Molecular Inhibitors of Growth Signals

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Signal transduction associated with growth factor receptors typically mediates the activation of cell cycle promoting gene products or the inactivation of cell cycle checkpoints. These signals are frequently transduced through G-Protein pathways, kinase receptor pathways, or nuclear receptor pathways (Fig. 4.1), are dependent on proto-oncogenic transcription factors, and lead to the expression of Cyclins and Cyclin-Dependent Kinases (CDKs), which are drivers of the cell cycle (Fig. 4.2). Physiologically, growth signals are transient and entirely depend on the engagement of a growth factor receptor by its cognate ligand. Upon termination of this interaction, the growth signal ceases. Gain-of-function mutations in cancer keep the growth signal active, regardless of the presence of the transient growth factor-receptor interaction. Such deregulated signaling cascades are appropriate drug targets in the treatment of cancer.

- Inhibitors of gene products with gain-of-function mutations may suppress the growth of cancer. Except in familial cancer syndromes, cancer causing mutations are somatic. Inhibitors of the mutant gene products selectively affect the cancer cells.
- Downstream signaling molecules are suitable drug targets, while inhibitors that act upstream of gain-of-function defects are ineffective. Drugs acting on immediate downstream molecules are more efficacious than drugs acting on far downstream molecules.
- Due to their specificity, molecular inhibitors of growth signals avoid the severe adverse effects of conventional chemotherapeutics on proliferating tissues (gastrointestinal, hematopoietic, hair).

The discovery that the genomes of some oncogenic viruses encode tyrosine kinases initiated research to identify drugs that suppress tyrosine kinase enzymes. Anti-proliferation activity was identified in herbimycin and staurosporine, but these compounds were insufficiently specific for individual kinases and exerted extensive toxicity. Intensive screening of chemical libraries for narrowly focused kinase inhibitors was initially viewed with scepticism, because the activity of these reagents relied on the competitive inhibition of ATP binding and the ATP binding domain was seen as a highly conserved motif in all kinases. The discovery of imatinib mesylate, which specifically inhibits BCR-ABL, the causative kinase for chronic myelogenous leukemia (CML), was a breakthrough to overcome this bias.

Drug Resistance Characteristically, molecularly targeted drugs are initially very successful but often loose efficacy after several months. It is likely that cells that are sensitive to the targeted drugs are eliminated, but the genetic instability of cancers leads to the presence of cells with mutations that confer drug resistance. Under the selective pressure of the drug treatment, the resistant cells preferentially proliferate.

Cancer cells exhibit a mutator phenotype, because defects in their DNA repair make their genomes instable. Therefore, considerable variation occurs in the spectrum of DNA alterations. Some of these genetic changes are "driver" mutations that play a causative role in transformation and constitute unique drug targets that are absent from normal cells (Table 4.1). However, most of the somatic mutations inside tumor cells are likely "passenger" mutations that are not involved in cancer formation. Their targeting with anti-cancer drugs has a low likelihood of success.

Inhibitors of gene products with gain-of-function mutations (or their downstream signaling targets) may suppress the growth of cancer.
Due to the genetic instability of cancer cells, molecularly targeted drugs often loose efficacy after several months.

4.1 Small Molecule Kinase Inhibitors

The coalescence of increasing insights into the molecular pathways associated with growth signals—specifically kinase cascades—with efforts to replace cancer therapy compounds that have intrinsic mutagenic potential have resulted in the development of small molecule kinase inhibitors. Most of their drug names end on -nib or -tinib.

- The majority of these agents block the ATP (adenosine triphosphate) binding site in their kinase drug target

G. F. Weber, *Molecular Therapies of Cancer,* DOI 10.1007/978-3-319-13278-5_4,

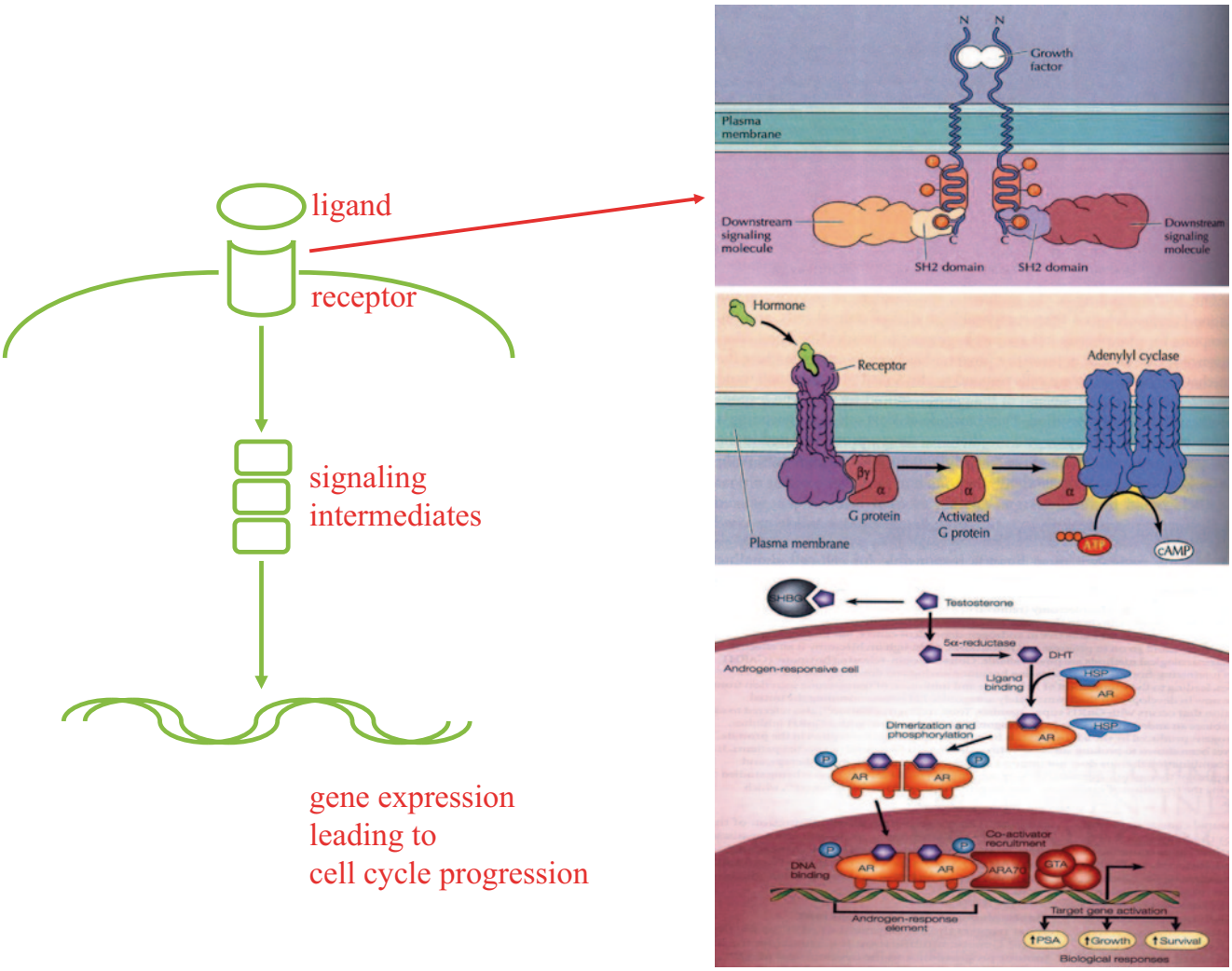

Fig. 4.1 Cellular growth pathways. (*Left*) the engagement of a growth factor receptor by its cognate ligand activates a pathway of signaling intermediates that causes changes in gene expression, which lead to cell cycle progression. (*Right*) most growth factor receptors belong to three families, comprising G-Protein Coupled Receptors (*top panel*), Receptor Tyrosine Kinases (*middle panel*), and Nuclear Hormone Receptors (*bottom panel*). (Parts adapted from Cooper 1997)

(type I inhibitors). Because all protein kinases have an ATP binding site that serves as the phosphate donor, there is potential for cross-reactivity and off-target effects. The close structural similarities among protein kinases can make achieving specificity for a particular kinase challenging, potentially leading to unwanted adverse effects. In clinical practice, this has not been a major problem. Despite the generic nature of the co-substrate ATP, the shapes of the binding pockets in various kinases are still sufficiently specific to allow for the development of unique kinase inhibitors.

- Type II inhibitors recognize the inactive conformation of the kinase.
- Some drugs are allosteric modulators that bind outside the ATP binding site.
- Covalent inhibitors are capable of forming irreversible, covalent bonds to the kinase active site, most frequently by reacting with a nucleophilic cysteine residue.

Drug Resistance Mechanisms of resistance to tyrosine kinase blockers include tyrosine kinase gene amplification and point mutations in the drug binding domain. Oncogenic switching is frequently induced as a resistance mechanism to tyrosine kinase inhibitors. The oncogenic switch allows cancer cells to signal through other receptor tyrosine kinases when one is blocked.

Most kinase inhibitors block the ATP binding site. Mechanisms of resistance to tyrosine kinase blockers include tyrosine kinase gene amplification and point mutations in the ATP binding domain.

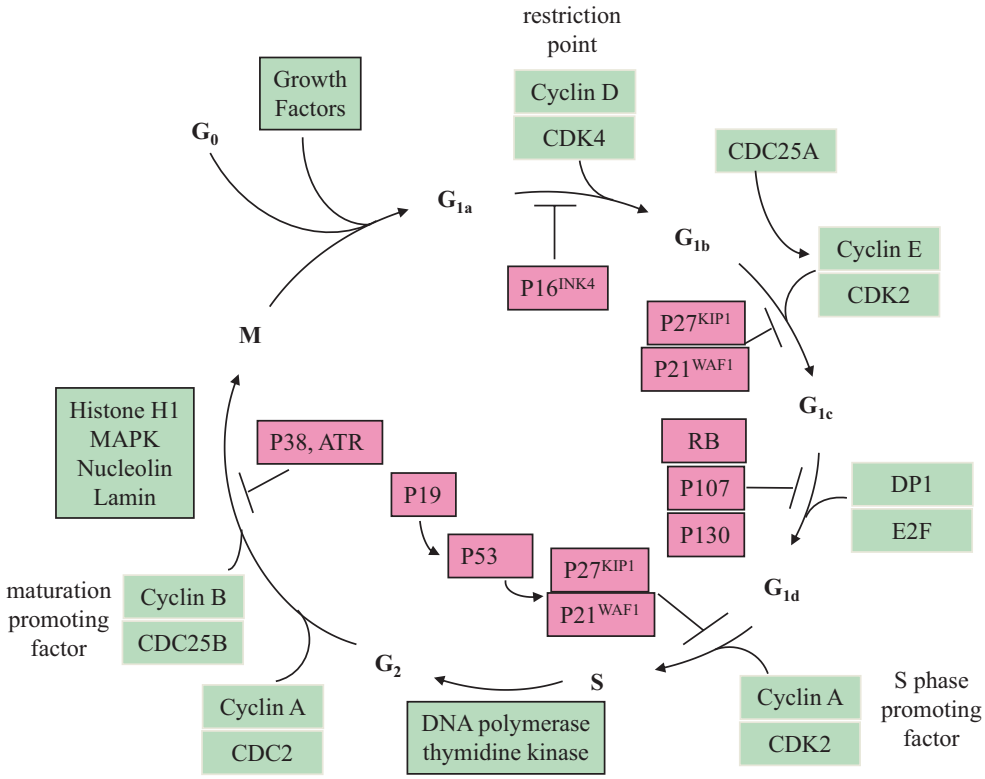

Fig. 4.2 Regulators of the cell cycle. The consecutive phases of the cell cycle are indicated between the *arrows* of the *circle*. Positive regulators mediate cell cycle progression and are shown on the outside in *green boxes*. Negative regulators activate cell cycle checkpoints or apoptosis and are shown in *red boxes* within the *circle*. The target points for action by the regulators are depicted. Three major checkpoints and their controlling complexes are named *unboxed*

4.1.1 EGFR Family Inhibitors

Cohen's discovery of Epidermal Growth Factor (EGF) in 1962 pioneered major progress in cell growth and differentiation research. In the early 1990s, the first clinical trials of anti-EGFR agents validated the drug target.

The EGFR (Epidermal Growth Factor Receptor, Human Epithelial Receptor, HER) family consists of receptor tyrosine kinases. Their excessive activity is common in solid tumors. It leads to a constitutive transduction of growth signals and correlates with decreased survival and a poor prognosis. ERBB1 (EGFR, HER1), ERBB2 (HER2/NEU), or ERBB3 may be over-expressed in lung, head and neck, breast, colon, and prostate cancers. ERBB2 is over-expressed in many adenocarcinomata, particularly in breast cancers. ERBB1 or ERBB2 may have gain-of-function mutations in lung cancer and glioblastoma multiforme.

The disruption of a transforming growth signal, caused by over-expressed ERBB gene products, can enforce remission. It may be accomplished with several strategies.

- Neutralization of the ligands before they can associate with their cognate receptors prevents them from initiating growth signals.
- Inhibition of the over-expressed receptors via blocking or disruption of their enzymatic activity can abrogate their excessive function.
- Inhibition of signaling by cytoplasmic second messengers, in particular serine-threonine kinases, can be therapeutic.

If there is a gain-of-function mutation, the affected receptor becomes independent of its ligand. Inhibitors acting upstream of the defect are ineffective. Inhibitors of the mutated receptors or their downstream targets may be therapeutic.

In addition to the growth promoting effects, ERBB1 protects malignant tumor cells from the cytotoxic effects of

Table 4.1 Molecular targets specific for cancer cells

1) Antigens encoded by *oncogenic viruses*. Epstein-Barr virus and Burkitt's lymphoma
2) *Mutations* of cancer-related genes. Expression of altered proteins
3) *Translocation* of proto-oncogenes. Frequent in leukemias. Unique domain at the junction
4) *Alternative transcripts*, including those from cryptic start sites, alternative reading frames, and pseudo-genes
5) *Post-translational modifications* of proteins differ between transformed and non-transformed cells, may open previously cryptic domains
6) Some *genes are silent* in normal tissue, *selectively expressed* on cells of a given differentiation. Oncofetal proteins
7) Malignancies of T- and B-lymphocytes express unique antigenic determinants in their T-cell antigen receptor or surface immunoglobulin. The *idiotype* of a given malignant clone provides a tumor specific marker

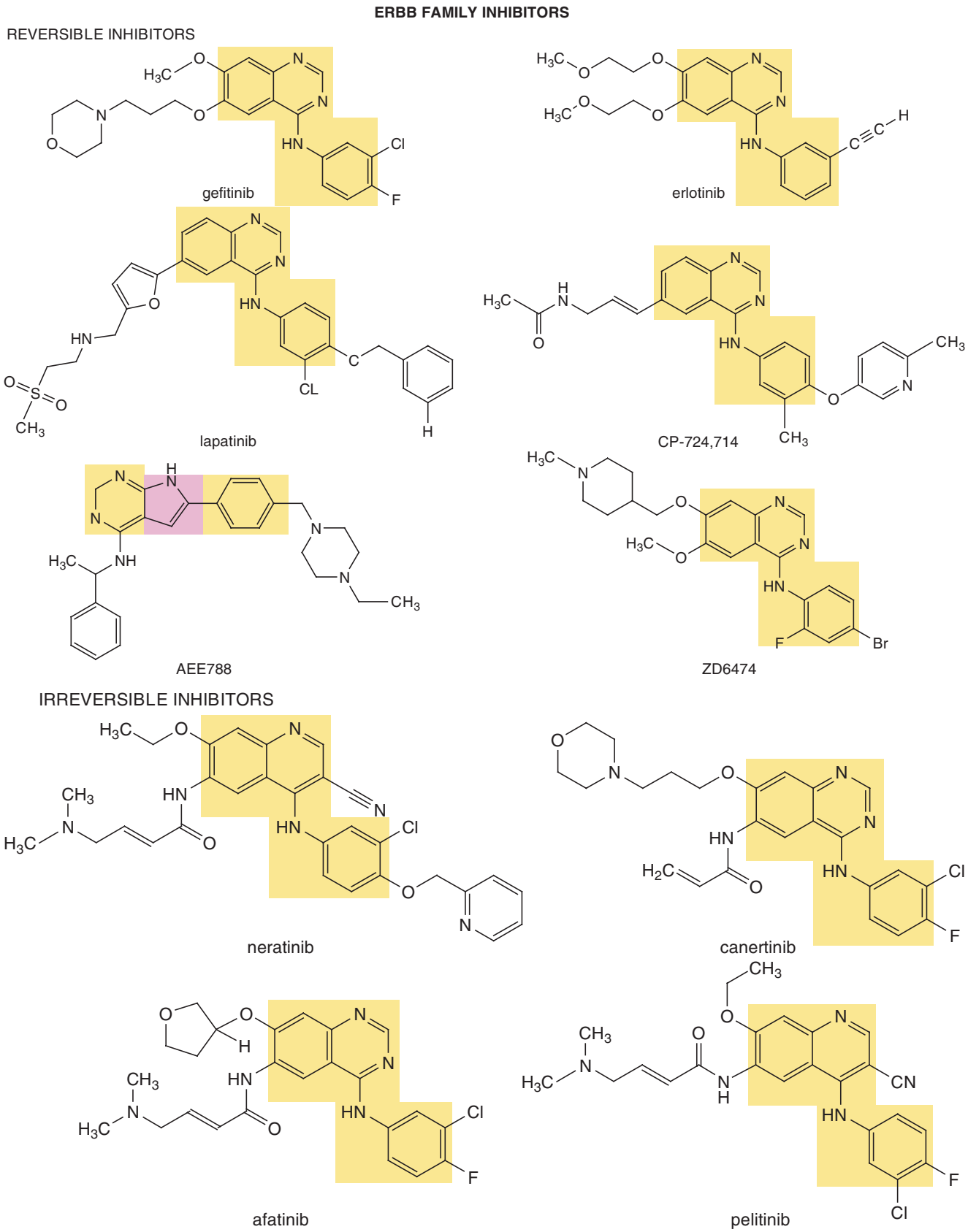

Fig. 4.3 Structures of ERBB family inhibitors. The 4-anilinoquinazoline core structure shared among all agents is highlighted in *yellow*, with one alteration in the core structure of AEE788 being marked in *pink*

Table 4.2 Treatment of EGFR mutations

Mutation	Cancers	Drug
EGFR mutation L858R (activation loop, kinase domain)	10 % of lung cancers	Gefitinib (high affinity for mutated activation loop)
Various EGFR mutations	Non-small cell lung cancer, pancreatic cancer	Erlotinib (reversible binding to catalytic domain)
ErbB1 deletion of exons 2–7 (leads to the truncated EGFRvIII, aka 801EGFR)	Glioblastoma	Neratinib (irreversible binding to ATP pocket)
ErbB2 mutation A775insYVMA	Non-small cell lung cancer	Neratinib
ErbB2 mutation G776insV_G/C	Non-small cell lung cancer	Neratinib
ErbB1 gene amplification (leads to over-expression, homo- and hetero- oligomerization)	Breast cancer, lung cancer	Lapatinib (binds ErbB1 in locked conformation)
ErbB1 gene amplification (leads to over-expression, ligand-independent oligomerization)		Cetuximab
ErbB2 gene amplification (leads to over-expression, ligand-independent oligomerization)	Breast cancer	Trastuzumab (binds juxtamembrane, receptor internalization)
ErbB2 gene amplification (leads to over-expression, homo- and hetero-oligomerization)	Ovarian cancer	Pertuzumab (binds domain II, blocks dimerization)

conventional chemotherapy and radio-therapy, making these treatments less effective. Compared to those regimens, the specific inhibition of ERBB1 (Fig. 4.3) is characterized by higher efficacy and fewer adverse effects. One aspect to this modality is the relatively high specificity of small molecule kinase inhibitors to select mutations (Table 4.2), which requires knowledge of the underlying molecular defect for the most suitable choice of drug therapy.

Adverse Effects Skin rashes are common adverse effects of EGFR family inhibitors.

Drug Resistance Reversible ERBB1 tyrosine kinase inhibitors are active in a subset of patients. However, most cancers develop resistance and progress within the course of 1 year. In nearly 50 % of the cases, acquired resistance is caused by the secondary ERBB1 mutation T790M.

Reversible specific inhibitors Gefitinib (Sirotnak 2000) is an anilinoquinazoline that inhibits the catalytic activity of multiple tyrosine kinases, including ERBB1 (EGFR, Human Epidermal Growth Factor Receptor 1, HER1). In initial clinical trials, there were no biomarkers available to predict outcome. In non-small cell lung cancer patients, gefitinib had only a 10 % response rate, but displayed dramatic effects in the responders. The individuals who benefited mostly belonged to an uncommon sub-population of lung cancer patients, comprising Japanese, females, and non-smokers. These individuals were affected by point mutations in the kinase domain of the EGF Receptor. Seven years after the start of clinical trials, a predictive biomarker was developed. The drug was approved by the U.S. FDA in 2003, with new labeling announced in 2005.

Gefitinib (*N*-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-ylpropoxy)quinazolin-4-amine) (ZD1839) <Iressa> competes with the binding of ATP to the tyrosine kinase domain of ERBB1, thereby inhibiting receptor auto-phosphorylation and resulting in the suppression of downstream signal transduction (Figs. 4.4, 4.5). The kinase inhibition may result in a suppression of tumor growth. The agent is indicated for the treatment of locally advanced or metastatic non-small cell lung cancer in patients who have previously received chemotherapy, but it is effective only in about 10 % of patients, depending on the nature of the cancer causing mutation. Gefitinib has durable anti-tumor activity in the treatment of cancers with mutations in the tyrosine kinase domain of ERBB1. In contrast, it has limited efficacy in cancers with other ERBB1 defects (such as lung cancers with deletions in the extracellular domain or glioblastomas with the distinct variant III (vIII) in-frame deletion of exons 2–7). The common dose is 250 mg/day.

Pharmacokinetics Gefitinib is absorbed slowly after oral administration, and has a bioavailability of 60 %. Elimination is by metabolism, primarily through CYP3A4. The molecule has three sites of biotransformation,

- metabolism of the N-propoxymorpholino-group,
- demethylation of the methoxy-substituent on the quinazoline,
- oxidative defluorination of the halogenated phenyl group.

Five metabolites accrue in the blood of patients. The elimination half-life is about 48 h. Daily oral administration of gefitinib to cancer patients results in a 2-fold accumulation compared to single dose administration. Steady state blood concentrations are achieved within 10 days. The drug is excreted in the feces.

Adverse Effects Adverse drug reactions include acne or other skin reactions, diarrhea, nausea and vomiting, asthenia or anorexia, conjunctivitis, blepharitis, corneal erosion, or aberrant eyelash growth. Asymptomatic elevations of liver Transaminases may arise. Interstitial lung disease is an infrequent, but serious adverse effect. About 1/3 of cases with interstitial lung disease are fatal. In the event of acute onset or worsening of pulmonary symptoms (dyspnea, cough, fever), gefitinib therapy should be interrupted, followed by a prompt investigation of the symptoms.

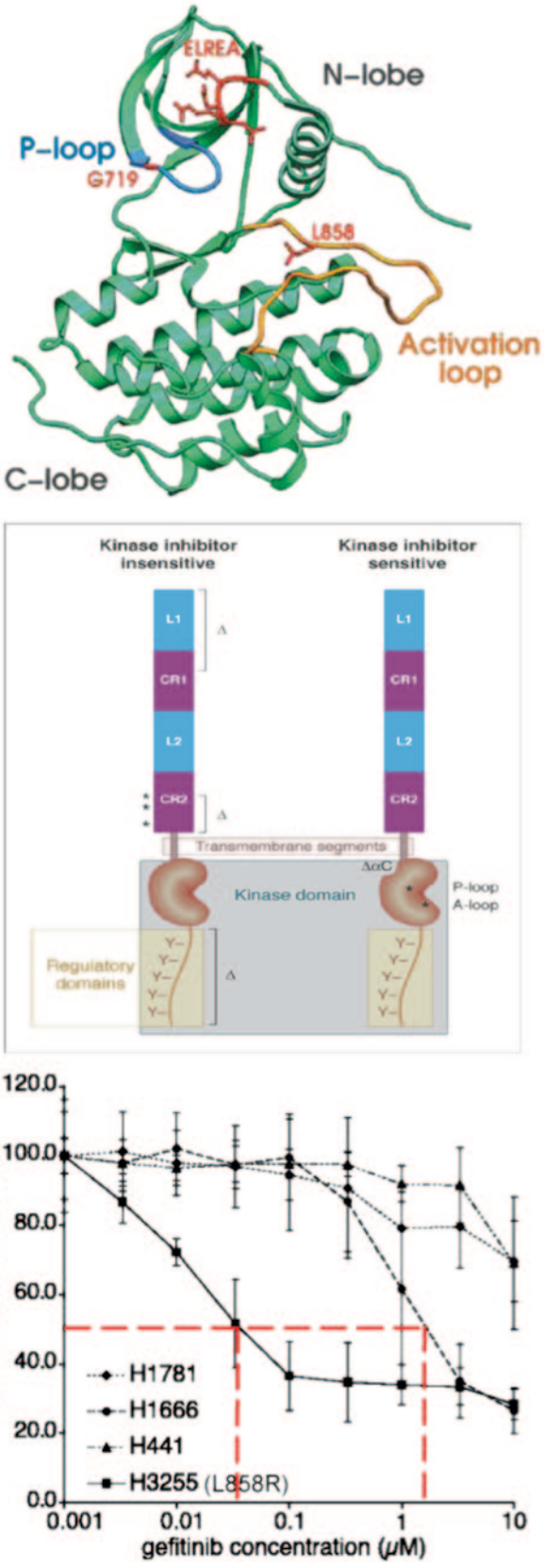

Fig. 4.4 Mode of action of gefitinib. (*Top panel*) Positions of missense mutations G719S and L858R and the Del-1 deletion in the 3-dimensional structure of the EGFR kinase domain. The *activation loop* is shown in *yellow*, the *P-loop* is in *blue*, and the *C-lobe* and *N-lobe* are as indicated. The residues targeted by mutation or deletion are highlighted in *red*. The Del-1 mutation targets the residues ELREA in codons 746–750. (*Middle panel*) EGFR mutations in tumors that are sensitive or insensitive to kinase inhibitors. Tumor mutations that are insensitive to kinase inhibitors, such as those in glioblastomata, include extensive deletions (Δ) or missense mutations (*) in the extracellular domain of EGFR, or deletions (Δ) of the regulatory intracellular domain (but not the kinase domain). In contrast, many non-small cell lung cancers carrying missense mutations (*) and deletions (Δ) in EGFR are sensitive to kinase inhibitors such as gefitinib. These mutations are in three distinct sites in the EGFR kinase domain: the P-loop, the activation loop (A-loop), and the αC helix. Tumor formation may require ligand binding to EGFR and receptor phosphorylation. The mutant EGFR allele also may be amplified (most notably with the ΔαC mutations). In kinase inhibitor-insensitive glioblastomata, tumors may be dependent or independent of ligand binding and the mutant EGFR locus is usually amplified. L1, L2: large (EGF binding) domain 1 and 2; CR 1, CR 2: cysteine-rich domain 1 and 2. Y, tyrosine residues in the regulatory domain that are available for phosphorylation. (*Bottom panel*) Cell survival after exposure to gefitinib at the indicated concentrations. Percentage of cell growth is shown relative to untreated controls. H3255 cells have the EGFR L858R mutation, whereas the three remaining cell lines have wild-type EGFR (WT). (Paez 2004; Minna 2004)

Drug Resistance Resistance to ERBB1 tyrosine kinase inhibitors, such as gefitinib, can arise through T790M mutation or activation of downstream pathways via *K- ras* mutation or *met* amplification.

Drug Interactions Gefitinib is sparingly soluble at pH 1, but is essentially insoluble above pH 7, with a sharp drop in the pH range of 4–6. Therefore, co-administration of high doses of ranitidine or sodium bicarbonate (which maintain the gastric pH above pH 5.0) reduces the mean gefitinib area under the concentration-time curve by 45%. Gefitinib has no inhibitory effect on CYP1A2, CYP2C9, and CYP3A4 activities up to concentrations of 5000 ng/mL. However, exposure to metoprolol, a substrate of CYP2D6, is increased by 30% when given in combination with gefitinib. The mean area under the concentration-time curve of gefitinib is reduced by 85% by rifampicin, an inducer of CYP3A4, and is increased by the concomitant administration of itraconazole, an inhibitor of CYP3A4.

Erlotinib (*N*-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy) quinazolin-4-amine) (OSI-77) <Tarceva> was developed by OSI Pharmaceuticals, Genentech, and Roche. It is the hydrochloride salt of a quinazoline derivative. Erlotinib is designed to bind to the intracellular catalytic domain of the tyrosine kinase ERBB1, competing with ATP, thereby reversibly inhibiting auto-phosphorylation and blocking the tumorigenic signal transduction events associated with uncontrolled ERBB1 activation. The orally bioavailable erlotinib has largely replaced gefitinib in the U.S. (except in patients where gefitinib has had a proven response). It is indicated to treat lung cancer and pancreatic cancer. The agent was the first ERBB1 inhibitor to display a survival benefit in lung cancer patients, it has durable activity in the treatment of non-small cell lung cancers with

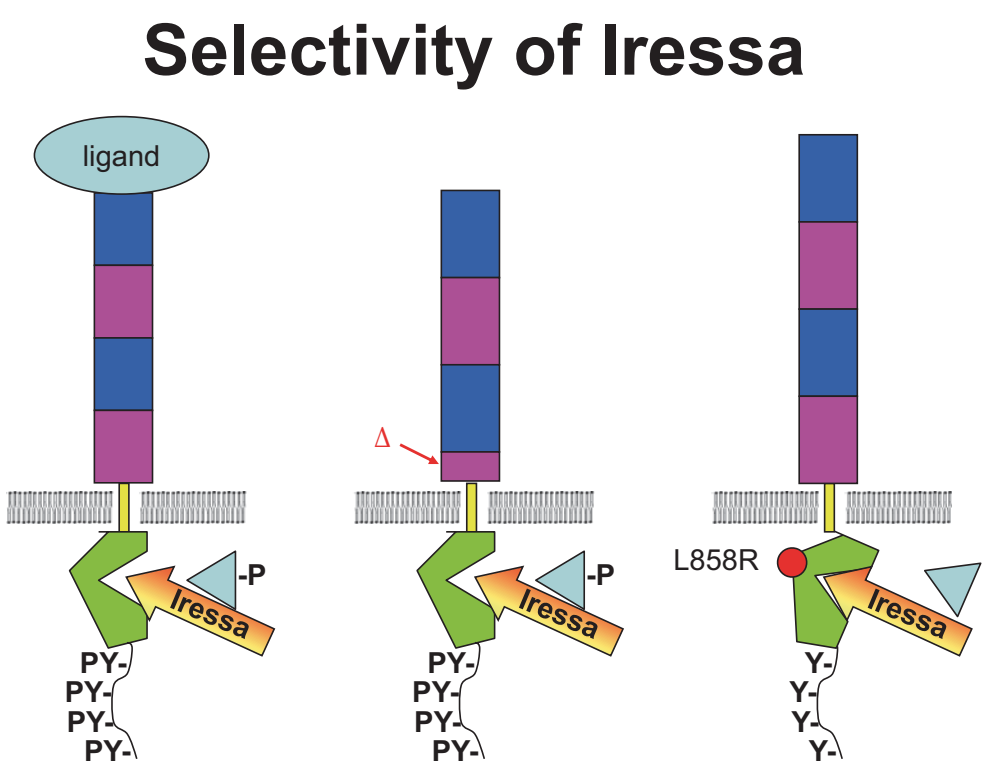

Fig. 4.5 Specificity of gefitinib to particular receptor mutations. Ligation of the EGF Receptor by its physiologic ligand leads to phosphorylation of the cytoplasmic tail and the transmission of a growth signal to the cell (*left*). Lung cancers may be caused by gain-of-function mutations of the EGF Receptor that lead to ligand-independent activation. Two such mutations include a deletion in the extracellular domain (*middle*), and a point mutation, L858R, in the catalytic domain (*right*). The anti-cancer drug gefitinib fits well into the altered conformation of the mutated catalytic domain (*right*) and blocks the phosphorylation of substrate. In contrast, the affinity of gefitinib to the unaltered catalytic domain (*left* and *center*) is low, thus allowing receptor kinase activity to persist

mutations in the tyrosine kinase domain of ERBB1. Erlotinib received U.S. FDA approval for the treatment of non-small cell lung cancer in 2004. European approval for treatment of non-small cell lung cancer in patients who have failed prior chemotherapy followed in 2005.

Adverse Effects Skin rashes occur in the majority of patients. They resemble acne and primarily involve the face and neck. In most cases, the rashes are self-limited and resolve. Other adverse effects comprise diarrhea, loss of appetite, fatigue, and ingrown hairs (such as eyelashes). Interstitial pneumonitis (characterized by cough and dyspnea) may become severe. Rare adverse effects may include serious gastrointestinal tract perforations, blistering and exfoliative skin conditions, or ocular disorders (such as corneal lesions).

Drug Resistance The T790M mutation of ERBB1 prevents erlotinib from binding to its drug target. The drug has limited efficacy in glioblastoma, where a distinct ERBB1 mutation, the variant III (vIII) in-frame deletion of exons 2–7, commonly arises. Drug resistance may occur through overexpression of MET.

Lapatinib ditosylate (GW572016) <Tykerb, Tyverb> is the salt of a synthetic, orally active quinazoline. The agent reversibly blocks the auto-phosphorylation of ERBB1, ERBB2 (HER2), and the kinases ERK-1, ERK-2, and PKB. It also suppresses the Cyclin D protein levels in tumor cells. Lapatinib is predominantly used with capecitabine in patients afflicted by advanced ERBB2-positive breast cancer with trastuzumab resistance. It is also approved with letrozole for the treatment of postmenopausal women with hormone receptor-positive metastatic breast cancer that over-expresses the ERBB2 receptor and for whom hormonal therapy is indicated. The drug is taken orally once a day.

Pharmacokinetics Absorption following oral administration of lapatinib is incomplete and variable. The systemic exposure is increased when administered with food. In the blood, more than 99 % of the drug is bound to Albumin and α-1 Acid Glycoprotein. Lapatinib is a substrate, and may act as an inhibitor, for the transporters ABCG2 and ABCB1, and the hepatic uptake transporter OATP1B1. The drug undergoes extensive metabolism, primarily by CYP3A4 and CYP3A5, with minor contributions from CYP2C19 and CYP2C8 to a variety of oxidized metabolites. The terminal phase half-life following a single dose is 14 h, which accumulates with repeated dosing to an effective half-life of 24 h. Elimination of lapatinib is predominantly through excretion in the feces, with renal excretion below 2 %.

Adverse Effects Cardiotoxicity is a potentially serious adverse effect. Lapatinib may cause liver damage and hepatitis, which can be severe or life-threatening. Liver damage may occur as soon as several days or as late as several months after the start of treatment.

Common adverse effects include nausea and vomiting, diarrhea, loss of appetite, heartburn, oral sores (lips, mouth, or throat), dysesthesias of hands and feet, pain (in arms, legs, or back), skin rashes, difficulty falling asleep or staying asleep.

CP-724,714 is an orally bioavailable quinazoline that selectively binds to the intracellular domain of ERBB2, reversibly inhibiting its tyrosine kinase activity and resulting in the suppression of tumor cell growth (Barbacci 2003). CP-724,714 suppresses ERBB2 auto-phosphorylation and selectively inhibits the growth of ERBB2 driven cells by inducing cell cycle arrest in G_1. The drug is under study for the treatment of metastatic breast cancer.

Reversible multi-kinase inhibitors AEE788 is an orally bioavailable, reversible multiple receptor tyrosine kinase inhibitor. It suppresses the phosphorylation of the tyrosine kinases of ERBB1 (EGFR), ERBB2 (HER2), and VEGFR2 (Vascular Endothelial Growth Factor Receptor 2), resulting in receptor inhibition, the inhibition of cellular proliferation, and the induction of tumor cell apoptosis and tumor associated endothelial cell apoptosis.

ZD6474 is an orally bioavailable inhibitor of EGFR and VEGFR2. It is in clinical trials for the treatments of non-small cell lung cancer and medullary thyroid carcinoma. The agent has shown efficacy in combination with cytotoxic chemotherapy.

Adverse Effects Adverse effects include diarrhea, hypertension, and skin rashes.

Irreversible inhibitors Neratinib (HKI-272) (Rabindran 2004) is an orally available, 6,7-disubstituted 4-anilinoquinoline-3-carbonitrile. It irreversibly binds to EGFR family kinases by targeting a cysteine residue in the ATP binding pocket, thereby reducing receptor auto-phosphorylation. Treatment with this agent results in an inhibition of downstream signal transduction events and cell cycle regulatory pathways, causing arrest at the G_1/S transition of the cell division cycle and ultimately decreasing cellular proliferation. Neratinib potently suppresses ERBB2 and is highly active against ERBB2 overexpressing breast cancer cells. It also inhibits ERBB1 and the proliferation of ERBB1 dependent cells. Anti-tumor activity is achieved at a daily oral dose of 10–40 mg/kg.

In approximately 2 % of primary non-small cell lung cancers, there are mutations in the *erbB2* gene, the most frequent one being A775insYVMA. While cells with this mutation are relatively resistant to erlotinib, they remain sensitive to neratinib (Minami 2007). The G776insV_G/C mutation in *erbB2*, present in a small percentage of non-small cell lung cancers, is also sensitive to neratinib (Shimamura 2006).

Adverse Effects When administered on a continuous, once-daily oral treatment schedule, the maximum tolerated dose of neratinib is 320 mg/day, with diarrhea as the most frequently occurring adverse effect.

Drug Resistance In glioblastoma, a characteristic EGFR mutation that commonly arises is the variant III (vIII) in-frame deletion of exons 2–7. EGFRvIII is also associated with 5 % of lung squamous cell carcinomata, but is not present in lung adenocarcinomata. Although this form of EGFR is resistant to multiple kinase inhibitors, treatment with neratinib may reduce the size of these EGFRvIII-driven tumors (Ji 2006). Neratinib is not a substrate for ABCG2.

Canertinib dihydrochloride (Cl-1033) is the salt of an orally bioavailable quinazoline, which binds to the intracellular domains of EGFR family tyrosine kinases. Along with the inhibition of tyrosine phosphorylation, small molecule kinase inhibitors enhance ubiquitylation, endocytosis, and subsequent intracellular destruction of ERBB molecules. Canertinib alkylates a cysteine specific to ERBB receptors and is particularly effective at inducing its degradation. Thus, it irreversibly inhibits ERBB associated signal transduction functions and results in tumor cell apoptosis and suppression of tumor cell proliferation. The kinase inhibition is selective for ERBB1, ERBB2, ERBB3, and ERBB4 without inhibiting the tyrosine kinase activity of receptors such as Platelet-Derived Growth Factor Receptor, Fibroblast Growth Factor Receptor, and Insulin Receptor, even at high concentrations. Canertinib effectively inhibits the growth of esophageal squamous cell carcinoma, which co-expresses ERBB1 and ERBB2, by mediating the inhibition of phosphorylation of both MAPK and AKT. It also acts as a radiosensitizing agent and displays synergistic activity with other chemotherapeutic agents.

Afatinib (BIBW 2992) <Gilotrif, Tomtovok, Tovok irreversibly inhibits ERBB1 and ERBB2. It is approved for the use in patients with metastatic non-small cell lung cancer, whose tumors have ERBB1 exon 19 deletions or exon 21 (L858R) substitution mutations. The drug is also in clinical trials for breast cancer, prostate cancer, and head and neck cancer. Afatinib is taken orally at least 1 h before or 2 h after a meal.

Adverse Effects Serious adverse effects that require permanent discontinuation of afatinib include life threatening skin lesions (bullous, blistering, or exfoliative), interstitial lung disease (may manifest as lung infiltration, pneumonitis, acute respiratory distress syndrome, or allergic alveolitis), severe drug-induced hepatic impairment, persistent ulcerative keratitis, left ventricular dysfunction.

