem et al. 2010). Further evaluation of CP-751871 in combination with chemotherapy or erlotinib is currently in progress for patients with advanced NSCLC. Other clinical trials in progress include phase I- II studies of CP-751871 as monotherapy or in conjunction with chemotherapy in patients with metastatic colorectal cancer, Ewing's sarcoma and in breast cancer (Atzori et al. 2009; Rodon et al. 2008).

R1507 (robatumumab, Roche), is a fully human IgG1 type monoclonal antibody also selective against IGFIR. Xenograft data has shown efficacy in osteosarcoma cancer models (Kolb et al. 2010). The results of a phase I study evaluating R1507 administered weekly in patients with advanced solid neoplasms in particular Ewing's sarcoma revealed partial responses and evidence of stable disease (Kurzrock et al. 2010).

AMG 479 (Amgen) is a fully IgG1 human monoclonal antibody selective to IGF1R that exhibited broad antitumor activity in xenograft models (Beltran et al. 2009). Furthermore, AMG 479 administration was proven safe in phase I clinical trials in patients with advanced solid tumors and demonstrated preliminary efficacy with one durable complete response and a partial response in two patients with Ewing-primitive neuroectodermal tumors (Tolcher et al. 2009a). Assessments of a combination of AMG 479 with panitumumab or gemcitabine in patients with advanced solid tumors, reported that the combination was well tolerated with very few side effects. There was a partial response and signs of stable disease were observed (Sarantopoulos et al. 2008; Puzanov et al. 2010). Further trials include evaluation of this agent in a Phase II double blind randomised study in hormone receptor positive metastatic breast, colorectal and lung cancer patients[2]. Results from a phase II clinical trial assessing safety, tolerability and maximum tolerated dose of a combination of AMG 479 with gemcitabine in patients with pancreatic cancer were promising with regard to tolerability. The second stage of this trial randomised the treatment between gemcitabine and AMG 479, versus gemcitabine and placebo, resulting in improved overall survival rate at six months (57 % in AMG 479 arm versus 50 % in gemcitabine plus placebo arm) (Kindler et al. 2010).

Sch717454 (Robatumumab), (19D12, Schering-Plough), a human IgG1 anti-IGFIR antibody demonstrated antitumor activity in solid tumor xenografts, including Ewing sarcoma, rhabdomyosarcoma, glioblastoma, neuroblastoma, and osteosarcoma panels (Kolb et al. 2008; Wang et al. 2010). This drug is currently under

[2] NIH's ClinicalTrials.gov. Available from (http://www.clinicaltrials.gov/) and ClinicalTrialsFeeds.org (http://www.clinicaltrialsfeeds.org/) web sites.

Table 27.4 Examples of novel IGF1R monoclonal antibodies

Monoclonal antibodies	Specificity	Selected tumor types
GSK 621659A (GSK)		Preclinical
CP 751–871 (Pfizer)	IgG2	Phase I–II in prostate, breast, colorectal and melanoma Phase III in NSLC with paclitaxel and carboplatin
IMC-A12 (ImClone)	Fully human IgG1	Phase I–II in prostate cancer, Ewing's sarcoma, colorectal cancer
AVE1642 (Sanofi-Aventis)		Phase I in patients with advanced solid tumors
MK 0646 (Merck)		Phase I in advanced solid tumors Phase–II in pancreatic cancer and colorectal cancer
AMG 479 (Amgen)		Phase I advanced solid tumors Sarcoma, breast cancer patients, colorectal cancer and lung cancer Phase II–II pancreatic cancer in combination with gemcitabine
R 1507 (Roche)	IgG1	Phase I in patients with advanced solid tumors and I–II in Ewing's sarcoma
SCH-717454 (19D12, Schering-Plough)		Phase I–II metastatic osteosarcoma

evaluation in phase II clinical trials in patients with metastatic relapsed osteosarcoma.[3]

MK-0646, dalotuzumab (Merck) is an anti-IGFIR antibody that was investigated in a phase I clinical trial which suggested favorable toxicity in patients with advanced solid tumors (Hidalgo et al. 2008). Further results and signs of antitumor activity were reported from a Phase I study of MK-0646, in combination with gemcitabine for advanced previously untreated pancreatic cancer (Javle et al. 2010). This agent is currently being evaluated in combination with cetuximab and irinotecan in an ongoing randomised phase II/III study in patients with refractory metastatic colorectal cancer. Preliminary data showed that the combination was tolerable with no overlapping toxicities (Watkins et al. 2009).

AVE1642, (Axelar), a humanized monoclonal antibody, specific for human IGF1R was reported to be well tolerated as a single agent in a phase I clinical trial in patients with advanced solid tumors (Tolcher et al. 2008).

A summary of novel IGF1R monoclonal antibodies therapies is given in Table 27.4.

27.4 Limitless Replicative Potential: Telomerase

Telomerase is an enzyme that maintains the ability of cancer cells to achieve limitless proliferation thus allowing them to divide an indefinite number of times.

This process is the result of the addition of TTAGGG nucleotide repeats onto the telomers of chromosomal DNAs maintaining their length. Telomerase activation

[3] (http://www.clinicaltrials.gov/)

is not found in somatic cells, however, is an early event during oncogenesis and has been detected in 85–90 % of tumors correlating with poor prognosis (Kim et al. 1994). Telomerase has important roles in angiogenesis, metastasis and cancer stem cells in addition to its classical function in telomere length maintenance (Dikmen et al. 2009). Therefore a growing number of anti-telomerase strategies have emerged against the RNA component hTERC (human telomerase RNA component) and the protein component of hTERT (human telomerase reverse transcriptase) (Blackburn et al. 2006). The main strategies are targeting the RNA component hTERC and hTERT by antisense oligonucleotides.