Common adverse effects include diarrhea (may result in dehydration with or without renal impairment), reduced ap-

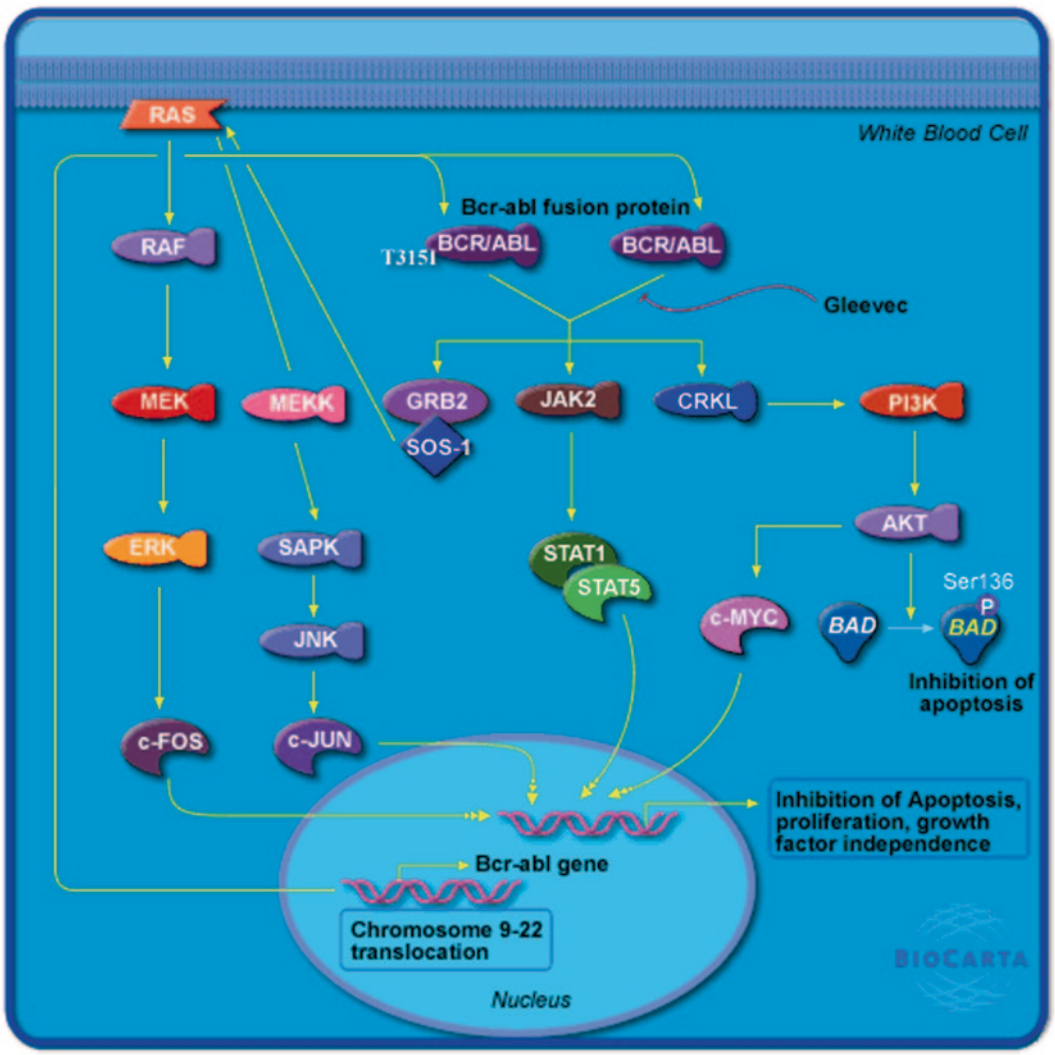

Fig. 4.6 BCR-ABL in cell proliferation. Chronic myelogenous leukemia (*CML*) is associated in most cases with a translocation between chromosomes 9 and 22 that occurs at the sites of *bcr* and the protein tyrosine kinase gene *abl*, creating the chimeric *BCR-ABL* protein. This leads to deregulation of the kinase activity of *ABL*, driving the uncontrolled cell growth in the disease. Blood cells containing the *BCR-ABL* fusion proliferate in the absence of growth factors they would normally require. In the cytoplasm, *BCR-ABL* is associated with a large signaling complex. Various of its cellular substrates may be involved in transformation, including PI-3 Kinase/PKB, the kinase JAK2, and STAT transcription factors. Specifically, STAT5 may contribute to the failure of apoptosis in *BCR-ABL* cells. The activation of *BCR-ABL* also represses apoptosis through the induction of *BAD*. *GRB-2* phosphorylation may play a regulatory role in tyrosine kinase signaling. Traditional cytotoxic anti-cancer drugs have serious adverse effects such as nausea, weight loss, hair loss and severe fatigue that result from their lack of specificity in killing cells. While such treatments kill all dividing cells, imatinib mesylate acts by a mechanism that is more specific to cancer cells. The drug was designed as a selective *BCR-ABL* inhibitor, and so produces less extensive adverse effects than other anti-cancer agents. (http://www.biocarta.com/pathfiles/h_gleevecpathway.asp)

petite, stomatitis, dermatitis, and paronychia. Afatinib may cause harm if administered during pregnancy.

Drug Interactions The concomitant treatment with ABCB1 inhibitors may cause increased systemic exposure to afatinib and require dose reduction. Concomitant ABCB1 inducers may require an afatinib dose increase.

Pelitinib (EKB-569) is a 3-cyanoquinoline that acts as an irreversible, covalent pan-ERBB tyrosine kinase inhibitor. It is under study for the treatment of colorectal cancer in combination with FOLFOX or FOLFIRI.

Adverse Effects The most common adverse effects are diarrhea and skin rashes.

Drug Resistance ABCG2 confers resistance to pelitinib.

Over-expression of EGFR family receptors is common in many solid tumors.
While reversible EGFR tyrosine kinase inhibitors are active in a subset of patients, most develop resistance within 1 year. In

nearly 50 % of the cases, acquired resistance is caused by the secondary ERBB1 mutation T790M.
In some cancers, the EGFR mutation variant III (vIII), an in-frame deletion of exons 2–7, commonly arises. Neratinib is effective against it.
Skin rashes are frequent adverse effects of EGFR inhibitors.

4.1.2 ABL Inhibitors

Physiologically, the tightly regulated activity of the tyrosine kinase ABL induces growth and resistance to apoptosis in hematopoietic cells. A common translocation[1] involving chromosomes 9 and 22 in chronic myeloid leukemia (CML) leads to the formation of the oncogenic, chimeric BCR-ABL, which transduces unregulated growth signals. The proto-oncogene product ABL signals through the PI 3-Kinase and JAK-STAT pathways, and it cross-talks to the RAS pathway (Fig. 4.6). The regulatory subunit of PI 3-Kinase binds directly to the SH3 domain of auto-phosphorylated, active ABL. Efficient transformation by ABL requires its protein tyrosine kinase activation and membrane association via NH_2-terminal myristoylation. BCR-ABL dependent JAK activation induces STAT5 to activate the expression of the anti-apoptotic protein BCL-x_L. The BCR-ABL oncoprotein also strongly associates with F-Actin. The formation of BCR-ABL oligomers is permissive for the simultaneous binding of multiple F-Actin filaments. BCR-ABL tyrosine kinase inhibitors (Fig. 4.7) are the first-line therapy for most patients with Philadelphia chromosome containing chronic myelogenous leukemia.

First generation BCR-ABL inhibitor Increasing insights into the molecular pathways associated with growth signals led to the development of small molecule inhibitors, including the salt imatinib mesylate <Gleevec, Glivec>(Druker 2000). Imatinib inhibits BCR-ABL, a fusion tyrosine kinase produced by chronic myelogenous leukemia cells, which harbor the Philadelphia chromosome. The drug suppresses ABL enzymatic activity by engaging with high specificity the ATP binding site of an inactive form of the kinase, thus preventing its auto-phosphorylation (Fig. 4.8). The interaction involves six hydrogen bond interactions at methionine 318, threonine 315, glutamine 286, asparagine 381, isoleucine 380, and histidine 361. This mechanism of action prevents ATP from reaching its binding site. Inhibition of the BCR-ABL kinase activity by imatinib induces cancer cell apoptosis. The agent was approved in 2001 in the U.S., Europe, and Japan for the treatment of chronic myelogenous leukemia.

Imatinib blocks auto-phosphorylation of the receptor tyrosine kinase CD117 (c-KIT, Stem Cell Factor Receptor, SCFR), as well as Stem Cell Factor stimulated downstream signaling events, such as the activation of ERK1, ERK2, and PKB (Heinrich 2000). It inhibits proliferation and induces apoptosis in cells that over-express the oncoprotein CD117. As CD117 is activated in gastrointestinal stromal tumor (GIST), imatinib was approved in 2002 by the U.S. FDA for the treatment of metastatic gastrointestinal stromal tumor, and as adjuvant treatment following the surgical resection of localized c-KIT positive-gastrointestinal stromal tumor. A fraction of melanomata harbor activating mutations or amplifications of *kit*. Some of the affected patients benefit from imatinib mesylate treatment.

Imatinib inhibits the receptor tyrosine kinase PDGFR (Platelet Derived Growth Factor Receptor). PDGF is a connective tissue cell mitogen with functions that include embryonal development, wound healing, and the control of interstitial fluid pressure in soft connective tissue. Paracrine PDGF signaling in the connective tissue tumor stroma plays a role in various types of solid tumors. It contributes to the regulation of interstitial fluid pressure. As most solid tumors have an increased interstitial fluid pressure, pharmacological reduction may be a way to increase the uptake of anti-cancer drugs. Through the inhibition of PDGFR, imatinib can significantly reduce the tumor interstitial fluid pressure.

Imatinib is under investigation as a treatment for additional cancers that have excessive activities of c-KIT or PDGFR. They include acute myelogenous leukemia, small cell lung cancer, ovarian cancer, testicular cancer, dermatofibrosarcoma protuberans, and glioma.

Pharmacokinetics At clinically relevant concentrations of imatinib, binding to plasma proteins is approximately 95 %, mostly to Albumin and α1-Acid Glycoprotein (AGP). The drug is metabolized by and is an inhibitor of the CYP3A4 enzyme system and caution needs to be exercised when substrates, inducers, or inhibitors of CYP3A4 are used concomitantly;. Additionally, imatinib mesylate is a substrate and inhibitor of CYP2D6 and the blood levels of CYP2D6 substrates can increase during imatinib mesylate therapy. CYPs 1A2, 2C9, and 2C19 are minor contributors to imatinib metabolism.

Adverse Effects While imatinib has been very successful in the treatment of chronic myelogenous leukemia and gastrointestinal stromal tumors, it is associated with uncommon, but severe cardiotoxicity[2]. The therapeutic effect on the kinase BCR-ABL is also linked to the cardiovascular adverse effects of the drug. Therefore, it is not possible to fully eliminate this toxicity. However, inhibition of the signaling intermediate JNK1 can ameliorate the imatinib effects on heart cells.

Drug Resistance Imatinib mesylate fails in approximately 20 % of patients within 5 years. Compromising factors in-

[1] The Philadelphia chromosome (Ph).

[2] Many receptor tyrosine kinase inhibitors may evoke cardiac dysfunction as potential adverse effects. They include lapatinib, imatinib, nilotinib, dasatinib, sorafenib, and sunitinib.

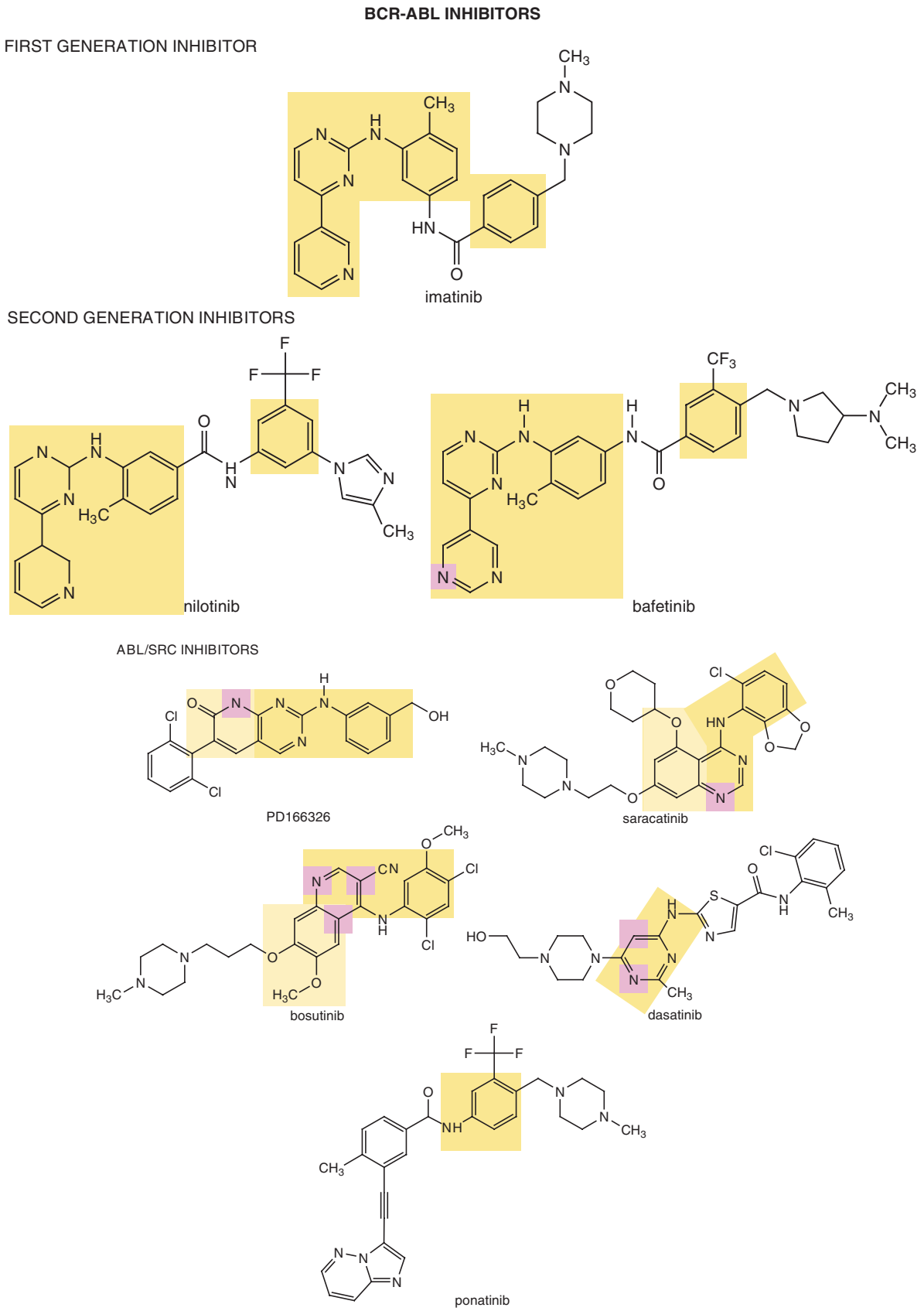

Fig. 4.7 Structures of BCR-ABL inhibitors. The subclasses comprise first and second generation BCR-ABL inhibitors, ABL/SRC inhibitors, and ABL/Aurora Kinase inhibitors. The core structure of the first generation inhibitor imatinib is common to the second generation inhibitors. Only in bafetinib a nitrogen is inserted (*pink*). The ABL/SRC inhibitors preserve some of the functional groups of the first and second generation inhibitors (*bright yellow*) and have subclass-specific elements in common (*light yellow*). Within the groups of ABL/SRC inhibitors and ABL/Aurora Kinase inhibitors, some core elements are common to several representatives

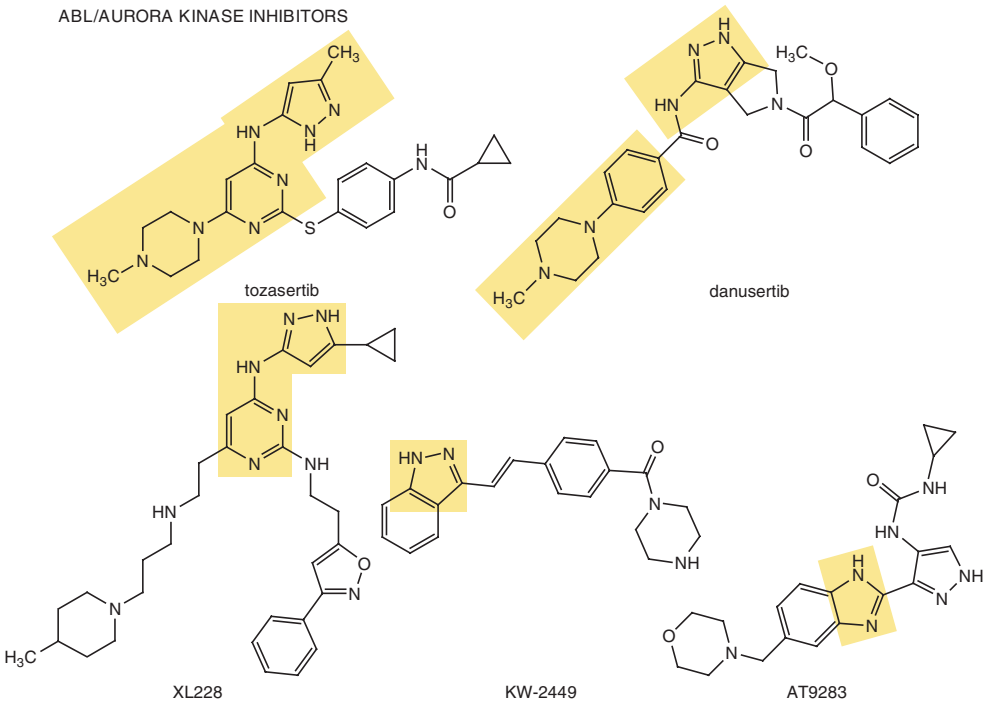

Fig. 4.7 (continued)

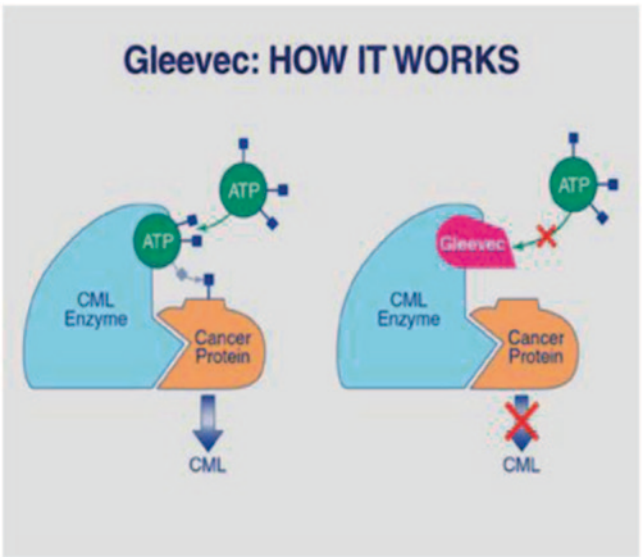

Fig. 4.8 Imatinib drug action. The drug targets the BCR-ABL protein in CML cells. (http://www.cancer.gov/PublishedContent/Images/images/documents/6ac3a026-8c6c-4a0f-97b0-df63d9f8ef92/poster_gleevec.gif) There are instances where we have been unable to trace or contact the copyright holder. If notified the publisher will be pleased to rectify any errors or omissions at the earliest opportunity

clude limited therapeutic activity and durability in advanced disease stages, persistence of minimal residual disease because of the limited effects of the drug on immature hematopoietic stem cells, and primary or acquired drug resistance (Gontarewicz 2008). In chronic myelogenous leukemia blast crisis, the initial rate of hematologic responses to imatinib is high. However, these responses are usually short-lived, most patients ultimately develop resistance and undergo disease progression. Mechanisms of resistance can be categorized into three main groups:

- BCR-ABL is reactivated and cell proliferation remains dependent on BCR-ABL signaling (amplification of the *bcr-abl* gene, mutations in the BCR-ABL kinase domain that prevent high affinity binding of imatinib). The emergence of transformed cell clones with resistance to imatinib and other small molecule inhibitors, mediated by mutations in BCR-ABL, has become a major challenge in the treatment of chronic myelogenous leukemia. Mutational frequencies increase as chronic myelogenous leukemia progresses from the chronic phase to the blast phase. More than 70 point mutations can be assigned to four major groups based on their location in the kinase domain, the ATP binding loop (40% of all mutations), the gatekeeper residue threonine 315 (25% of all mutations), the catalytic domain (25% of all mutations), or the activation loop (5% of all mutations). BCR-ABL contains the ATP binding P-loop and the activation loop, which stabilize the basal conformation. Mutations in these loops destabilize the protein structure, such that the kinase domain cannot assume the inactive conformation required for imatinib binding. Mutations in the P-loop region are the most common (Fig. 4.9). The mutation T315I is completely resistant to multiple BCR-ABL directed therapies. This substitution eliminates a critical oxygen molecule needed for

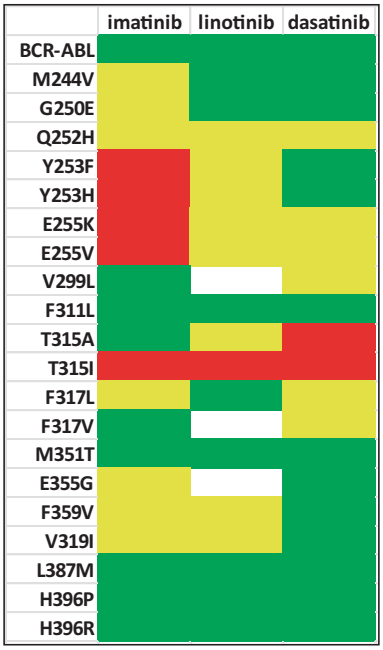

Fig. 4.9 Resistance to small molecule BCR-ABL inhibitors. The kinase inhibitors imatinib, nilotinib, and dasatinib are compared for their efficacy against BCR-ABL mutations (shown *top* to *bottom* in *N*-terminal to *C*-terminal order). The color coding indicates sensitivity (*green*), intermediate sensitivity (*yellow*), or resistance (*red*) according to IC_{50} values for cell proliferation (the IC_{50} value is the concentration of inhibitor resulting in a 50% reduction in cell viability). Imatinib: sensitive (up to 1000 nM), intermediate (1001–3000 nM), insensitive (>3000 nM). Nilotinib: sensitive (up to 50 nM), intermediate (51–500 nM), insensitive (>500 nM). Dasatinib: sensitive (up to 3 nM), intermediate (4–60 nM), insensitive (>60 nM). The graph highlights the problematic nature of T315I. (Redrawn from (O'Hare 2007))

hydrogen bonding between imatinib and the ABL kinase. It also creates steric hindrance to the binding of most small molecule inhibitors. The development of an inhibitor for BCR-ABL^{T315I} has been challenging, because most ATP competitive inhibitors directly interact with threonine 315 via hydrogen bonding and with the specificity imparting hydrophobic pocket, for which this residue serves as a gatekeeper. Several BCR-ABL mutations in the active site, such as Y235F/H or E255K/V, also confer imatinib mesylate resistance. Small molecule inhibitors of Aurora Kinase, including MK-0457 (VX-680), XL228, and AT9713, can effectively inhibit a mutant enzyme (Table 4.3).

- The BCR-ABL protein re mains inhibited by imatinib, but alternative or downstream signaling pathways become activated, often involving excessive activity of SRC kinases.
- The drug disposition is altered through increased efflux via up-regulated export transporters such as ABCB1, compromised influx by down-regulation of the organic cation transporter OCT1, or enhanced protein binding of imatinib to circulating α1-Acid Glycoprotein (AGP).

Approaches to overcome resistance include dose escalation, the use of alternative BCR-ABL inhibitors, combination chemotherapy (possibly comprising multiple BCR-ABL inhibitors), and inhibitors of downstream BCR-ABL targets (such as Farnesyl Transferase inhibitors).

In systemic mastocytosis, most cases have a point mutation in codon 17 of CD117, which results in a D816V amino acid substitution in the kinase-2 domain of CD117. This mutated CD117 is resistant to inhibition by imatinib (Frost 2002).

Second generation BCR-ABL inhibitors Nilotinib and bafetinib belong to the 2-phenylamino-pyrimidine based sub-class of inhibitors.

Although imatinib is an effective, frontline therapy for chronic phase chronic myelogenous leukemia, accelerated phase or blast crisis phase chronic myelogenous leukemia patients and Philadelphia chromosome positive acute lymphocytic leukemia, patients often relapse, resulting from the emergence of imatinib-resistant point mutations within the BCR-ABL tyrosine kinase domain.

Nilotinib monohydrate monohydrochloride (AMN107) <Tasigna> is an orally available phenylamino-pyrimidine, which was derived from the imatinib scaffold (Weisberg 2006). Nilotinib exhibits a similar binding mode as its model drug, however, its potency is increased by approximately 30-fold because of an improved topologic fit to the enzyme. Designed to overcome imatinib resistance, nilotinib binds to the ATP site in the inactive conformation of the ABL kinase domain, largely through lipophilic interactions. This agent inhibits (with decreasing potency) the auto-phosphorylation of DDR1, BCR-ABL, PDGFR, CSF-1R, and c-KIT. Nilotinib interrupts the downstream signaling targets of these tyrosine kinases, resulting in decreased cellular proliferation and the induction of apoptosis. The drug is indicated for the treatment of chronic phase and accelerated phase Philadelphia chromosome-positive (Ph^+) chronic myelogenous leukemia in adult patients—newly diagnosed or resistant of or intolerant to prior therapy that included imatinib.

Pharmacokinetics The recommended dose is 300–400 mg twice daily. As the bioavailability of nilotinib is variably increased with food, patients should avoid eating 2 h before and 1 h after taking a dose. Peak concentrations of nilotinib are reached 3 h after oral administration. The unaltered drug is the main circulating component in the blood. The main metabolic pathways are oxidation and hydroxylation, with none of the products contributing to the pharmacological activity. The apparent elimination half-life with daily dosing is approximately 17 h. Over 90% of the drug is eliminated in the feces; the unmetabolized parent drug accounts for about 70% of the eliminated agent. Dose reduction may be required in patients with hepatic impairment. Since nilotinib and its

metabolites are not renally excreted, a decrease in total body clearance is not anticipated in patients with renal impairment.

Adverse Effects Nilotinib prolongs the QT interval, possibly leading to sudden deaths. The agent should not be used in patients with hypokalemia, hypomagnesemia, or long QT syndrome. As nilotinib causes myelosuppression, it may be given in combination with hematopoietic growth factors such as erythropoietin or G-CSF if indicated. The most common non-hematologic adverse drug reactions—occurring in over 10 % of patients—are rash, pruritus, headache, nausea, fatigue, and myalgia. Less common and of mild to moderate severity are upper abdominal pain, constipation or diarrhea, dry skin, alopecia, muscle spasms, arthralgia, peripheral edema, and asthenia. They are manageable and generally do not require dose reduction. Pleural and pericardial effusions may arise in 1 %, gastrointestinal hemorrhage in 0.5 % of patients. Because nilotinib can increase serum Lipase it requires caution in patients with a history of pancreatitis. If Lipase elevations are accompanied by abdominal symptoms, it necessitates dose interruption and diagnostics to exclude pancreatitis. The drug is Pregnancy Category D.

Nilotinib is a competitive inhibitor of UGT1A1 and can thus increase bilirubin levels. The largest increases in bilirubin arise in (TA)7/(TA)7 genotype (UGT1A1*28) patients relative to the (TA)6/(TA)6 and (TA)6/(TA)7 genotypes.

Drug Resistance In acute phase or blast crisis of chronic myelogenous leukemia, 30–60 % of patients are refractory to nilotinib. Resistance is associated with a limited spectrum of BCR-ABL kinase mutations that mostly affect the P-loop and T315I. As the drug is not a substrate for the efflux transporter ABCB1, its over-expression does not lead to resistance. Nilotinib influx is also independent of the uptake transporter OCT1.

Drug Interactions Nilotinib undergoes metabolism by CYP3A4, and concomitant administration of strong inhibitors or inducers of CYP3A4 can increase or decrease the nilotinib concentrations. The agent also acts as a competitive inhibitor of CYP3A4, CYP2C8, CYP2C9, CYP2D6. It may potentially increase the concentrations of drugs that are eliminated by these enzymes. Conversely, nilotinib can induce CYP2B6, CYP2C8, and CYP2C9, and may potentially decrease the concentrations of drugs which are eliminated by these enzymes. As nilotinib inhibits ABCB1, its administration with drugs that are substrates of this export transporter likely causes increased concentrations of the substrate drugs, and caution should be exercised. Drugs that prolong the QT interval need to be avoided in conjunction with nilotinib treatment. Due to decreased nilotinib solubility at increasing pH, drugs that suppress gastric acid secretion to elevate the gastric pH (such as proton pump inhibitors) may decrease the solubility of nilotinib and reduce its bioavailability.

The orally available, dual BCR-ABL/LYN inhibitor bafetinib (*N*-[3-([5,5′-Bipyrimidin]-2-ylamino)-4-methylphenyl]-4-[[(3S)-3-(dimethylamino)-1-pyrrolidinyl]methyl]-3-(trifluoromethyl)benzamide) (INNO-406, NS-187) was developed at Kyoto University Hospital in collaboration with Nippon Shinyaku. Its activity profile may overcome imatinib resistance. In addition to the BCR-ABL inhibitory properties, Bafetinib acts as a specific inhibitor of LYN kinase. Up-regulation of

Table 4.3 Specificities of ABl inhibitors

BCR-ABL inhibition										
	BCR-ABL	BCR-ABL^{T315I}	BCR-ABL^{T315N}	BCR-ABL^{V229L}	BCR-ABL$^{Y235F/H}$	BCR-ABL$^{E255K/V}$	BCR-ABL^{Q252H}	BCR-ABL^{M351T}	BCR-ABL^{F359V}	BCR-ABL^{H396P}
First generation										
Imatinib	S	R	R	S	R	R	S	S	S	
Second generation										
Nilotinib	S	R								
Bafetinib	S									
ABL/SRC inhibitors										
PD166326	S									
Saracatinib	S									
Bosutinib	S	R	R							
Dasatinib	S	R	S	R	S	S	S	S	S	S
Ponatinib	S	S								
ABL/Aurora Kinase inhibitors										
Tozasertib	S	S	S	S			S	S	S	S
Danusertib	S	S				S		S		
XL228	S	S								
KW-2449	S	S								
AT9283	S									

S sensitive, *R* resistant

Table 4.3 (continued)

	Off-target inhibition																
	SRC	SRC^{Y530F}	LYN	Auroa A	Aurora B	KIT	KIT^{D816V}	PDGFR	IGF-1R	FGFR	DDR1	DDR2	CSF-1R	JAK2	FLT3	EPHA2	GFR
First generation																	
Imatinib				R	R	S	R	S							R		
Second generation																	
Nilotinib						S		S			S		S				
Bafetinib			S														
ABL/SRC inhibitors																	
PD166326	S																
Saracatinib	S	S	S														
Bosutinib	S					R	R	R									
Dasatinib	S			R	R	S		S				S			R	S	S
Ponatinib	S																
ABL/Aurora Kinase inhibitors																	
Tozasertib				S	S										S		
Danusertib				S	S												
XL228	S			S	R				S	S							
KW-2449				S	S										S		
AT9283				S	S									S	S		

S sensitive, *R* resistant

LYN kinase activity is a common cause of imatinib resistance, and LYN activation is associated with a variety of solid tumors, including prostate cancer. Bafetinib has been granted orphan drug status by the U.S. FDA for the treatment of Philadelphia chromosome-positive chronic myelogenous leukemia.

ABL/SRC inhibitors SRC family protein tyrosine kinases interact with a variety of cell surface receptors and participate in intracellular signal transduction pathways to support growth. Tumorigenic forms can arise through altered regulation or expression of SRC. Over-expression of specific SRC kinases may be active in chronic myelogenous leukemia, and may contribute to the resistance by tumor cells to specific BCR-ABL inhibitors, such as imatinib. Therefore, dual ABL/SRC kinase inhibitors have the potential to suppress chronic myelogenous leukemia cells more effectively than ABL specific inhibitors.

PD166326 is a pyridopyrimidine type inhibitor of receptor tyrosine kinases that inhibits ABL induced or BCR-ABL induced cell growth with half maximal inhibitory concentrations in the low milimolar to sub-milimolar concentration range (Wolff 2005). PD166326 also potently inhibits SRC. Orally administered PD166326 is well tolerated.

Drug Resistance PD166326 can reduce the growth of some imatinib resistant forms of chronic myelogenous leukemia, such as $BCR\text{-}ABL^{H396P}$ and $BCR\text{-}ABL^{M351T}$.

Saracatinib (AZD0530) is a 5-, 7-substituted anilinoquinazoline that inhibits SRC and ABL by blocking the ATP binding site. Saracatinib sensitively inhibits SRC^{Y530F} as well as the SRC tyrosine kinase family members c-YES, FYN, LYN, BLK, FGR, and LCK. The orally available small molecule exerts anti-tumor and anti-invasive activities, as it has inhibitory effects on proliferation and survival, cell motility and invasion. In metastatic cancer, saracatinib inhibits SRC kinase-mediated osteoclast bone resorption.

Bosutinib (SKI-606) <Bosulif> is a synthetic 7-alkoxy-3-quinolinecarbonitrile and a dual kinase inhibitor that targets both ABL and SRC kinases, with minimal activity against PDGFR and c-KIT. It also inhibits select serine/threonine kinases. Bosutinib suppresses the auto-phosphorylation of ABL and SRC kinases, resulting in the inhibition of cell growth and the induction of apoptosis. Because of the dual mechanism of action, this agent may have activity in imatinib resistant chronic myelogenous leukemia, other myeloid malignancies, and solid tumors.

Adverse Effects Bosutinib causes nausea, vomiting and diarrhea, which diminish over time. Myelosuppression affecting platelets, white and red blood cells arises in 1–10% of patients. Fluid retention, particularly in the lungs, is possible. The symptoms of adverse events tend to be less severe than with other tyrosine kinase inhibitors.

Drug Resistance The T315 mutation is completely resistant to bosutinib. The drug is not an efficient substrate for multi-drug resistance export transporters.

Dasatinib hydrochloride (BMS-354825) <Sprycel> is a thiazolylaminopyrimidine dual BCR/ABL and SRC family kinase inhibitor that suppresses the growth promoting activities of these enzymes. In contrast to most other tyrosine

kinase inhibitors, dasatinib binds to the active conformation of its target enzymes. At nanomolar concentrations, the drug inhibits BCR-ABL, SRC family (SRC, LCK, YES, FYN), c-KIT, EPHA2, PDGFRβ, and GFR. Mutations in the gene *discoidin domain receptor 2* (*ddr2*) are present in 4% of lung squamous cell carcinomata. These kinase mutations are associated with sensitivity to dasatinib. The drug has more than 300-fold increased potency to inhibit BCR-ABL compared with imatinib. Because it binds to ABL with less stringent conformational requirements than imatinib it exhibits increased potency but reduced selectivity. Likely because of its less stringent binding characteristics, dasatinib overcomes the resistance to imatinib of chronic myelogenous leukemia cells that harbor BCR-ABL kinase domain point mutations. In the U.S., dasatinib is approved for use in

- patients with chronic myelogenous leukemia who no longer benefit from imatinib treatment
- patients with Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph^+ ALL) who no longer benefit from, or cannot tolerate prior treatment.

Dasatinib is given at 100–140 mg orally once per day. Dose escalation is permissible in patients who do not achieve a hematologic or cytogenetic response at the recommended starting dosage.

Pharmacokinetics Dasatinib is best absorbed from the stomach due to the presence of gastric acid. Therefore, medicines that reduce stomach acid or increase gastric pH should be avoided within 2 h before or after dasatinib administration. Following oral administration, maximum blood concentrations of dasatinib are reached after 0.5–6 h. The agent exhibits dose-proportional increases in blood concentration and linear elimination characteristics over the range of 15–240 mg/day. Binding of dasatinib and its major active metabolite to plasma proteins is in excess of 90%, however, the drug is abundantly distributed in the extravascular space. Dasatinib is extensively biotransformed, primarily by CYP3A4, the primary enzyme responsible for the formation of the active metabolite. Flavin-Containing Monooxygenase 3 (FMO-3) and Uridine Diphosphate-Glucuronosyltransferase (UGT) are also involved in the formation of dasatinib metabolites. However, dose adjustment is not necessary in patients with hepatic impairment. The overall mean terminal half-life is 3–5 h. 85% of dasatinib is eliminated in the feces.

Adverse Effects Dasatinib has a toxicity profile that includes nausea and vomiting, diarrhea, headache, musculoskeletal pain, and rash. Myelosuppression may require dose reduction or discontinuation of dasatinib treatment. Bleeding due to thrombocytopenia or platelet dysfunction can be serious. Severe brain hemorrhages may be fatal. Fluid retention can manifest in pleural effusions, pericardial effusions, ascites, superficial localized edema, or generalized edema and can lead to congestive heart failure. Dasatinib has the potential to prolong cardiac ventricular repolarization (the QT interval). The agent may increase the risk for developing pulmonary arterial hypertension, which may occur anytime after initiation, including after more than 1 year of treatment. The drug is Pregnancy Category D.

Drug Resistance In acute phase or blast crisis of chronic myelogenous leukemia, 20–40% of patients are refractory to dasatinib. Drug resistance to dasatinib occurs in patients with the BCR-ABL mutation T315I. The dasatinib resistant mutation V299L may develop under treatment. The resulting BCR-ABL^{V299L} regains sensitivity for imatinib, and the mutated leucine enhances the affinity to the Aurora Kinase inhibitor tozasertib.

Since dasatinib is an inhibitor of ABL and SRC family kinases, it can overcome imatinib resistance due to SRC activation. Dasatinib is also not a substrate of multi-drug resistance efflux pumps.

Drug Interactions Similar to other tyrosine kinase inhibitors, dasatinib has extensive drug-drug interactions with agents metabolized by the enzyme CYP3A4. Drugs that inhibit CYP3A4 increase the blood levels of dasatinib (including ketoconazole, itraconazole, ritonavir, atazanavir sulfate, indinavir, nelfinavir, saquinavir, telithromycin, erythromycin, and clarithromycin). Grapefruit juice may also increase the blood concentrations of dasatinib and should be avoided. The use of concomitant strong CYP3A4 inducers (including dexamethasone, phenytoin, carbamazepine, rifampin, and phenobarbital) may decrease dasatinib concentrations. St. John's Wort may decrease dasatinib plasma concentrations unpredictably and should be avoided. Conversely, dasatinib may alter the blood levels of cyclosporine, alfentanil, fentanyl, pimozide, sirolimus, tacrolimus, and ergotamine. Antacids or proton pump inhibitors may reduce the absorption of dasatinib from the stomach.

Ponatinib (AP24534) <Iclusig> is an orally active BCR-ABL kinase inhibitor that is effective against the T315I mutation. The imidazo[1,2b]pyridazine core rests in the adenine pocket of the enzyme, the methylphenyl group occupies a hydrophobic pocket behind I315, the ethynyl linkage forms van der Waals interactions with the amino acid, and the trifluoromethyl group binds to a pocket of the inactive conformation kinase. Ponatinib was derived from the lead compound AP23464, which does not inhibit BCR-ABL^{T315I}.

Adverse Effects The most common adverse effects are diarrhea or constipation, abdominal pain, skin rashes, hypertension, fatigue, headache, dry skin, arthralgia, nausea, and pyrexia. Hematologic adverse reactions include thrombocytopenia, anemia, neutropenia, and leukopenia. Discontinuation may be required in cases of arterial thrombosis or hepatic toxicity. Ponatinib is Pregnancy Category D.

Drug Interactions The administration with strong CYP3A inhibitors may require dose reduction. Ponatinib inhibits ABCB1. It may affect the systemic exposure to drugs that are substrates for this export transporter. Drugs that elevate the gastric pH may reduce the bioavailability of ponatinib.

ABL/Aurora Kinase inhibitors The dual ABL/Aurora Kinase inhibitors are active against the mutant form BCR-ABL^{T315I}, which is associated with drug resistance against other BCR-ABL inhibitors. Therefore, they may serve as rescue treatments.

The pan-Aurora Kinase inhibitor tozasertib lactate (VX-680, MK-0457) is a synthetic small molecule drug that inhibits its wild-type ABL, BCR-ABL^{Y253F}, and BCR-ABL^{T315I} at half-maximal concentrations below 50 nM. It anchors at the hinge region engaging asparagine-381 but does not reach as deep into the kinase domain as imatinib, which allows tozasertib to avoid the steric constraints imposed by the T315I mutations. Under dasatinib treatment, the resistant mutation BCR-ABL^{V299L} may develop. It remains sensitive to tozasertib because the mutated leucine enhances the drug affinity. The agent also induces apoptosis in tumor cells, in which Aurora Kinases are over-expressed.

Adverse Effects The main toxicities consist of myelosuppression, alopecia, and mucositis. QTc elongation in the electrocardiogram is a possible complication.

The pyrrolo-pyrazole danusertib (PHA-739358) is an ATP competitive pan-Aurora Kinase (Aurora Kinase A, B, and C) inhibitor that also has activity against BCR-ABL^{T315I}. Danusertib binds to the active conformation of BCR-ABL^{T315I} in a mode that accommodates the substitution of isoleucine for threonine, thus avoiding steric hindrance. Exposure of imatinib-resistant CD34^{+}, BCR-ABL^{T315I} carrying cells to danusertib decreases the phosphorylation of Histone H3 on serine-10 (reflective of Aurora Kinase inhibition) and CRKL (a downstream target of BCR-ABL). A regimen under study is the administration of 250–330 mg/m^2/day as a weekly 6-h infusion for 3 consecutive weeks, every 4 weeks.

Adverse Effects Adverse events comprise high grade neutropenia and infusion related reactions.

XL228 is an Aurora Kinase A inhibitor that also suppresses wild-type ABL and BCR-ABL^{T315I} at low nanomolar concentrations. It further acts as a kinase inhibitor of IGF1R, FGFR, and SRC. In sensitive cells, XL228 inhibits the phosphorylation of STAT5, a downstream target of BCR-ABL.

KW-2449 is a multi-kinase inhibitor of ABL, BCR-ABL^{T315I}, Aurora Kinase, and FLT3. As an ABL inhibitor, the drug may be of benefit in imatinib resistant leukemia. KW-2449 is also growth inhibitory for leukemia cells with FLT3 gain-of-function mutations, resulting in G_1 arrest, and apoptosis. In FLT3 wild-type leukemia cells, it induces G_2/M arrest and apoptosis. The orally available KW-2449 is under investigation to treat leukemia patients.

The pan-Aurora, ABL, FLT3, and JAK2 kinase inhibitor AT9283 (1-cyclopropyl-3[5-morpholin-4yl methyl-1*H*-benzomidazol-2-yl]-urea) induces a reduction in the phosphorylation of Histone H3, CRKL, and STAT5 (downstream targets of ABL, FLT3, and JAK2 signaling). AT9283 is in clinical trials and has activity in patients with refractory acute or chronic myelogenous leukemia (AML or CML).

Adverse Effects The maximum tolerated dose of AT9283, given as a 72-h intravenous infusion, is 100 mg/m^2/day. Dose limiting toxicities include myelosuppression and alopecia, tumor lysis syndrome may occur. Elevated Transaminases, non-cardiac Creatine Kinase, and Lactate Dehydrogenase rises can be reflective of muscle or liver damage.