Other methods target the telomerase associated proteins TP1(telomerase associated protein 1) and TRF1 (human tekomeric-repeat binding factor) (Burger 2007).

Imetelstat (GRN163L, Geron) is a 13-mer oligonucleotide that targets the active site of the enzyme TERC- RNA template. It has exhibited promising anti-tumor effects including antiangiogenic and anti-metastatic effects. Imetelstat was effective in preclinical studies in breast and lung cancer tumor cell lines and xenograft models (Dikmen et al. 2005; Hashizume and Gupta 2010). It entered phase I and II clinical trials in patients with chronic lymphocytic leukemia, multiple myeloma, and advanced solid tumors (NSCL and breast cancer).

Several small molecules, BRACO19 and RHPS4 that target single stranded telomeric repeat sequences (G-quadruplex), have shown very promising anticancer activity in tumour xenograft models (Neidle 2010).

27.5 Resistance to Apoptosis

Evasion of apoptosis is yet another crucial step in the overall process of tumor development. Anti-apoptotic mechanisms are up-regulated in tumors due to overexpression of anti-apoptotic proteins. Additionally, resistance to apoptosis and anoikis are important characteristics of metastatic cells. Apoptosis is the result of several key events that include inactivation of p53, activation of survival pathways (PI3k), and the upregulation of MMPs (which down-regulate death receptors, release growth factors, and prepare the extracellular matrix for invasion). Overexpression of anti-apoptotic proteins such as BCL-2, BCL-XL or focal adhesion kinase (FAK) also play a role (Vaux et al. 1988; Cory and Adams 2002).

The BCL-2 family is an important regulator of the mitochondria-dependent apoptotic pathways. It consists of pro-apoptotic proteins such as the BH3 family, two multi-domain pro-apoptotic proteins BAX and BAK as well as several multi-domain anti-apoptotic proteins (BCL-2, BCL-XL, BCL-W, MCL-1 and A1) (Cotter 2009). The anti-apoptotic BCL-2 promotes cell survival by impeding the activation of pro-apoptotic caspase proteins thereby contributing to the pathogenesis and progression of human cancers. Increased expression of BCL-2 is common in a number of tumors such as melanoma, lung, renal, colo-rectal, head and neck and brain cancer. Increased expression has also been seen in B cell lymphomas, NHL and chronic myelogenous

leukemia (CML) (Cotter 2009; Maurer et al. 1998; Ravandi et al. 2001; Sharma et al. 2004; Shabnam et al. 2004; Sharma et al. 2005; Gradilone et al. 2003). Overexpression of BCl-2 in tumors has a negative impact on anticancer therapy as a result of increased resistance to drugs and radiotherapy (Sartorius and Krammer 2002).

Alterations in the expression and function of BCL-2 occur for various reasons. These include chromosomal abnormalities, gene hypomethylation, altered epigenetic regulation of the *BCL-2* gene (Hanada et al. 1993) and down-regulation of inhibitory mechanisms of the microRNAs *miR-15* and *miR-16* (Cimmino et al. 2005). Other factors, such as p53 mutation contribute to anti-apoptotic mechanisms in tumors through regulation of pro-apoptotic targets in the BCL-2 family including BAX and the BH3 proteins PUMA (p53 up-regulated modulator of apoptosis) and NOXA (Cotter 2009; Yu et al. 2001; Nakano and Vousden 2001; Miyashita and Reed 1995). Additionally, deregulations in many signal-transduction pathways in cancers affect the expression of the BCL-2 family members (e.g. RAS pathway, PI3-K and nuclear factor-κB (NF-κB) transcription factors) (Mayo and Baldwin 2000; Cox and Der 2003). Each of the biological steps of the apoptotic process has been therapeutically targeted resulting in the development of specialized apoptosis-modulatory therapy. These agents are currently under investigation in various clinical trials.

Therapeutic opportunities Inactivation of BCL-2 has been shown to induce apoptosis in malignant cells and to increase their sensitivity to chemotherapy (Guo et al. 2003). BCL-2 antisense oligonucleotide therapy showed anti-tumor responses and increased apoptosis in melanoma biopsies (Jansen et al. 1988).

Oblimersen sodium (G3139, Genasense) is an antisense phosphorothioate oligodeoxynucleotide (ODN) that is designed to be complementary to the first six codons of the human BCL-2 mRNA sequence. It is currently being extensively evaluated in clinical trials in CLL, AML, advanced melanoma (Patel et al. 2009). This therapy induces pro-apoptotic effects through an increase in BAX and PARP as well as through the release of cytochrome **c** with subsequent activation of the caspase cascade (Nicholson 2000). Furthermore, several studies have indicated that this compound has modulatory effects on the immune system. Results from phase I and III clinical trials using this agent in combination with classic chemotherapeutic agents demonstrated modest anti-tumor responses (Jansen et al. 1988; Nicholson 2000; Kang and Reynolds 2009).

Addition of BCL-2 anti-sense therapy to dacarbazine was evaluated in a randomized phase III clinical trial in patients with cutaneous melanoma, and revealed an improvement in clinical outcomes (Bedikian et al. 2006). BCl-2 antisense drug therapy has shown chemosensitizing effects in CLL patients when combined with cyclophosphamide (O'Brien et al. 2007, 2009). In metastatic prostate cancer it has been used in combination with mitoxantrone (Tolcher et al. 2005). In breast cancer it has been used as an adjuvant to docetaxel (Moulder et al. 2008) and in colorectal cancer in combination with irinotecan (Mita et al. 2006). However, addition of oblimersen to etoposide did not improve overall clinical outcome in patients with SCLC (Rudin et al. 2008).