A translocation involving chromosomes 9 and 22 generates the constitutively active fusion kinase BCR-ABL and causes chronic myelogenous leukemia (CML).
BCR-ABL inhibitors are drugs of choice for the disease.
While initially successful, small molecule kinase inhibitors of BCR-ABL loose effectiveness—often because of mutations in the drug target.
Second generation BCR-ABL inhibitors, ABL/SRC inhibitors or BCR-ABL^{T315I} inhibitors (also inhibit Aurora Kinase) are second line of defense.
In contrast to most tyrosine kinase inhibitors, dasatinib binds to the active conformation of the target enzyme.
BCR-ABL inhibitors are soluble at low pH and are absorbed in the stomach.
Many BCR-ABL inhibitors exert cardiotoxicity and cause fluid retention.

4.1.3 RAS Pathway Inhibitors

Signals transduced by various proto-oncogenic growth factors proceed through the small GTPase RAS. RAS functions as a cell membrane-associated switch that relays signals from ligand stimulated receptors to cytoplasmic cascades. These receptors include G-Protein coupled seven transmembrane spanning receptors, tyrosine kinase receptors, and cytokine receptors that cause the stimulation of associated non-receptor tyrosine kinases. The signal transduction may be initiated via the cascade Growth Factor Receptor→GRB→SOS→RAS and proceed through the canonical RAS→RAF→MEK→ERK signaling pathway (Fig. 4.10).

Kinases of the RAS family and some of their downstream targets are frequently up-regulated in neoplasms (Weber 2007). K-RAS mutations have the highest incidence in adenocarcinomata of the pancreas (90%), colon (50%), and lung (30%). N-RAS is predominantly mutated in myeloid

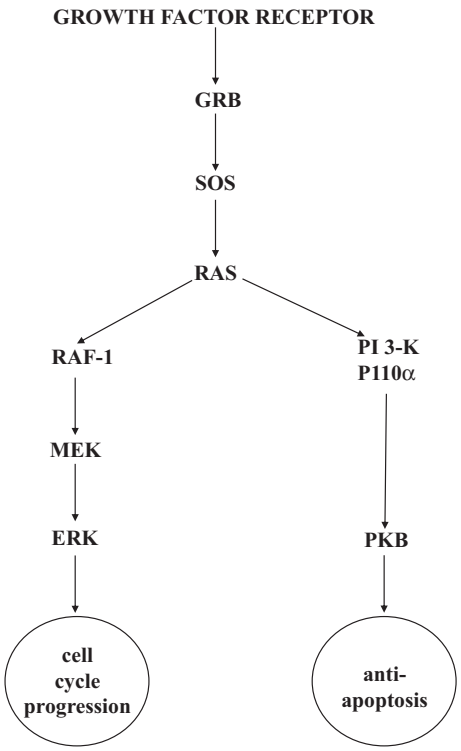

Fig. 4.10 RAS signaling pathways. RAS may mediate signals transduced by growth factor receptors. The latter may proceed through the cascade SHC→GRB-2→SOS. RAS induces cell growth through two principal downstream targets, namely the *ERK* (MAPK) pathway and the *PI3-K* pathway

leukemia (30 %) (Bos 1989). Inhibition of RAS signaling has the potential to cure those cancers by overcoming the excessive, RAS dependent cell growth (Table 4.4).

RAF inhibitors RAF is a serine/threonine kinase that is involved in the RAS dependent transduction of mitogenic signals from the cell membrane to the nucleus. There are three active *raf* genes (*A-raf*, *B-raf-1*, and *C-raf-1*), 2 of which have related pseudo-genes (*B-raf-2* and *C-raf-2*). *c-raf-1* RNA is present in many tissues, while *A-raf* and *B-raf-1* expression is restricted. *A-raf* and *c-raf* encode cytoplasmic serine/threonine protein kinases of 68 and 74 kD, which contain three conserved regions (CR). CR1 and CR2 are in the NH_2-terminal half with CR1 comprising the presumed ligand binding site, and CR3 represents the COOH-terminal kinase domain. RAF acts as a MAP Kinase Kinase Kinase and counteracts apoptosis by suppressing the activation of MST-2. RAF inhibitors may inhibit the excessive growth of some cancers. 40–60 % of melanomata carry a B-RAF mutation, in roughly 90 % of cases the specific mutation V600E.

RAF inhibitors suppress ERK signaling in mutant B-RAF cells but enhance ERK signaling in cells harboring wild-type B-RAF. RAF inhibitors bind to one member of the C-RAF/B-RAF hetero-dimer or C-RAF/C-RAF homo-dimer. While inhibiting one protomer, this allows transactivation of the other, drug-free binding partner.

Drug Resistance The development of drug resistance is a major limitation for the efficacy of small molecule B-RAF inhibitors. Mechanisms that mediate drug resistance include:

- expression of mutant N-RAS activates the signaling of MEK-1 and MEK-2 in some resistant tumors,
- an increase in RAS activation or RAF dimerization may be sufficient to cause drug resistance,
- over-expression of PDGF-β (Platelet Derived Growth Factor β) enables tumor cell survival,
- over-expression of MAP3K8 leads to resistance by activating MEK-1 and MEK-2 signaling.

Sorafenib tosylate (4-[4-[[4-chloro-3-(trifluoromethyl) phenyl]carbamoylamino] phenoxy]-*N*-methyl-pyridine-2-carboxamide) <Nexavar> is a synthetic compound that inhibits wild-type and mutant forms of the kinase RAF (Fig. 4.11[3]). The kinases KIT, FLT3, and RET are also targets. Sorafenib inhibits the VEGFR2/PDGFRβ signaling cascade, thereby blocking tumor angiogenesis. The drug is active orally and it is available as tablets. It is taken without food, 1 h before or 2 h after a meal, typically twice daily. Sorafenib was approved by the U.S. FDA in 2005, it received E.U. marketing authorization in 2006 to treat unresectable hepatocellular carcinoma and advanced renal cell carcinoma. The administration of sorafenib prolongs progression-free survival in patients with advanced clear cell renal carcinoma, in whom previous therapy has failed (Escudier 2007). Further, the agent is in testing for the treatment of soft tissue sarcoma.

Pharmacokinetics Following oral administration, sorafenib reaches peak blood levels after 3 h. With continuous exposure, steady state concentrations are achieved within 7 days, with a peak-to-trough concentration ratio below 2. The agent undergoes oxidative metabolism by hepatic CYP3A4, as well as glucuronidation by UGT1A9. Of eight sorafenib metabolites, five accumulate in the blood. The pyridine *N*-oxide is the main circulating product, comprising 10–15 % at steady state. The mean elimination half-life of sorafenib is 24–48 h. Neither renal impairment nor hepatic impairment substantially affects the pharmacokinetics.

Adverse Effects Common adverse effects of sorafenib include fatigue, weight loss, diarrhea, arthralgia or myalgia, alopecia, mucositis or stomatitis, hand-foot skin reactions, and skin rash or desquamation (in rare serious cases Stevens-Johnson syndrome and toxic epidermal necrolysis). Hypertension, congestive heart failure, and cardiac ischemia are rare serious adverse events. QT prolongation is rare, but increases the risk

[3] The structure of the representative XL281 has not been disclosed.

Table 4.4 RAS pathway inhibitors

	RAF	B-RAFV600E	PDGFR	VEGFR2	KIT	FLT3	RET	FGFR	MEK	ERK1	ERK2	CDKs	PKB	GSK3β	PKC
RAF inhibitors															
Sorafenib	S		S	S	S	S	S								
CHIR-265	S	S	S	S			S								
CHIR-258	S		S	S				S							
Vemurafenib	R	S													
PLX4032 and PLX4720	R	S	R												
Dabrafenib	R	S													
XL281	B and C, not A														
MEK/ERK inhibitors															
PD0325901									S						
Trametinib										S	S				
PKC inhibitors															
7-hydroxystaurosporine												S	S		S
Midostaurin			S	S		S									S
Enzastaurin				R									S	S	S
Safingol															S

S sensitive, *R* resistant, *CDKs* Cyclin Dependent Kinases

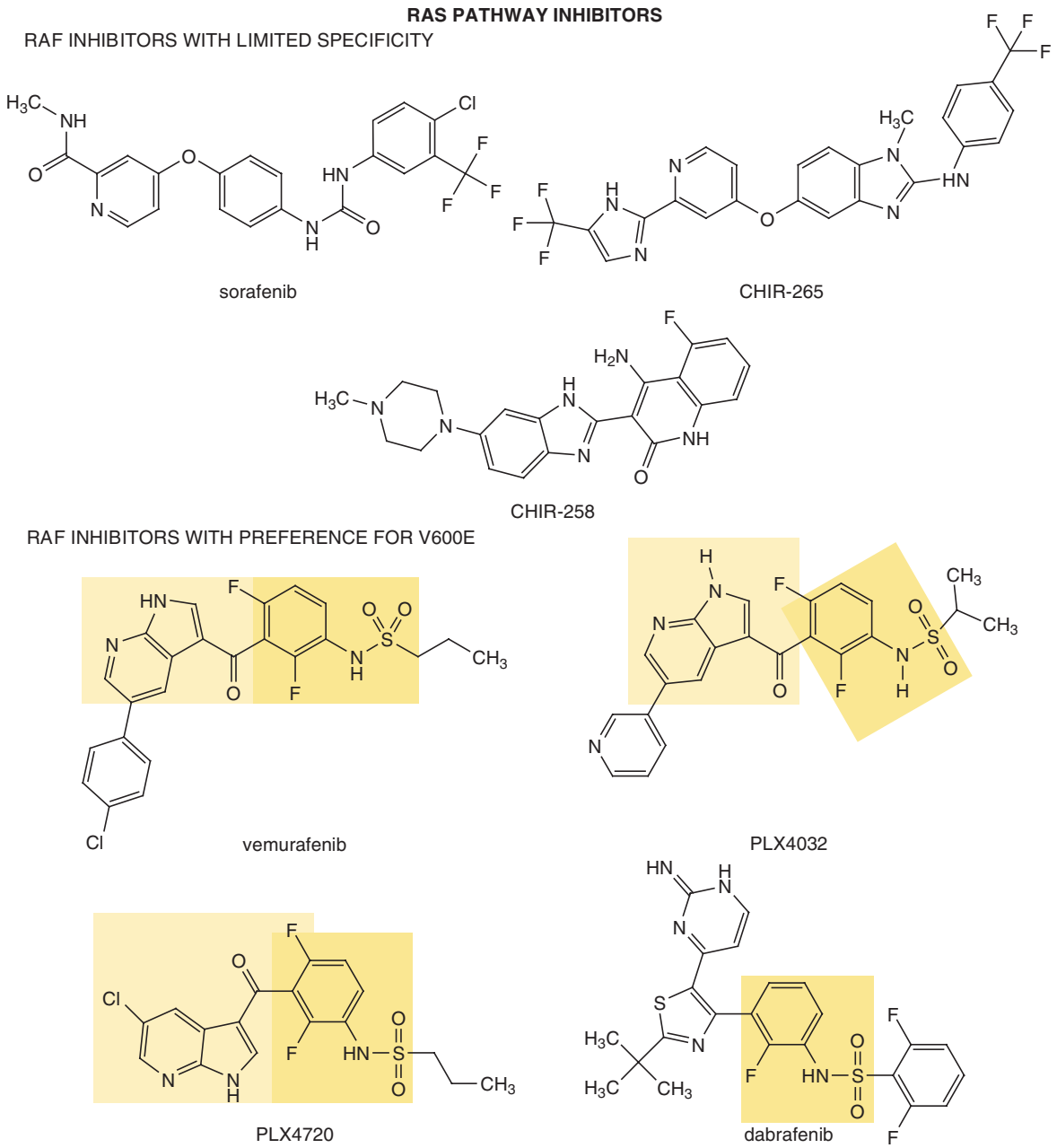

Fig. 4.11 Structures of RAS pathway inhibitors. Three sub-classes of RAF inhibitors comprise inhibitors with limited specificity, inhibitors with a preference for the mutant V600E, and (not shown) selective B-RAF/C-RAF inhibitors. The highlighted core structure (*bright yellow*) is common to all RAF^{V600E} inhibitors. The partially overlapping motif of joined 5- and 6-rings, a (C=O based) bridge, and a 6-ring (*light yellow*) bears similarity to the joined 6- rings, a (N-H based) bridge, and a 6-ring in the ERBB family inhibitors (see Fig. 4.3). MEK inhibitors suppress the RAS pathway downstream of RAF. Among the PKC inhibitors, the three staurosporin derivatives have an identical core structure, which is shared with indolocarbazoles (see Fig. 2.33 and 4.13). Their differences are highlighted in *pink*

for ventricular arrhythmias. An elevated risk of bleeding may arise following sorafenib administration. Gastrointestinal perforation is an uncommon adverse reaction in less than 1% of patients. The drug is Pregnancy Category D.

Drug Interactions Concomitant use of strong CYP3A4 inducers (such as, carbamazepine, dexamethasone, phenobarbital, phenytoin, rifampin, rifabutin, St. John's Wort) is to be avoided, because they can decrease the systemic exposure to sorafenib. Neomycin can reduce the sorafenib bioavailability by as much as 50%. Sorafenib inhibits glucuronidation catalyzed by UGT1A1 and UGT1A9 and transport mediated by ABCB1. It could increase the systemic exposure of simultaneously administered drugs that are substrates for these molecules.

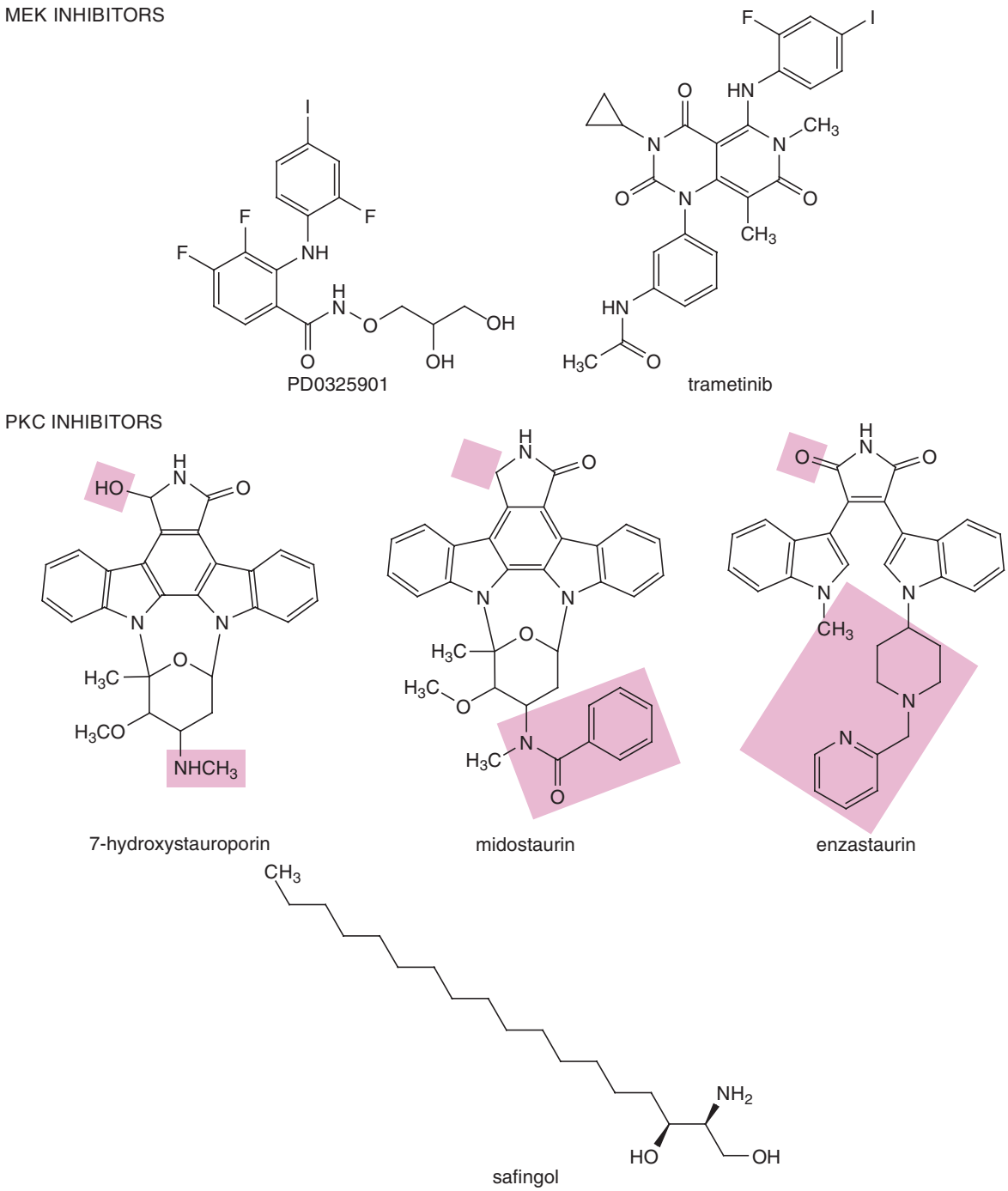

Fig. 4.11 (continued)

CHIR-265 (RAF265) is an orally active small molecule that binds to and inhibits RAF family kinases (C-RAF, B-RAF, B-RAF^{V600E}) and abrogates downstream ERK signaling. It also inhibits RET activity. This drug action results in a suppression of tumor cell proliferation and tumor cell death. In addition, CHIR-265 inhibits VEGFR-2, thereby disrupting tumor angiogenesis. Combination with the PI 3-K/mTOR (Phosphoinositol 3-Kinase/mammalian Target of Rapamycin) inhibitor BEZ235 achieves a synergistic effect leading to decreased cell proliferation. CHIR-265 is in clinical trials for the treatment of locally advanced or metastatic melanoma. The maximum tolerated oral dose is 48 mg daily, an intermittent schedule at higher dose may be possible.

- The related compound CHIR-258 is a small molecule with inhibiting properties against the kinases B-RAF, PDGFR-β, VEGFR-2, and FGFR. It is in clinical trials for the treatment of melanoma.

Adverse Effects Dose limiting toxicities within the first 28-day cycle include pulmonary embolism, visual disturbances, hyperlipasemia, diarrhea, and ataxia. Grade III and IV thrombocytopenia may also become dose limiting.

Vemurafenib (PLX4032) <Zelbroraf> blocks mutated B-RAF. It engages the ATP-binding domain of mutant B-RAF monomer and, under the condition that RAS is not constitutively activated, can block MEK and ERK signaling. The drug received U.S. FDA approval in 2011 for the treatment of unresectable or metastatic melanoma with the B-RAF^{V600E} mutation. It is not suitable for use in patients with wild-type B-RAF melanoma. The recommended dose is 960 mg twice daily without a meal.

Pharmacokinetics The vemurafenib concentration-time profile is adequately described by a one-compartment disposition model with first order absorption and first order elimination. At steady state, the drug exhibits linear pharmacokinetics within the 240–960 mg dose range. In the blood, over 99% of vemurafenib is bound to Albumin and α1 Acid Glycoprotein. Over 95% of a dose is excreted in the feces, only 1% in the urine. The elimination half-life is 55 h.

Adverse Effects Serious hypersensitivity reactions, including anaphylaxis, may arise upon initial vemurafenib administration as well as upon re-initiation of treatment. Skin manifestations are common, including palmar-plantar erythrodysesthesia syndrome, keratosis pilaris, erythema nodosum, and rarely Stevens-Johnson syndrome. Patients have an increased risk for developing B-RAF-negative squamous cell skin cancers or basal cell carcinomata that are readily treatable. General adverse effects comprise arthritis, dizziness, folliculitis, vasculitis, uveitis, and mild photosensitivity. QT prolongation resulting in atrial or ventricular fibrillations may occur. The drug is Pregnancy Category D.

Drug Interactions Vemurafenib is a moderate CYP1A2 inhibitor, a weak CYP2D6 inhibitor, and a CYP3A4 inducer. Its concomitant use with agents with narrow therapeutic windows that are metabolized by CYP1A2, CYP2D6, or CYP3A4 is not recommended as vemurafenib may alter their concentrations. Co-administration of vemurafenib results in an increase in S-warfarin blood levels.

PLX4032 (RG7204, RO5185426) and PLX4720 are selective RAF inhibitors with a more than 10-fold higher selectivity for B-RAF^{V600E} than for wild-type B-RAF. Mutant B-RAF inhibition may lead to programmed cell death in the cancer cells. The orally bioavailable agents are in clinical trials for metastatic melanoma.

Adverse Effects Dose limiting toxicities include rash, fatigue, and joint pains. Serious adverse events can occur in some patients after long-term treatment, including the possibility of drug related cutaneous squamous cell carcinoma.

Drug Resistance Mechanisms of drug resistance, which is encountered in 40% of patients after 2–18 months, include the over-expression of PDGFR-β, which creates an alternate survival pathway, and reactivation of the normal B-RAF survival pathway via N-RAS.

The orally bioavailable drug dabrafenib mesylate (GSK2118436) <Tafinlar> is a reversible, selective, ATP competitive B-RAF inhibitor with more than 100-fold selectivity for mutant B-RAF over wild type B-RAF. It is approved to treat patients with metastatic or unresectable melanoma whose tumors express the B-RAF^{V600E} gene mutation. There is a risk of paradoxical activation of MAP Kinase signaling and increased cell proliferation in B-RAF wild-type cells, which are exposed to B-RAF inhibitors. Dabrafenib has efficacy in shrinking brain metastases. The recommended dose is 150 mg orally taken twice daily, approximately 12 h apart, at least 1 h before or at least 2 h after a meal.

Pharmacokinetics Dabrafenib is primarily metabolized by CYP2C8 and CYP3A4. As it contains a sulfonamide moiety, dabrafenib confers a potential risk of hemolytic anemia in patients with Glucose-6-Phosphate Dehydrogenase (G6PD) deficiency.

Adverse Effects Cutaneous squamous carcinoma arises in 10% of the patients. Hyperkeratosis, alopecia, palmar-plantar erythrodysesthesia syndrome, rash, headache, pyrexia, arthralgia or myalgia, back pain, constipation, or upper respiratory tract infections may occur. Serious febrile drug reactions[4] afflict fewer than 5% of patients treated with dabrafenib. Hyperglycemia requiring an increase in the dose of, or initiation of insulin or oral hypoglycemic agent therapy is possible under treatment. The drug is Pregnancy Category D.

Drug Interactions Strong inhibitors or inducers of CYP3A4 or CYP2C8 may affect the concentrations of dabrafenib. Drugs that alter the pH of the upper gastrointestinal tract (such as proton pump inhibitors, H_2-receptor antagonists, or antacids) may alter the solubility of dabrafenib and reduce its bioavailability. Because dabrafenib induces CYP3A4 and CYP2C9 it decreased the systemic exposures of the CYP3A4 substrate midazolam, the CYP2C9 substrate S-warfarin, and the CYP3A4/CYP1A2 substrate R-warfarin.

XL281 (BMS-908662) is a highly selective inhibitor of B-RAF and C-RAF, but not A-RAF. The agent is orally bioavailable. The maximum tolerated dose is 150 mg/day.

Pharmacokinetics The time to peak blood concentration is 2 h. The half-life is 5–20 h. Steady-state levels after continuous administration are reached by day 8.

[4] Serious febrile drug reactions are defined as serious cases of fever or fever of any severity accompanied by hypotension, rigors or chills, dehydration, or renal failure in the absence of other identifiable causes (such as infections).

Adverse Effects Grade 3 adverse events include fatigue, vomiting, diarrhea, nausea, and arthralgia. Dose limiting toxicities occurring at 225 mg/day are grade 4 nausea, grade 3 fatigue, grade 3 diarrhea, and grade 3 vomiting.

MEK/ERK inhibitors MEK (MAP Kinase Kinase, MAPKK) is phosphorylated and activated by RAF in a growth factor signaling pathway that is frequently overactive in cancer cells.

PD0325901 (*N*-[(2R)-2,3-dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]-benzamide) is a synthetic derivative of the selective, non-competitive MEK inhibitor CI-1040. PD0325901 has improved oral bioavailability and induces a longer duration of target suppression. The agent selectively binds to and inhibits MEK, thereby preventing the phosphorylation and activation of the downstream kinases ERK -1 and -2, which may result in the inhibition of tumor cell proliferation.

Trametinib (GSK-1120212) <Mekinist> is a reversible MEK-1/MEK-2 inhibitor approved for the treatment of patients with unresectable or metastatic melanoma harboring the B-RAF^{V600E} or B-RAF^{V600K} mutations. It is not indicated for the treatment of patients who have received prior B-RAF inhibitor therapy. The drug is also under study to treat leukemia, lymphoma, and pancreatic cancer. The recommended dose is 2 mg per day, 1–2 h before a meal, until disease progression or unacceptable toxicity is encountered.

Pharmacokinetics More than 95% of the drug in circulation is protein-bound. Metabolism occurs via deacetylation with or without mono-oxygenation or glucuronidation.

Adverse Effects Rash, diarrhea, and lymphedema are common adverse effects. Discontinuation is required if retinal pigment epithelial detachments, retinal vein occlusion, or an intolerable rash occur. Interstitial lung disease or pneumonitis require permanent discontinuation. An asymptomatic decrease of the left ventricular ejection fraction of the heart is possible it may be a sign of cardiomyopathy. The drug is Pregnancy Category D.

PKC inhibitors Protein Kinase C (PKC) may activate RAS via the nucleotide exchangexe factor RAS-GRP. RAS-GRP activity also depends on the local availability of the signaling intermediate diacylglycerol. Abnormally high levels of RAS-GRP can lead to malignant transformation through excessive RAS pathway activity.

The indolocarbazole 7-hydroxystaurosporine (UCN-01) is a synthetic derivative of staurosporine that inhibits several kinases, including calcium dependent Protein Kinase C, the serine/threonine kinase PKB (AKT), and Cyclin-Dependent Kinases (CDKs). 7-hydroxystaurosporine promotes the accumulation of the negative cell cycle regulators P21 and P27, arresting tumor cells in the G_1/S phase of the cell cycle. The agent prevents nucleotide excision repair by inhibiting the G_2 checkpoint kinase CHK1, which results in apoptosis. Thus, it may enhance the effects of DNA damaging drugs. 7-hydroxystaurosporine is under study for treating melanoma and lymphoma.

Adverse Effects The maximum tolerated dose is 43 mg/m^2/day for 3 days. Dose limiting toxicities comprise nausea and vomiting, hypoxemia, and hyperglycemia.

Midostaurin ((9S,10R,11R,13R)-2,3,10,11,12,13-hexahydro-10-methoxy-9-methyl-11-(methylamino)-9,13-epoxy-1*H*,9*H*-diindolo[1,2,3-gh:3′,2′,1′-lm]pyrrolo[3,4-j][1,7]benzodiamzonine-1-one) (PKC412) is a synthetic indolocarbazole that acts as a non-specific inhibitor of Protein Kinase C family enzymes. Midostaurin inhibits oncogenic PKC signal transduction pathways involved in the regulation of the cell cycle in tumor cells. It also inhibits VEGF Receptors, Platelet-Derived Growth Factor Receptor, FLT3, and other receptor tyrosine kinases. Midostaurin thereby initiates tumor cell apoptosis. The agent is in clinical development.

Enzastaurin hydrochloride (3-(1-Methylindol-3-yl)-4-[1-[1-(pyridin-2-ylmethyl)piperidin-4-yl]indol-3-yl]pyrrole-2,5-dione) (LY317615) is the salt of a synthetic macrocyclic bisindolemaleimide. It acts as a potent inhibitor of PKCβ, PKCα, PKCγ and PKCε. Enzastaurin inserts into the ATP binding site and blocks catalytic activity. The drug also suppresses the phosphorylation of GSK3β and AKT. It may decrease tumor blood supply and so tumor burden. Enzastaurin has orphan drug status in Europe for the treatment of diffuse large B-cell lymphoma. In 2013 it failed a phase III clinical trial for lymphoma.

Safingol (L-threo-dihydrosphingosine, (2S,3S)-2-aminooctadecane-1,3-diol) <Kynacyte> is a saturated derivative of sphingosine. As an inhibitor of PKC, it competitively inhibits the regulatory, phorbol binding domain of PKC. This agent acts synergistically with other chemotherapeutic drugs, such as doxorubicin or cisplatin, and may potentiate chemotherapy-induced apoptosis.

Pharmacokinetics Despite its non-natural stereochemistry, safingol is metabolized preferentially by enzymes of the sphingolipid biosynthetic pathway. The cytotoxic potential of safingol is reduced via its metabolic conversion.

Excessive growth signals through constitutively active RAS pathways are common in some cancers.
RAF inhibitors may be first-line treatments of choice.
Some RAF inhibitors have low specificity, others bind preferentially to the RAF^{V600E} mutant in cancer cells.
Many RAF inhibitors exert cardiotoxicity, some increase the risk for cutaneous secondary malignancies.

Table 4.5 The phosphatidylinositol 3′OH kinase gene family. (Adapted from Wikipedia)

Group	Gene	Protein	Acronym	EC number
Class 2	PIK3C2A	PI3K, class 2, α polypeptide	PI3K-C2α	2.7.1.154
	PIK3C2B	PI3K, class 2, β polypeptide	PI3K-C2β	
	PIK3C2G	PI3K, class 2, γ polypeptide	PI3K-C2γ	
Class 3	PIK3C3	PI3K, class 3	VPS34	2.7.1.137
Class 1 catalytic	PIK3CA	PI3K, catalytic, α polypeptide	P110-α	2.7.1.153
	PIK3CB	PI3K, catalytic, β polypeptide	P110-β	
	PIK3CG	PI3K, catalytic, γ polypeptide	P110-γ	
	PIK3CD	PI3K, catalytic, δ polypeptide	P110-δ	
Class 1 regulatory	PIK3R1	PI3K, regulatory subunit 1 (α)	P85-α	(None)
	PIK3R2	PI3K, regulatory subunit 2 (β)	P85-β	
	PIK3R3	PI3K, regulatory subunit 3 (γ)	P55-γ	
	PIK3R4	PI3K, regulatory subunit 4	P150	
	PIK3R5	PI3K, regulatory subunit 5	P101	
	PIK3R6	PI3K, regulatory subunit 6	P87	

Inhibitors of various RAS pathway signaling molecules are in development.

4.1.4 Phosphoinositide 3-Kinase Pathway Inhibitors

PI3K inhibitors The Phosphoinositide 3′OH-Kinase (PI 3-Kinase, PI3K) family of gene products consists of three classes of lipid kinases that have a regulatory subunit (P85) and a catalytic subunit (P110), which combine to form an enzyme that phosphorylates the 3′ OH group of phosphatidylinositols (Table 4.5). The signaling pathway activated downstream of PI3K via PKB (AKT) and mTOR protects cells from apoptosis.

Whereas in healthy cells the Phosphoinositide 3′OH-Kinase pathway is tightly controlled, the inappropriate activation of PI3K is important in the pathogenesis of multiple cancers, in which activating somatic mutations may arise. Mutations in the catalytic subunit P110α (PIK3CA) are associated with approximately 30% of epithelial cancers (Engelman 2009). Kinase domain and helical domain mutants comprise about 80% of PI 3-Kinase dependent cancers. Kinase domain mutants, such as H1047R, have increased lipid kinase activity. Helical domain mutants, such as E545K or E542K, disrupt the inhibitory inter-molecular interaction between P85 and P110, thus excessively activating the kinase domain (Samuels 2004). Each type of mutant promotes uncontrolled cell expansion via anti-apoptosis. This renders the enzyme and its associated downstream signaling intermediates drug targets (Table 4.6).

Several agents that target the kinase domain of PI 3-Kinase (including BGT226, BKM120, XL765, XL147, GDC0941, GSK1059615) have entered early phase clinical trials. BEZ235, BGT226, PWT33597, and XL765 also possess mTOR inhibitory properties because the P110 kinase domain shares similar structural features with the serine/threonine kinase domain of mTOR.

PWT33597 mesylate[5] is a dual inhibitor of PI3Kα and mTOR with IC_{50} values around 20 nM. It is approximately 10-fold more selective for PI3Kα compared to PI3Kγand PI3Kδ. There is little or no cross-reactivity with either serine/threonine or tyrosine kinases. PWT33597 is in clinical trials for advanced solid tumors.

Pharmacokinetics PWT33597 is not extensively metabolized and shows little potential for interaction with Cytochrome P450 enzymes. There is high compound distribution into tumors. Also, administration of PWT33597 is associated with transient increases in Insulin blood levels, consistent with an effect on PI3K→PKB signaling.

Unlike other isoforms of PI3K, PI3Kδ is expressed primarily in hematopoietic lineage cells. The targeted inhibition of PI3Kδ is designed to suppress hematologic malignancies while preserving PI3K signaling in non-neoplastic cells. CAL101 (Fig. 4.12) is an oral small molecule inhibitor that targets the δ isoform of the 110 kD catalytic subunit of class IA PI3K. CAL-101 inhibits the production of the second messenger phosphatidylinositol-3,4,5-trisphosphate (PIP_3), thus preventing the activation of the PI3K signaling pathway and inhibiting tumor cell proliferation and survival. The agent is in clinical trials for certain forms of late-stage leukemias.

Semafore (SF1126) consists of the PI3K inhibitor LY294002 conjugated to an arginine-glycine-asparagine-serine (RGDS) peptide fragment. This peptide binds Integrin receptors in the tumor vasculature such that the LY294002 moiety inhibits PI3K dependent angiogenesis specifically in the tumor environment. SF1126 was granted orphan drug

[5] In veterinary use: VDC-597.

Table 4.6 PI3K pathway inhibitors

	PI3K	$PI3K^{H1047R}$	$PI3K^{E545K}$	$PI3^{KE542K}$	PKB	PKC	mTOR	FKBP12	Calcineurin
PI3K inhibitors									
PWT33597	PI3Kα						S		
CAL101	PI3Kδ								
Semafore	S								
NVP-BEZ235	S						S		
PX-866	S								
PKB inhibitors									
Miltefosine	S				S	S			
Perifosine	S	S	S		S				
MK-2206					S				
mTOR inhibitors									
Sirolimus							S	S	
Temsirolimus							S	S	S
Everolimus								S	
Ridaforolimus							S		

S sensitive, *R* resistant

status by the U.S. FDA in 2010 for the treatment of chronic B-cell lymphocytic leukemia.

The imidazoquinoline-derived dual PI3K and mTOR inhibitor NVP-BEZ235 is effective as a single agent and in combination with established cancer drugs. It is in clinical trials for advanced solid tumors. As gain-of-function mutations of RAS activate multiple pathways, including the PI3K pathway and the MEK→MAPK pathway, clinical trials combine NVP-BEZ235 with a MEK inhibitor.

PX-866 is a synthetic derivative from the fungal metabolite wortmannin (a product of Penicillium wortmannin). Compared to wortmannin, it has increased stability, reduced toxicity, and stronger drug activity. PX-866 is the only pan-isoform, irreversible PI3K inhibitor in clinical development (it forms a covalent bond with the PI3K molecule.). Due to its mechanism of action the agent results in sustained inhibition of the PI3K pathway. It is potent when delivered orally at 2–4 mg/kg.

Pharmacokinetics PX-866 is metabolized to produce an active metabolite, 17-OH, that is a more potent PI3K inhibitor than the parent drug and retains the same irreversible mechanism of action. PX-866 and the 17-OH metabolite inhibit all 4 PI3K family members and have the greatest potency for PI3Kα and β, the family members that are strongly linked to certain solid tumors.

PKB inhibitors The serine/threonine kinase PKB (AKT) is located at a critical signaling node downstream of Phosphatidylinositol 3′OH-Kinase. It is important in promoting cell survival and inhibiting apoptosis.

The PKB inhibitor miltefosine (hexadecylphosphocholine) <Impavido, Miltex> is an orally and topically active alkyl-phosphocholine compound that inhibits the anti-apoptotic MAPK (Mitogen-Activated Protein Kinase) pathway and modulates the balance between the MAPK and pro-apoptotic Stress-Activated Protein Kinase (SAPK/JNK) pathways, thereby inducing apoptosis. Synthetic phospholipids may exert anti-tumor activity through the inhibition of Protein Kinase C, an essential mediator in growth factor signal transduction, and m Miltefosine also targets cellular membranes, modulating cell membrane permeability, membrane lipid composition, and phospholipid metabolism. This results in cell differentiation and inhibition of cell growth. Acting further as an anti-protozoal immunomodulator, miltefosine stimulates T-lymphocytes, macrophages and the expression of IL-3 (Interleukin-3), GM-CSF (Granulocyte-Macrophage Colony Stimulating Factor), and IFN-γ (Interferon-γ).

Pharmacokinetics Administration can be oral or topical. Miltefosine is used as a 6% solution in an aqueous 3-alkyloxypropylene glycol mixture.

Adverse Effects With oral administration, hyperplastic gastrointestinal tract changes, nausea and vomiting, gonadal atrophy, hair loss, and ocular toxicity may arise. No systemic effects arise after dermal application, which may be indicated for breast cancer that is metastatic to the skin. The drug is teratogenic and should not be given to pregnant women.

Perifosine (octadecyl-(1,1-dimethyl-4-piperidylio) phosphate) (KRX-0401) is an alkylphospholipid that is structurally related to miltefosine. Perifosine inhibits PI3K and PKB. It is in clinical trials for colorectal cancer and multiple myeloma. It has orphan drug status for the treatment of multiple myeloma and neuroblastoma in the U.S., and for multiple myeloma in the E.U.

MK-2206 is a selective, allosteric inhibitor of PKB1, PKB2, and PKB3. The drug is activated by the pleckstrin homology domain and inhibits the auto-phosphorylation of both T308 and S473. This action results in an inhibi-

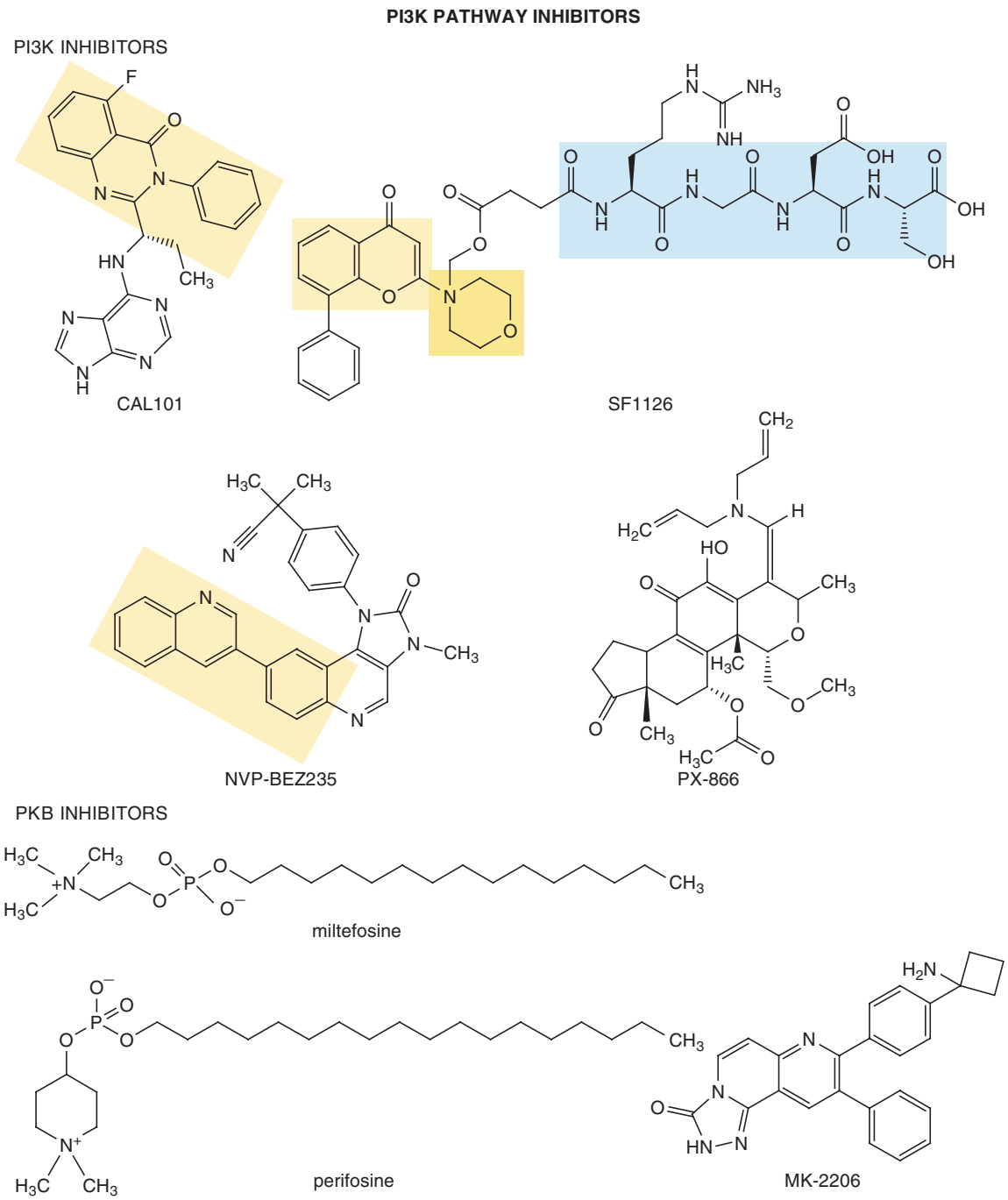

Fig. 4.12 Structures of PI3K pathway inhibitors. Subclasses include PI3K inhibitors, PKB inhibitors, and mTOR inhibitors. The loosely conserved core structure in a subset of PI3K inhibitors is highlighted in *light yellow*, the peptide bonds in the RGDS (arginine-glycine-aspara-gine-serine) of semaphore are shown on *blue* background. The mTOR inhibitors are derivatives of sirolimus. Structural differences among the representatives are highlighted in *pink*

tion of downstream signaling molecules, including TSC2, PRAS40 and Ribosomal S6 proteins. The orally bioavailable MK-2206 is in clinical trials for the treatment of ovarian cancer, primary peritoneal cancer, and fallopian tube cancer.

mTOR inhibitors mTOR (mammalian Target of Rapamycin, FRAP, FKBP-Rapamycin Associated Protein) is a serine/threonine kinase that controls cell growth and metabolism in response to nutrients, growth factors, and cellular energy levels. It plays a role in the PI3K→PKB→mTOR→RSK pathway that is up-regulated in several types of cancer. When the kinase activity of mTOR is activated, its downstream effects, entailing the synthesis of Cyclin D and HIF-1α, are increased. Cyclin D then stimulates cell cycle progression. HIF-1α (Hypoxia-Inducible Factor-1α) then stimulates VEGF (Vascular Endothelial Growth Factor), which results in angiogenesis.

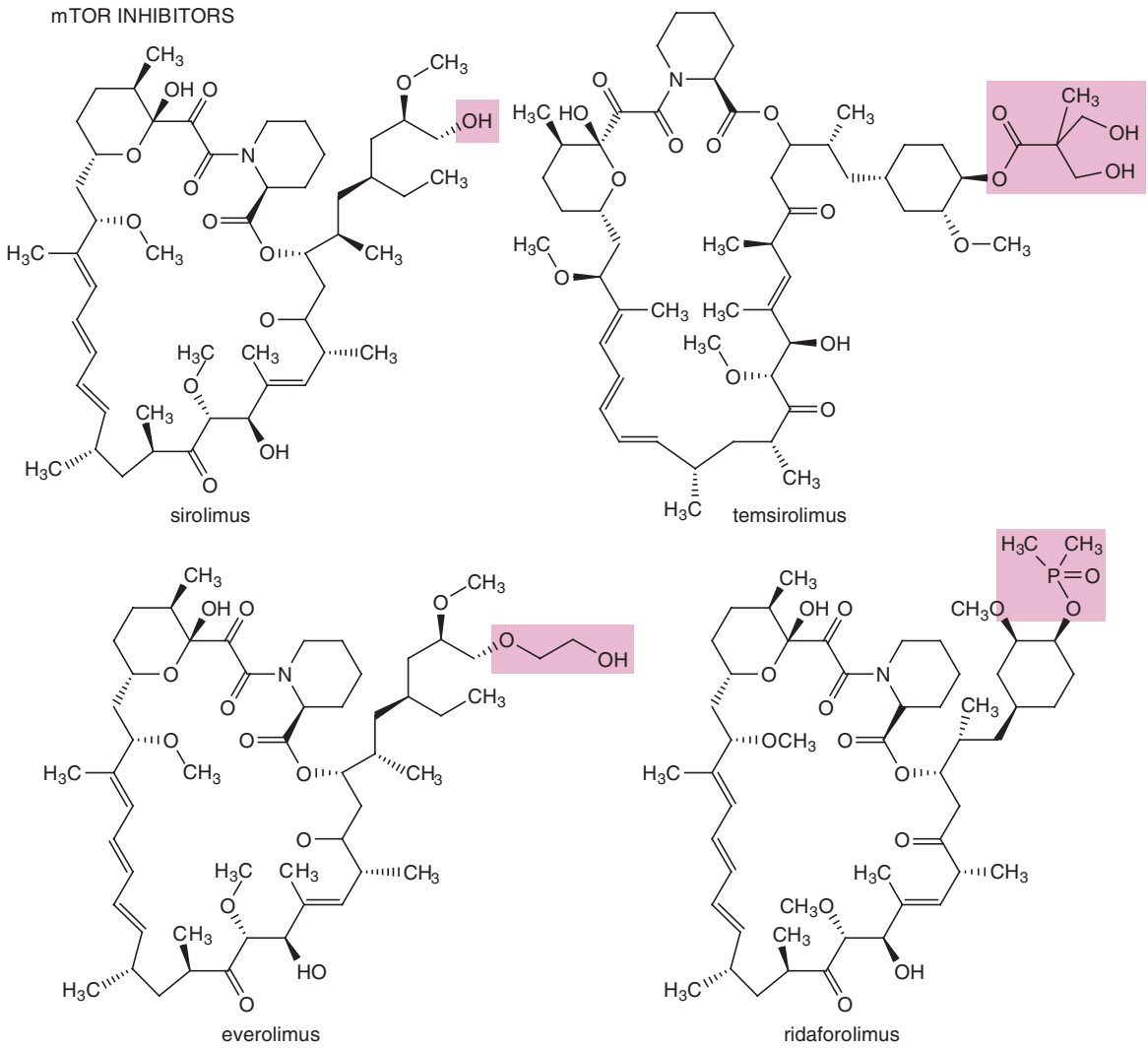

Fig. 4.12 (continued)

There are two mTOR multi-protein kinase complexes that may form. mTOR Complex-1 (mTORC1; comprised of mTOR, mLST8, AKT1S1, and RAPTOR) is the central component of a pathway that promotes growth in response to Insulin, energy levels, and amino acids. It activates protein synthesis, ribosome biogenesis, nutrient transport, and lipid synthesis, all resulting in cell growth. mTOR Complex-2 (mTORC2; consisting of mTOR, mLST8, PROTOR1, MAPKAP1, RICTOR) mediates Actin cytoskeletal organization via a mechanism involving PKB, SGK1, and PKC. Its dysregulation is implicated in tumor cell survival, motility, and invasiveness. The mTORC1 mediated consequences on cell cycle and cell size are distinct form the mTORC2 effects.

mTOR is particularly important in the biology of renal cell carcinoma, where HIF-1α plays a pathogenetic role. Mutation or loss of the tumor suppressor gene *vhl* (*von Hippel Lindau*) is common in this cancer and leads to a reduced degradation of HIF-1α. Activated mTOR further exacerbates the accumulation of the transcription factor HIF-1α by increasing its synthesis. There are familial cancer syndromes, in which mutations in negative regulators of the mTOR pathway give rise to transformation, including lymphangioleiomyomatosis and tuberous sclerosis complex (loss of TSC1 or TSC2), Peutz-Jeghers syndrome (loss of STK11), and Cowden syndrome (loss of PTEN).

- Derivatives of rapamycin (sirolimus, temsirolimus, everolimus, ridaforolimus) are allosteric mTOR inhibitors, and are sometimes referred to as rapalogs. Sirolimus inhibits only TORC1.
- ATP-competitive mTOR inhibitors are ATP analogs that inhibit mTOR kinase activity by competing with ATP for binding to the kinase domain in mTOR. The ATP analogs inhibit both mTORC1 and mTORC2. (Because of the similarity between the kinase domains of mTOR and the PI3Ks, mTOR inhibition by some of these compounds overlaps with PI3K inhibition. These drugs are discussed under PI3K inhibitors above.)

Sirolimus (rapamycin) was first discovered in the 1970s as a product of the bacterium Streptomyces hygroscopicus, present in a soil sample from Easter Island (Rapa Nui). The name of the compound was derived from that island. The macrolide sirolimus <Rapamune> binds the cytosolic immunophilin protein FKBP-12 (FK-Binding Protein 12). The bound molecules then engage the mTOR Complex-1 and inhibit it. Sirolimus strongly suppresses Interleukin-2 stimulated T-lymphocyte proliferation. It is mainly used as an immunosuppressant, but it possesses anti-proliferative properties. The agent suppresses the progression of dermal Kaposi sarcoma in patients with renal transplants. It also has modest clinical benefit in patients with tuberous sclerosis who have angiomyolipomata or sporadic lymphangioleiomyomatosis.

Adverse Effects Common adverse effects include stomach pain, headache, constipation, diarrhea, nausea, and joint pain. Lung toxicity is a potentially severe complication associated with sirolimus therapy. Allergic reactions may be serious.

Temsirolimus (CCI-779) <Torisel> is an ester analog of rapamycin that inhibits mTOR, causing decreased expression of genes necessary for cell cycle progression and resulting in consecutive cell cycle arrest in G_1. Temsirolimus is used to treat advanced renal cell carcinoma. It is also efficacious in the treatment of refractory mantle cell lymphoma. The intravenous dose is 25 mg over 30–60 min once weekly. The drug may also be administered orally. Temsirolimus received approval from the U.S. FDA and the European Medicines Agency (EMEA) in 2007.

Pharmacokinetics Cytochrome P450 3A4 is the primary enzyme responsible for the formation of five metabolites. Sirolimus, an active form of temsirolimus, is the principal metabolite following intravenous injection, while the other metabolites account for less than 10% of the drug in the blood. Elimination is primarily via the feces (80%) and less in the urine (5%).

Adverse Effects Premedication with an anti-histamine, such as 25–50 mg diphenhydramine, 30 min before the start of each dose is appropriate. Despite pretreatment, serious allergic reactions (anaphylaxis, dyspnea, flushing, chest pain) can occur.

The most common adverse reactions are rash, asthenia, mucositis, nausea, edema, and anorexia. There is the possibility of abnormal wound healing, bowel perforation, or intracranial bleeding. The use of temsirolimus may result in immunosuppression. Serious interstitial lung disease can develop. Increased blood glucose levels may require the initiation of Insulin therapy, while elevated triglycerides or cholesterol may require lipid lowering agents. The drug is Pregnancy Category D.

Drug Interactions The concomitant use of strong CYP3A4 inhibitors (including anti-retroviral protease inhibitors, ketoconazole, clarithromycin, nefazodone, telithromycin, voriconazole) or inducers (including dexamethasone, phenytoin, carbamazepine, rifampin, rifabutin, rifampacin, phenobarbital) should be avoided. Grapefruit juice may also increase the blood concentrations of sirolimus, a major metabolite of temsirolimus, and should be avoided. If the co-administration of strong CYP3A modulators is unavoidable, dose reduction (with CYP3A inhibitors) or dose increase (with CYP3A inducers) of temsirolimus need to be considered.

Everolimus (RAD001) <Zortress, Certican, Afinitor> is the *O*-(2-hydroxyethyl) derivative of sirolimus in position 40. Everolimus binds to FKBP-12, resulting in an inhibitory complex formation with mTORC1 and thus inhibition of mTOR. The effect of everolimus is solely on the mTORC1 protein complex and not on the mTORC2 complex. The drug was initially approved for use in Europe and Australia. It can be administered orally for use in

- advanced kidney cancer,
- subependymal giant cell astrocytoma (associated with tuberous sclerosis in patients who are not eligible for surgical intervention),
- progressive or metastatic pancreatic neuroendocrine tumors that are not surgically removable.

Pharmacokinetics Peak blood concentrations are reached 1–2 h after oral administration. Food may reduce the systemic exposure to oral everolimus. The drug is a substrate of CYP3A4. While the parent compound is the main circulating component in the blood, six main metabolites of everolimus are also present. They include three monohydroxylated metabolites, two hydrolytic ring-opened products, and one phosphatidylcholine conjugate. Everolimus is a substrate of ABCB1. 80% of the administered dose is excreted in the feces, while 5% is excreted in the urine. The agent should not be given to patients with severe hepatic impairment.

Adverse Effects Non-infectious pneumonitis (manifest in hypoxia, pleural effusion, cough, or dyspnea) is a class effect of rapamycin derivatives, including everolimus. The most common adverse reactions comprise stomatitis (leading to oral ulceration), rash, edema, diarrhea, abdominal pain, nausea, fatigue, fever, and headache. Everolimus has immunosuppressive properties and may predispose patients to bacterial, fungal, viral, or protozoal infections, including infections with opportunistic pathogens. The drug is Pregnancy Category D.

The selective inhibition of mTORC1 can lead to a hyperactivation of the kinase PKB via inhibition on the mTORC1 negative feedback loop, while not inhibiting the mTORC2-positive feedback to PKB. Because this effect may result in hypercholesterolemia and hypertriglyceridemia, monitoring of blood lipid levels is recommended.

Drug Interactions The concomitant use of strong CYP3A4 inhibitors or inducers should be avoided as they affect the blood concentrations of everolimus.

Ridaforolimus (deforolimus) (AP23573, MK-8669) contains a substitution of the secondary alcohol moiety at C-43 with a phosphate group. The drug is designed for improved aqueous solubility and oral delivery. Ridaforolimus improves the progression-free survival in patients with soft tissue or bone sarcomata.

Cell signaling via PI3K, PKB (AKT), and mTOR protects cells from apoptosis.
The inappropriate activation of PI3K pathways is an important step in the pathogenesis of multiple cancers.
PI3K inhibitors with various levels of specificity are in development.
mTOR inhibitors cause cell cycle arrest in G_1.
Sirolimus derivatives are substrates for CYP3A4.
As the mTOR pathway is important for glucose metabolism mTOR inhibitors increase blood glucose.
Non-infectious pneumonitis is a class effect of sirolimus derivatives.
Allergic reactions to sirolimus derivatives may be serious.
mTOR inhibitors are immunosuppressant.

4.1.5 FLT3 Inhibitors

The members of the proto-oncogene family *flt* encode receptors with protein tyrosine kinase activity that is important for the control of cell proliferation and differentiation. Expression of the cell surface receptor FLT3 (FMS Like Tyrosine Kinase 3, STK1, FLK2, CD135) is primarily restricted to the most undifferentiated hematopoietic progenitor cells. FLT3 Ligand is a member of a small family of growth factors that stimulate the proliferation of hematopoietic cells at select stages of their development and differentiation.

FLT3 is present on 80% of myeloid leukemia blast cells. Internal tandem duplication (FLT3 ITD[6]) is a common genetic defect in acute myelogenous leukemia (AML). Nearly 1/3 of AML patients have a gain-of-function mutation in the gene *flt3*, which is linked to an increased risk for relapse and reduced prospects for survival. For these cells, an excessive sensitivity to FLT3 dependent growth signals underlies their transformation.

FLT3 inhibitors may provide successful treatments for these cancers. These drugs fall into the categories of indolinones, and indolocarbazoles, FLT3/Aurora Kinase inhibitors, and others (Fig. 4.13). They all have cross-reactivities with multiple kinases and receptors. Members of the indolinone subgroup of inhibitors also block VEGFR2 (FLK1, KDR) and therefore have anti-angiogenic properties. Indolocarbazoles also inhibit Topoisomerase 1 and PKC. The FLT3/Aurora Kinase inhibitors also inhibit KIT (CD117) and ABL. They may be effective against tumors that over-express these molecules (Table 4.7).

[6] The duplicated stretch of DNA contains 18–108 nucleotides; it is always in frame and located in the juxtamembrane region of this class III tyrosine kinase receptor.

Drug Resistance In acquired resistance to FLT3 inhibitors, point mutations in the FLT3 molecule are rarely the cause. Much more common is anti-apoptosis through the up-regulation of MCL-1 (induced by FLT3 ITD627E, a non-juxtamembrane internal tandem duplication integrated into the first kinase domain), BCL-2 family proteins (may be overcome by adding the BH3 mimetic ABT-737), Survivin, or STAT signaling pathways. The resistance profiles of the FLT3 kinase inhibitors PKC412, SU5614, and sorafenib are non-overlapping, which suggests that combinations of FLT3 inhibitors may be therapeutically beneficial.

Indolinones Semaxanib (semoxind) (SU5416) is an intravenous indolinone that targets FLT3, KIT (CD117), PDGFR, and VEGFR (FLK-1, KDR). Twice weekly infusions of SU5146 administered on a 4-week cycle can reduce marrow blasts in AML or myeloproliferative disorders. In clinical trial, the agent displayed limited efficacy and substantial toxicity.

Sunitinib (SU11248) <Sutent> is an oral indolinone that acts as a more potent target inhibitor than semaxanib. The drug has higher success rates in patients with FLT3 mutations than in patients with wild-type FLT3. However, the duration of clinical responses is short, ranging 4–16 weeks. Sunitinib also inhibits c-KIT. The drug is used for the treatment of acute myelogenous leukemia, advanced renal cell carcinoma and gastrointestinal stromal tumors. Low dose treatment of 50 mg may be effective in combination chemotherapy with cytotoxic drugs.

Adverse Effects Target inhibition is achieved at dose levels of 75 mg at the expense of poor tolerability. Adverse effects include hand-foot syndrome, mucositis or stomatitis, and skin discoloration. Systemic adverse effects comprise fatigue, diarrhea, nausea, anorexia, and hypertension.

Indolocarbazoles Midostaurine (PKC412) is a semi-synthetic derivative of the alkaloid staurosporine, which is produced by Streptomyces staurosporeus. It is a multi-target protein kinase inhibitor that also inhibits FLT3. The drug is used for the treatment of acute myeloid leukemia. In 2004, orphan designation was granted by the European Commission to midostaurin for the treatment of AML.

Lestaurtinib (CEP-701, KT-5555, SPM-924) is an orally bioavailable indolocarbazole derivative. It inhibits the autophosphorylation of FLT3, resulting in a suppression of FLT3 activity and induction of apoptosis in tumor cells that over-express this kinase. The drug also acts as an inhibitor of RET, JAK2, TRK-A, TRK-B and TRK-C. Lestaurtinib is used to treat myeloproliferative disorders and AML in sequence with induction chemotherapy. The drug is administered in an oral dose of 80 mg twice daily for 16 weeks, beginning 2 days after the end of chemotherapy.

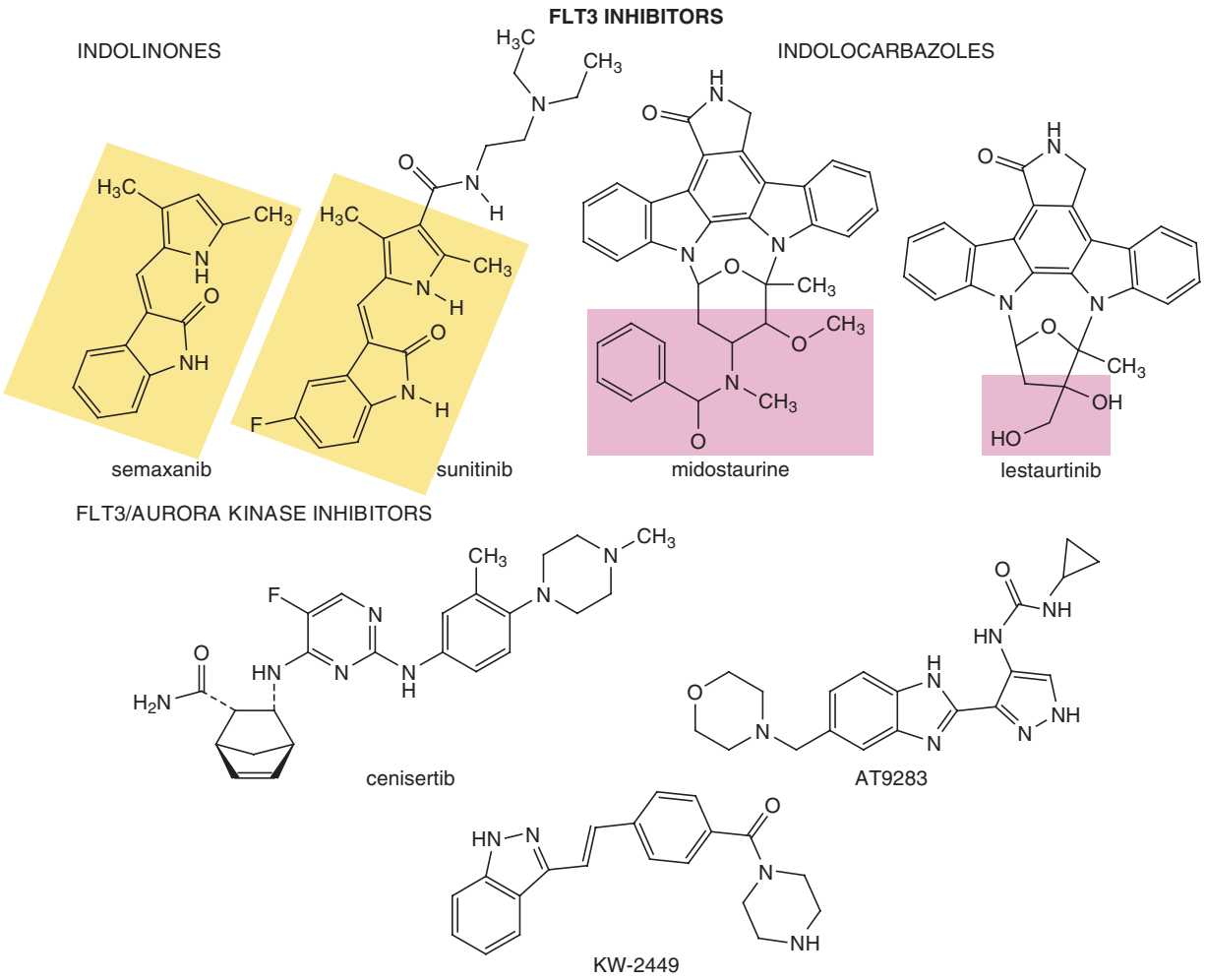

Fig. 4.13 Structures of FLT3 inhibitors. Sub-classes include indolinones (the indolinone-containing core moiety is highlighted in *yellow*), indolocarbazoles (the structural differences are shown on *pink* background), FLT3/Aurora Kinase inhibitors, and other inhibitors

Adverse Effects Most toxicities affect the gastrointestinal tract.

FLT3/Aurora Kinase inhibitors Some FLT3 inhibitors also inhibit Aurora Kinase. Cenisertib (AS703569, R-763) is an oral multi-kinase inhibitor that suppresses FLT3, Aurora A, Aurora B, and ABL kinases. The compound is in clinical trials for primary acute myelogenous leukemia (AML), secondary AML, chronic myelogenous leukemia (CML), myelodysplastic syndrome, and myeloproliferative disease.

Adverse Effects Common toxicities include neutropenia, thrombocytopenia, infection, mucositis and diarrhea.

The FLT3, pan-Aurora, ABL, and JAK2 kinase inhibitor AT9283 induces a reduction in the phosphorylation of Histone H3, CRKL, and STAT5 (a downstream target of ABL, FLT3 and JAK2 signaling). AT9283 is in clinical trials and has activity in patients with refractory acute and chronic myelogenous leukemias.

Adverse Effects The maximum tolerated dose of AT9283 as a 72-h intravenous infusion is 100 mg/m^2/day. Dose limiting toxicities include myelosuppression and alopecia, as well as tumor lysis syndrome. Elevated Transaminases, non-cardiac Creatine Kinase, and Lactate Dehydrogenase may occur.

KW-2449 is an orally available multi-kinase inhibitor of Aurora Kinases A and B, FLT3, ABL, and BCR-ABL^{T315I}. KW-2449 is growth inhibitory for leukemia cells with FLT3 gain-of-function mutations, resulting in G_1 arrest, and apoptosis. In FLT3 wild-type leukemia cells, it induces G_2/M arrest and apoptosis. KW-2449 is under investigation to treat leukemia patients.

Others Cabozantinib (XL184) <Cometriq> is an orally bioavailable, small molecule kinase inhibitor that strongly binds to and inhibits several receptor tyrosine kinases. This agent inhibits FLT3, KIT, TIE2 (TEK), MET (HGF Receptor), and VEGFR2. Cabozantinib was approved by the U.S. FDA in

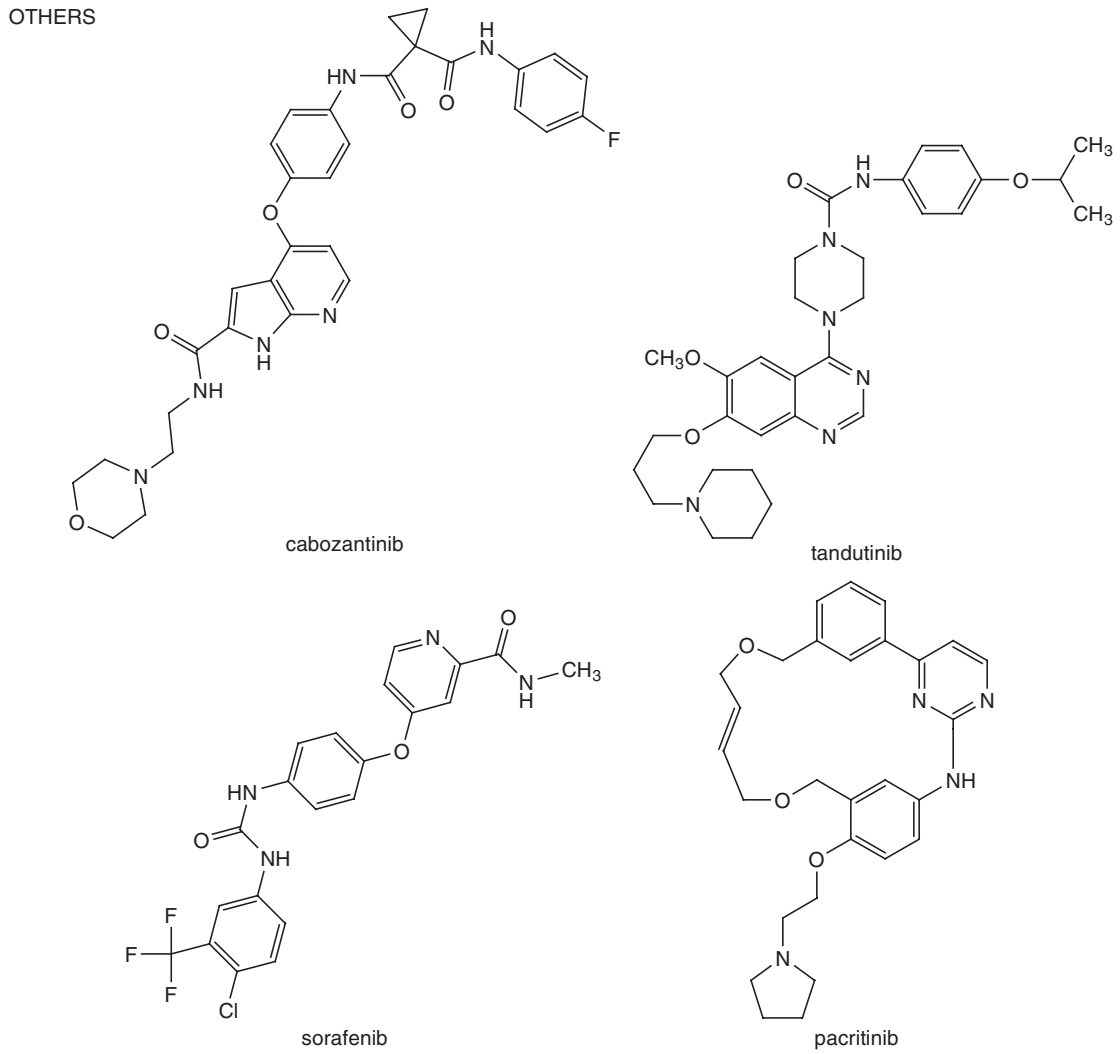

Fig. 4.13 (continued)

2012 for the treatment of medullary thyroid cancer. There is single agent activity in castration resistant prostate cancer, lung cancer, and ovarian cancer. The drug is in clinical trials for melanoma, brain, breast, non-small cell lung, hepatocellular, and kidney cancers.

Pharmacokinetics Cabozantinib has a long half-life of 60–120 h.

Adverse Effects Grade 3 dose limiting toxicities are palmar-plantar erythema, transaminitis, and mucositis. Permanent discontinuation of cabozantinib is required in cases of development of visceral perforation or fistula formation, severe hemorrhage, serious arterial thromboembolic event (such as myocardial or cerebral infarction), nephrotic syndrome, malignant hypertension or hypertensive crisis, persistent uncontrolled hypertension despite optimized medical management, osteonecrosis of the jaw, or reversible posterior leukoencephalopathy syndrome.

Tandutinib (*N*-(4-isopropoxyphenyl)-4-(6-methoxy-7-(3-(piperidin-1-yl) propoxy)quinazolin-4-yl)piperazine-1-carboxamide) (MLN518, CT53518) is a piperazinyl quinazoline that inhibits the auto-phosphorylation of receptor tyrosine kinases, thereby suppressing cellular proliferation and inducing apoptosis. Tandutinib acts as an ATP-competitive and reversible inhibitor of type III receptor tyrosine kinases, including FLT3 (and its cancer causing mutant W51 that results in a duplication of the amino acids 596–602, REYEYDL), PDGFRβ, and c-KIT, which are equally inhibited. The agent is much less active against CSF-1R. Tandutinib has no appreciable activity against EGFR, FGFR, or KDR. Non-receptor tyrosine kinases, serine-threonine kinases and MAP Kinases are not inhibited. The agent is under investigation in the treatment of glioblastoma and advanced androgen independent prostate cancer. Because of the correlation between FLT3 internal tandem duplication mutations and

Table 4.7 FLT3 inhibitors

	FLT3	FLT3(mut.)	KIT	KIT^{D816V}	PDGFR	VEGFR2	TIE2	RAF	RET	MET	PKC	Topoiso-merase 1	ABL	BCR-ABL^{T315I}	JAK2	TRK	Aurora Kinase A	Aurora Kinase B
Indolinones																		
Semaxanib	S		S		S	S												
Sunitinib	(S)	S	S	S		S												
Indolocarbazoles																		
Midostaurine	S		S								S	S						
Lestaurtinib	S								S		S	S			S	S		
FLT3/Aurora Kinase inhibitors																		
Cenisertib	S												S				S	S
AT9283	S												S		S		S	S
KW-2449	S												S	S			S	S
Others																		
Cabozantinib	S		S			S	S			S								
Tandutinib	S		S		S													
Sorafenib	S		S					S	S									
Pacritinib	S														S			

S sensitive, *R* resistant, (*mut.*) mutated

poor prognosis in acute myelogenous leukemia (AML), tandutinib may be beneficial against this form of leukemia.

Pharmacokinetics The major driver of tandutinib disposition are transporters, mainly ABCB1 (P-Glycoprotein) and ABCG2 (BCRP), with metabolism playing an insignificant role in its clearance.

Adverse Effects The principal dose limiting toxicity of tandutinib is reversible generalized muscular weakness or fatigue, occurring at doses of 525–700 mg (DeAngelo 2006).

Sorafenib tosylate (4-[4-[[4-chloro-3-(trifluoromethyl) phenyl]carbamoylamino] phenoxy]-*N*-methyl-pyridine-2-carboxamide) <Nexavar>is a synthetic, orally active compound that inhibits wild-type and mutant forms of the kinases FLT3, KIT, RAF, and RET (see Sect. 4.1.3). The drug also inhibits the VEGFR2/PDGFRβ signaling cascade, thereby blocking tumor angiogenesis.

Pacritinib (SB-1518) is a pyrimidine-based, ATP-competitive macrocyclic compound that inhibits FLT3 and JAK2. It also inhibits the JAK2 mutant V617F. As a consequence, the drug has anti-proliferative effects on myeloid and lymphoid cells driven by FLT3 or mutant or wild-type JAK2. Moreover, pacritinib is active against primary erythroid progenitor cells in myeloproliferative disease. The agent is in clinical studies in patients with myelofibrosis or lymphoma. Pacritinib has favorable pharmacokinetic properties after oral dosing and is well tolerated.

Acute myelogenous leukemia patients with gain-of-function mutations in flt3 have increased risk for relapse and reduced prospects for survival.
FLT3 inhibitors can treat these forms of AML.
FLT3 inhibitors have limited specificity. The indolinones also block VEGFR2. Indolocarbazoles also inhibit Topoisomerase 1 and PKC. Others also inhibit KIT.

4.1.6 Cell Cycle Kinase Inhibitors

The cell cycle describes the time period between two consecutive cell divisions and consists of four strictly regulated phases, referred to as gap 1 (G_1) for cell growth, DNA synthesis (S) for duplication of the genetic material, gap 2 (G_2) for preparation for cell division (G_1, S, and G_2 are collectively named interphase), and mitosis (M) for the execution of cell division. The cell cycle is driven by Cyclins and Cyclin-Dependent Kinases.

- The levels of Cyclins fluctuate with the phases of the cell cycle, due to changes in synthesis and degradation. Furthermore, their sub-cellular localization into the nucleus or the cytoplasm is important in cell cycle control. Cyclins serve as the regulatory subunits of cell cycle kinases. A Cyclin can assemble with an appropriate catalytic CDK subunit.
- Cyclin-Dependent Kinases (CDKs) are serine/threonine kinases. The regulation of their activities constitutes a rate limiting step in cell cycle progression, so that activation and inhibition of these kinases pace the progression through the cell cycle. While the expression levels of Cyclin-Dependent Kinases remain fairly constant, their activities are highly regulated.

DNA synthesis phase (S phase) occurs when the protein RB is phosphorylated by active CDK4 or CDK6, which releases the transcription factor E2F1 from RB and allows the free E2F1 to bind and activate E2F responsive genes necessary for progression to S phase. A majority of neoplasias have abnormalities in some component of the RB pathway.

During mitosis, Cyclin A is degraded. Cyclin B1, which is in the cytoplasm during S and G_2, translocates to the nucleus. The maturation promoting factor (MPF), consisting of Cyclin B and CDK1, initiates mitosis through the breakdown of the nuclear envelope, chromatin condensation, spindle formation, and fragmentation of the Golgi complex and endoplasmatic reticulum. The activation of M phase Cyclin-Dependent Kinases promotes the formation of the Ubiquitin Ligase complex called anaphase promoting complex (cyclosome, APC/C), which induces the loss of sister chromatid cohesion. The anaphase promoting complex is inactivated by the accumulation of G_1 Cyclin-Dependent Kinases at the end of M phase.

In prophase, the first mitotic stage, the Aurora Kinases phosphorylate Histone proteins (thereby promoting chromosome condensation) and interact with a number of chromosomal passenger proteins, including Inner Centrosome Proteins (INCEPs) and Survivin. Microtubules of the cytoskeleton, responsible for cell shape, motility, and attachment to other cells during interphase, dis-assemble. The building blocks of these microtubules are used to grow the mitotic spindle from the region of the centrioles.

Polo-Like Kinases, comprising PLK1, PLK2 (SNK), and PLK3 (FNK, PRK), are serine/threonine kinases that are required at several points during mitosis, including for the activation of the anaphase promoting complex, centrosome maturation, and mitotic exit. Polo-Like Kinases are localized to the kinetochore of chromosomes. The multi-protein complex Cohesin (SMC1, SMC3, SCC1/MCD1) creates physical links between sister chromatids. The phosphorylation of Cohesin by Polo-Like Kinases enhances its propensity to be cleaved by Separin (Separase), which then leads to chromatid separation (Fig. 4.14). The inhibition of Separin is released by the degradation of Securin (PDS1). APC/C acts as a Ubiquitin Protein Ligase, triggering the degradation of the anaphase inhibiting protein Securin.

Cell cycle kinase inhibition targets an essential step in tumor cell proliferation. However, the drug targets in this case are so far downstream in signal transduction that transformed and untransformed cells are affected alike. There-

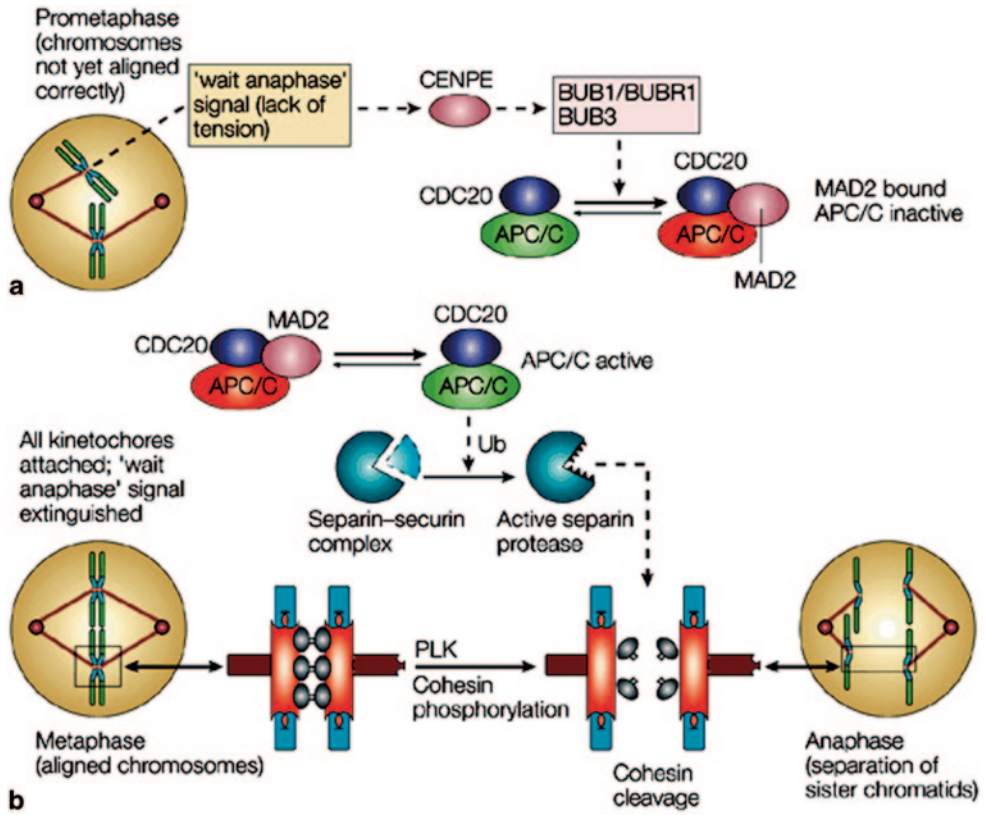

Fig. 4.14 Sister chromatid separation. **a** In prometaphase, cells contain condensed chromosomes that actively establish bipolar attachments to the mitotic spindle. Unattached chromosomes generate a signal that delays progress to anaphase until all sister chromatids are attached to the spindle apparatus. This signal is transduced by a relay of spindle checkpoint proteins that include CENPE and the MAD/BUB proteins. This ultimately results in inhibition of the anaphase promoting complex/cyclosome (APC/C), which is associated with the mitotic co-factor CDC20. **b** Following attachment of the last kinetochore to the mitotic spindle, the "wait anaphase" signal is extinguished. This allows APC/C and CDC20 to become active, resulting in the Ubiquitin dependent degradation of Securin and liberation of active Separin. This protease catalyses the cleavage of Cohesin complexes that bridge the aligned sister chromatids. The newly separated sister chromatids can then migrate to the poles along the spindle axis during anaphase. *PLK* Polo Like Kinases, *Ub* Ubiquitin. (Jallepalli 2001 with permission)

fore, adverse effects on rapidly proliferating, healthy tissues (hematopoietic system, gastrointestinal lining, hair roots) are likely. Further, while inhibition of an oncogenic pathway immediately downstream of a gain-of-function mutation is efficacious, the inhibition far downstream will capture only a fraction of the transforming activity as signaling pathways are branched, not linear.

A potential benefit of treatment with cell cycle kinase inhibitors may be the enhancement of concomitant cytotoxic therapy. The efficacy of cytotoxic agents is limited when cell cycle arrest and DNA repair reverse the drug induced damage. In the absence of cell cycle arrest, DNA repair cannot take place. Hence, the likelihood of cancer cell death is increased when cell cycle kinase inhibitors are used in conjunction with cytotoxic agents.

CDK inhibitors First generation ATP-site small molecule inhibitors (flavopiridol, bryostatin, seliciclib, 7-hydroxystaurosporin, BMS387032, E7070) and the second generation ATP-site small molecule inhibitors (PD-0332991, AT7519, SNS-032, P276-00, ZK304709, R-547, AG-24322, JNJ-7706621, GPC-286199, Bay80-3000) are in clinical trials. In general, second generation CDK inhibitors are more specific to individual Cyclin-Dependent Kinases (CDKs) (Table 4.8) and have improved pharmacokinetic and pharmacodynamic profiles compared to first generation inhibitors (Fig. 4.15).

Flavopiridol (HMR-1275, L86-8275) <Alvocidib> is derived from rohitukine, an alkaloid produced by an Indian medicinal plant that has been used in indigenous medicines. The drug acts as a pan-CDK inhibitor. It suppresses the activities of CKDs -1, -2, and -4 through competition with ATP. Flavopiridol blocks cell cycle progression at the G_1/S and G_2/M boundaries. This leads to apoptosis, which is correlated with the down-modulation of Cyclin D_1, up-regulation of P21, and induction of Caspase 3/7 activities. Flavopiridol has efficacy in hematologic malignancies, renal, colorectal, prostate, and gastric cancers.

Pharmacokinetics Because of the pharmacokinetic properties of flavopiridol, administration starts with a loading dose followed by continuous intravenous infusion.

Adverse Effects Main adverse effects are secretory diarrhea and a pro-inflammatory syndrome associated with hypotension.

In 1973, Lionel Rebhun observed that the puromycin analog 6-dimethylaminopurine prevents cell division without blocking protein synthesis. The compound was later found to inhibit the CDK1/Cyclin B complex. Consecutive screens identified 2,6,9-trisubstituted purines to represent a class of CDK inhibitors.

Seliciclib (R-roscovitine) (CYC202) is a 2,6,9-substituted purine analog that acts as a CDK inhibitor by competing with ATP. It has activity against CDK2/Cyclin E, CDK7/Cyclin H, and CDK9/Cyclin T, and also inhibits CDK1/Cyclin B and CDK5. The drug induces programmed cell death in various cell types independently of functional P53. Cell death is a consequence of the inhibition of CDK7/CDK9 dependent transcription (Fig. 4.16). Seliciclib reduces the level of the anti-apoptotic protein XIAP by down-regulating *xiap* mRNA expression. It also decreases the tyrosine phosphorylation and consequent activation of STAT5a, an upstream regulator of XIAP. Further, seliciclib down-regulates Survivin, which contributes to the activation of Caspase cascades (Kim 2004). Seliciclib is in clinical trials for B-cell malignancies, lung cancer, and breast cancer. Exposure to the drug overcomes glioma resistance to TRAIL mediated apoptosis. It can be given orally or by injection.