Table 27.5 Examples of BCL-2 inhibitors that are currently in clinical development

Drug	Target	Clinical Trial
Oblimersen	Anti-sense BCL-2	CLL, AML, multiple myeloma, SCLC, non-Hodgkin's lymphoma and melanoma
Gossypol (AT-101)	BCL-2 small molecule inhibitor BH3 mimetic	Phase I/II CLL, prostate cancer
ABT-737 (ABT-263)	BCL-2, BCL-XL, BCL-W BH3 mimetic	Phase I in chronic myelogenous leukemia and (SCLC)
GX15–070	Pan apoptotic inhibitor BCL-2, BCL-XL, BCL-W, MCL-1	Phase I in SCLC and NSCLC

An alternative strategy inclusive of the BCL-XL antisense oligonucleotide targeting a specific BCL-XL sequence has been shown to induce even further chemosensitization of the tumor cells (Zangemeister-Wittke et al. 2000).

Other therapeutic modalities affecting gene or protein expression are small molecules that act as BH3 mimetics and bind to BCL2 neutralizing its activity and inducing pro-apoptotic effects. Several agents targeting the BCL-2 family and demonstrating inhibition of BCL-2, BCL-XL, and MCL-1 are currently being evaluated in clinical trials. An example is Gossypol which is a drug that entered Phase II clinical trials in CLL and in prostate cancer (Kang and Reynolds 2009; MacVicar et al. 2008) and was also tested in patients with advanced cancers (Saleh et al. 2009). Other pan-apoptotic inhibitors have been developed, for example the ABT-737 (A-779024, Abbott Laboratories), a small-molecule inhibitor of BCL-2, BCL-XL and BCL-W (Oltersdorf et al. 2005). Yet another example is ABT-263 which is an oral compound of ABT-737 that was shown to induce tumor regression in xenograft models of SCLC and acute lymphoblastic leukemia (ALL) (Tse et al. 2008). Lastly, GX15-070 (Obatoclax, Gemin X), also an inhibitor of BCL-2 family is currently in preliminary trials in patients with small cell lung cancer (SCLC) (Chiappori et al. 2009). A summary of BCL-2 inhibitors that are currently in preclinical and clinical development is shown in Table 27.5 (Nicholson 2000; Kang and Reynolds 2009).

27.6 Abnormalities in Growth-Stimulatory Signaling Pathways

27.6.1 RAS/RAF/MEK/ERK

Advancements in the field of molecular and genomic technology have led to the identification of various pathways that are deregulated in human cancers. This has paved the way for further investigation of additional targets for anticancer therapy.

The *RAS* family of oncogenes (*HRAS*, *KRAS*, and *NRAS*) encodes 21-kDa plasma membrane–associated G-proteins that regulate signaling cascades involved in normal cellular differentiation, proliferation, and survival (Downward 2003). Activating

Table 27.6 A summary of novel therapies that s target Ras-Raf-MEK-ERK pathway

Inhibitor	Specificity	Selected tumor types
Tipifarnib (Zarnestra™; Ortho Biotech Products, Lonafarnib (Sarasar™; Schering-Plough), BMS-214662 (Bristol-MyersSquibb, FTI-277 (Calbiochem). L744832 (Biomol International L.P., Biosciences.)	Inhibitors of the farnesyl-transferase enzyme	NSCLC, HNSCC, breast, ovarian, prostate, glioma, pancreatic, colorectal cancer
Sorafenib (BAY 43–9006, Nexavar®)	Raf-1, wild-type B-Raf, and *b-raf* V600E RAF kinase, VEGFR2, PDGFR-α and PDGFR-β, FLT3 and c-Kit	Metastatic RCC, HCC, melanoma, NSCLC, breast, ovarian, prostate, pancreatic, colorectal, glioma
PLX4032, PLX4720	B-Raf	Melanoma
Selumetinib (AZD6244; ARRY-142886)	MEK inhibitor	Melanoma
Tanespimycin (KOS-953, 17-AGG) Hsp90 inhibitor	B-Raf, AKT/PKB, ERBB2, CDK4, HER2, HIF-1α	Melanoma, breast cancer
Vaccination with mutant KRAS peptides		Pancreatic adenocarcinoma
RAS antisense treatment, ISIS2503, ISIS5132	HRAS, c-RAF1	NSCLC

oncogenic mutations in the all three *RAS* genes are common in several human cancers (Lowy and Willumsen 1993; Davies et al. 2002) and approximately 50 % of metastatic tumors contain RAS mutations (Chambers and Tuck 1993). RAS oncogenes contribute to tumor growth, invasion, angiogenesis and metastasis through Ras binding to Raf protein kinases, Raf-MEK-extracellular signal-regulated kinase family, and PI3K pathway. Additionally, RAS oncogenes function through Ral-specific guanine nucleotide exchange factors (RalGEFs), (Chambers and Tuck 1993; Shields et al. 2000; Ward et al. 2001) RAC, RHO and NFkB pathways (Downward 2003). A number of drugs that specifically target KRAS function have been developed and are currently under investigation in clinical trials (Table 27.6) (Downward 2003; Bos 1989).