Pharmacokinetics After injection, seliciclib undergoes rapid passage into the blood, distribution into tissues, and metabolism. In accordance with a 2-compartment open model, the drug is subject to biphasic elimination with a 5-min and a 30-min half-life. It undergoes a rapid loss of the isopropyl group, several oxidations, and conjugation of a glucose residue. The carboxylated derivative is the main metabolite formed.

Palbociclib (PD-0332991) is an orally available, pyridopyrimidine derived CDK inhibitor that is selective for CDK4/Cyclin D_1 and CDK6. It may thus block RB protein phosphorylation and cause a down-regulation of genes under the transcriptional control of E2F. This prevents RB expressing tumor cells from entering the S phase of the cell cycle, leading to arrest in G_1. The drug results in a suppression of DNA reduplication, and consecutively in decreased tumor cell proliferation. The presence of a functional RB protein is a prerequisite for palbociclib efficacy.

In healthy cells, CDK4 and CDK6 are under the negative control of P16. However, in glioblastoma, P16 is often deleted. Thus, CDK4 and CDK6 are promising drug targets. Palbociclib can pass through the blood-brain barrier and is under study for the treatment of glioblastoma.

AT7519 is an orally available, multi-targeted inhibitor of CDKs -1, -2, -4, -5, and -9. In addition to its direct effects on the cell cycle (via CDKs 1–5), AT7519 also suppresses RNA Polymerase II dependent transcription (via inhibition of CDK9). The agent is under study for non-small cell lung cancer and pancreatic cancer. It is also under development for relapsed or refractory chronic lymphocytic leukemia (CLL), where the target is CDK9. As the survival of these transformed cells is very dependent on the cellular levels of certain anti-apoptotic proteins, which require RNA Polymerase II activity for their generation, AT7519 induces rapid cell death in leukemia cells.

Pharmacokinetics Over a wide dose range, AT7519 encounters multi-phasic elimination with a long terminal half-life of 8–12 h. There is only modest interpatient variation.

Adverse Effects The maximum tolerated dose is 30 mg/m^2 with the dose limiting toxicity being QTc prolongation. Adverse events are cyclical neutropenia[7], mucositis, and fatigue.

PNU-151807 (see Sect. 2.2.2.) is a synthetic minor groove binding antibiotic. Unlike other drugs in its class, PNU-151807 also abolishes the kinase activities of the CDK2/Cyclin A, CDK2/Cyclin E, and CDC2/Cyclin B complexes. PNU-151807 induces apoptosis via the activation of Caspases. While it may further induce the activation of P53, a disruption of P53 function does not substantially affect the cytotoxic activity of the drug. This property may make it useful for treating tumors with inactivating *p53* mutations.

Drug Resistance Mismatch DNA repair deficiency is associated with resistance to certain anti-cancer drugs. It may occur as a consequence of loss of MLH1 expression. By contrast, loss of mismatch repair does not mediate resistance to PNU-151807.

7-hydroxystaurosporine (UCN-01) is a synthetic derivative of staurosporine that arrests tumor cells in the G_1/S phase of the cell cycle by inhibiting Cyclin-Dependent Kinases (CDKs). This arrest leads to apoptosis. It promotes the accumulation of the cell cycle inhibiting proteins P21 and P27. The agent inhibits many kinases, including the G_2 checkpoint kinase CHK1, calcium dependent Protein Kinase C, and the serine/threonine kinase PKB (AKT). 7-hydroxystaurosporine is under study for treating melanoma and lymphoma.

CHK inhibitors In the presence of DNA damage or incomplete DNA reduplication, cells activate cell cycle checkpoints that temporarily halt the cell division cycle to permit DNA repair or the completion of DNA reduplication to take place. In the presence of extensive damage or absence of a timely repair, these checkpoint signaling pathways can also

[7] Cyclical neutropenia (cyclic neutropenia) is a form of the condition that tends to occur every 3 weeks and lasts 3–6 days at a time due to changing rates of cell production by the bone marrow.

trigger apoptosis. The physiologic functions of Checkpoint Kinases (CHKs) involve the initiation of cell cycle arrest and the up-regulation of gene transcription involved with DNA excision repair and deoxy-nucleotide synthesis.

Healthy tissues have a functioning G_1 checkpoint signaling pathway, allowing for DNA repair and cell survival. A large fraction of tumors are deficient in the G_1 DNA damage checkpoint, resulting in a dependence on the G_2 checkpoint for survival during cell reduplication. Specifically, high rates of *p53* loss-of-function mutations in cancer cells result in reliance on the S and G_2 checkpoints to repair DNA damage and promote cell survival. The S and G_2 checkpoints are regulated by the serine/threonine kinase CHK1, which is activated in response to DNA damage. Thus, inhibition of CHK1 signaling impairs DNA repair and increases tumor cell death (Fig. 4.17). G_2 checkpoint abrogation may be a relatively cancer cell specific therapy.

Checkpoint kinase inhibitors are in clinical development as combination therapies with DNA damaging cytotoxic agents. By preventing checkpoint activation, these drugs enhance the cell killing effects of the cytotoxic compounds. Potentiation of the effects of DNA damage is driven primarily through CHK1 inhibition rather than CHK2 inhibition. CHK2 inhibition may lead to the enhanced effect of sensitizing cancer cells that lack P53 while protecting normal cells. CHK inhibitors cause an increase in the levels of phospho-Histone 2A.X (p-H2A.X), which reflects double-stranded DNA damage. Tumor types that are particularly sensitive to CHK1 inhibition include triple negative breast cancer, where CHK1 is up-regulated, and colorectal cancer, where CHK1 levels are elevated in the tumor mucosa over healthy mucosa.

The oncogenic protein MYC induces DNA damage and results in genetic instability. This defect alone would lead to cell cycle arrest or apoptosis in early tumorigenesis, never allowing a clinically detectable cancer to form. For full transformation, loss of P53 or of DNA repair molecules is required. Further, ATR and CHK1 suppress a Caspase dependent apoptotic response following DNA reduplication stress induced by MYC. CHK1 inhibitors can effectively treat those cells in single agent regimens.

XL844 (EXEL-9844) is an orally available synthetic small molecule inhibitor of the checkpoint kinases CHK1

Table 4.8 Specificities of cell cyle kinase inhibitors

	CDK1	CDK2	CDK3	CDK4	CDK5	CDK6	CDK7	CDK9	CHK1	CHK2	Aurora Kinase A	Aurora Kinase B	PLK	ABL
CDK inhibitors														
Flavopiridol	S	S		S										
Seliciclib	S	S			S		S	S						
Palbociclib				S		S								
AT7519	S	S		S	S			S						
7-hydroxystaurosporine	S	S	S	S										
CHK inhibitors														
XL844									S	S				
AZD7762									S	S				
PF-00477736									S					
VRX0466617										S				
4,4′-diacetyl-diphenylurea-bis(guanylhydrazone)										S				
Aurora Kinase inhibitors														
Cenisertib											S	S		S
Tozasertib											S	S		S
Danusertib											S	S		
KW-2449											S	S		S
AT9283											S	S		S
MLN 8237											S			
XL228											S			S
Hesperadin												S		
AZD1152												S		
ZM447439												S		
PLK inhibitors														
BI2536													S	

S sensitive, *R* resistant, (*mut.*) mutated

Table 4.8 (continued)

	BCR-ABL^{Y253F} BCR-ABLY253F	BCR-ABL^{V-299L} BCR-ABLV299L	BCR-ABL^{T315I} BCR-ABLT315I	FLT3	FLT3 (mut.)	FGFR	IGF-1R	PDGFR	RET	TRK-A	LYN	SRC	JAK2
CDK inhibitors													
Flavopiridol													
Seliciclib													
Palbociclib													
AT7519													
7-hydroxystaurosporine													
CHK inhibitors													
XL844				S				S					
AZD7762													
PF-00477736													
VRX0466617													
4,4′-diacetyldiphenylurea-bis(guanylhydrazone)													
Aurora Kinase inhibitors													
Cenisertib				S									
Tozasertib	S	S	S										
Danusertib			S						S	S			
KW-2449			S	S	S								
AT9283				S									S
MLN 8237													
XL228			S			S	S				S	S	
Hesperadin													
AZD1152													
ZM447439													
PLK inhibitors													
BI2536													

S sensitive, *R* resistant, (*mut.*) mutated

and CHK2. XL844 also inhibits FLT-3, PDGFR, VEGFR2 and VEGFR3. The agent inhibits cell cycle arrest and DNA repair, which ultimately leads to tumor cell apoptosis. XL844 increases cancer cell radio-sensitivity through the promotion of mitotic catastrophe.

Checkpoint Kinase inhibitors have the potential to enhance the efficacy of both conventional chemotherapy and radio-therapy, and increase patient response rates. AZD7762 inhibits CHK1 and CHK2 via competition with ATP, and abrogates the DNA damage induced S and G_2 checkpoints. In combination with DNA damaging agents, the agent dose dependently potentiates the remission of cancers, in which the inhibition of Checkpoint Kinases results in an abrogation of DNA damage induced cell cycle arrest. AZD7762 enhances the efficacies of gemcitabine and topotecan.

The small molecule, ATP-competitive kinase inhibitor PF-00477736 has high selectivity for CHK1 over CHK2. PF-00477736 abrogates the S and G_2/M checkpoints and prevents the cell cycle arrest that is required for subsequent repair in DNA damaged tumor cells. The checkpoint abrogation results in sensitization to apoptosis. PF-00477736 displays chemopotentiation when given with DNA damaging agents. It is in clinical trials in combination with gemcitabine.

The selective CHK2 inhibitor VRX0466617 reduces the phosphorylation of the serines 19 and 33–35 on its target protein, but not of threonine 68 which is phosphorylated by ATM. VRX0466617 blocks the activation of CHK2 and downstream degradation of MDM4 in response to ionizing radiation, and may thus act as a radio-sensitizer. The agent is in preclinical development.

The inhibition of CHK2 sensitizes P53 deficient cells and may protect normal tissue after exposure to DNA damaging agents. 4,4′-Diacetyldiphenylurea-bis(guanylhydrazone) (NSC 109555) is a reversible, ATP-competitive CHK2 inhibitor that is under development.

Polo Like Kinase inhibitors PLK1 is a serine/threonine protein kinase that regulates cell division. It is important for the proliferation of tumor cells. Several Polo Like Kinase

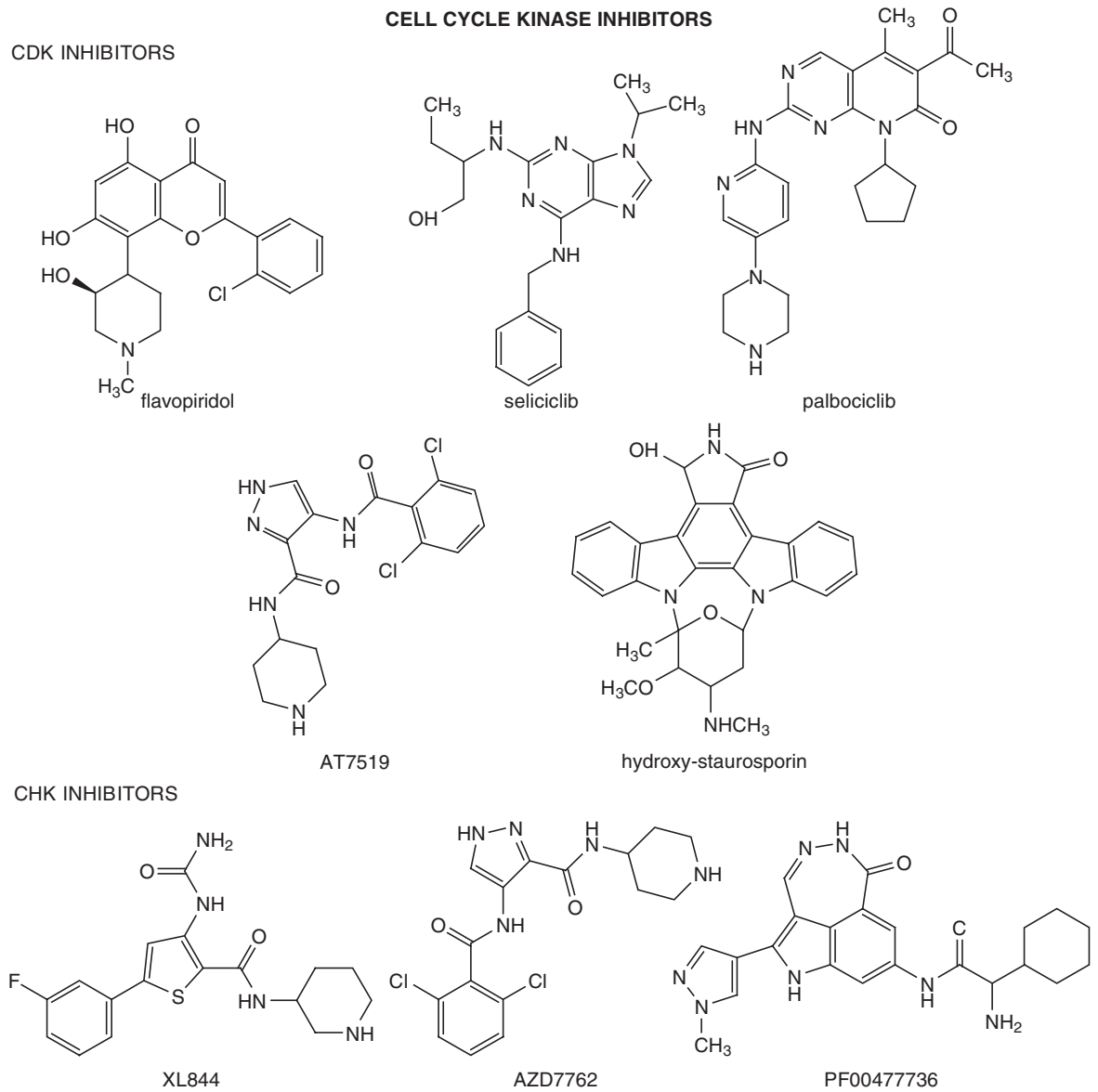

Fig. 4.15 Structures of cell cycle kinase inhibitors. Sub-classes include Cyclin Dependent Kinase (*CDK*) inhibitors, Checkpoint Kinase (*CHK*) inhibitors, and Polo-Like Kinase (*PLK*) inhibitors. The structural similarities among the representatives of a class are limited

small molecule inhibitors are in early clinical trials (BI2536, BI6727, GSK461364, ON 019190.Na, HMN-214) or in preclinical development (ZK-thiazolidinone, NMS-1, CYC-800, DAP-81, LC-445).

BI2536 is an anti-mitotic compound with anti-proliferative effects. BI2536 inhibits cell division by acting as a small molecule inhibitor of Polo Like Kinase 1 (PLK1). The inhibition of PLK1 by the drug results in mitotic arrest, disruption of cytokinesis and apoptosis in tumor cell populations. BI2536 induces the regression of large tumors with well tolerated intravenous dose regimens.

Aurora Kinase inhibitors Aurora kinases regulate cell cycle transit from G_2 through cytokinesis. They belong to a family of serine/threonine kinases that is essential for mitotic progression, spindle formation, centrosome maturation, chromosomal segregation, and cytokinesis. Aurora A is enriched at centrosomes, whereas Aurora B and its binding partners INCENP and Survivin act as chromosomal passenger proteins. Aurora Kinases are over-expressed or amplified in various types of cancer. Such up-regulation may result in drug resistance, as it disrupts the spindle checkpoint that is activated by paclitaxel or nocodazole treatment, thus inducing the cells to become unresoponsive to these drugs.

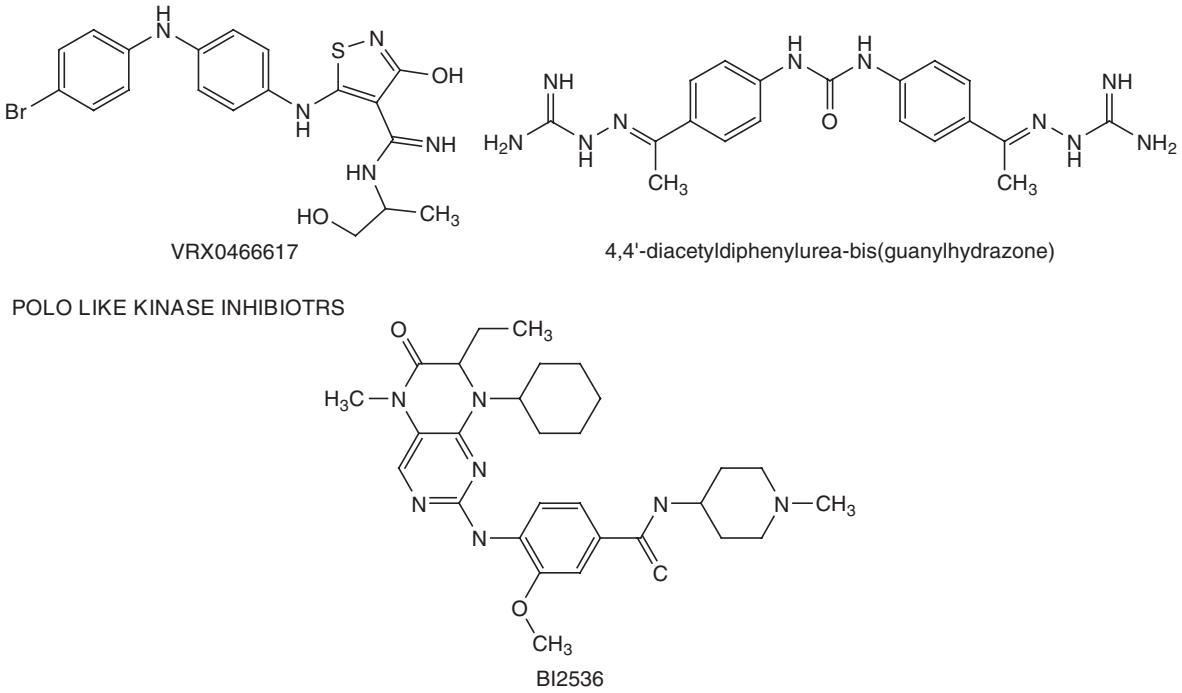

Fig. 4.15 (continued)

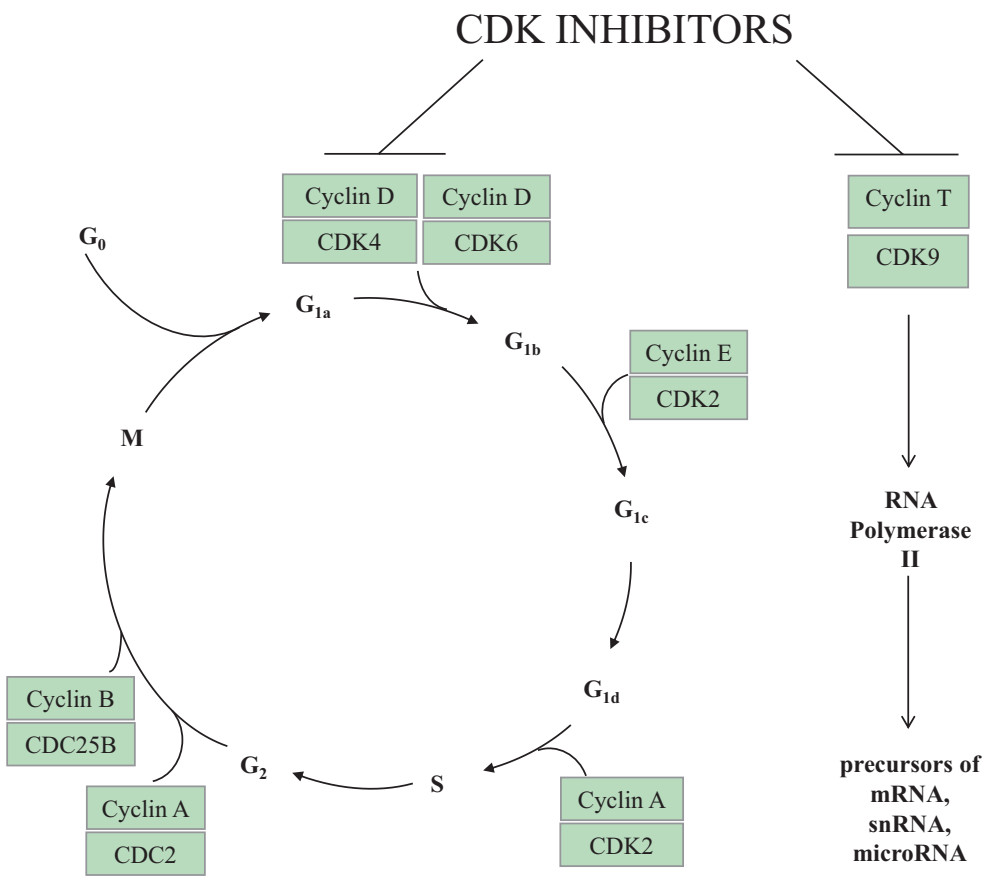

Fig. 4.16 Inhibition of CDK actions. CDK inhibitory drugs suppress the actions of Cyclins and Cyclin Dependent Kinases (*CDKs*), which entail furthering the progression of the cell division cycle (CDKs -2, -4, -6) at all phases and activating RNA Polymerase II (CDK9) to induce mRNA synthesis (and some small nuclear RNA and microRNA synthesis)

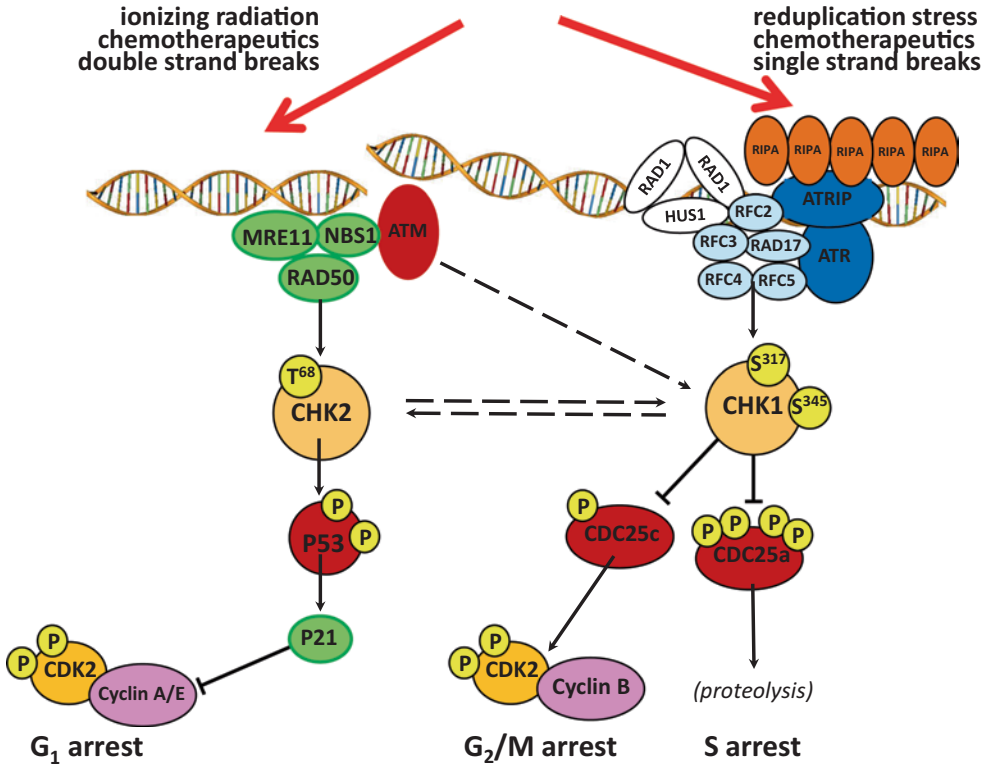

Fig. 4.17 DNA damage checkpoints. *CHK1* and *CHK2* are serine/threonine kinases that are activated in response to DNA damage by the kinases *ATM* and *ATR*. The checkpoint kinases are transducers of the DNA damage signal and both phosphorylate a number of substrates involved in the DNA damage response. *CHK1* and *CHK2* share a number of overlapping substrates, although it is clear that they have distinct roles in directing the response of the cell to DNA damage. Checkpoint kinases are involved not only in cell cycle regulation but also in other aspects of the cellular response to DNA damage. The G_1 checkpoint is modulated primarily by the ATM-CHK2-P53 pathway, as expression of *ATR*, *CHK1*, and *CDC25A* is limited until the cell passes this restriction point. At this point, levels of *ATR*, CHK1, and *CDC25A* all increase. If DNA damage is detected, *CHK1*/*CHK2* are activated, CDC25A is phosphorylated, and thus destabilized, resulting in a P53 independent S arrest. In S phase, the same cascade can result in an intra-S arrest in response to stalled replication forks. The G_2/M checkpoint prevents entry into mitosis with unrepaired DNA lesions. Initiation of this checkpoint is mediated by the ATM/ATR/CHK1/CHK2 cascades as shown, which ultimately suppresses the pro-mitotic activity of Cyclin B/CDC2. Along with their pivotal roles in the modulation of the cell cycle checkpoints, CHK1 and CHK2 are also involved in other aspects of the DNA damage response, including DNA repair, induction of apoptosis, and chromatin remodeling. (Redrawn from (Ashwell 2008))

Inhibition of Aurora Kinases does not arrest the cell cycle but leads to failure of cytokinesis, resulting in polyploidy and ultimately in cell death. Pan-Aurora Kinase inhibitors comprise tozasertib, AT9283, SNS-314, and PHA-739358. Inhibitors selective for Aurora Kinase A are MLN8054 and MLN8237. Aurora Kinase B inhibitors are hesperadin and AZD1152 (Fig. 4.18).

Some Aurora Kinase inhibitors also inhibit ABL, JAK2, or FLT3. Several of these compounds have promising applications in acute myelogenous leukemia with mutated FLT3, and as second line of defense in chronic myelogenous leukemia with imatinib resistant BCR-ABL.

Adverse Effects Among the most frequent adverse events caused by Aurora Kinase inhibitors are myelosuppression, febrile neutropenia, and gastrointestinal effects (nausea, diarrhea, mucositis).

Cenisertib (AS703569, R-763) is an oral multi-kinase inhibitor that suppresses Aurora A, Aurora B, FLT3 and ABL kinases. The compound is in clinical trials for primary or secondary acute myelogenous leukemia, chronic myelogenous leukemia, myelodysplastic syndrome, and myeloproliferative diseases.

Adverse Effects Common toxicities include neutropenia, thrombocytopenia, infection, mucositis, and diarrhea. Gastrointestinal bleeding can arise. Potential late effects from Aurora Kinase C inhibition on spermatogenesis and fertility are yet unknown.

Danusertib (PHA-739358) is a pyrrolo-pyrazole pan-Aurora Kinase (Aurora Kinase A, B, and C) inhibitor. The drug also inhibits BCR-ABL^{T315I}, RET, TRK-A, and (at a 4-fold higher half-maximal inhibitory concentration) FGFR-1. A regimen under study in the clinic is the administration of

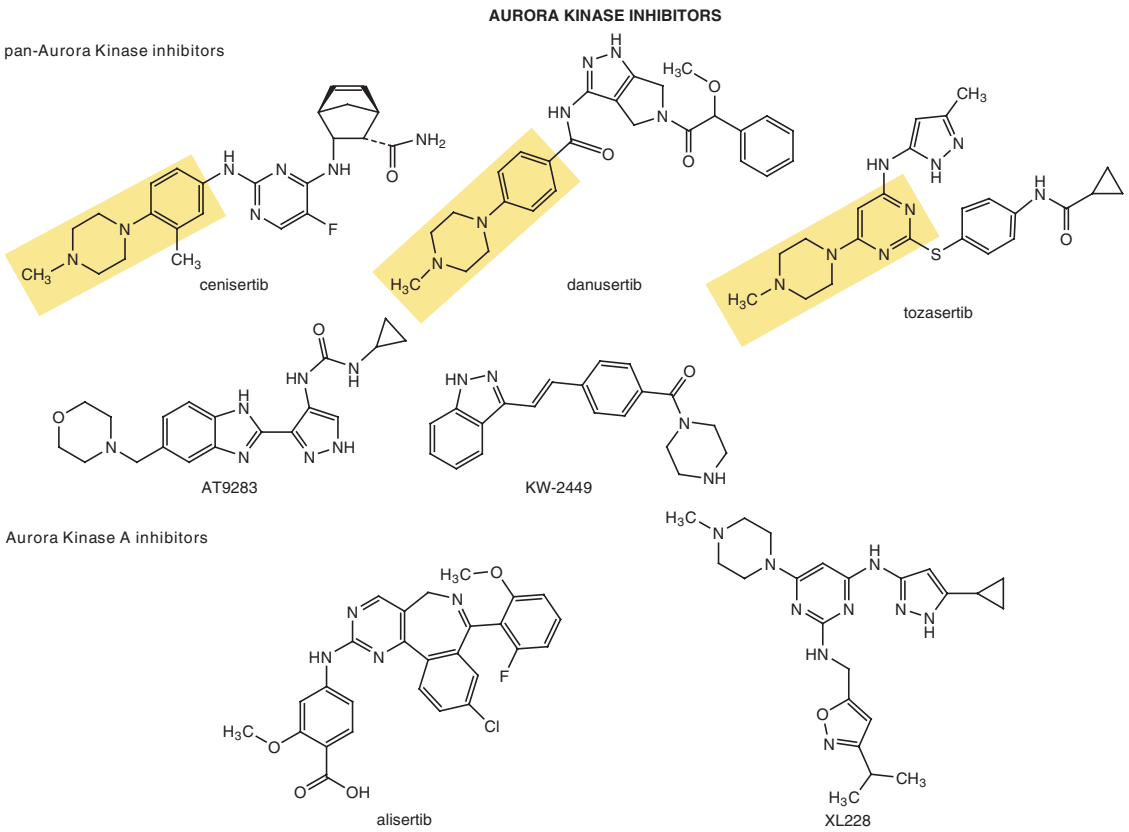

Fig. 4.18 Structures of Aurora Kinase inhibitors. Sub-classes include pan-Aurora Kinase inhibitors, Aurora Kinase A inhibitors, and Aurora Kinase B inhibitors. Common structural characteristics among the pan-Aurora Kinase inhibitors are highlighted in yellow. The Aurora Kinase B inhibitors AZD1152 and ZM447439 are related, and their differences are shown on *pink* background

250–330 mg/m^2/day as a weekly 6-h infusion for 3 consecutive weeks, every 4 weeks.

Adverse Effects Adverse events comprise high grade neutropenia and infusion related reactions.

The pan-Aurora Kinase inhibitor tozasertib (L-001281814, VX-680, MK-0457) is a synthetic small molecule drug that binds to and inhibits Aurora Kinases, thereby inducing apoptosis in tumor cells, in which Aurora Kinases are over-expressed. Tozasertib also inhibits wild-type BCR-ABL, BCR-ABL^{Y253F}, BCR-ABL^{V299L}, and BCR-ABL^{T315I}.

Adverse Effects The main toxicities consist of myelosuppression, alopecia, and mucositis. Long QT syndrome (QTc prolongation)[8] is a possible complication. Therefore, tosazertib (MK-0457) has been replaced in clinical trials by MK-5108 (VX-689).

The pan-Aurora, ABL, FLT3, and JAK2 kinase inhibitor AT9283 induces a reduction in the phosphorylation of Histone H3, CRKL, and STAT5 (a downstream target of ABL, FLT3 and JAK2 signaling). AT9283 is in clinical trials and has activity in patients with refractory acute or chronic myelogenous leukemia.

Adverse Effects The maximum tolerated dose of AT9283 as a 72-h intravenous infusion is 100 mg/m^2/day. Dose limiting toxicities include myelosuppression and alopecia. Tumor lysis syndrome may occur. Transaminases, non-cardiac Creatine Kinase, and Lactate Dehydrogenase may be elevated.

KW-2449 is a multi-kinase inhibitor of Aurora Kinases A and B, FLT3, ABL, and BCR-ABL^{T315I}. It is under investigation to treat leukemia patients. KW-2449 is growth inhibitory for leukemia cells with FLT3 gain-of-function mutations, resulting in G_1 arrest, and apoptosis. In FLT3 wild-type leukemia cells, it induces G_2/M arrest and apoptosis. As an ABL inhibitor, KW-2449 may be of benefit in imatinib resistant leukemia. KW-2449 is orally available.

Aurora Kinase A is required for cells to divide. In epithelial ovarian cancer, its frequent over-expression or up-reg-

[8] The QT interval describes the time between the start of the Q wave and the end of the T wave in the electrical cycle of the heart beat. It represents the electrical depolarization and repolarization of the ventricles. In long QT syndrome, delayed repolarization of the heart following a beat increases the risk of ventricular tachyarrhythmias. Such episodes may lead to fainting and sudden death.

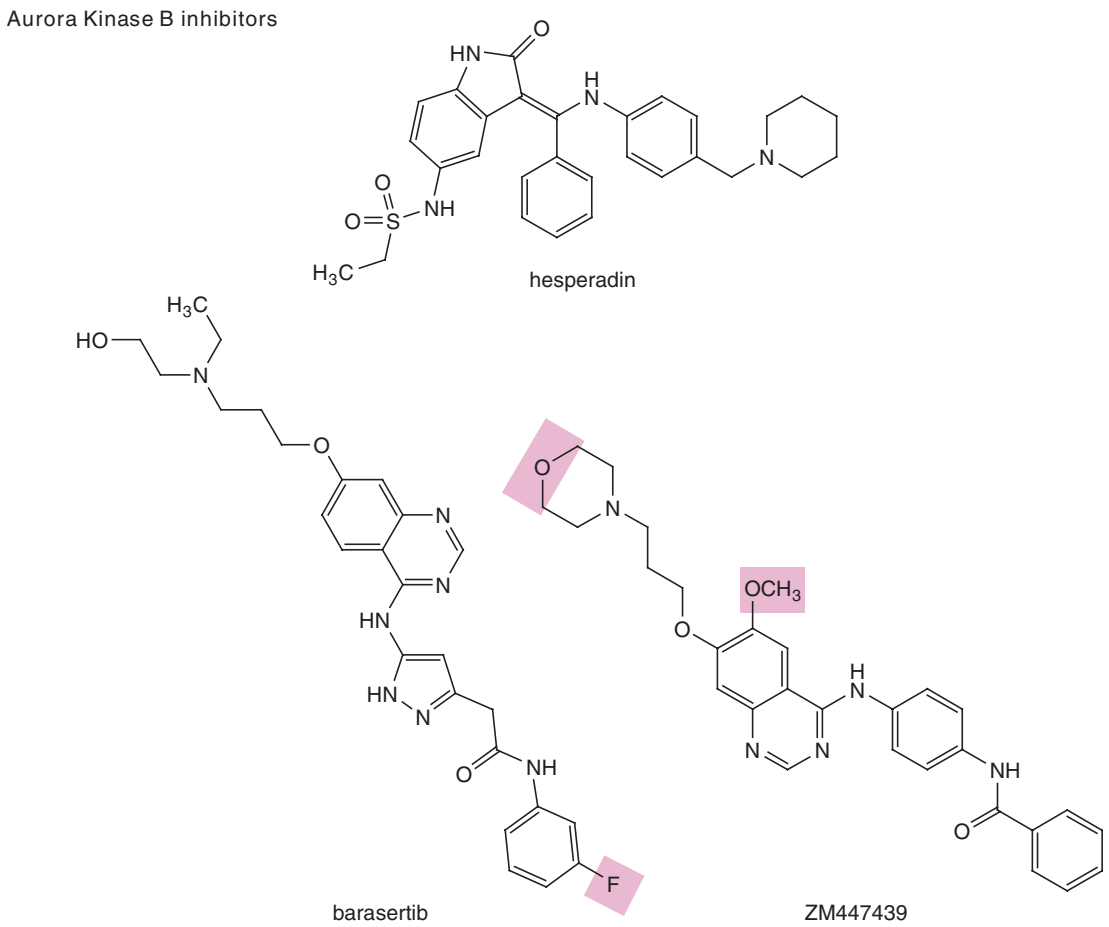

Fig. 4.18 (continued)

ulation is oncogenic. Alisertib (MLN8237) inhibits Aurora Kinase A. It was designed as a second line of defense in platinum resistant or refractory ovarian cancer.

Adverse Effects The predecessor lead compound MLN8042 was discontinued due to the induction of somnolence and lack of objective tumor responses in clinical trials. It has been replaced by the second generation agent alisertib (MLN8237).

XL228 is an Aurora Kinase A inhibitor that also suppresses wild-type ABL and BCR-ABL^{T315I} at low nanomolar concentrations. XL228 also inhibits the kinases FGFR, IGF-1R, LYN and SRC. It is in clinical trials.

Pharmacokinetics XL228 exhibits a long terminal half-life after administration through a 1-h infusion.

Adverse Effects Neutropenia may manifest as a dose limiting toxicity.

The indolinone hesperadin (Hauf 2003) is an inhibitor of Aurora Kinase B. Exposed cells enter anaphase in the presence of numerous mono-oriented chromosomes, many of which may have both sister kinetochores attached to 1 spindle pole. This effect prevents chromosome alignment and segregation, induces polyploidy, and overrides the spindle assembly checkpoint. In cancer cells treated with monastrol or taxol, hesperadin may induce mitotic exit.

High Aurora Kinase B activity may be associated with hepatocellular carcinoma, breast cancer, and some forms of leukemia. The dihydrogen phosphate prodrug barasertib (AZD1152-hydroxyquinazoline pyrazol anilide, AZD1152-HQPA) is under investigation. It is rapidly converted in the blood. The active drug AZD1152 is a selective inhibitor of Aurora Kinase B that suppresses downstream Histone phosphorylation and induces apoptosis in a dose dependent manner. A therapeutic dose is 1200 mg, administered as a continuous 7-day intravenous infusion every 21 days.

Adverse Effects Neutropenia and febrile neutropenia are the most common adverse events.

The quinazoline derivative ZM447439 (*N*-[4-[[6-Methoxy-7-[3-(4-morpholinyl)propoxy]-4-quinazolinyl]amino]phenyl] benzamide) is an ATP competitive Aurora Kinase inhibitor. It has higher affinity for Aurora Kinase B than for the forms A or C. Cells exposed to ZM447439 progress through interphase, enter mitosis and assemble bipolar spindles, but fail to properly align and segregate their chromosomes.

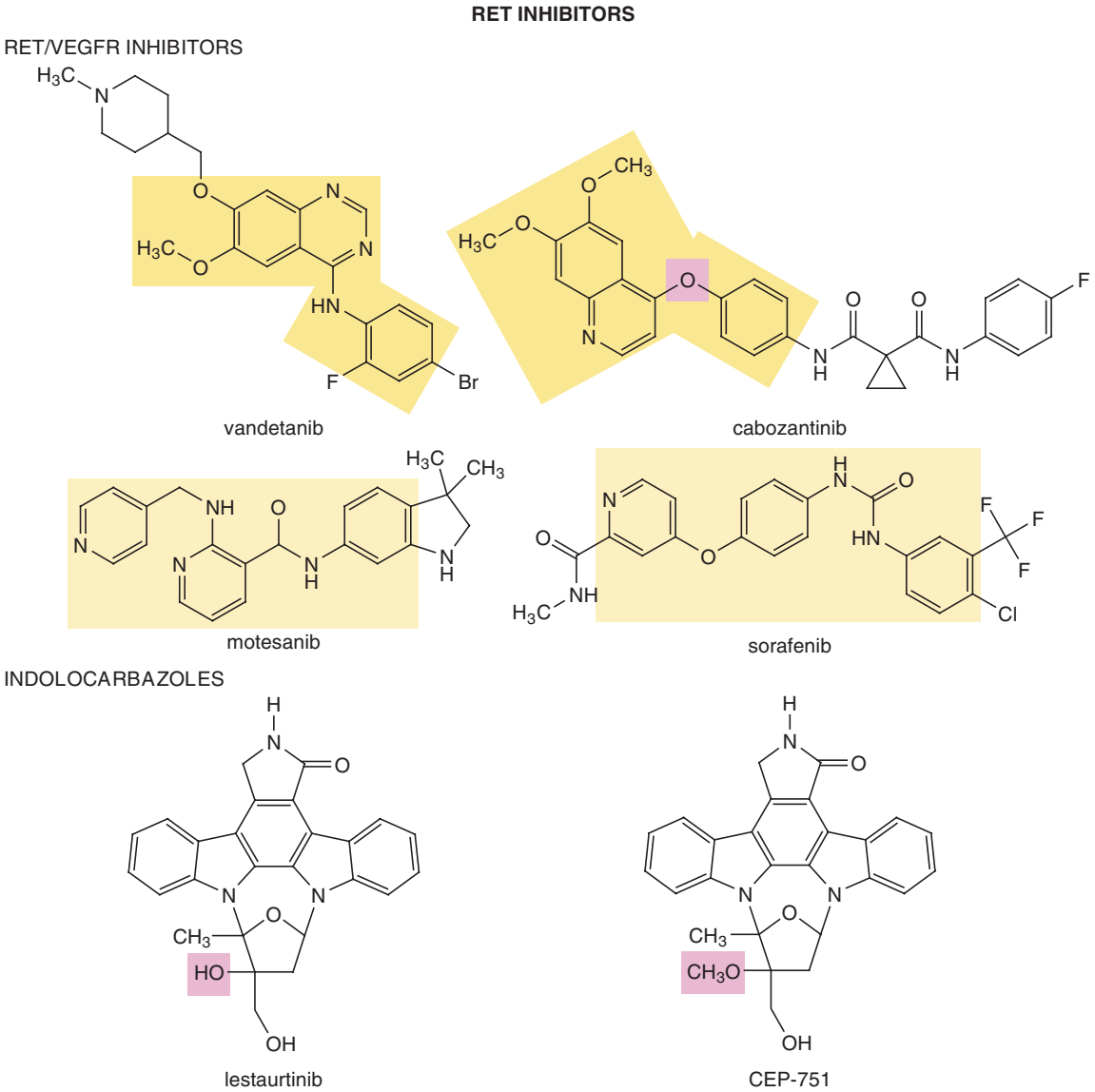

Fig. 4.19 Structures of RET inhibitors. Sub-classes include RET/VEGEF inhibitors and indolocarbazoles. The 4-anilinoquinazoline domain is highlighted in *yellow* in vandetanib and (with the modification of the bridging oxygen in *pink*) in cabozantinib. *Light yellow* shading shows a loosely conserved structure of 3 6-rings connected by bridges between motesanib and sorafenib. The difference in one chemical group between the indolocarbazoles is shown on *pink* background

The drug targets for cell cycle kinase inhibitors are far downstream signaling molecules.
A potential benefit of treatment with cell cycle kinase inhibitors may be the enhancement of concomitant cytotoxic therapy.
Adverse effects on rapidly proliferating normal tissues and incomplete inhibition of oncogenic pathways are likely.