Maturation of Ras proteins is a process that relies on farnesylation through covalent attachment of the enzyme coupling a 15-carbon isoprenyl group to Ras proteins. (Adjei et al. 2000). Inhibitors of the farnesyl-transferase enzyme are currently being investigated as potential therapeutic agents in the treatment of various cancers (Johnston 2001). Farnesyl-transferase enzyme inhibitors (FTIs) mimic the carboxy-terminal motif of RAS and compete for binding to farnesyltransferase. These compounds for example tipifarnib (Zarnestra™; Ortho Biotech Products),lonafarnib (Sarasar™; Schering-Plough Corporation), BMS-214662 (Bristol-Myers Squibb), FTI-277(Calbiochem),EMD and L744832 (Biomol International L.P., Biosciences) were demonstrated to have apoptotic and anti-angiogenic effects. They were also effective in achieving inhibition of tumor cell growth in various cancers such as that of colon, bladder, lung, prostate, and pancreas (Johnston 2001). FTIs initially showed

significant promise in preclinical studies (Appels et al. 2005) and were subsequently tested in combination with cytotoxic drugs in clinical trials for lung cancer (Isobe et al. 2005). However, the results gathered from other phase II clinical trials revealed only moderate effects. Further studies are required for a complete understanding of the biological activities of FTIs (Brunner et al. 2003).

The RAF–MEK–ERK signaling cascade has an important role in tumor pathogenesis, and aberrant signaling through RAF (a downstream effector of the RAS pathway) occurs in approximately 30 % of human cancers (Bos 1989). Activating mutations of *BRAF* occur in approximately 8 % of human tumors, most frequently in melanoma (66 %), colorectal, and thyroid cancers. The three *RAF* somatic missense mutations code for cytoplasmic serine/threonine kinases which were shown to be related to proliferation and resistance to apoptosis. Therefore BRAF protein serine/threonine kinase could be used as an important and specific therapeutic target (Davies et al. 2002).

A. Kinase inhibitors targeting RAS effector pathway Sorafenib (BAY 43–9006, Nexavar®), is a multikinase inhibitor which was designed as an inhibitor for Raf-1, wild-type B-Raf and *b-raf* V600E. Sorafenib also inhibits several receptor tyrosine kinases on the intracellular domain of VEGFR1, VEGFR2, VEGFR3, PDGF receptors FMS-like tyrosine kinase 3 (Flt-3), stem cell factor receptor (KIT), and the glial cell-line derived neurotrophic factor receptor (RET) (Downward 2003). Sorafenib demonstrated good safety, tolerability and clinical activity in several tumor types particularly in patients with RCC and HCC (Strumberg et al. 2007; Lombardi et al. (in press); Escudier et al. 2007; Llovet et al. 2008). Further phase II and III studies evaluating sorafenib demonstrated an increased median overall survival and delayed the median time to progression in patients with advanced HCC and metastatic RCC (Lombardi et al. (in press); Cheng et al. 2009; Keating and Santoro 2009; Reeves and Liu 2009). However, a phase III clinical trial of sorafenib in combination with carboplatin and paclitaxel in patients with metastatic melanoma did not have an impact on improvement in overall survival (Hauschild et al. 2009).

Other small-molecule inhibitors of Raf kinases including Raf265 (Novartis), XL281 (Exelixis/Bristol Myers Squibb), AZ628 (AstraZeneca), SB-590885 (GlaxoSmithkline) and PLX-4032 (Plexxikon/Roche) a highly selective inhibitor for BRAF(V600E) has demonstrated a greater selectivity and antitumor activity in preclinical trials and phase I studies (Wellbrock and Hurlstone 2010; Pratilas and Solit 2010).

PLX-4032 is currently under clinical investigation as a single agent in metastatic cutaneous melanoma. The results from a phase I trial, reported good oral bioavailability, tumor regression and a median increased survival in metastatic melanoma patients (Flaherty et al. 2009). This therapeutic agent is currently being investigated in phase III clinical trials. However, PLX4032 may paradoxically enhance the proliferation of tumors through ERK activation in tumor cells that co-express BRAF(V600E) and mutant RAS (Pratilas and Solit 2010; Poulikakos et al. 2010).

Other drugs targeting the RAS/RAF- ERK–MAPK pathway include inhibitors of Hsp90 and its target proteins. Some of these client proteins such as RAF, AKT,

ERK, PI3K, VEGF, uPA, and MMPs are involved in promoting cancer invasion and angiogenesis. Inhibition of Hsp90 results in destabilization of the client proteins with antitumor effects (Koga et al. 2009). Tanespimycin (KOS-953) an inhibitor of Hsp90, was evaluated in a phase II clinical trial in cutaneous melanoma (Solit et al. 2008) and in combination with trastuzumab in breast cancer (Modi et al. 2007). The combination of sorafenib and tanespimycin resulted in pharmacodynamic activity in kidney cancer and melanoma meeting the criteria for further evaluation (Vaishampayan et al. 2010).

B. MEK inhibitors Mitogen-activated protein kinase (MAPK) pathway activation can result from mutations of *BRAF* and *RAS* oncogenes or upstream deregulation of growth factor receptors.

Inhibitors of the RAF–MEK–ERK signaling could modulate tumor cells growth, differentiation, and proliferation. MEK inhibitor, PD0325901(Pfizer), significantly suppresses pERK levels in certain tumors in preclinical studies (Barrett et al. 2008) and showed preliminary clinical activity in patients with advanced cancers (LoRusso et al. 2010)**.** The specific MEK 1/2 inhibitor AZD6244 (ARRY-142886) (AstraZeneca)) is an ATP noncompetitive, allosteric inhibitor of MEK1/MEK2 and has shown tumor suppressive activity in pre-clinical models including melanoma, pancreatic, colon, lung, and breast cancers (Pratilas and Solit 2010; Bennouna et al. 2010). The results reported from a phase II clinical trial in cutaneous melanoma have shown lasting remissions in patients with BRAF mutations and this agent is currently being evaluated in Phase II clinical trials (Bennouna et al. 2010; Board et al. 2009). However, the activity of this agent was comparable to disease-specific standard chemotherapy. AZD6244 is currently undergoing evaluation in Phase II trials in combination with other chemotherapeutic agents in selected patients with active mutations in BRAF and/or RAS.