CDK inhibitors are in clinical trials.

Many tumors are deficient in the G_1 DNA damage checkpoint pathway, which results in reliance on the S and G_2 checkpoints. These checkpoints are regulated by the serine/threonine kinase CHK1.
By preventing checkpoint activation, CHK inhibitors enhance the cell killing effects of cytotoxic therapy or radio-therapy.

Adverse events caused by Aurora Kinase inhibitors are myelosuppression, febrile neutropenia, and gastrointestinal effects.

4.1.7 Others

RET The proto-oncogene *ret* encodes a transmembrane receptor of the tyrosine kinase family. Activation of RET prompts its tyrosine phosphorylation. The ensuing signal transduction engages GRB2, SHC, and Phosphatidylinositol 3-Kinase. Oncogenic RET also activates RAS, RAF, MEKK1, and I-κB Kinase β. This leads to the phosphorylation and degradation of I-κB and results in the activation of NF-κB, which may promote cell expansion through its anti-apoptotic effects.

Gain-of-function mutations or translocations in the RET proto-oncogene are causative for medullary thyroid carci-

Table 4.9 Inhibitors of RET

	RET	VEGFR2	PDGFR	c-KIT	c-MET	EGFR	FLT3	RAF	RAF (mut.)	JAK	TRK
RET inhibitors											
Vandetanib	S	S				S					
Cabozantanib	S	S			S						
Motesanib	S	S	S	S							
Sorafenib	S	S	S	S			S	S	S		
Lestaurtinib	S						S			S	S
CEP-751	S										S

S sensitive, R resistant, *Raf (mut.)* mutant RAF

noma. Therefore, several therapies directed at this tyrosine kinase are in early phase clinical trials for thyroid cancer (Fig. 4.19; Table 4.9).

Vandetanib (*N*-(4-bromo-2-fluorophenyl)-6-methoxy-7-[(1-methylpiperidin-4-yl)methoxy]quinazolin-4-amine) (AZD6474, ZD6474) <Caprelsa, Zactima> targets RET, VEGFR2, and EGFR. It is an orally bioavailable 4-anilinoquinazoline[9] that is taken once per day. The drug has a half-life of more than 120 h.

Adverse Effects Vandetanib is well tolerated at doses of up to 300 mg/d. The main adverse effects are nausea, diarrhea, rash, and hypertension. Asymptomatic QTc prolongation of the heart beat may arise.

Cabozantinib (XL184) <Cometriq> is an oral multi-targeted inhibitor of RET, VEGFR2, and c-MET. It also binds to and inhibits FLT3, KIT, and TIE2 (TEK). Cabozantinib was approved by the U.S. FDA in 2012 for the treatment of medullary thyroid cancer. There is single agent activity in castration resistant prostate cancer, lung cancer, and ovarian cancer.

Motesanib (AMG706) is a multi-targeted tyrosine kinase inhibitor that suppresses RET, VEGFR, c-KIT, and PDGFR activity. It is in clinical trials for locally advanced and metastatic medullary thyroid cancer, and has resulted in partial responses.

Sorafenib tosylate <Nexavar> is a synthetic compound that inhibits RET. The kinases KIT, FLT3, and wild-type as well as mutant forms of the kinase RAF are also sorafenib targets. The drug inhibits the VEGFR2/PDGFRβ signaling cascade, thereby suppressing tumor angiogenesis.

Sorafenib is active orally and it is available as tablets. The drug is taken without food, 1 h before or 2 h after a meal, typically twice daily.

Indolocarbazoles with two bonds between the glycoside and the indolocarbazole heterocycle may act as inhibitors of Protein Kinases. Lestaurtinib (CEP-701) is an orally bioavailable indolocarbazole derivative that inhibits RET at nanomolar concentrations. The drug also acts as an inhibitor of FLT3, JAK2, TRK-A, TRK-B, and TRK-C.

Adverse Effects Most toxicities affect the gastrointestinal tract.

CEP-751 (KT-6587) is an indolocarbazole derivative that preferentially inhibits auto-phosphorylation and signaling of TRK family receptors. CEP-751 also inhibits constitutively active RET. It is effective at nanomolar concentrations.

MET The receptor tyrosine kinase MET (c-MET, Hepatocyte Growth Factor Receptor, HGFR) is amplified in some gastric cancers. It is mutationally activated in some head and neck cancers, is activated or over-expressed in glioblastomata, breast carcinomata, and non-small cell lung cancers. Single allele germline missense mutations in the *c-met* gene are oncogenic in hereditary papillary renal cell carcinoma type I and sporadic papillary renal cell carcinoma (trisomy 7 with non-random duplication of mutant *c-met*).

Most small molecule inhibitors of the MET intracellular kinase domain target the ATP binding site (crizotinib, MK2461, JNJ38877605, foretinib, BMS777607, cabozantinib, SGX523, MP470). They associate with the kinase hinge region by mimicking the purine in ATP (for this purpose, BMS777607 and crizotinib have a 2-amino-pyridine group, AMG-458 has a quinoline group, and MK2461 has a tricyclic aromatic group). MET inhibitors can be divided into two classes. Class I drugs (crizotinib, PF04217903, MK-2461, SU-11274) bind adjacent to the hinge region with a U-shaped conformation, whereas class II drugs (foretinib, cabozantinib, AM7) bind in an extended conformation that spans the area from to the C-helix of c-MET. Few small molecule drugs are allosteric c-MET inhibitors (ARQ197) (Fig. 4.20; Table 4.10).

The aminopyridine crizotinib (PF2341066) <Xalkori> is an orally bioavailable ATP-site small molecule inhibitor of c-MET, ALK (Anaplastic Lymphoma Kinase), and ROS1. The crizotinib derivative PF04217903 has a *N*-hydroxyethyl pyrazole group tethered to *C*-7 of the triazolopyrazine and

[9] Anti-cancer kinase inhibitors in the 4-anilinoquinazoline class include most ERBB inhibitors.

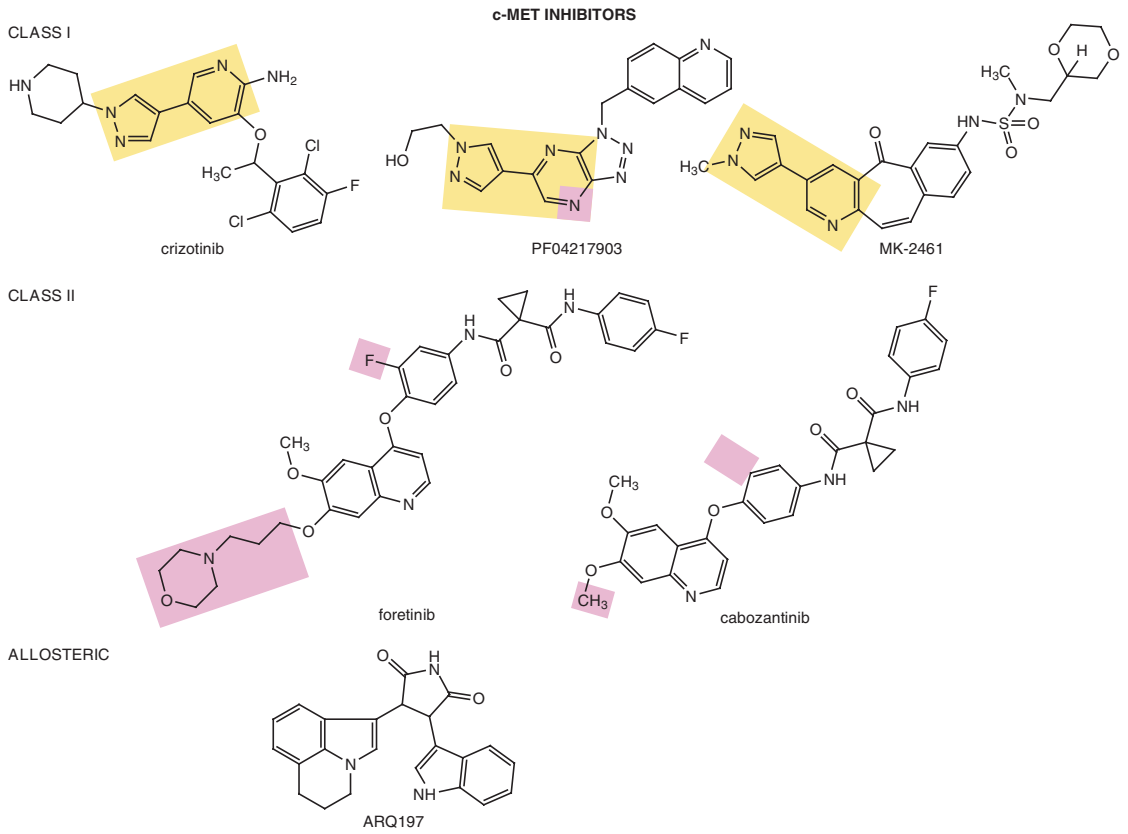

Fig. 4.20 Structures of MET inhibitors. Inhibitors of c-MET are categorized as class I inhibitors, class II inhibitors, and allosteric inhibitors. Class I inhibitors contain heterocyclic 5-and 6-rings connected by a bridge. The structural differences between the class II inhibitors are shown in *pink*

Table 4.10 Inhibitors of MET

	c-MET	VEGFR2	PDGFR	RON	KIT	TIE2	KDR	FLT-1	FLT-4	ALK	ROS1	RET
c-MET inhibitors												
Crizotinib	S									S	S	
MK-2461	S											
Foretinib	S	S	S	S	S	S	S	S	S			
Cabozantinib	S	S										S
ARQ197	S											

S sensitive, *R* resistant

possesses high selectivity for c-MET. The agent is under study for lung cancer and myofibroblastic tumors.

Adverse Effects At the maximum tolerated dose of 250 mg twice a day, the drug causes fatigue.

MK-2461 is an ATP-competitive multi-targeted inhibitor. The pyridine nitrogen in the molecule is necessary for inhibitory activity, and planarity of the molecule is essential for maximum potency. While the drug has low potency as an inhibitor of c-MET auto-phosphorylation at the kinase activation loop, MK-2461 binds preferentially to activated, phosphorylated c-MET. It inhibits the phosphorylation of c-MET substrates and its downstream signaling to the PI 3-K→PKB and RAS→ERK pathways. MK-2461 also displays inhibitory activities against Fibroblast Growth Factor Receptor (FGFR) and Platelet Derived Growth Factor Receptor. A well tolerated oral regimen of MK-2461 administered at 100 mg/kg twice daily effectively suppresses c-MET signaling and tumor growth.

Drug Resistance Mutations of c-MET are not likely to cause drug resistance to MK-2461.

Class II MET inhibitors are usually not as selective as class I inhibitors. The first ATP-site specific c-MET inhibitor to enter clinical trials was the orally bioavailable foretinib (XL880, GSK1363089), a class II ATP competitive antagonist. Foretinib also inhibits VEGFR2, PDGFR, RON, KIT, TIE2, KDR, FLT-1, and FLT-4.

Table 4.11 Inhibitors of PDGFR

	PDGFR	KIT	FLT3	BCR-ABL	VEGFR2	SRC	RAF	RET	CSF-1R	GFR	EPHA2	DDR1	DDR2	PKC	Topoisomerase 1	FGFR
PDGFR inhibitors																
Imatinib	S	S	R	S												
Nilotinib	S	S		S					S			S				
Dasatinib	S	S	R	S		S				S	S		S			
Semaxanib	S	S	S		S											
Sorafenib	S	S	S				S	S								
Tandutinib	S	S	S													
Midostaurin	S	S	S											S	S	
Sorafenib	S	S	S		S		S	S								
CHIR-258	S				S		S									S

S sensitive, *R* resistant

Adverse Effects Foretinib is well tolerated with adverse events mostly limited to grade 1 or 2 hypertension, nausea, anorexia, fatigue, and asymptomatic liver function abnormalities.

Cabozantinib (XL184) <Cometriq> is an orally bioavailable, small molecule kinase inhibitor that strongly binds to and inhibits several receptor tyrosine kinases. Specifically, cabozantinib has a strong affinity for MET, which may result in the inhibition of tumor growth and possible tumor regression. The drug is also an inhibitor of RET and VEGFR2.

ARQ197 is a non-ATP-site competitive, selective small molecule inhibitor of the c-MET intracellular domain, which prevents auto-phosphorylation. ARQ197 selectively targets the inactive form of the kinase between the N- and C- lobes and occupies the ATP binding site. After prolonged treatment (12 weeks or longer), it may induce stable disease in otherwise refractory solid tumor patients, implicating potential anti-invasive activity. The agent is orally bioavailable.

PDGFR The cytokine Platelet Derived Growth Factor (PDGF) acts as a connective tissue cell mitogen with functions that include embryonal development, wound healing, and the control of interstitial fluid pressure in soft connective tissue. Paracrine PDGF signaling in the connective tissue stroma plays a role in various types of solid tumors. Further, gain-of-function mutations in *platelet-derived growth factor receptor* (*pdgfr*) or *c-kit* are the drivers in the pathogenesis of gastrointestinal stromal tumor (GIST). These properties render PDGF and its cognate receptor (PDGFR) potential drug targets.

Multi-kinase inhibitors that suppress PDGFR also inhibit ABL (imatinib, nilotinib, dasatinib; see Sect. 4.1.2.), FLT3 (semaxanib, sorafenib, tandutinib, midostaurin; see Sect. 4.1.5.), or RAF (sorafenib, CHIR-258) (Table 4.11).

Imatinib inhibits the receptor tyrosine kinase PDGFR. The agent also suppresses proliferation and induces apoptosis in cells that over-express the oncoproteins c-KIT or ABL. Imatinib is under investigation as a treatment for cancers that have excessive activity of PDGFR.

PDGF contributes to the regulation of interstitial fluid pressure. As most solid tumors have an increased interstitial fluid pressure, pharmacological reduction may be a way to increase the uptake of anti-cancer drugs. Through the inhibition of PDGFR, imatinib can substantially reduce tumor interstitial fluid pressure. In combination therapy, it may thus improve the pharmacokinetics of other agents.

Nilotinib monohydrate monohydrochloride (AMN107) <Tasigna> is an orally available phenylamino-pyrimidine, which was derived from the imatinib scaffold (Weisberg 2006). This agent inhibits the auto-phosphorylation of PDGFR, DDR1, BCR-ABL, CSF-1R, and c-KIT. Nilotinib interrupts the phosphorylation of these tyrosine kinases and their downstream signaling targets, resulting in decreased cellular proliferation or in the induction of apoptosis.

Dasatinib hydrochloride (BMS-354825) <Sprycel> inhibits PDGFRβ, BCR-ABL, SRC family kinases (SRC, LCK, YES, FYN), c-KIT, EPHA2, and GFR at nanomolar concentrations. In contrast to most tyrosine kinase inhibitors, dasatinib binds to the active conformation of the target enzyme. The agent is given at 100–140 mg orally once per day.

Semaxanib (SU5416) is an intravenous indolinone that targets PDGFR, FLT3, KIT, and VEGFR. Twice weekly infusions of semaxanib administered on a 4-week cycle can reduce marrow blasts in AML or myeloproliferative disorders. In clinical trials, the agent displayed substantial toxicity.

Midostaurine (PKC412) is a semi-synthetic derivative of the alkaloid staurosporine, which is produced by Streptomyces staurosporeus. It is a multi-target protein kinase inhibitor that inhibits PDGFR and FLT3. In 2004, orphan designation was granted to midostaurin for the treatment of acute myeloid leukemia (AML) by the European Commission.

Sorafenib tosylate <Nexavar> is a synthetic compound

Table 4.12 Inhibitors of KIT

	KIT	PDGFR	FLT3	BCR-ABL	VEGFR2	SRC	RAF	RET	CSF-1R	GFR	EPHA2	DDR1	DDR2	MET	TIE2
c-KIT inhibitors															
Imatinib	S	S	R	S											
Nilotinib	S	S		S					S			S			
Dasatinib	S	S	R	S		S				S	S		S		
Bosutinib	(S)	(S)		S		S									
Semaxanib	S	S	S		S										
Sorafenib	S		S				S	S							
Cabozantinib	S		S		S									S	S
Tandutinib	S	S	S												

S sensitive, *R* resistant

that inhibits the VEGFR2/PDGFRβ signaling cascade, thereby blocking tumor angiogenesis. The kinases RAF, KIT, FLT3, mutant and wild-type RAF, and RET are also drug targets. Sorafenib is active orally. It is taken without food, 1 h before or 2 h after a meal, typically twice daily.

Tandutinib (MLN518, CT53518) is a piperazinyl quinazoline that inhibits the auto-phosphorylation of receptor tyrosine kinases, thereby inhibiting cellular proliferation and inducing apoptosis. Tandutinib acts as a small molecule ATP competitive and reversible inhibitor of the type III receptor tyrosine kinases[10], including PDGFRβ, FLT3 (and its cancer causing mutant W51), and c-KIT, which are all equally suppressed. Non-receptor tyrosine kinases, serine-threonine kinases and MAP Kinases are not inhibited. The agent is under investigation in the treatment of glioblastoma and advanced androgen independent prostate cancer.

CHIR-265 is an orally active small molecule that binds to and inhibits PDGFR-β and RAF family kinases. The drug action results in a suppression of tumor cell proliferation and tumor cell death. In addition, CHIR-265 inhibits VEGFR-2, thereby disrupting tumor angiogenesis. This agent is in clinical trials for the treatment of locally advanced or metastatic melanoma.

The related compound CHIR-258 is a small molecule with inhibiting properties against the kinases B-RAF, PDGFR-β, VEGFR-2, and FGFR. It also is in clinical trials for the treatment of melanoma.

c-KIT c-KIT (Stem Cell Factor, SCF, CD117) is a cell surface receptor tyrosine kinase that is stimulated by the cognate ligand HGF (Hepatocyte Growth Factor, Scatter Factor, SCF). Gain-of-function mutations in *c-kit* are the drivers in the pathogenesis of a large fraction of gastrointestinal stromal tumors (GISTs). Multi-kinase inhibitors that suppress c-KIT (Table 4.12) also target ABL (imatinib, nilotinib, dasatinib, bosutinib; see Sect. 4.1.2.) or FLT3 (semaxanib, sorafenib, cabozantinib, tandutinib; see Sect. 4.1.5.).

Imatinib mesylate inhibits proliferation and induces apoptosis in cells that over-express the oncoproteins c-KIT, or ABL, or PDGF Receptor. The drug blocks c-KIT auto-phosphorylation, as well as downstream signaling events, such as the activation of ERK1, ERK2, and PKB (Heinrich 2000). In 2002, imatinib was approved by the U.S. FDA for the treatment of metastatic gastrointestinal stromal tumor. Other indications include adjuvant imatinib following surgical resection of localized c-KIT-positive gastrointestinal stromal tumor. A fraction of melanomas harbor activating mutations or amplifications of *kit*. Some of the affected patients benefit from imatinib mesylate treatment. The agent is under investigation for additional cancers that have excessive activities of c-KIT, including acute myelogenous leukemia, small cell lung cancer, ovarian cancer, testicular cancer, dermatofibrosarcoma protuberans, and glioma.

Drug Resistance Imatinib eventually fails in approximately 50 % of patients, who are then given salvage therapy with sunitinib malate. Sunitinib achieves a good response from cancers with exon nine mutants and wild-type KIT. However, patients with exon 11 mutations, in whom imatinib mesylate has failed, do not respond well to sunitinib because of secondary mutations within the activation loop of the kinase domain that cause resistance.

Nilotinib monohydrate monohydrochloride (AMN107) <Tasigna> is an orally available phenylamino-pyrimidine, which was derived from the imatinib scaffold (Weisberg 2006). This agent inhibits the auto-phosphorylation of DDR1, BCR-ABL, PDGFR, CSF-1R, and c-KIT. Nilotinib interrupts the phosphorylation of these tyrosine kinases and their downstream signaling targets, resulting in decreased cellular proliferation and the induction of apoptosis.

Dasatinib hydrochloride (BMS-354825) <Sprycel> inhibits c-KIT, BCR-ABL, SRC family kinases (SRC, LCK, YES, FYN), EPHA2, PDGFRβ, and GFR at nanomolar con-

[10] Close to 20 types of receptor tyrosine kinases have been identified. Type III comprises the PDGFR family of receptors. It also includes c-KIT, FMS, and FLT3.

centrations. In contrast to most other tyrosine kinase inhibitors, dasatinib binds to the active conformation of the target enzyme. The drug is given at 100–140 mg orally once per day.

Bosutinib (SKI-606) is a synthetic 7-alkoxy-3-quinolinecarbonitrile. While it targets ABL and SRC kinases, it has some activity against c-KIT and PDGFR. Bosutinib suppresses cell growth and apoptosis.

Semaxanib (SU5416) is an intravenous indolinone that targets PDGFR, FLT3, KIT, and VEGFR. Twice weekly infusions of SU5146 administered on a 4-week cycle can reduce marrow blasts in acute myelogenous leukemia (AML) or myeloproliferative disorders. In clinical trial, the agent displayed substantial toxicity.

The kinases KIT, FLT3, and RET, as well as wild-type and mutant forms of the kinase RAF are targets for sorafenib tosylate <Nexavar>. The drug also inhibits the VEGFR2/PDGFRβ signaling cascade, thereby blocking tumor angiogenesis. Sorafenib is active orally. It is taken without food, 1 h before or 2 h after a meal, typically twice daily.

Cabozantinib (XL184) <Cometriq> is an orally bioavailable, small molecule inhibitor that strongly binds to and suppresses several receptor tyrosine kinases. This agent inhibits KIT, FLT3, TIE2 (TEK), MET (HGF Receptor), and VEGFR2. There is single agent activity in medullary thyroid cancer, castration resistant prostate cancer, lung cancer, and ovarian cancer.

Tandutinib (MLN518, CT53518) is a piperazinyl quinazoline that inhibits the auto-phosphorylation of receptor tyrosine kinases, thereby suppressing cellular proliferation and inducing apoptosis. Tandutinib acts as a small molecule, ATP-competitive, reversible inhibitor of the type III receptor tyrosine kinases, including c-KIT, FLT3 (and its cancer causing mutant W51), and PDGFRβ, which are equally inhibited. The agent is much less active against CSF-1R. It is under investigation in the treatment of glioblastoma and advanced androgen independent prostate cancer. Because of the correlation between FLT3 internal tandem duplication mutations[11] and poor prognosis in acute myelogenous leukemia (AML), tandutinib may be beneficial against this form of leukemia.

[11] In FLT3 internal tandem duplication mutations, partial sequences in the juxtamembrane domain through the first tyrosine kinase domain are tandemly duplicated, generating a longer protein product. These mutations result in ligand independent dimerization and tyrosine autophosphorylation, as well as activation of downstream signaling pathways. The constitutive kinase activation confers factor-independent growth.

JAK/STAT Janus Kinases (JAKs) are universally required for signal transduction from cytokine receptors. Because receptors of this family do not have intrinsic kinase activity JAKs serve as the receptor associated tyrosine kinases. Cytokine receptor ligation and dimerization recruits two molecules of JAK, (Janus Kinase), which then bind to two molecules of STAT (Signal Transducer and Activator of Transcription), in turn leading to JAK/STAT dimerization and transmission of a growth signal. Similarly, the ligation of receptor tyrosine kinases, such as the EGF Receptor, can recruit JAK and SRC, which phosphorylate and activate two molecules of STAT, inducing their dimerization. JAK2 is the most commonly mutated gene in BCR-ABL negative myeloproliferative disorders.

Atiprimod (*N, N*-diethyl-8,8-dipropyl-2-azaspiro(4.5) decane-2-propanamine) (SK&F106615) (Fig. 4.21) was initially developed as a potential treatment for rheumatoid arthritis. The drug is an orally bioavailable small molecule belonging to the azaspirane class of cationic amphiphilic agents. Atiprimod inhibits the phosphorylation of STAT3, thus down-regulating the anti-apoptotic proteins BCL-2, BCL-X_L, and MCL-1. This induces cell cycle arrest and leads to apoptosis. The agent lowers the blood levels of IL-6, VEGF, TNF-α, and IL-1. Atiprimod is under evaluation for the treatments of multiple myeloma, metastastic carcinoid cancer, and hepatocellular carcinoma. It has activity against mantle cell lymphoma.

Lestaurtinib (CEP-701) is an orally bioavailable indolocarbazole derivative that inhibits JAK2 as well as RET, FLT3, TRK-A, TRK-B, and TRK-C.

Adverse Effects Most toxicities affect the gastrointestinal tract.

Pacritinib (SB-1518) is a pyrimidine based macrocycle that acts as an ATP competitive small molecule JAK2 kinase inhibitor. It also inhibits the mutant $JAK2^{V617F}$. The drug blocks JAK2 tyrosine phosphorylation on Y221, and dose dependently suppresses JAK2/STAT5 signaling. Pacritinib also suppresses FMS-Like Tyrosine Kinase-3 (FLT3). As a consequence, it has potent anti-proliferative effects on myeloid and lymphoid cells driven by mutant or wild-type JAK2 or FLT3. JAK1, JAK3, and TYK2 are not effectively inhibited. Pacritinib is active against megakaryoblastic leukemia and primary erythroid progenitor cells in myeloproliferative disease. The agent is in clinical studies in patients with myelofibrosis and lymphoma.

JNK The Stress-Activated Protein Kinase (SAPK) pathway is activated in response to noxious influences, including radiation, heat shock, reactive oxygen metabolites, and growth factor deprivation. c-JUN N-Terminal Kinases (JNK)

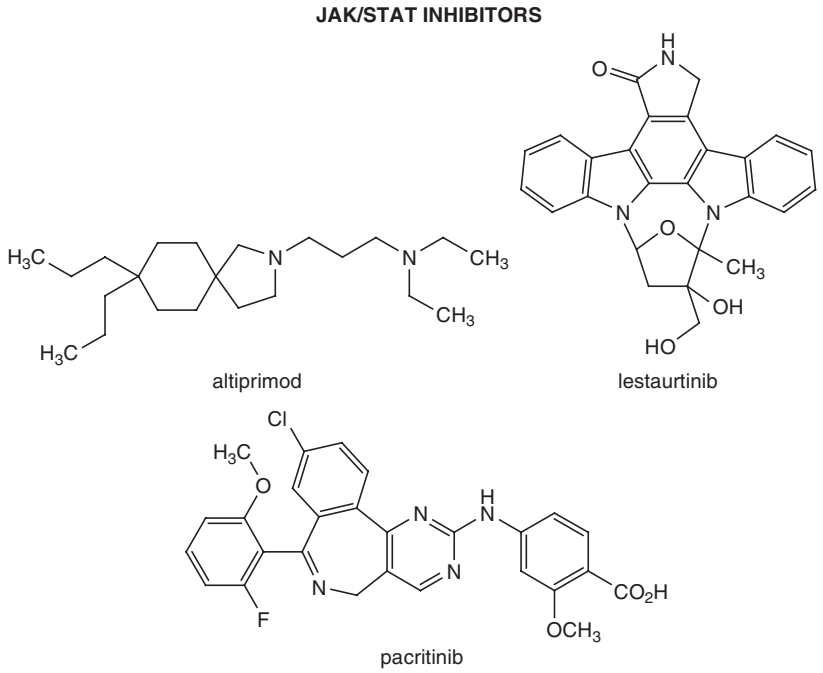

Fig. 4.21 Structures of JAK/STAT inhibitors

constitute a family of stress activated protein kinases derived from the genes, *jnk1*, *jnk2*, and *jnk3*. JNK-1 is required for the survival of the transformed cells in the absence of stromal support.

Robust over-expression of the JNK substrates and transcription factors JUN and JUN-B arises in Hodgkin lymphoma and anaplastic large cell lymphoma (ALCL), but not in other lymphoma types. This is associated with the proliferation of Hodgkin cells and suppression of apoptosis in ALCL cells. Furthermore, the oncogenic BCR-ABL fusion protein activates the JNK signaling pathway in hematopoietic cells. JNK therefore has promise as a drug target (Fig. 4.22).

1,9-pyrazoloanthrone (SP600125) is an anthrapyrazolone first generation, reversible, ATP competitive inhibitor with high selectivity for JNK over other kinases. The agent dose dependently inhibits the phosphorylation of JUN. Its half-maximal inhibitory dose for JNK1 and JNK2 is half that for JNK3. The compound is poorly water soluble, but cell permeable.

CC-401 (3-(3-(2-(piperidin-1-yl)ethoxy)phenyl)-5-(1*H*-1,2,4-triazol-3-yl)-1*H*-indazole hydrochloride) is a second generation, ATP competitive anthrapyrazolone JNK inhibitor. Similar to 1,9-pyrazoloanthrone, CC-401 competitively binds the ATP site of JNK. This leads to an inhibition of phosphorylation of the c-JUN NH_2-terminal activation domain, resulting in decreased transcriptional activity of c-JUN, and consequently decreased cellular proliferation.

ALK About 5% of patients with non-small cell lung carcinoma have a chromosomal rearrangement that generates a fusion protein between EML4 and ALK (Anaplastic Lymphoma Kinase), which results in constitutive ALK kinase activity that contributes to carcinogenesis. Patients with this gene fusion are typically non-smokers, who do not have mutations in the *egfr* or *K-ras* genes. ALK gain-of-function mutations also may be important in driving the malignant phenotype in about 15% of neuroblastomata.

Crizotinib ((R)-3-[l-(2,6-Dichloro-3-fluorophenyl) ethoxy]-5-[1-(piperidin-4-yl)-1H-pyrazol-4-yl]pyridin-2-amine) (PF2341066) <Xalkori> (see Fig. 4.22) is an orally bioavailable, ATP site competitive small molecule inhibitor of ALK. Crizotinib also inhibits the tyrosine kinases MET, which is involved in the oncogenesis of a number of other forms of cancer, and ROS1, which also may act as a proto-oncogene. The drug is approved in the U.S. for the treatment of non-small cell lung carcinoma. It is also undergoing clinical trials in anaplastic large cell lymphoma, neuroblastoma, myofibroblastic tumors, and other advanced solid tumors.

Pharmacokinetics Following oral single dose administration, crizotinib reaches a peak blood concentration in 4–6 h. With twice daily administration, steady state is reached within 15 days. Although the drug can be administered with or without food, a high-fat meal reduces crizotinib absorption by about 15%. There is extensive distribution from the blood into tissues. Over a wide drug concentration the binding to

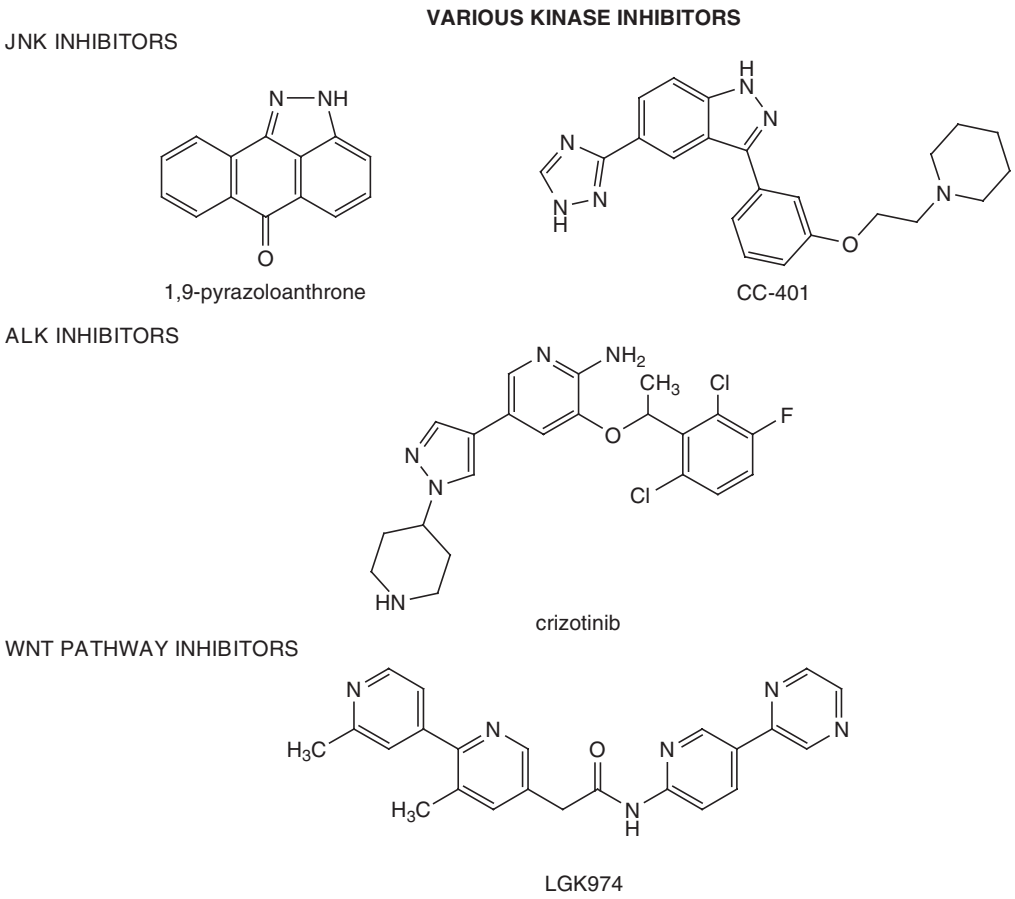

Fig. 4.22 Structures of various signal transduction kinase inhibitors

plasma proteins is 90%. Following a single dose of crizotinib, the mean apparent terminal half-life is 40 h. 65 and 20% of the administered are excreted in the feces and urine, respectively. About half of the fecal elimination is constituted by the unchanged drug, whereas the urine excretes mostly metabolites.

Crizotinib is predominantly metabolized by CYP3A4 and CYP3A5. The primary metabolic pathways are oxidation of the piperidine ring to crizotinib lactam and *O*-dealkylation, with subsequent conjugation of *O*-dealkylated metabolites. After multiple dosing, crizotinib acts as a time-dependent inhibitor of CYP3A.

Adverse Effects At the maximum tolerated dose of 250 mg twice a day, the drug causes increased fatigue. Other adverse events may include vision disorder (65%), nausea or vomiting (50%), diarrhea (50%), constipation (40%), esophageal discomfort (20%), abdominal pain (15%), or stomatitis (10%). Severe pneumonitis and QTc prolongation are potentially dangerous complications. Drug induced hepatotoxicity with fatal outcome has occurred in fewer than 1% of patients.

Drug Resistance Crizotinib is a substrate for the export transporter ABCB1 (P-Glycoprotein, MDR).

Drug Interactions Co-administration with strong CYP3A inhibitors (HIV protease inhibitors, triazole antifungals, macrolide antibiotics, ketoconazole, nefazodone) increases the crizotinib blood concentrations. Grapefruit or grapefruit juice should be avoided as they may slow the disposition of crizotinib. Co-administration of crizotinib with strong CYP3A inducers (carbamazepine, phenobarbital, phenytoin, rifabutin, rifampin, St. John's Wort) decreases its blood concentrations. As crizotinib itself inhibits CYP3A, dose reduction may be needed for co-administered drugs that are predominantly metabolized by CYP3A.

WNT The success of WNT pathway inhibitors is limited by the narrow therapeutic window afforded by the requirement for WNT signaling in healthy tissue homeostasis. The selective, orally bioavailable Porcupine inhibitor LGK974 (see Fig. 4.22) was the first small molecule WNT signal suppressor in clinical trials (Fig. 4.23). It limits the growth of WNT pathway driven tumors by reducing the expression of WNT target genes, such as *axin2*. LGK974 is efficacious at well tolerated doses against cancers that have NOTCH1 and mutations in RNF43. Head and neck cancer cells with loss-of-function mutations in the NOTCH signaling path-

way have a high response rate to LGK974. All LGK974 sensitive pancreatic cancer cells carry inactivating mutations of RNF43.

Excessive activities of diverse kinases can be transforming, causing the development of various cancers.
Drug targets include RET, MET, PDGFR, KIT, JAK, JNK, ALK, WNT.
Drugs that inhibit these kinases have limited specificities.

4.2 Inhibitors of Oncogene Functions

4.2.1 Farnesyl Transferase Inhibitors

Prenylation (the attachment of a farnesyl group or a geranylgeranyl group) provides a mechanism for the membrane localization of proteins that lack a transmembrane domain. RAS proteins participate in numerous signaling pathways related to proliferation and cytoskeletal organization (see Sect. 4.1.3.). One function of RAS is to facilitate the localization of its cytosolic effectors to the plasma membrane, to which it is anchored via a COOH-terminal farnesyl group[12]. To support this function, a key step in the post-translational processing of RAS is its farnesylation by the enzyme Farnesyl Protein Transferase (Farnesyl Transferase). Some RAS proteins (H-RAS1, N-RAS, and K-RAS2) are further lipidated by palmitoylation at 1–2 cysteines near the farnesylated COOH-terminus.

Farnesyl Transferase is not specific for RAS. Other proteins are also subject to farnesylation. The centromeric proteins CENP-E and CENP-F depend on farnesylation for their association with the microtubules, which is important for mitosis. All farnesylated proteins end in a CAAX sequence (C = cysteine, A = aliphatic amino acid, X = methionine, glutamine, serine, or threonine).

Because constitutively activated forms of RAS contribute to a wide range of cancers, Farnesyl Transferase inhibitors were designed to block RAS signal transduction (Reuter 2000). However, they can prevent the farnesylation of various targets (Fig. 4.24) and exert their effects in part through inhibiting the functions of proteins other than RAS. Tumor cells that are sensitive to this class of drugs accumulate in G_2/M, except for cells with activated H-RAS, which accumulate in G_1. Based on the mechanism of action, there are three groups of Farnesyl Transferase inhibitors,

- compounds that act competitively with farnesylpyrophosphate,
- peptidomimetic and non-peptidomimetic compounds that compete with CAAX,
- bisubstrate analogs that combine features of both.

[12] farnesyl is a 15-carbon acyclic sesquiterpene alcohol.

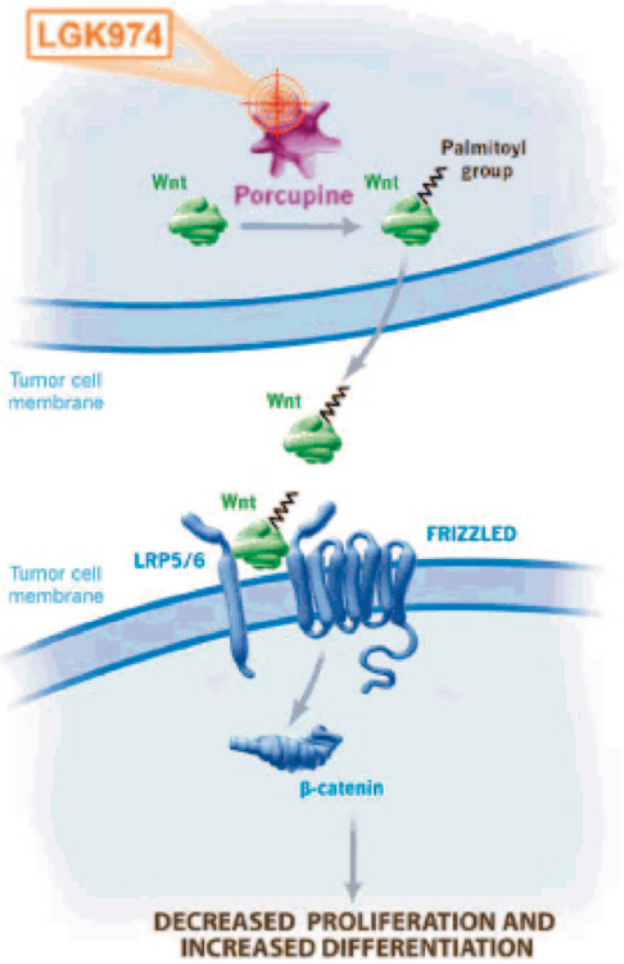

Fig. 4.23 Small molecule inhibitor of Porcupine. The WNT pathway regulates cellular morphology, differentiation, apoptosis, proliferation, and stem cell self-renewal. Dysregulation of this pathway plays a critical role in the development of melanoma, breast cancer, and squamous cell carcinoma. The canonical WNT signaling pathway is initiated by an interaction between WNT ligands and the co-receptors Frizzled LRP5/6. Activation of this pathway prevents the proteosomal degradation of β-Catenin, which translocates into the nucleus and activates the TCF/LEF family of transcription factors to activate specific WNT-target genes. This mechanism results in cell proliferation. WNT ligands require post-translational palmitoylation in order to be secreted and to be functionally active. The key enzyme regulating this process is the membrane-bound O-Acyl Transferase Porcupine, which is required and specific for palmitoylation of WNT ligands. LGK974 is a selective Porcupine inhibitor under development for the treatment of cancers that are driven by the WNT pathway in a WNT ligand-dependent manner ((http://www.novartisoncology.com/research-innovation/pipeline.jsp) with permission, ©2014 Novartis AG)

Adverse Effects The Farnesyl Transferase inhibitor group of drugs has mild toxicities, including neutropenia, thrombocytopenia, diarrhea, rash, neuropathy, and Transaminase elevations.