Another therapeutic approach is the development of antisense synthetic oligonucleotides that are specific for sequences in the mRNAs for HRAS (ISIS2503) or c-RAF1 (ISIS5132). These agents are now being evaluated for clinical activity in phase II trials NSCLC (Sato et al. 2007). However, their high level of specificity for one target is likely to be less effective in a tumor modulated by pleiotropic mechanisms.

Immunotherapy via vaccination with mutant KRAS peptides induced a transient Ras-specific T-cell response, a long-term immune response and increased survival in patients with pancreatic carcinoma following surgical resection (Wedén et al. 2010)

27.6.2 Phosphatidylinositide 3-Kinase (PI3K) Pathway

The PI3K pathway is a major cellular signal transduction pathway involved in cell growth, survival, angiogenesis and metabolism (Vivanco and Sawyers 2002). Activation of the PI3K pathway occurs through stimulation of RTKs which results in the assembly of receptor–PI3K complexes. Based on their structure PI3Ks are classified as class I (class IA p110α, p110β, p110δ and class IB, p110γ), class II (PI3KC2α,

PI3KC2β and PI3KC2γ) and class III (lipid kinases VPS34; homologue of the yeast vacuolar protein sorting-associated protein 34) which mediates signaling through mammalian target of rapamycin (mTOR) (Cantley 2002; Workman et al. 2010). The activation of the catalytic subunit of class I- PI3Ks is followed by the phosphorylation of phosphatidylinositol-4, 5-bisphosphate (PIP2) to phosphatidylinositol-3, 4, 5-trisphosphate (PtdIns(3,4,5)P_3).They recruit PDK1 and AKT to the plasma membrane followed by AKT phosphorylation at Thr308 by PDK1 and at Ser473 by mammalian target of rapamycin (mTOR) complex 2 (TORC2), (Wullschleger et al. 2006; Sarbassov et al. 2005). PTEN is a major limiting factor of this step and antagonizes this process by dephosphorylating PIP_3 to inhibit activation of AKT (Zhang and Yu 2010; Blanco-Aparicio et al. 2007). *PTEN* tumor suppressor gene is frequently inactivated in cancers by mutation, resulting in accumulation of PIP3 thus triggering the activation of its downstream effectors PDK1 and AKT/PKB (Yuan and Cantley 2008). One of the consequences of AKT activation is mTOR activation. The signaling complex downstream mTOR include ribosomal protein S6 kinase 1(p70S6K) and eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1) which are important factors in protein synthesis, cell growth, metabolism and angiogenesis (Wang et al. 2006; Sabatini 2006). Phosphorylated AKT mediates the activation and inhibition of several targets, promoting cell cycle progression, proliferation and inhibition of apoptosis through various mechanisms (Yuan and Cantley 2008). Mutations in both PI3K and mTOR pathway are critical for tumor growth and survival and are involved in a wide range of tumors including breast, prostate, colon carcinomas and malignant brain tumors (Blume-Jensen and Hunter 2001).

The signalling of the PI3K pathway triggers tumor progression through multiple effects on cellular growth, proliferation, survival, motility and modulates tumor drug resistance (Vivanco and Sawyers 2002).The PI3K–AKT pathway also modulates angiogenic effects through upregulation of hypoxia-inducible factor (HIF)-1α and VEGF (Eccles and Welch 2007; Kong and Yamori 2008).

PI3K -AKT activation in cancer can occur at multiple points including activation of receptors or oncogenes upstream of PI3K or accumulation of PtdIns(3,4,5)P_3. Additionally this pathway can be deregulated through mutation or loss of the tumor suppressor PTEN, PI3K or of the downstream elements such as AKT and mTOR (Yuan and Cantley 2008; Abdel-Rahman et al. 2006; Watters and Huang 2009). Several studies indicated that targeting the PI3K-AKT pathway caused a reduction of tumor cell proliferation as well as their migratory and invasive capacity (Vivanco and Sawyers 2002). Therefore, the PI3K/AKT/mTOR pathway is considered an attractive target for novel anti-cancer therapeutic strategies. Several pathway components including AKT, PI3K and mTOR represent potential therapeutic targets. Many of these inhibitors are currently being evaluated preclinically or in early clinical trials (Liu et al. 2009).

A. PI3K inhibitors All PI3K isoforms are mutated in several cancers (Samuels et al. 2004) and are proven to induce oncogenic transformation in xenograft animal models. They are involved in cancer cell proliferation, growth, apoptosis, cytoskeletal rearrangement and tumor angiogenesis while also being a therapeutic target in tumors with PI3K mutations (Kang et al. 2006).

Table 27.7 Examples of several PI3K inhibitors currently being evaluated in preclinical and patient trials