Drug Resistance Under conditions of inhibited Farnesyl Transferase, the related enzyme Geranyl-Geranyl Transferase 1 can catalyze the activation of RAS (Appels 2005). This

may underlie the limited therapeutic effectiveness of the Farensyl Transferase inhibitors.

Tipifarnib (R115777) <Zarnestra> is a non-peptidomimetic quinolinone that binds to and inhibits Farnesyl Protein Transferase. By suppressing the farnesylation of proteins, this agent prevents the activation of RAS, inhibits cell growth and induces apoptosis. Tipifarnib may be useful in the treatment of multiple myeloma, where RAS mutations are common. Although protein farnesylation is inhibited in all patients exposed to the drug, the targeting of Farnesyl Transferase does not correlate with disease stabilization. Tipifarnib may also target a survival pathway, reflected in reduced levels of phosphorylated PKB and STAT3 in bone marrow from patients, in whom these tumor survival pathways are constitutively active. The reduced phosphorylation correlates with disease stabilization. The compound can be given orally. Dosing regimens frequently administer 300–600 mg twice daily for 21 days, followed by 1 week of rest.

Pharmacokinetics Maximal blood concentrations are reached at 2–4 h after administration of tipifarnib. There is extensive metabolism, with approximately 15 % of tipifarnib and its metabolites being excreted in the urine, whereas approximately 80 % is excreted in the feces (Garner 2002).

Adverse effects The dose limiting toxicity is reversible myelosuppression.

Lonafarnib (SCH66336) <Sarasar> is a synthetic tricyclic derivative of carboxamide that binds to and inhibits Farnesyl Transferase. The centromeric proteins CENP-E and CENP-F are substrates for Farnesyl Transferase, but not for Geranyl-Geranyl Transferase 1. Their prenylation is completely inhibited by lonafarnib. While this does not affect their localization to the kinetochores, it alters the association between CENP-E and the microtubules, resulting in the alteration of microtubule-centromere interactions during mitosis and leading to the accumulation of cells prior to metaphase (Ashar 2000). Lonafarnib is administered at 200 mg orally twice daily on a continuous regimen. The compound has oral bioavailability and metabolic stability, with maximal plasma concentrations being reached at 6–8 h.

Adverse Effects The main toxicity is diarrhea, but nausea, vomiting, and fatigue also frequently arise.

BMS-214662 is a non-sedating benzodiazepine derivative that belongs to the class of non-peptidomimetic Farnesyl Transferase inhibitors. The drug inhibits H-RAS and K-RAS. Its action is highly selective for Farnesyl Transferase over Geranyl-Geranyl Transferase.

Adverse Effects Because the oral formulation exhibits dose dependent gastrointestinal toxicity, intravenous administration is preferred. The maximum tolerated dose is around 200 mg/m^2, with dose limiting toxicities consisting of nausea, vomiting, and diarrhea. Besides, creatinine elevation, renal failure and acute pancreatitis can become dose limiting (Ryan 2004; Agus 2005).

Other Farnesyl Transferase inhibitors in development include L-778123, L744832, and FTI-277.

Farnesyl Transferase inhibitors suppress the prenylation of proteins, thus preventing their membrane association. This blocks signal transduction.
Multiple proteins may be affected.
The mechanism is not specific for cancer cells.

4.2.2 Inhibitors of Protein Turnover

Inhibitors of protein synthesis Inhibition of tumor cell proliferation by L-Asparaginase was described by Kidd, who observed that the growth of leukemia cells in culture was supported by horse serum or rabbit serum, but not by guinea pig serum. Guinea pig serum turned out to contain an enzyme that degrades asparagine in the medium, thus eliminating a required exogenous nutrient for the growth of leukemia cells.

L-asparagine is a non-essential amino acid that can be synthesized by most human cells, except for those of certain lymphoid malignancies, which lack or have very low levels of the Synthetase enzyme required for L-asparagine formation. L-Asparaginase (Colaspase) <Elspar> is an enzyme produced by various bacteria, such as Escherichia coli. It deaminates L-asparagine to aspartate and ammonia. The depletion of exogenous L-asparagine supplies and inhibition of protein synthesis results in tumor cell blockage of proliferation, especially in the G_1 phase of the cell cycle. L-Asparaginase also induces apoptosis in tumor cells. The drug is used in combination chemotherapy of childhood acute lymphocytic leukemia (ALL), particularly in patients who are refractory to first-line treatments. It has also been used to treat malignant mesothelioma.

- Pegaspargase <Oncaspar> is a complex of polyethylene glycol conjugated with L-Asparaginase. PEGylation decreases the antigenicity of the enzyme.

Drug Resistance Increased L-Asparagine Synthetase activity within tumor cells causes resistance to L-Asparaginase treatment (Kurtzberg 2003).

Ranpirnase is a natural homolog of Ribonuclease A isolated from the eggs of the frog Rana pipiens. Ranpirnase primarily degrades cellular transfer RNA (tRNA) with a substrate specificity for uridine-guanidine base pair sequences, resulting in the inhibition of protein synthesis and cytotoxicity. This agent also activates Caspase-9 in the mitochondria,

Treatment of Pancreatic Carcinoma

1. **Chemotherapy**

The high rate of relapse of pancreatic adenocarcinoma after surgery (85%) makes the combination of adjuvant postoperative therapy an important strategy. Postoperative adjuvant chemo-radiation with fluorouracil was the standard of care for more than 25 years (Kalser 1985; Abbruzzese 2008). However, the benefit of the radiation component was called into doubt by the EPAC-1 (European Study Group for Pancreatic Cancer-1) clinical trial. The treatment of pancreatic cancer depends on the stage. Fluorouracil, gemcitabine (approved by the U.S. FDA in 1996), and erlotinib (or other ERBB targeting drugs) are the chemotherapeutic agents of choice.

Fluorouracil-based regimens 5-fluorouracil is suitable for patients who cannot tolerate gemcitabine. Although pancreatic cancer is highly resistant to chemotherapy, response rates of approximately 25% can be achieved with 5-fluorouracil as a single agent or in combinations with other drugs. However, these treatments provide temporary relief with only small effects on overall survival.

Adjuvant postoperative therapy with fluorouracil and leucovorin (20 mg/m^2 folinic acid intravenous bolus, followed by 425 mg/m^2 fluorouracil intravenous bolus for 1–5 days every 28 days over 6 months) results in a survival benefit. Continuous infusion of 5-fluorouracil is associated with higher survival than bolus injection.

FAM (5-fluorouracil, doxorubicin <Adriamycin> , mitomycin C), FAM-S (5-fluorouracil, doxorubicin, mitomycin C with streptozocin), and the Mallinson regimen (sequential 5-fluorouracil, cyclophosphamide, methotrexate, vincristine, and mitomycin C) have response rates of 20–40% and median survival times up to 10 months. However, no survival advantages has been documented over single-agent 5-fluorouracil, and the toxicities are substantial.

First-line treatment for metastatic pancreatic adenocarcinoma with FOLFIRINOX (folinic acid (leucovorin), 5-fluorouracil, irinotecan, oxaliplatin) is superior to monotherapy with gemcitabine. The combination is associated with an improved response rate and increased progression free survival.

Adverse Effects Common adverse effects include vomiting, fatigue, diarrhea, neuropathy, myelosuppression, and febrile neutropenia. Because of the substantial adverse events, this regimen is limited to patients with very good performance status.

Gemcitabine-based treatments Adjuvant gemcitabine may have a slightly higher survival benefit compared to fluorouracil/leucovorin. Gemcitabine (1 g/m2 intravenous infusion once per week for 3 of every 4 weeks) also has a lower incidence of stomatitis and diarrhea. Monotherapy with this drug is common. While numerous gemcitabine based combination chemotherapy protocols (comprising 2–4 drug combinations) have been tested, none has yet evolved as superior.

Adverse Effects The use of gemcitabine is associated with an increased risk of myelosuppression and resulting hematologic toxicity (O'Reilly 2010).

The combination of gemcitabine with 5-fluorouracil is well tolerated, despite some additional toxicity over single-agent gemcitabine (grade 3/4 leukopenia and diarrhea are more frequent). However, it is not documented whether this combination has any advantage over single-agent gemcitabine.

GemCap is a combination chemotherapy treatment for pancreatic cancer that uses gemcitabine <Gemzar> and capecitabine <Xeloda>. Gemcitabine is given as an infusion over about 30 min on days 1 and 8. Capecitabine is taken in tablet form for 14 days, usually as a combination of 500 and 150 mg tablets. A rest period of 1 week completes the cycle. A usual regimen covers 4–8 cycles of treatment over a period of 3–4 months.

Gemcitabine/cisplatin combinations have modest additional benefits compared with outcomes for gemcitabine as a single agent. This therapy is tolerated without major compromises in the quality of life. Response rates and a longer median survival may favor the gemcitabine/cisplatin doublet over monotherapy.

Several Topoisomerase I inhibitors, including irinotecan, exatecan, and the oral drug rubitecan (9-nitrocamptothecin, 9NC), are undergoing clinical development in an effort to identify novel, improved gemcitabine based combinations.

ERBB-targeting treatments EGFR is overexpressed in a large fraction of pancreatic tumors. Resulting from a phase III randomized controlled trial, that demonstrated improved survival rates, improved tumor responses, and improved progression-free survival rates, the U.S. FDA has licensed the use of erlotinib <Tarceva> in combination with gemcitabine as a palliative agent for pancreatic cancer. The survival

improvement with the combination is on the order of less than four weeks, causing the incremental value of adding erlotinib to gemcitabine treatment to be questioned. Erlotinib has now secured approval for the treatment of advanced pancreatic cancer in combination with gemcitabine in chemotherapy-naïve patients in both the U.S. and Europe.

As an alternative to erlotinib, the antibody cetuximab <Erbitux> (loading dose of 400 mg/m2 then 250 mg/m2 weekly) has been evaluated in conjunction with gemcitabine. The combination leads to disease stabilization in about 50 % of patients and an increase in overall survival.

Adverse Effects The main adverse effects are dermatological, including acneiform rash (40 %) and folliculitis (15 %).

Approximately 20 % of pancreatic tumors overexpress ERBB2 (HER-2/neu). Trastuzumab (loading dose of 4 mg/kg for the first week and then 2 mg/kg weekly) in combination with gemcitabine (1000 mg/m2 weekly) has a survival benefit in patients with metastatic, ERBB2-positive pancreatic cancer.

Adverse Effects Grade 3 hematologic toxicities and cardiotoxicity may occur.

Other drugs Blocking RAS signaling is highly promising for therapy since ras mutations arise in approximately 90 % of pancreatic cancers. RAS inhibition may be achieved by small molecule kinase inhibitors or by blocking the posttranslational farnesylation of K-RAS. SCH66336, R1115777, and BMS 214662 are Farnesyl Transferase inhibitors in clinical development. They are available for oral and intravenous administration. Mostly, they are under study in combination with gemcitabine.

Leukocytes actively infiltrate the stroma of pancreatic ductal adenocarcinoma and orchestrate an immune response that is—in its result—immunosuppressive and leads to fibrosis. Activation of the TNF receptor CD40 is a key regulator in reversing this immunosuppression. Tumor regression requires macrophages, but not T-lymphocytes.

The fully human agonistic anti-CD40 antibody CP-870,893 may facilitate tumor regression in surgically incurable patients (Beatty 2011). The most common adverse effect is mild to moderate cytokine release syndrome (characterized by chills, fever, and rigors), which can be managed by supportive care.

TNFerade for the treatment of pancreatic cancer has orphan drug designation from the U.S. FDA. TNFerade is an adenovector, which contains the gene for tumor necrosis factor-α (tnf-α), an immune system protein with potent anti-cancer effects, for the direct injection into tumors. After administration, TNFerade stimulates the production of TNF-α in the tumor. The agent did not prove efficacious in clinical trials.

Endocrine cancer targets Z-360 is a selective, orally available, 1,5-benzodiazepine derivative that acts as a Gastrin/Cholecystokinin-2 Receptor antagonist. By binding to the receptor, Z-360 prevents its activation by Gastrin, a peptide hormone frequently associated with the proliferation of gastrointestinal and pancreatic tumor cells.

Streptozotocin is approved by the U.S. FDA for treating metastatic cancer of the pancreatic islet cells. The agent is generally limited to patients whose cancer cannot be resected surgically. Streptozotocin can reduce the tumor burden and ameliorate symptoms, such as hypoglycemia due to excessive Insulin secretion by insulinomata.

2. **Pain management**

The typical pain of locally advanced pancreatic cancer is a dull, fairly constant pain of visceral origin localized to the region of the middle and upper back. It results from tumor invasion of the celiac and mesenteric plexi (neuropathic and inflammatory type of pain). Some patients complain of vague, intermittent epigastric pain. The etiology of this discomfort may be secondary to pancreatic duct obstruction and resultant pancreatic insufficiency (obstructive and inflammatory types). In the early stages of the disease, only 10 % of patients have severe pain.

Comprehensive pain management consists of a combination of anti-tumor therapy, analgesic drug therapy, anesthetic blocks, and behavioral approaches.

- Initially, pain associated with unresectable pancreatic cancer can be controlled with non-steroidal anti-inflammatory drugs (NSAIDs) or oral or transdermal narcotic analgesics. Upon disease progression, opioids alone may not be sufficient.
- The next major modality for pain control is an anesthetic block of the celiac plexus, using injection of a solution of 50–100 % alcohol or a phenol solution. This offers pain control for a duration of 3–4 months in 80–90 % of patients. Celiac block can be performed percutaneously (under radiologic guidance) or intraoperatively.
- While external beam radiation therapy with or without chemotherapy may be used to palliate pain asso-

ciated with pancreatic cancer, pain control may take several weeks to be achieved with radiation.
- Chemotherapy also can achieve pain control in pancreatic cancer patients, as is the case with the use of gemcitabine. Over 20% of patients treated with gemcitabine experience a clinical benefit with regard to improved pain or fatigue (el-Kamar 2003).

3. **Obstruction**

About 70% of pancreatic cancers are located in the head of the pancreas, and obstructive jaundice is often the first symptom leading to diagnosis. Biliary decompression can be accomplished by surgical bypass (including cholecystojejunostomy, choledochojejunostomy, or hepaticojejunostomy) or endobiliary stenting. Endoscopic stent placement has a higher success rate and lower 30-day mortality rate than percutaneous stenting and represents the procedure of choice. The choice of surgery versus stenting should be made in the context of the patient's overall treatment plan. For obstructive pain, pancreatic duct stenting or pancreatic enzyme replacement may be effective.

Gastric outlet obstruction is a late complication of advanced pancreatic cancer affecting approximately 10–20% of patients who survive more than 15 months. However, fewer than 3% of these patients require surgical bypass. 60% of patients with advanced pancreatic cancer have no evidence of gastric or duodenal invasion but have abnormal gastric motility with delayed emptying. Also, nausea and vomiting may develop secondary to tumor infiltration of the nerve plexi of the stomach and duodenum. After gastric outlet and small bowel obstructions have been ruled out, empiric prokinetic drugs, such as metoclopramide, can be used to manage these symptoms (el-Kamar 2003).

4. **Cachexia**

Cachexia in the advanced pancreatic cancer patient results not only from loss of appetite and malnutrition but also from hyper-catabolism of lean tissue and anorexia/cachexia syndrome. The condition is reflected in weakness, fatigue, and poor quality of life. Weight loss also contributes to depression and is predictive of poor outcome and greater morbidity.

– The management of pancreatic exocrine insufficiency is especially important for managing cachexia. Pancreatic exocrine insufficiency is common but usually moderate in patients with pancreatic cancer. Approximately 65% of patients have some degree of fat malabsorption and 50% have some degree of protein malabsorption. These may contribute to weight loss, epigastric discomfort, and malabsorption symptoms such as excessive flatus, bloating, diarrhea, and steatorrhea. Pancreatic enzyme replacement improves malabsorption, bloating, and diarrhea and helps prevent further weight loss. Pancreatic enzyme therapy may sometimes be associated with gastrointestinal adverse effects such as nausea, vomiting, cramps, constipation, or diarrhea that can be ameliorated by adjusting doses or brands. Empiric Pancreolipase replacement should be considered for most patients.
– The initial management usually entails (preferably oral) supportive nutrition, comprising caloric supplementation and hydration.
– Anorexia is well treated with appetite stimulants, such as progestational drugs and corticosteroids. These agents are potent anti-emetics, act rapidly, and increase the non-fluid body weight in advanced cancer patients with anorexia. The most used agents are megesterol acetate, medroxyprogesterone acetate, and dexamethasone. Dexamethasone can be administered at doses ranging 3–8 mg/day and megesterol acetate doses range 400–800 mg/day. They are comparable in efficacy and produce an improvement in appetite with subsequent weight gain in approximately 15% of patients. Dexamethasone has a short-lived duration of action (4 weeks) and a substantial adverse effect profile, including myopathy, mood changes, and hyperglycemia.
– A class of drugs in use is orexigenic (appetite stimulant) agents, such as the prokinetic medication metoclopramide and the cannabinoid dronabinol. Dronabinol at doses of 2.5–7.5 mg/day improves chemotherapy-induced nausea and vomiting in 65% of cancer patients.
– An agent that may reverse cancer-associated cachexia is eicosapentaenoic acid (EPA, contained in fish oil pills) at doses of 1–6 g/day, which may result in weight stabilization after 3–4 weeks. Eicosapentaenoic acid works by suppressing the effect of the glycoprotein Proteolysis-Inducing Factor (PIF).
– Thalidomide at 100–200 mg/day at bedtime, provides some weight stabilization but no weight gain. It may shorten the half-life of *tnf-α* mRNA. Because of mixed results in clinical trials, thalidomide is not widely used.
– Ibuprofen has a role in abrogating the catabolic process and may lead to modest increases in weight. The combination of ibuprofen with megestrol acetate can reverse weight loss and improve the quality of life (el-Kamar 2003).

Treatment of Lung Cancer

Small cell lung cancer is treated primarily with chemotherapy, as surgery has no demonstrable influence on survival. Primary chemotherapy is also given in metastatic non-small cell lung cancer. The combination regimen depends on the tumor type.

- For non-small cell lung cancer, cisplatin or carboplatin, in combination with gemcitabine, paclitaxel, docetaxel, etoposide, or vinorelbine are used.
- For small cell lung cancer, cisplatin and etoposide are most commonly used. Combinations with carboplatin, gemcitabine, paclitaxel, vinorelbine, topotecan, and irinotecan are also applicable.

Adjuvant chemotherapy refers to the use of chemotherapy after surgery to improve outcome. In stage 2 or 3 disease (afflicted lymph nodes), adjuvant chemotherapy may improve survival by up to 15%. Standard practice is to offer platinum based chemotherapy, such as cisplatin and vinorelbine.

Small cell lung cancer Chemotherapy has been used in patients with small cell lung cancer (SCLC) for more than 25 years.

- Lung cancers may be treated with CAP, a combination chemotherapy of cyclophosphamide, doxorubicin <Adriamycin>, and cisplatin (platinum alkylating agents).
- CAV is named after cyclophosphamide, doxorubicin <Adriamycin>, and vincristine. The chemotherapy drugs are then given separately, often starting with doxorubicin, followed by vincristine, and then cyclophosphamide. The cycle is completed with a rest period of 3 weeks. A course of the treatment comprises 4–6 cycles that are given over a period of 3–4 months.
- The COPE regimen includes cyclophosphamide, vincristine <Oncovin>, cisplatin (platinum alkylating agents), and etoposide.
- The PE (cisplatin, etoposide) regimen was developed in the early 1980s. For many years, it was a standard chemotherapy program most extensively used in clinical practice. Various modalities exist, in which cisplatin and etoposide are administered.

Non-small cell lung cancer Non-small cell lung cancer is more common than small cell lung cancer, accounting for around 80% of all lung cancers. It is an aggressive disease, for which the overall 5 years survival rates are below 10%. Cytotoxic chemotherapy is the standard of care for patients with unresectable stage 3 and stage 4 cancer and a good performance status. For the treatment of locally advanced or metastatic non-small cell lung cancer, platinum-based and taxane-based chemotherapies are the first line of treatment. Doublet chemotherapy including cisplatin or carboplatin with another drug (vinorelbine, paclitaxel, docetaxel, or gemcitabine) is recommended.

- Chemotherapy is initially conducted with docetaxel, paclitaxel, vinorelbine, gemcitabine, or pemetrexed.
- Second to third line therapy for non-small cell lung cancer includes small molecule tyrosine kinase inhibitors, such as gefitinib and erlotinib. In initial clinical trials, erlotinib was used as monotherapy in patients with chemo-refractory non-small cell lung cancer. 50% of these achieved disease stabilization and 40% survived for at least 12 months.
- The addition of bevacizumab to doublet chemotherapy improves the 1-year survival in selected patients with advanced non-small cell lung cancer. The drug is used in combination with carboplatin and paclitaxel as first-line treatment of patients with unresectable, locally advanced, recurrent or metastatic non-squamous non-small cell lung cancer.

GemCarbo chemotherapy (consisting of gemcitabine and carboplatin) is used to treat non-small cell lung cancer. It is usually given as a day patient treatment, involving a blood test the day before infusion of the drugs. The regimen is administered as a 21 day cycle. On the first day of treatment, the patient is given both agents. On day 8, there is an infusion of gemcitabine only. There then follows a rest period of 2 weeks which completes one cycle of chemotherapy. The next cycle of treatment is given after a rest period, which will be 3 weeks after the first injection. Usually 4–6 cycles of treatment are given over a period of 3–4 months and this makes up a course of treatment. This regimen may prevent the further spread of the cancer or in some cases may reduce the size of the tumor between 20–80% depending upon the individual.

Inhibiting ERBB1 (EGFR) signaling is an effective strategy for treating non-small cell lung cancer

patients. The first generation tyrosine kinase inhibitors, gefitinib and erlotinib, have temporary success. Second generation agents that aim to further improve patient outcomes are in preclinical and clinical trials. They include EKB-569, HKI-272, CI-1033, and ZD6474 (Sequist 2007).

Drug Resistance About half of all patients develop the ERBB1 mutation T790M, for which existing inhibitors do not work well. Over-expression of MET by the cancer cells may also cause resistance to small molecule ERBB1 inhibitors.

Adenocarcinoma 10–15% of lung cancers involve patients who never smoked. In 70% of the cases, these tumors are adenocarcinomata. These lung cancers constitute a distinct disease from lung cancers in smokers. Two mutations commonly occur.

- Many non-smoker type lung cancers have mutations in ERBB1 (EGFR). An activating mutation in exons 19 and 21 may be causative. Many patients initially respond to tyrosine kinase inhibitors, such as erlotinib, but resistance eventually develops. In relapsed patients, an activating mutation in exon 20 often arises. It may be selected for by treatment with tyrosine kinase inhibitors. The exon 20 mutation changes the structure of the tyrosine kinase inhibitor binding site, thus rendering the drugs ineffective. Amplification of *c-met* can be another mechanism to cause resistance to tyrosine kinase inhibitors by activating a rescue growth and survival pathway.
- A tumor driver in non-smoker type lung cancer can be mutated K-RAS. Until targeted therapies are developed, only conventional chemotherapy can be offered to those patients.

Mesothelioma Pemetrexed <Alimta> is a multi-target agent that inhibits several folate dependent enzymes. It is used in combination with cisplatin for the treatment of pleural mesothelioma. The average survival and time to cancer progression can be further improved by giving a vitamin supplementation of B12 and folic acid.

The chemotherapy regimen MVP includes mitomycin, vinblastine, and cisplatin. The drugs are given as consecutive injections or infusions over 8–12 h. Because the treatment contains a platinum drug, the ample consumption of fluids is recommended to protect kidney function. The cycle is completed by a rest period of 3 weeks.

resulting in tumor cell apoptosis. The agent is under study for the treatment of pleural and peritoneal mesothelioma.

KRN5500 (Fig. 4.25) is a semi-synthetic derivative of the nucleosidic antibiotic spicamycin, which was originally isolated from the bacterium Streptomyces alanosinicus. KRN5500 inhibits protein synthesis by interfering with endoplasmic reticulum and Golgi apparatus functions. This agent also induces cell differentiation and Caspase dependent apoptosis. It is under investigation for B-cell chronic lymphocytic leukemia

Homoharringtonine (omacetaxine mepesuccinate, cephalotaxine 4-methyl (2R)-2-hydroxy-2-(4-hydroxy-4-methylpentyl)butanedioate) <Synribo> is a cytotoxic plant alkaloid isolated from the evergreen trees Cephalotaxus fortuneii and Cephalotaxus hainanensis. The molecule binds to the ribosome. It suppresses the elongation phase of translation by preventing substrate binding to the acceptor site on the 60S ribosomal subunit and blocking aminoacyl-tRNA binding[13] and peptide bond formation.

Homoharringtonine induces differentiation or apoptosis in some cancer cell types. It has been used in China to treat adults with chronic or accelerated phase chronic myeloid leukemia (CML) who are no longer responding to, or who could not tolerate, at least two tyrosine kinase inhibitors. Homoharringtonine is also active in patients carrying the BCR-ABL^{T315I} mutation. The recommended starting schedule for induction is 1.25 mg/m^2 administered subcutaneously twice daily for 14 consecutive days over a 28-day cycle. Cycles should be repeated until the patient achieves a hematologic response. The recommended maintenance schedule is 1.25 mg/m^2 administered subcutaneously twice daily for 7 consecutive days over a 28-day cycle. Treatment should continue as long as the patient is clinically benefiting from the therapy.

Adverse Effects In chronic and accelerated phase patients, the most common adverse reactions (over 20% of patients) include thrombocytopenia, anemia, neutropenia, diarrhea, nausea, fatigue, asthenia, injection site reaction, pyrexia, infection, and lymphopenia. The drug is Pregnancy Category D.

Didemnins are cyclic depsipeptides, originally isolated in 1978 from a tunicate of the genus Tridemnum collected in the Caribbean Sea. A didemnin was the first marine peptide that entered clinical trials in U.S. for the treatment of cancer. Didemnins inhibit protein synthesis and arrest cells in G_1.

[13] The interaction of homoharringtonine with the ribosomal A site prevents the correct positioning of amino acid side chains of incoming aminoacyl-tRNAs.

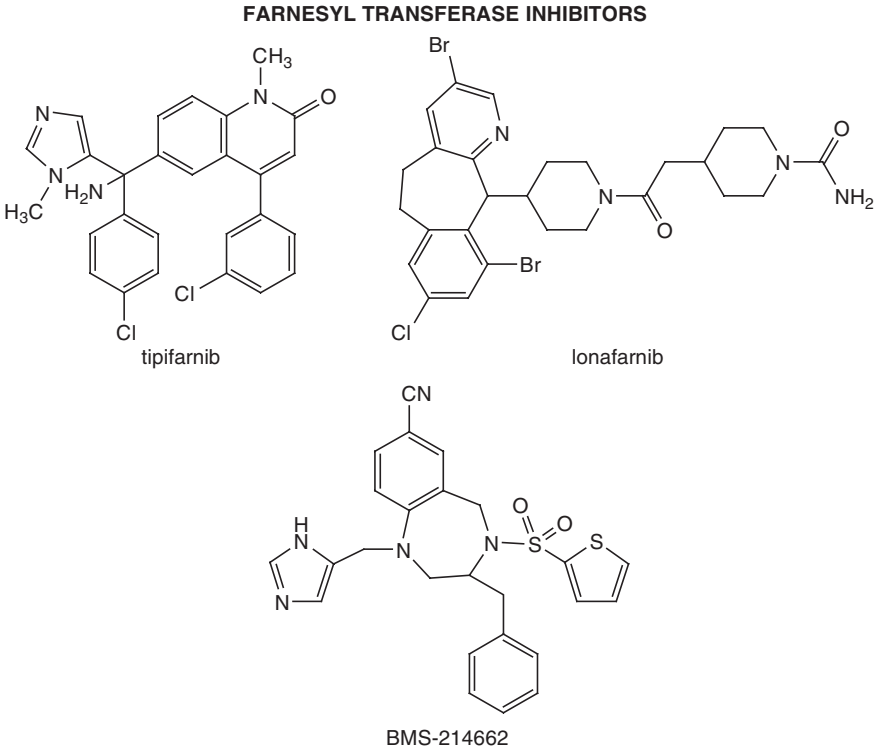

Fig. 4.24 Structures of Farnesyl Transferase inhibitors

They display GTP dependent binding to the translation elongation factor EF1α. They also bind to Palmitoyl Thioesterase (the enzyme removes palmitate from H-RAS and from the $G\alpha_s$ subunits of hetero-trimeric GTP binding proteins). Didemnins are under study against renal adenocarcinoma, advanced epithelial ovarian cancer, and metastatic breast cancer.

- Dihydrodidemnin B (aplidine) is produced by the ascidian Aplidium albicans. It is in clinical trials. In 2003, aplidine was granted orphan drug status by the European Medicines Agency for treating acute lymphoblastic leukemia.

Adverse Effects Didemnins are immunosuppressive. Didemnin B exhibits a high incidence of anaphylactic reactions, which have led to the termination of its clinical development.

Proteasome inhibitors The proteasome is a multi-catalytic protease complex that degrades most endogenous proteins, including those that are misfolded or damaged, to ensure normal cellular function. The attachment of Ubiquitin moieties to a protein typically marks it for transport to the proteasome. The Ubiquitin-proteasome degradation pathway plays essential roles in multiple cellular processes, including cell cycle progression and proliferation. Cancer cells are more sensitive than untransformed cells to proteasome inhibition. Healthy cells often are able to recover from intermittent inhibition of proteasome function, but many types of cancer cells undergo apoptosis when their proteasomes are inhibited longer. The drug names of proteasome inhibitors end on -zomib.

Bortezomib (PS-341, MLN341, LDP-341) <Velcade> is a first-in-class reversible proteasome inhibitor. It exerts its effects in part through its ability to block the activation of the transcription factor NF-κB by preventing the proteasomal degradation of the inhibitor I-κB. Upon activation, NF-κB sends a message for cells to increase the expression of various adhesion molecules, which allow myeloma cells to attach to cells in the bone marrow. This interaction stimulates the bone marrow cells to produce growth and survival factors, such as IL-6 and VEGF. Therefore, by blocking NF-κB, bortezomib blocks myeloma cell growth, inhibits the interactions between myeloma and bone marrow cells, and suppresses angiogenesis.

In 2005, bortezomib was approved by the U.S. FDA. In the same year, the European Commission also approved it for use in mono-therapy for myeloma patients who have received at least one prior therapy and who have already undergone or are unsuitable for stem cell transplantation. The recommended dose of bortezomib for treating multiple myeloma or mantle cell lymphoma is 1.3 mg/m^2 administered as an intravenous injection twice weekly for 2 weeks (days 1, 4, 8, and 11), followed by a 10-day rest period (days 12–21) to complete the cycle. For extended therapy of more than eight cycles, bortezomib may be administered on this standard schedule or on a maintenance schedule of once weekly injections for 4 weeks (days 1, 8, 15, and 22) followed by a 13-day rest period (days 23–35). At least 70–72 h should elapse between consecutive doses.

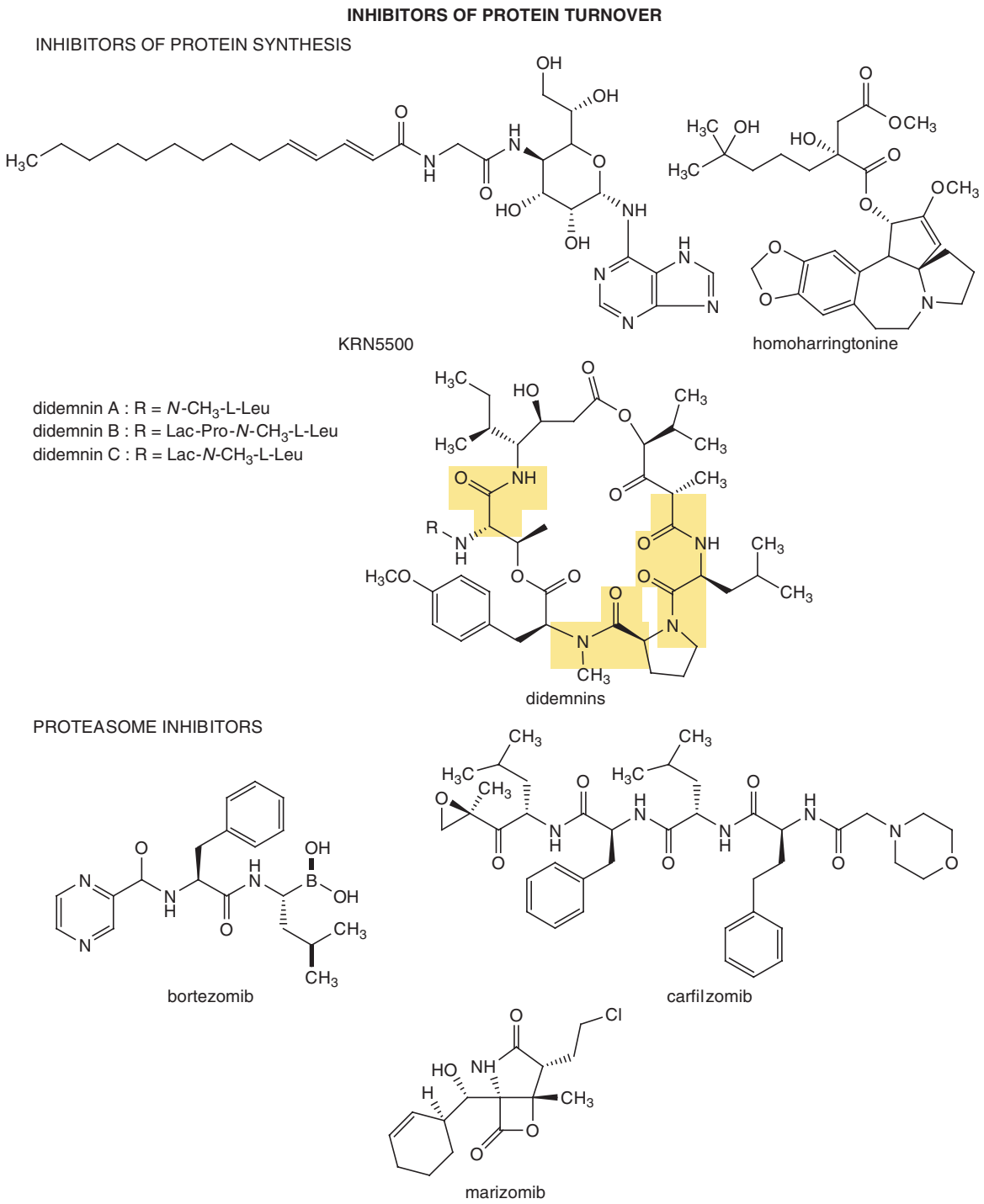

Fig. 4.25 Structures of inhibitors of protein turnover. The peptide bonds in didemnins are highlighted in *yellow*

Pharmacokinetics Bortezomib undergoes hepatic metabolism primarily via the Cytochromes P450 CYP3A4, CYP2C19, and CYP1A2. Its pharmacokinetics is not affected by renal impairment, however, dialysis may reduce the bortezomib blood concentrations.

Adverse Effects Common adverse effects comprise asthenic conditions (fatigue, malaise, weakness) in about 60%, digestive irregularity (60% diarrhea, 40% constipation), nausea (55%) and vomiting (35%), peripheral neuropathy (35%), fever (35%), thrombocytopenia (35%), psychiatric disorders (35%), and decreased appetite or weight loss in 35% of cases. Patients who experience neuropathic pain or peripheral neuropathy (abnormal or decreased sensation, burning, or tingling) should have their dose and schedule

adjusted. Bortezomib is contraindicated in patients with hypersensitivity to bortezomib, boron, or mannitol.

Carfilzomib <Kyprolis> is derived from epoxomicin, a natural product that was found in the laboratory of Craig Crews at Yale University to inhibit the proteasome. The drug is approved for the treatment of patients with multiple myeloma, who have already received at least two other treatments including bortezomib and an immunomodulatory agent (lenalidomide or thalidomide), and whose disease has progressed on or within 60 days of their last therapy. Carfilzomib for injection has efficacy as single agent therapy, however, improvement in survival has not been proven.

Adverse Effects Anemia and thrombocytopenia are the most common adverse effects. Carfilzomib can cause heart problems or worsen pre-existing heart conditions, and death due to cardiac arrest may occur. Shortness of breath arises in 35 % of patients. There is a risk for pulmonary hypertension. Potential infusion reactions include fever, chills, joint or muscle pain, facial flushing or swelling, nausea, weakness, low blood pressure, fainting, and chest tightness. These symptoms can occur immediately following infusion or up to 24 h after administration.

The β-lactone-γ-lactam proteasome inhibitor marizomib ((-)-salinosporamide A) (NPI-0052) was discovered from the marine-obligate actinomycete Salinispora tropica. The agent is distinct from bortezomib in its chemical structure and mechanism of action. It covalently modifies the active site threonine residues of the 20S proteasome. Inhibition of Ubiquitin-proteasome mediated proteolysis leads to an accumulation of poly-ubiquitinated proteins, which may result in the disruption of cellular processes, the induction of apoptosis, and the inhibition of tumor growth. Marizomib can be given orally. It is in clinical trials.

Several natural compounds, including the microbial metabolite lactacystin, green tea polyphenols, and traditional medicinal triterpenes, are potent proteasome inhibitors. This suggests the potential use of natural proteasome inhibitors as chemopreventive and chemotherapeutic agents, but also as tumor sensitizers to conventional radio-therapy and chemotherapy.

HSP90 inhibitors Chaperones, such as HSP90 (90 kD Heat Shock Protein) are critical to maintaining the normal protein folding environment. The HSP90 protein family consists of four members (HSP90α, HSP90β, GRP94, TRAP1). HSP90 comprises three conserved domains, the N-terminal lobe that contains an adenine nucleotide binding pocket (responsible for the ATPase activity), the highly charged middle region (modulates the ATPase activity), and the C-terminal lobe (contains an additional ATP binding pocket). HSP90 mediated protein folding requires energy that depends on the hydrolysis of ATP by the N-terminal ATP binding pocket. This nucleotide binding domain is unique compared to other ATP binding proteins because the nucleotide is bound in a bent conformation[14] as opposed to the extended conformation utilized by most proteins. Activation of HSP90 results in its association with a series of co-chaperones, which form a complex that interacts with client proteins. The increased expression of chaperones enhances cell survival in tissues damaged by a variety of stressors. Such conditions are common within tumors, and there is increased expression of chaperone proteins in several types of solid tumors. Several HSP90 client proteins play important roles in the regulation of the cell cycle and cell survival.

Specific inhibitors of HSP90 have entered clinical trials for metastatic cancer. Inhibition of the HSP90 ATPase activity with high affinity natural product inhibitors results in proteasome mediated degradation of the client proteins (including oncogenic signaling proteins that are over-expressed by tumor cells) and subsequent interruption of their signaling function. Cancers that are not driven by HSP90 client proteins are not sensitive to HSP90 inhibitors.

HSP90 inhibitors may be useful in combination with DNA damaging agents, because of their ability to abrogate S and G_2/M cell cycle checkpoint controls by promoting the degradation of the client kinases CHK1 and WEE1. The effect is independent of the P53 status in the tumor, which is advantageous as about 50 % of cancers are defective in P53 function.

Inhibition of the molecular chaperone HSP90 promotes the transcription of both Major Histocompatibility (MHC) class I encoding genes (*mica* and *micb*) in myeloma cells. MICA and MICB are ligands for a receptor on natural killer cells, which is important for tumor cell recognition and lysis by the immune system. Consequently, HSP90 inhibitors increase myeloma cell stimulation of natural killer cell degranulation, possibly enhancing host immune surveillance.

Ansamycins were discovered in 1959 in the actinomycete Amycolatopsis mediterranei. The ansamycin (ansa macrolide) class of antibiotics is structurally characterized by a medium sized to large macrolide or macrolactam handle fused to a mono- or bicyclic aromatic core. Representatives of this class differ mainly in the aromatic moiety, which can be a naphthalene ring (in rifamycin, naphthomycins), a naphthoquinone ring (in hygrocins), a benzene or benzoquinone ring system (in geldanamycin, ansamitocin). Ansamycins (Fig. 4.26) bind to the N-terminal domain and inhibit the cytosolic chaperone functions of HSP90 via blocking the essential ATPase activity.

[14] This binding conformation is shared by only four proteins, DNA Gyrase, HSP90, Histidine Kinase, and MUT-L.