Inhibitor	Specificity	Selected tumor types
PX-866 (Oncothyreon, Bellevue, WA, USA)	PI3 K (p110α, -δ and -γ)	Ovarian and lung carcinoma, colon xenografts Phase I clinical trial in patients with advanced metastatic solid cancer
CAL-101	PI3 K δ	Non-Hodgkin's lymphoma, mantle cell lymphoma, and CLL
PI-103(Novartis, Basel)	Class I PI3 K and mTOR	Preclinical studies in ovarian, breast, glioblastoma
XL765	Dual class I PI3K and mTOR	Patients with advanced tumors
SF1126 (Semafore, Indianapolis, IN, USA)	PI3K class I mTORC1/2	Antitumor and antiangiogenic effects in preclinical studies Phase I clinical trials
GDC-0941	pan PI3 K inhibitor	Breast, ovarian, lung, prostate xenografts Phase I clinical trials
GSK1059615	PI3K inhibitor	Phase I clinical trial
XL114	Pan PI3K inhibitor	Preclinical studies in breast, lung, ovarian, prostate and glioma tumors Phase I clinical trials
ZSTK474 (Zenyaku Kogyo, Tokyo, Japan)	Pan PI3 K inhibitor	Tumor xenografts of prostate adenocarcinoma, colorectal carcinoma and lung adenocarcinoma
XL184	PI3 K (p110α, -δ and –γ) and (TORC1, TORC2) MET, VEGFR2, and RET	Phase I-III clinical trials in patients with patients with progressive glioblastoma and medullary thyroid cancer
NVP-BEZ235 (Novartis)	Pan-PI3K/mTOR	Phase I and II clinical trials in patients with advanced breast, prostate, and brain cancers
NVP-BGT226	Dual class I PI3K and mTOR inhibitor	Phase I

The first PI3K inhibitors to be extensively researched were the fungal metabolite wortmannin (Arcaro and Wymann 1993) and LY294002 (Vlahos et al. 1994) which block the enzymatic activity of PI3Ks through an ATP-binding competitive mechanism (Liu et al. 2009).These compounds showed dose-dependent cell growth inhibition and antitumor and antiangiogenic efficacy in preclinical studies, but high levels of toxicity (dermal and liver toxicity), combined with poor solubility and low bioavailability, prevented their evaluation in clinical trials. However, wortmannin and LY294002 were widely used as tools for further elucidating the biological roles of PI3Ks in tumorigenesis (Workman et al. 2010). Several PI3K inhibitors have been developed and are currently being evaluated in preclinical and patient trials (Table 27.7)

New generation of PI3K inhibitors include PX-866 (Oncothyreon, Bellevue, WA, USA), a compound similar to wortmanin which demonstrated activity as an oral irreversible PI3K inhibitor with selectivity for class I PI3K isoforms α, γ and δc in

lung carcinoma, ovarian and colon carcinoma xenografts (Ihle et al. 2004). This drug is currently being investigated in a phase I clinical trial in patients with advanced metastatic cancers and preliminary results indicated signs of disease stabilization (Jimeno et al. 2009).

Pan-specific PI3K inhibitors (for example PI-103, NVP-BEZ235, GDC-0941 and ZSTK474), occupy the ATP-binding site of the enzyme and have improved properties to modulate PI3K kinases.

GDC-0941 is a pan PI3K inhibitor that demonstrated signs of antitumor activity in multiple xenograft models such as breast, ovarian, lung and prostate cancer (Folkes et al. 2008). In a phase I clinical trial in patients with advanced solid tumors this agent was well tolerated, with signs of biological activity (Hoff et al. 2010). Recent studies with GDC-0941 have shown promising results by combining this agent with trastuzumab (Yao et al. 2009) and MEK inhibitors (Hoeflich et al. 2009).

ZSTK474 (Zenyaku Kogyo, Tokyo, Japan) is a triazine derivative with selective pan-PI3K inhibitory activity that showed favorable responses in preclinical studies with tumor xenografts of prostate adenocarcinoma, colorectal carcinoma and lung adenocarcinoma, (Yaguchi et al. 2006).

PI-103 (Novartis) is a synthesized molecule of the pyridofuropyrimidine that shares a similar structure with LY294002 and has the ability to target both PI3K-p110α and mTOR. It demonstrated antiproliferative and antitumor effects in preclinical studies in, breast and ovarian cells xenografts and enhanced chemotherapy-induced cell death of glioblastoma GBM cells (Raynaud et al. 2007; Westhoff et al. 2009). Further studies are ongoing to determine the efficacy and the pharmacological properties of PI-103 agent to target both mTOR and PI3Ks in cancer (Raynaud et al. 2007; Fan et al. 2006).

SF1126 (Semafore) is a LY294002 pro-drug that targets all PI3 K class I isoforms including mTORC1/2 and has proven antitumor and antiangiogenic responses in preclinical studies of brain, neuroblastoma, NSCLC, prostate, myeloma, RCC. It is currently being evaluated in phase I and dose escalation clinical studies (Garlich et al. 2008).

Encouraging results have been described for XL765 compound which is a dual PI3K and mTOR inhibitor which is currently in phase I studies in patients with solid tumors. Preliminary results showed that XL765was well tolerated and demonstrated pharmacodynamic modulation of PI3K and ERK pathway with evidence of stable disease in patients with advanced cancer (Papadopoulos et al. 2008; Brana et al. 2010). Other multikinase PI3K inhibitors, XL184, XL147, XL765 and XL147 (Exelixis) are currently in development. Clinical data from patients treated with XL184 a MET, VEGFR2, and RET inhibitor, has demonstrated activity in phase I-III clinical trials in patients with progressive glioblastoma and medullary thyroid cancer (Wen et al. 2010; Sugawara et al. 2009).

XL-765, a pan-class I- PI3K inhibitor has an inhibitory effect also on DNA-PK and MTOR and has the ability to induce delays in tumor growth in xenograft models. This agent has been well tolerated as monotherapy in a phase I clinical trial when administered orally to patients with advanced solid tumors, (Papadopoulos et al. 2008) or in combination with temozolomide (TMZ), (Nghiemphu et al. 2010).

Interim analyses of an ongoing phase I clinical trial in patients with advanced cancer showed that the XL147 compound was well tolerated and induced prolonged stable disease in several cases (Shapiro et al. 2009). Also, preliminary results of a trial evaluating the combination of XL147 and erlotinib resulted in clinical activity and simultaneous inhibition of PI3K and EGFR signaling (Moldovan et al. 2010).

NVP-BEZ235 (Novartis) is an imidazo-quinoline derivative, which exhibits dual pan-PI3 K/mTOR inhibition. Preclinical data show that NVP- BEZ235 has strong anti-proliferative activity in cell lines and tumor xenografts with abnormal PI3K signalling. This therapeutic agent has entered Phase I and II clinical trials in patients with advanced breast, prostate, and brain cancers (Maira et al. 2008). Other PI3K inhibitors that have entered phase I clinical trials include: NVP-BGT226 (a dual class I PI3K and mTOR inhibitor) and NVP-BKM120 (a selective pan-class I PI3K inhibitor) (Brachmann et al. 2009).

Several other phase I studies investigating PI3K inhibitors are ongoing. Two examples of the study drugs are GSK1059615 (GlaxoSmithKline) (Brachmann et al. 2009) and CAL-101 (Calistoga Pharmaceuticals). CAL-101 is a selective agent targeting p110δ. Interim results from a phase I trial with CAL-101 demonstrated favourable clinical results in patients with haematological malignancies such as non-Hodgkin's lymphoma (NHL), mantle cell lymphoma, and chronic lymphocytic leukemia (CLL) (Lannutti 2010)

B. PDK inhibitors Phosphorylation of the threonine residue in the activation loop of the three AKT isoforms and PKC (protein kinase C) is modulated by PDK1. This process stimulates cell growth, proliferation and survival, as well as promoting angiogenesis. Several anti-PDK 1 inhibitors such as UCN-01 were tested in phase I and II clinical trials, however they did not have significant antitumor activity (Welch et al. 2007). Further development of an indoline-based series of PDK1 inhibitors such as BX-517, demonstrated a potent inhibitory effect through binding to the ATP pocket of PDK1 (Islam et al. 2007a, b).

C. AKT inhibitors AKT amplification and activation occurs in a variety of tumors such as melanoma, breast, ovarian and pancreatic cancers. It is critical for phosphorylation of many downstream substrates involved in tumor survival as well as organization of the actin cytoskeleton and invasion (Liu et al. 2009; Carpten et al. 2007). Over expression of AKT2 was reported in late-stage colorectal cancer and metastases suggesting that AKT2 promotes metastatic disease (Rychahou et al. 2008). The involvement of AKT in these processes supports a role for selective targeting of the PI3K/AKT pathway as a strategy for metastasis (Table 27.8) (Vivanco and Sawyers 2002).

Perifosine (Keryx) is a lipid-based phosphatidylinositol analogue that inhibits AKT by targeting the pleckstrin homology (PH) domain of AKT thus blocking AKT membrane translocation. This drug has the end result of reduction of proliferation while also inhibiting AKT. This effect has been shown in a variety of tumors cells such as melanoma, lung, prostate, colon, and breast cancer (Crul et al. 2002). Results from a phase I clinical trial in patients with advanced solid tumors showed that the drug was well tolerated (Unger et al. 2010) with evidence of stable disease in sarcoma (Bailey

Table 27.8 AKT inhibitors that are currently under investigation for various malignancies

Inhibitor	Specificity	Selected tumor types
Perifosine (Keryx)	AKT	Phase I and II in advanced solid tumors
GSK690693	AKT, GSK3 beta, PRAS40, Forkhead	Preclinical studies in ovarian, breast, prostate carcinoma
API-2	AKT	Phase I in advanced solid tumors NSCLC, leukemia
XL418 (Exelixis)	AKT	Phase I in advanced solid tumors
MK2206 (Merck)	AKT	Phase I in advanced solid tumors
Tanespimycin (KOS-953, Kosan)	Hsp90 inhibitor AKT	Phase II metastatic breast cancer Multiple myeloma

et al. 2006) and renal cell carcinoma. However, these results were not clinically validated in phase II clinical studies in breast, pancreas, prostate, head and neck, and lung cancer (Kondapaka et al. 2003; Ummersen et al. 2004; Gills and Dennis 2009).

Other AKT inhibitors that are phosphatidylinositol ether lipid analogues (PIA) which interfere with the PH domain of AKT inhibit the translocation of AKT to the plasma membrane (Hu et al. 2000). Lipid analogues and PH domain-targeting inhibitors were shown to have AKT inhibitory effects (Gills et al. 2007) in addition to reducing tumor cell growth in preclinical studies (Powis et al. 1992).

Inositol polyphosphates such as InsP5, a novel inhibitor of the PI3K/AKT pathway, can compete with PtdIns(3,4,5)P_3 by binding to AKT- PH domain. InsP_5 has anti-AKT and antiangiogenic effects resulting in xenograft tumor growth inhibition (Maffucci et al. 2005). A derivative of InsP_5, 2-*O*-Bn-InsP_5, resulted in enhanced pro-apoptotic and anti-tumor activity through inhibition of PDK1 and mTOR (Falasca et al. 2010). The recent development of the aminofurazan AKT series of inhibitors has led to the identification of GSK690693, a compound that causes dephosphorylation of targets downstream of AKT, including GSK3 beta, PRAS40, and Forkhead (Heerding et al. 2008). Xenograft studies resulted in antitumor activity in ovarian, prostate, and breast carcinoma (Rhodes et al. 2008).

Several AKT antagonists have been identified using high throughput screening. API-1 inhibits AKT by binding to the PH domain and blocking AKT membrane translocation (Kim et al. 2010). API-2 (triciribine phosphate), a water-soluble tricyclic nucleotide selectively induces apoptosis and inhibits cell growth in tumors with PTEN mutations and AKT amplification. This drug is currently being tested in Phase I clinical trials in patients with both solid and haematological malignancies. (Yang et al. 2004)

MK2206 (Merck), an orally active allosteric AKT inhibitor is under evaluation for the treatment of patients with locally advanced or metastatic solid tumors (Tolcher et al. 2009b). Preclinical data showed enhanced anticancer activity for MK-2206 in combination with several anticancer agents (erlotinib, lapatinib) (Hirai et al. 2010) as well as in combination with MEK inhibitor, AZD6244.

XL418 (Exelixis), a small molecule that inhibits the activity of AKT and S6 Kinase (S6K). It has shown inhibitory effects on tumor growth in preclinical studies, including breast and lung adenocarcinomas and has currently entered Phase I clinical trials.

Table 27.9 Summary of mTORC1 and mTORC2 inhibitors currently in clinical trials

Inhibitor	Specificity	Selected tumor types
Rapamycin (Wyeth)	mTOR	Approved advanced RCC Phase I and II in advanced solid tumors
Temsirolimus (Torisel; Wyeth)	mTORC1	Approved advanced RCC and mantle cell lymphoma Phase I-III in advanced solid tumors, ovarian, endometrial carcinoma, NSCLC, melanoma
Everolimus (Afinitor; Novartis)	mTORC1	Approved advanced RCC Phase I-II in advanced breast cancer, lung cancer, pancreatic carcinoma, melanoma or glioma
Ridaforolimus (Merck/Ariad)	mTORC1	Approved soft-tisse and bone sarcomas Phase I clinical trial in patients with advanced malignancies
WYE-132 (Wyeth)	Dual mTORC1 and mTORC2	Preclinical studies in breast, glioma, lung, renal tumors

Hsp90 inhibitors: Both AKT and its activating kinase 3-phosphoinositide-dependent kinase-1 rely on Hsp90 for stability. Hsp90 and its co-chaperones modulate tumor cell apoptosis through formation of AKT-Hsp90 complexes, thus stabilizing the AKT kinase activity and phospho-AKT dephosphorylation (Sato et al. 2000). Several studies indicated that targeting the PI3K-AKT pathway with 17-AAG caused inhibition of AKT phosphorylation, induction of apoptosis and downregulation of multiple AKT and RAF dependent pathways (Workmann et al. 2007; Basso et al. 2002; Georgakis et al. 2006; Hostein et al. 2001; Solit et al. 2002).

D. mTOR inhibitors mTOR has a critical effect through regulation of several intracellular functions including cell growth, cell cycle progression, actin cytoskeleton organization, angiogenesis and apoptotic cell death. Several downstream compounds targeting mTOR have been designed (Faivre et al. 2006) (Table 27.9).

Rapamycin (sirolimus, Wyeth) is a macrolide antibiotic which binds to mTORC1 via FKBP12-rapamycin binding domain adjacent to the catalytic site of mTORC1. It suppresses mTOR-mediated phosphorylation. Analogues of rapamycin, such as temsirolimus (Torisel; Wyeth), everolimus (RAD001/Afinitor) and ridaforolimus (Ariad Pharamceuticals/Merck) have demonstrated antiproliferative activity against a diverse range of malignancies in preclinical studies, and have also been evaluated in multiple clinical trials. Results from phase III clinical trials showed improved clinical outcomes for everolimus in patients with RCC that had progressed after sunitinib or sorafenib therapy. Also, temsirolimus improved overall survival when compared with interferon in patients with metastatic RCC leading to FDA approval (Motzer et al. 2008; Hudes et al. 2007; Motzer et al. 2010). Temsirolimus is also approved for the treatment of mantle-cell lymphoma following results from a phase III clinical trial which reported improved progression free survival (PFS) and objective responses (Hess et al. 2009). Partial response rates were reported in patients with

soft-tissue sarcoma, neuroendocrine tumors and endometrial carcinoma and led to phase III trials evaluating everolimus and ridaforolimus in neuroendocrine and soft-tissue sarcoma. However, low response rates have been seen in trials of patients with advanced breast, lung, and pancreatic cancer as well as melanoma and glioma (Dancey 2010).

In addition to inhibiting tumor growth, mTOR inhibitors also act as anti-angiogenic agents, interfering with HIF-1α (hypoxia inducible factor), VEGF and PDGF signalling cascades (Faivre et al. 2006; Thomas et al. 2006). These agents can therefore be effective when used in combination with anti-angiogenic drugs. Evidence of this was seen in a phase II clinical trial investigating the efficacy of the combination of bevacizumab and everolimus which revealed biological activity and good tolerability in the treatment of advanced clear cell renal cancer. This combination had moderate activity in patients with metastatic melanoma (Hainsworth et al. 2010a, b).

Ridaforolimus is an analog of rapamycin that has been shown to inhibit mTOR activity, as evidenced by reduced phosphorylation of 4E-BP1 and S6. This drug inhibits the proliferation of multiple tumor cell lines including breast, colon, lung, prostate, glial, and those of pancreatic origin. This drug was well tolerated with favorable antitumor activity in a phase I clinical trial in patients with advanced malignancies including NSCLC, RCC, and Ewing sarcoma (Mita et al. 2008). Ridaforolimus is currently being evaluated in a phase III clinical trial in patients with advanced sarcoma[4].

Dual ATP-competitive inhibitors of both mTORC1 and mTORC2 are emerging. They have been reported to reduce cancer cell proliferation in vitro and tumor xenograft formation in vivo. In preclinical studies, oral administration of WYE-132 inhibited mTORC1 and mTORC2 and resulted in antitumor activity against breast, glioma, lung, and several renal tumor cell lines (Yu et al. 2010).

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