- Geldanamycin (geldenamycin) was first described in 1970. It is a naturally occurring benzoquinone ansamycin. Geldanamycin preferentially induces the degradation of proteins that are mutated in tumor cells such as v-SRC, BCR-ABL, and P53 over their normal cellular counterparts.
- Tanespimycin (17-allylamino,17-demethoxygeldanamycin, 17-AAG) (NSC 330507) is a benzoquinone derived from geldanamycin. In 1999, it was the first HSP90 inhibitor to enter clinical trials. The drug has possible clinical activity in metastatic melanoma, prostate cancer, and multiple myeloma with manageable toxicity. In clinical trials, it has shown signs of effectiveness against ERBB2-positive breast cancers[15]. However, tanespimycin has unfavorable physiochemical properties, including poor water solubility, making its formulation for clinical delivery difficult.
- Alvespimycin hydrochloride (17-DMAG) (KOS-1022) is a tansepimycin derivative with improved water solubility that may be given intravenously or orally.
- Retaspimycin hydrochloride (IPI-504) is the hydroquinone hydrochloride salt of alvespimycin. It is administered intravenously.
- 17-Aminogeldanamycin (17-AG) (IPI-493) is the major metabolite of tanespimycin (17-AAG) and retaspimycin (17-DMAG). It can be administered as an orally available HSP90 inhibitor.
- The rifamycins are a group of ansamycins, in which the aromatic moiety is a naphthalene ring. They are synthesized either naturally by the bacterium Amycolatopsis mediterranei or synthetically. Beside acting as HSP90 inhibitors, rifamycins may inhibit RNA tumor viruses via inhibition of their DNA Polymerase.
- Maytansinoid anti-tumor agents are members of the ansamycin group. They are 19-membered macrocyclic lactams that target the microtubules (see Sect. 3.1.6.) and thus inhibit cell proliferation.

Adverse Effects Hepatotoxicity (due to the presence of a reactive quinone moiety) limits the use of geldanamycin and its derivatives.

Drug Resistance The co-chaperone of HSP90, Prostaglandin E Synthase 3 (PTGES3, P23), modulates the rate of the HSP90 cycle by affecting the conformational dynamics of the chaperone. Over-expression of PTGES3 protects cells from geldanamycin. NQO1 (NAD(P)H/Quinone Oxidoreductase 1) reduces the quinone moiety of geldanamycin to its dihydroquinone form, which enhances the HSP90 binding properties. A loss of the enzyme NQO1 leads to tanespimycin resistance. The cytotoxic activity of ansamycin antibiotics is compromised by over-expression of BCL-2 in the tumor cells. Ansamycin antibiotics are ABCB1 substrates, and are inactive against cells that express high levels of the export transporter.

HSP90 client proteins play important roles in the regulation of the cell cycle. A pocket region in the N-terminal domain of HSP90 serves as an ATP/ADP-binding site. The macrocyclic antifungal radicicol (monorden) is a natural product that binds to this site. Like ansamycins, radicicol inhibits the binding of the accessory protein P23. It interferes with the assembly of the mature Progesterone Receptor complex and depletes cells of ERBB2, RAF1, and mutant P53. Radicicol does not diminish cellular HSP90, but rather increases synthesis and steady-state levels of this protein, similar to a stress response. Through an ATPase domain common to HSP90, radicicol also binds to and inhibits DNA Topoisomerase 2 and GRP94.

Novobiocin (albamycin, cathomycin), first reported in 1955 under the name streptonivicin (Hoeksema 1955), is an aminocoumarin antibiotic that is produced by the actinomycetes Streptomyces niveus and Streptomyces spheroides, members of the order Actinobacteria. The HSP90 inhibitor engages a C-terminal motif[16]. Whereas small molecule inhibitors of the canonical N-terminal nucleotide binding site (ansamycins, radicicol) have half-maximal inhibitory concentration values in the low nanomolar range, novobiocin exhibits half-maximal inhibition at 700 μM, which is substantially less potent. The agent also inhibits Topoisomerase 2[17], increases the cytotoxicity of several alkylating agents by the formation of lethal DNA-DNA inter-strand cross-links, perhaps by decreasing the repair of drug mono-adducts.

Representatives of the Pu class (purine based molecules) of HSP90 inhibitors specifically bind to the HSP90 N-terminal regulatory pocket. They include Pu24FCl (8-(2-chloro-3,4,5-trimethoxybenzyl)-2-fluoro-9-(pent-4-ynyl)-9Hpurin-6-amine), Pu-H58 (8-(6-bromobenzo[d][1,3]dioxol-5-ylthio)-9-(pent-4-ynyl)-9H-purin-6-amine), and Pu-H71 (8-(6-iodobenzo[d][1,3]dioxol-5-ylthio)-9-(3-(isopropylamino)propyl)-9H-purin-6-amine).

[15] ERBB2 (HER2/NEU) is a HSP90 client protein.

[16] Other coumarin antibiotics, such as clorobiocin and coumermycin A1, also target the HSP90 C-terminal domain. However, the poor affinity of these compounds for HSP90 in conjunction with their high affinity for Topoisomerase 2 has precluded their further development as clinically useful HSP90 inhibitors.

[17] Due to similarities to the ATP binding sites between HSP90 and DNA Gyrase (an enzyme present in prokaryotes and some eukaryotes), novobiocin is also a DNA Gyrase inhibitor.

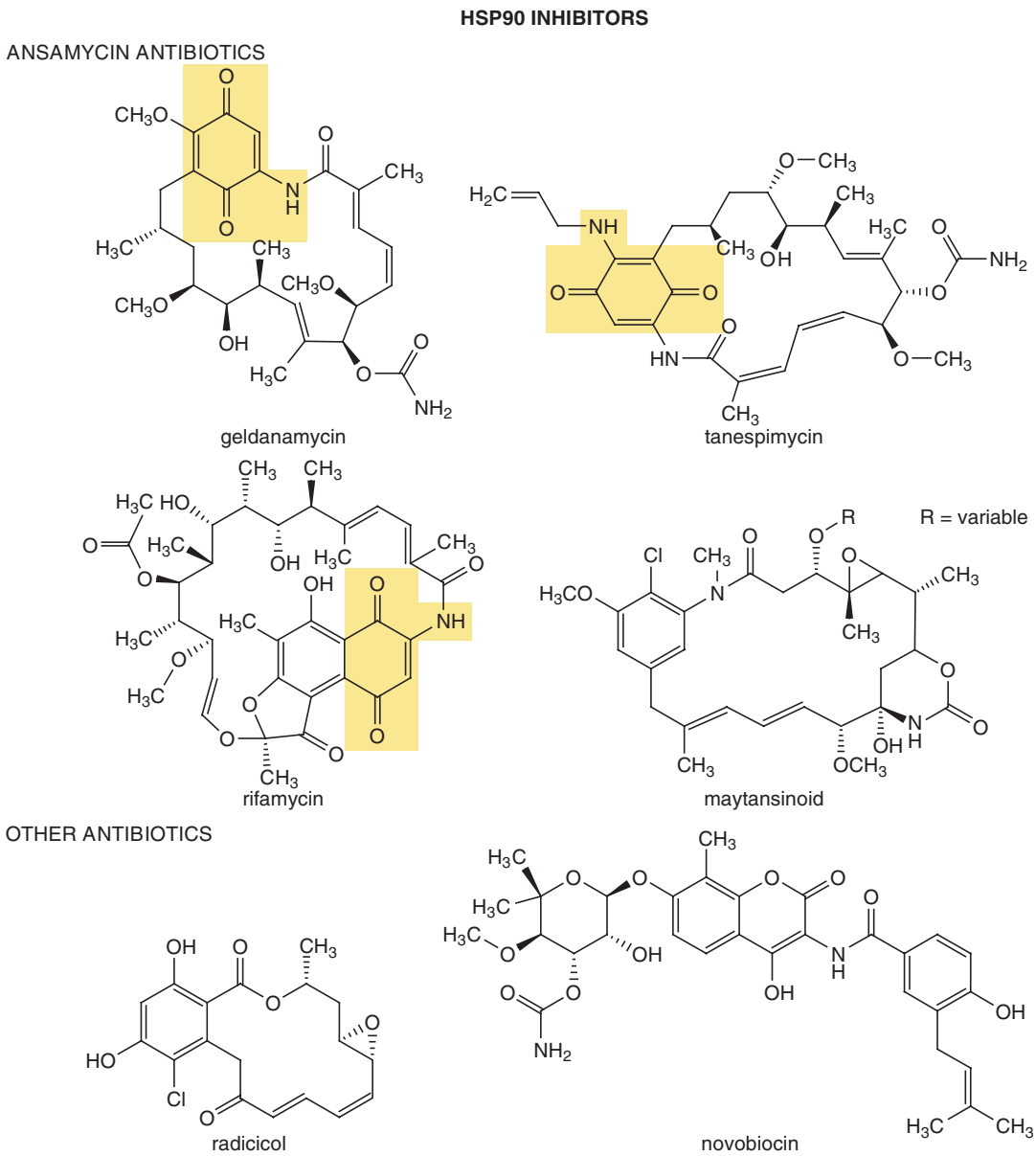

Fig. 4.26 Structures of HSP90 inhibitors. Sub-classes include ansamycin antibiotics, other antibiotics, PU class inhibitors, various inhibitors, and peptidomimetic inhibitors. The amioquinone domain in ansamycins is highlighted in *yellow* (not present in the ansamycin analog mytansinoid), as is the purine domain in the PU class inhibitors

- Pu-H71 exerts durable anti-cancer effects in triple negative breast cancers. It causes the down-regulation of various HSP90 client proteins, including components of the RAS-RAF-MAPK pathway, the PKB pathway, cell cycle regulators, and anti-apoptotic factors. Due to the critical role of HSP90 in the disease, small cell lung cancer is particularly susceptible to HSP90 inhibitors, including Pu-H71, Pu24FCi and Pu-H58.
- BIIB021 (CNF2024) is an orally active 2-amino-6-halopurine HSP90 inhibitor (Kasibhatla 2007) that depletes NF-κB. It may sensitize tumors to radio-therapy or immune surveillance. The cytotoxic activity of BIIB021 is also not influenced by loss of NQO1 or BCL-2 over-expression.

Pharmacokinetics The Pu class of compounds exhibits favorable pharmacological features, including negligible micro-environmental inactivation.

Drug Resistance Pu inhibitors are not subject to ABCB1 mediated export.

Treatment with AUY922 (5-(2,4-dihydroxy-5-isopropylphenyl)-N-ethyl-4-[4-(morpholinomethyl)phenyl]isoxazole-3-carboxamide) results in the binding of HSP90 to client proteins and setting them up as targets for degradation by the proteasome. AUY922 causes P23 dissociation from HSP90 and can then recruit HSP70 to the HSP90 complex. The agent reduces the expression of VEGFR-1, -2, -3, PDGFR-α,

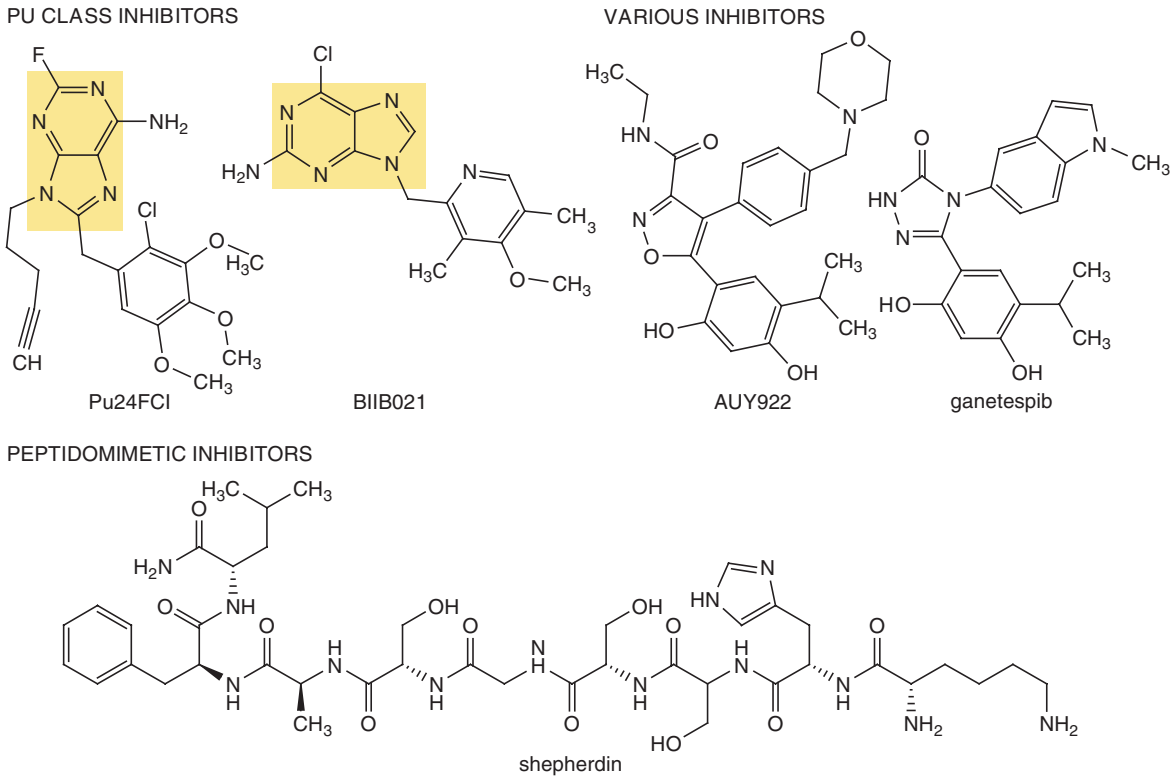

Fig. 4.26 (continued)

and PKB (AKT). AUY922 does not influence the expression of HSP90, but like other HSP90 inhibitors it leads to a compensatory increase in expression of HSP70. The agent is administered intravenously.

Ganetespib (STA-9090) is an intravenous HSP90 inhibitor that also inhibits multiple kinases with comparable potency. The drug suppresses c-KIT and EGFR, which as client proteins depend on functional HSP90 for maintenance. In chronic myelogenous leukemia (CML) caused by the chimeric oncoprotein BCR-ABL, the secondary mutation BCR-ABL^{T315I} is cross-resistant to several tyrosine kinase inhibitors, however, the transformed cells remain sensitive to HSP90 inhibition. Compensatory elevation of HSP72 levels occurs. Treatment with ganetespib has shown objective responses in clinical trials.

Adverse Effects The most common adverse event is diarrhea, which is manageable with standard supportive care. There is no evidence of the serious bone marrow toxicities and neuropathy that are associated with conventional chemotherapy, or of the severe liver and common ocular toxicities associated with other HSP90 inhibitors.

Shepherdin (L-lysyl-L-histidyl-L-seryl-L-serylglycyl-L-cysteinyl-L-alanyl-L-phenylalanyl-L-leucine) is a peptidomimetic drug. It was modeled on the binding interface between the molecular chaperone HSP90 and the anti-apoptotic and mitotic regulator Survivin. The agent makes extensive contacts with the ATP pocket of HSP90, destabilizes its client proteins (specifically Survivin), and induces death of tumor cells by apoptotic and non-apoptotic mechanisms. The fusion of its N-terminus to either helix III of the Antennapedia homeodomain protein or the human immunodeficiency virus TAT sequence has generated membrane permeant forms of Shepherdin. They accumulate in the mitochondria, bind to HSP90 and TRAP1, and rapidly trigger Cyclophilin D mediated mitochondrial pore transition and cell death, independently of P53 and BCl-2 expression levels.

Protein synthesis inhibitors do not target cancer specific mechanisms, but they reduce the levels of proteins that are over-abundant in cancers.
Protein turnover can be targeted with inhibitors of protein synthesis, proteasome inhibitors, or HSP90 inhibitors.
The proteasome is a clinically relevant target for the treatment of hematologic malignancies.
Only cancers that are driven by HSP90 client proteins are sensitive to HSP90 inhibitors.
The exposure to HSP inhibitors may lead to a compensatory up-regulation of HSP proteins.

4.2.3 Others

The Notch, Hedgehog, and WNT pathways may facilitate the expansion of the stem-like cancer initiating cells. This

sub-population is often responsible for the aggressiveness of a tumor. It also tends to be highly treatment resistant against conventional chemotherapy. Somatostatin is an endocrine suppressor. It may exert growth inhibitory effects on some tumors.

Notch signaling The Notch signaling pathway importantly contributes to cell fate determination, cell survival, and cell proliferation. However, the outcome of Notch signaling is highly context dependent. The engagement by one of the Notch ligands, Delta Like-1 (DLL-1), Delta Like-3 (DLL-3), Delta Like-4 (DLL-4), Serrate/Jagged-1 (JAG-1), or Serrate/Jagged-2 (JAG-2), presented on adjacent cells, initiates a proteolytic cascade that leads to its cleavage and release of the Notch intracellular domain, which then translocates to the nucleus and functions as a transcriptional activator. This setting causes the gene expression of *hes-1* and may lead to the regulation of programmed cell death (Fig. 4.27).

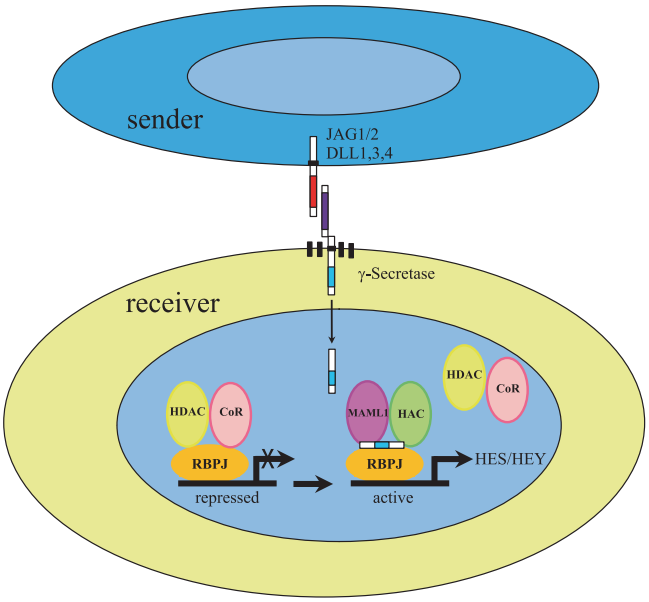

Fig. 4.27 Core components of the canonical Notch signaling pathway. Ligands of the Jagged (*JAG1* and *JAG2*) and Delta-Like (*DLL1*, *DLL3*, *DLL4*) families (*upper* cell, shown in *green*) interact with Notch family receptors (NOTCH1 through to NOTCH4) on an adjacent cell (*lower* cell, shown in *yellow*). The Notch receptor exists at the cell surface as a proteolytically cleaved hetero-dimer consisting of a large ectodomain and a membrane-tethered intracellular domain. The receptor-ligand interaction induces two additional proteolytic cleavages that free the Notch Intracellular Domain (*NICD*) from the cell membrane. The NICD translocates to the nucleus (*blue*), where it forms a complex with the *RBPJ* protein, displacing a Histone Deacetylase (*HDAc*) co-repressor (*CoR*) complex from the *RBPJ* protein. Components of an activation complex, such as *MAML1* and Histone Acetyl Transferases (*HAc*), are recruited to the NICD-RBPJ complex, leading to the transcriptional activation of Notch target genes. (Redrawn from Gridley 2007)

Ligand binding leads the release of NICD (Notch Intracellular Domain) from the endothelial cell plasma membrane by γ-Secretase dependent proteolytic cleavage of Notch. NICD translocates to the nucleus, where it binds to the transcription repressor CSL thus activating the transcription of target genes. Inhibitors of γ-Secretase can suppress Notch signaling.

MK0752 (Fig. 4.28) is a synthetic small molecule that inhibits the Notch signaling pathway by inhibiting γ-Secretase[18]. The agent may cause growth arrest and apoptosis in tumor cells, in which the Notch signaling pathway is over-activated. The drug can be administered orally.

Adverse Effects Dose limiting toxicities include grade 3 diarrhea or constipation, nausea, and abdominal cramping. Grade 2–3 fatigue may require dose reduction.

RO4929097 is a selective inhibitor of γ-Secretase. The drug suppresses Notch signaling. While it does not block tumor cell proliferation or induce apoptosis, it produces a less transformed, slower growing phenotype. The agent is in clinical studies for advanced cancer.

Hedgehog pathway Signaling by the Hedgehog family of secreted glycoproteins contributes to the determination of embryonic cell fate, the maintenance of somatic cell fate, the specification of organ size, and the patterning of skin, lung, brain, bone, and blood. Hedgehog (HH) signaling promotes the expression of G_1/S Cyclins, including Cyclins D and E, and results in the growth of cells. It also opposes epithelial cell cycle arrest through $P21^{CIP1/WAF1}$ (Fig. 4.29).

Vismodegib <Erivedge> inhibits the Hedgehog signaling pathway. The agent acts as a cyclopamine-competitive antagonist of the receptor SMO (Smoothened) which is part of the hedgehog signaling pathway. SMO inhibition causes the transcription factors GLI1 and GLI2 to remain inactive, which prevents the expression of oncogenes within the Hedgehog pathway. In 2012, vismodegib gained U.S. FDA approval. The drug is indicated for patients with basal cell carcinoma, which has spread, relapsed after surgery, or cannot be treated with surgery or radiation. Vismodegib is also undergoing clinical trials for basal cell naevus syndrome (Grolin syndrome), metastatic colorectal cancer, small cell lung cancer, advanced stomach cancer, pancreatic cancer, medulloblastoma and chondrosarcoma.

Adverse Effects Vismodegib has a high rate of adverse effects, including dysgeusia (loss of taste), gastrointestinal disorders (nausea and vomiting, diarrhea or constipation), hair loss, muscle cramps, and fatigue, which lead to frequent of non-compliance.

[18] The γ-Secretase complex is composed of four proteins, Presenilin, Nicastrin, APH-1, and PEN-2.

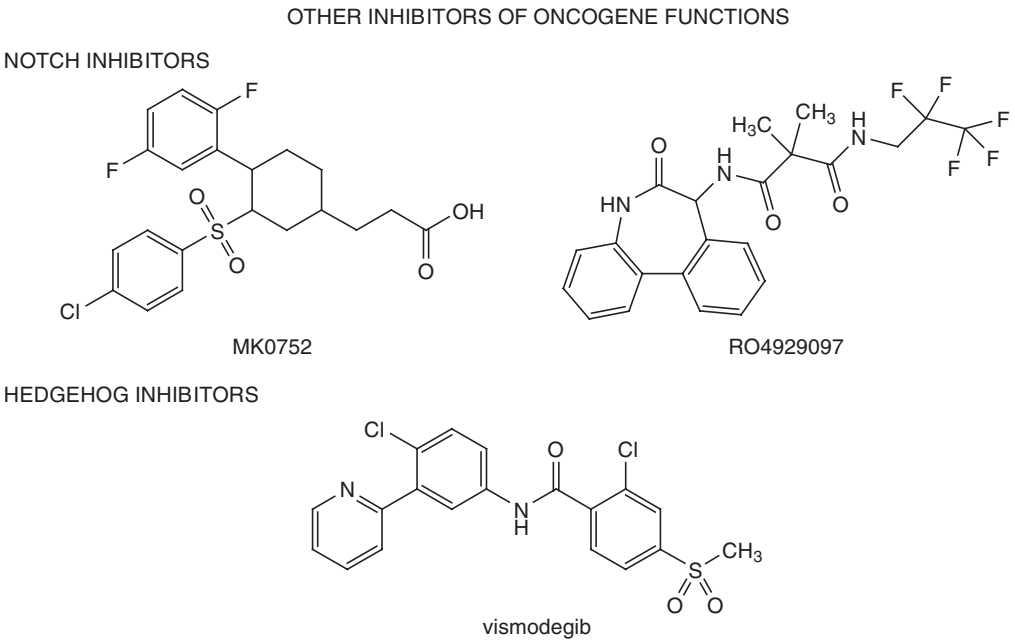

Fig. 4.28 Structures of various inhibitors of oncogene function

Somatostatin Somatostatin is an endogenous cyclic tetradecapeptide that inhibits the release of growth hormone and all gastrointestinal hormones. Somatostatin Receptors are expressed by some neuroendocrine and non-neuroendocrine tumor cells.

The mode of action of Somatostatin analogs is the suppression of hormone production, particularly Growth Hormone and serotonin. It helps to control the diarrhea and skin flushing associated with carcinoid syndrome[19]. Reducing these hormones also has anti-proliferative effects on some tumors, involving the hyper-phosphorylation of RB with resulting G_1 cell cycle arrest in addition to Somatostatin Receptor mediated apoptosis. Somatostatin analogs may enhance the therapeutic effects of hormonal intervention in patients with breast cancer, prostate cancer, or pancreatic cancer. However, only a small number of patients treated with Somatostatin analog therapy experience partial tumor regression.

Pharmacokinetics The clinical utility of native Somatostatin has been limited by its short half-life of 1–3 min. This has led to the development of Somatostatin analogs that are long-acting and more potent than native Somatostatin-14. The typical duration of treatment with Somatostatin analogs is approximately 12 months because of the development of tachyphylaxis (less frequent with long-acting formulations than short-acting formulations) or disease progression.

Octreotide pamoate (SMS 201–995) <Sandostatin> is a synthetic, long-acting octapeptide analog of Somatostatin. It binds to Somatostatin Receptors, thereby initiating apoptosis. Other possible anti-neoplastic activities of this agent include the suppression of the tumor growth promoting effects of IGF-1 (Insulin Like Growth Factor 1). The main indication is carcinoid syndrome. The drug is administered up to three times per day subcutaneously. The half-life is 40–60 min after intravenous administration and close to 2 h when given subcutaneously.

Adverse Effects The main adverse effects are loss of appetite, nausea, fatigue, bloating and stomach pain, diarrhea or steatorrhea, and soreness at the injection site. Over the course of months, gallstones may develop.

Drug Interactions Drugs that prolong the QT interval of the electrocardiogram or that slow down the heart (β−blockers, digoxin), certain blood pressure medicines (calcium channel blockers), and anti-arrhythmic drugs can cause dangerously slow heartbeats. Agents that lower the blood potassium or magnesium levels (such as diuretics) can worsen this effect. As somatostatin analogs affect the blood sugar levels, diabetic patients may need to reduce their medication or Insulin. Octreotide may lower the levels of cyclosporine and thus require an increase in cyclosporine dose.

Lanreotide <Somatuline LA> is a long acting (slow release) drug which is given as an intramuscular injection every 2 or 4 weeks. The effects of lanreotide on symptom relief are comparable to those of octreotide, with 75–80% of carcinoid syndrome patients having decreased diarrhea

[19] Carcinoid syndrome may be caused by over-abundant endogenous secretion of serotonin and kallikrein. It manifests in flushing and diarrhea, as well as possibly heart failure and bronchoconstriction.

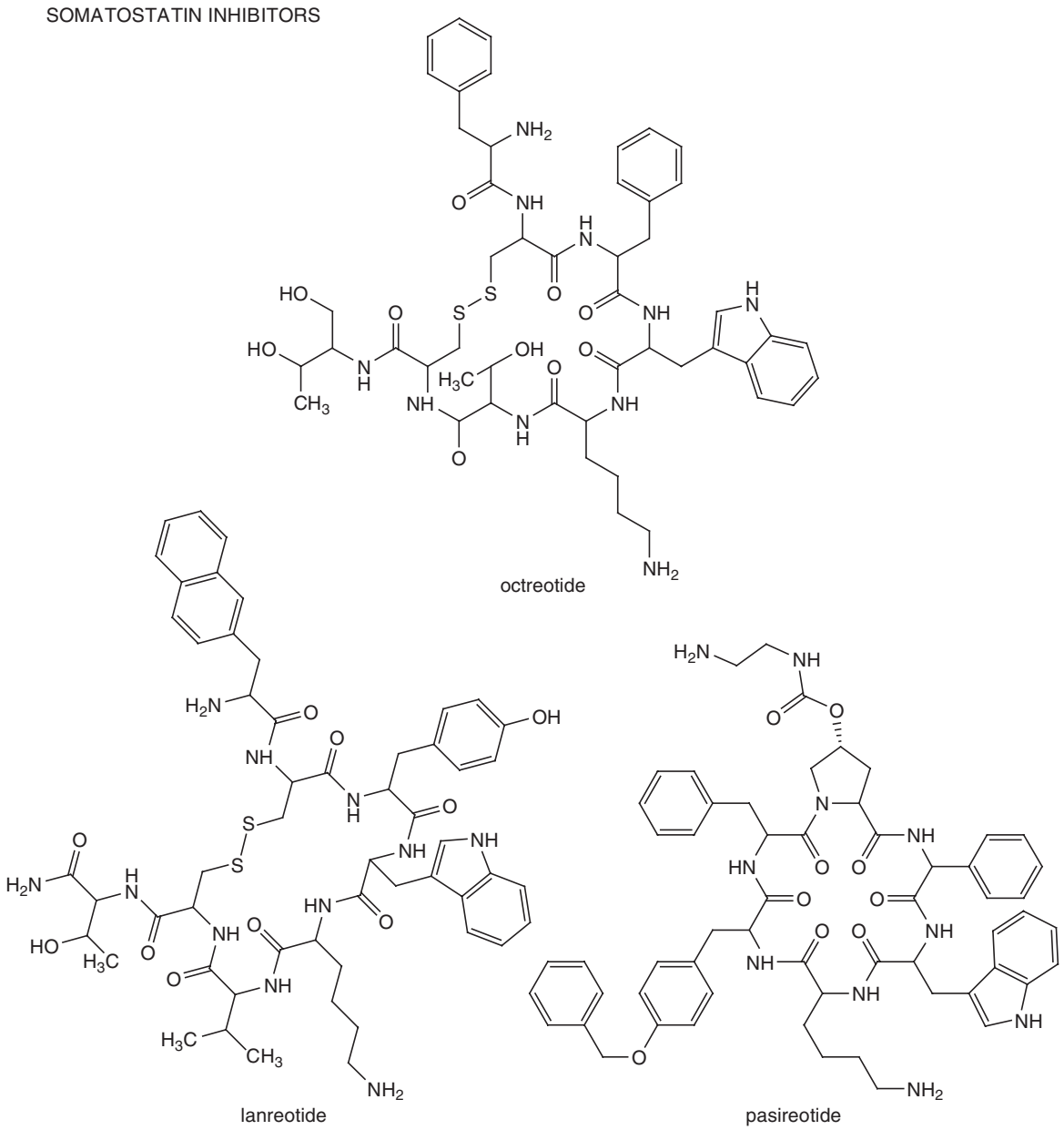

Fig. 4.28 (continued)

and flushing. However, there is little improvement in tumor responses over octreotide.

Pasireotide <Signifor> is injected subcutaneously 1–2 times per day. It is used to treat Cushing disease, a common syndrome associated with pituitary tumors. The drug is also under study to treat carcinoid tumors.

Vapreotide (octastatin) (RC-160) is an octapeptide analog of Somatostatin. It is under study for various palliative applications.

Adverse Effects Abscess formation at the infusion site, erythema, and discomfort have occurred in clinical studies.

Ligand and receptors that contribute to cell fate decisions may also facilitate the expansion of cancer stem cells. These pathways are suitable drug targets in cancer therapy.

4.3 Antibodies

The investigation of antibodies began in 1890, when Emil von Behring and Shibasaburo Kitasato described antibody activity against diphtheria and tetanus toxins. They put forward the theory of humoral immunity, proposing that a mediator in serum could react with a foreign antigen. In the 1920s, Michael Heidelberger and Oswald Avery observed that an-

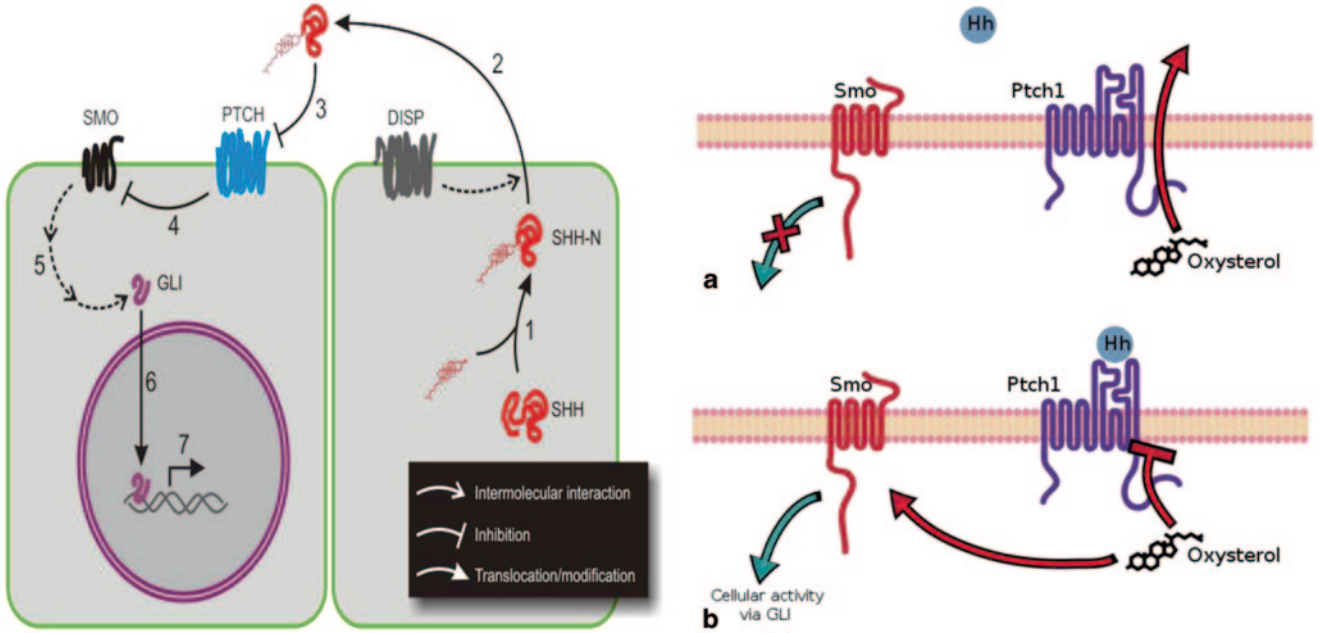

Fig. 4.29 The Hedghog signaling pathway. (*Left panel*) Overview of Sonic Hedgehog signaling. (*Right panel*) Pathway of Smoothened (*Smo*) regulation via Hedgehog (*Hh*) and Patched (*Ptch1*) When the protein Hedgehog is absent (*A*), the transmembrane Patched (*Ptch1*) removes oxysterols, thus preventing them from interacting with the transmembrane protein Smoothened (*Smo*). When Hedgehog is bound to Patched (*B*), *Ptch1* no longer removes oxysterols, allowing them to accumulate and activate *Smo*. (http://en.wikipedia.org/wiki/Hedgehog_signaling_pathway)

tibodies are proteins that can precipitate antigens. In 1948, Astrid Fagreaus discovered that plasma cells (differentiated B-lymphocytes) were responsible for generating antibodies. Consecutively in the 1950s and 1960s, many efforts to devise "magic bullets" for targeted cancer therapy were undertaken[20], but stalled due to technical limitations. In 1975, Köhler and Milstein develoepd hybridoma technology for the essentially unlimited generation of monoclonal antibodies. These agents quickly became valuable diagnostic tools (Yelton 1981). The specificity of binding also made antibodies attractive as potential therapeutics and reignited thoughts in the cancer research community about the potential of therapeutic antibodies being used to treat cancer, as illustrated by the 1984 publication of the book "Magic Bullets" (Fjermedal 1984). The development of antibodies as therapeutic agents, however, was hampered by the immune response of patients, which readily inactivated murine antibodies. A fast development of human anti-mouse antibody (HAMA) responses in the treated patients accounted for an accelerating clearance rate, resulting in ineffectiveness of the treatment after about 3 weeks[21]. HAMA responses could also be associated with symptoms that can range from mild (fever, chills, nausea, rash) to severe, life threatening anaphylaxis with cardiopulmonary collapse. Technical advances in antibody humanization were therefore required to gain safety and efficacy. To this end, the first chimeric antibodies were generated in the late 1980s (Liu 1987; Riechmann 1988), later followed by CDR grafting (Fig. 4.30). In 1997, the first therapeutic anticancer antibody, rituximab, was approved by the U.S. FDA for the treatment of B-cell non-Hodgkin lymphoma[22]. Since then, monoclonal antibody based therapies have become a major strategy in cancer treatment. The humanization of antibodies has allowed them to function as drug molecules. They are generally very well tolerated and lead to clinical results. However, none of them are able to cure cancer as single agents.

The fundamental structure of all antibodies is identical and consists of two heavy chains and two light chains joined by disulfide bonds. The constant region (Fc fragment) determines the effector function of the antibody. The Fab portion is composed of heavy (V_H) and light chains (V_L), which are

[20] The metaphoric term "magic bullets" to describe ideal therapeutic agents goes back to Paul Ehrlich, who reasoned that the selective targeting of a disease causing agent would provide a therapeutic with high efficacy and low toxicity.

[21] The immunologic memory generated in the HAMA response renders ensuing treatments with xenogeneic antibodies of the same species ineffective for the life of the patient.

[22] The first monoclonal antibody to be approved as a drug was muromonab (anti-CD3), which received U.S. FDA approval in 1986 as an immunosuppressant to reduce the acute rejection of organ transplants.

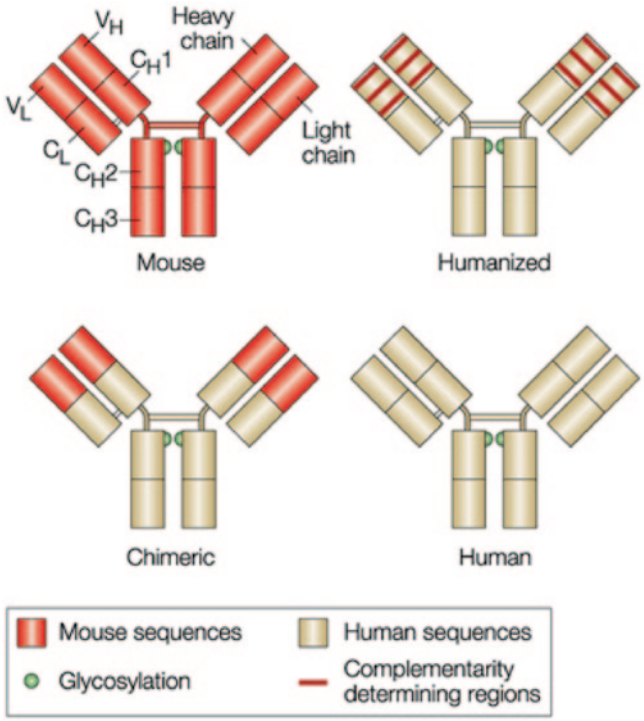

Fig. 4.30 Antibody humanization. Murine antibodies: Derived by hybridoma technology following immunization of mice or, less commonly, rats. Chimeric antibodies: Obtained by joining the antigen-binding variable domains of a mouse monoclonal antibody (*mAb*) to human constant domains: mouse *VL* to human *CL* and mouse *VH* to human *CH1–CH2–CH3* for *light* and *heavy* chains, respectively. Humanized antibodies: In the simplest case, these are created by grafting the antigen-binding loops, known as complementarity-determining regions (*CDRs*), from a mouse mAb into a human IgG. The generation of high-affinity humanized antibodies generally requires the transfer of one or more additional residues from the so-called framework regions (*FRs*) of the mouse parent mAb. Several variants of the humanization technology have been developed. Human antibodies: These have high affinity for their respective antigens and are routinely obtained from very large, single-chain variable fragments (*scFvs*) or Fab phage display libraries. Moreover, the difficulty in obtaining antibodies to self-antigens that are highly conserved between mouse and humans using hybridoma technology is readily overcome using phage display technology. High-affinity human antibodies have also been obtained from transgenic mice that contain some, or preferably many, human immunoglobulin genes and genetically disrupted endogenous immunoglobulin loci. Immunization elicits the production of human antibodies recoverable using standard hybridoma technology. A human anti-epidermal growth factor (*EGF*) receptor mAb obtained using transgenic mice eradicates large, established tumours in some preclinical xenograft models10, auguring well for ongoing oncology trials. (Carter 2001 with permission)

responsible for binding to antigens. Within the Fab fragment, the variable region of antibodies differs greatly and is composed of three complementarity determining regions (CDRs) on each arm. Most of the therapeutic antibodies used in oncology are of the IgG_1 sub-class, which is most effective at engaging Fcγ receptors on natural killer cells, macrophages, and neutrophils.

Antibodies can suppress tumor growth in various ways.

- Antibodies may induce anti-tumor immune responses by complement dependent cytotoxicity (CDC). It results when the Fc portion of the antibody activates the complement system leading to tumor cell lysis.
- Antibodies can stimulate antibody dependent cellular cytotoxicity (ADCC). Effector cells that contain Fc Receptors bind to the Fc portion of the antibody and either lyse or phagocytose the antibody bound cells. Natural killer cells, monocytes, and macrophages are all capable of mediating ADCC.
- Most therapeutic antibodies neutralize growth factor receptors on the tumor cell surface by blocking their interactions with their cognate ligands.
- Activating antibodies may be therapeutic if they induce signals that lead to cell cycle arrest or apoptosis.

Several of these mechanisms may synergize to produce the therapeutic effect.

The nomenclature of monoclonal antibodies assigns generic (non-proprietary) names to therapeutic monoclonal antibodies. The World Health Organization's International Nonproprietary Names defines this scheme. In general, suffixes are used to identify classes of medicines; all monoclonal antibody pharmaceuticals end with the suffix -mab. It is preceded by an infix that denotes the animal origin of the antibodies. The infix preceding the source of the antibodies refers their targets. Most of these consist of a consonant, vowel, then another consonant. For ease of pronunciation, the final consonant is dropped if the following infix begins with a consonant. The prefix carries no special meaning and should be unique for each antibody. A second word may be added if there is another substance attached or linked to the antibody (Table 4.13).

Pharmacokinetics Antibodies are protein drugs of about 150 kD molecular mass. They cannot be given orally and are administered by injection. The half-life of antibody drugs is comparatively long. This is in part due to the minor role played by drug-metabolizing enzymes in the liver. Antibodies are internalized in cells of the monocyte-phagocyte system (MPS, reticuloendothelial system, RES), either directly or by phagocytosis after binding to their target cells. This is followed by catabolism via proteolysis. Antibody half-life and degradation are also regulated by binding to the neonatal receptor FcRn, mostly on cells of the monocyte-phagocyte system. This binding initially protects from clearance. After dissociation, the IgG is taken up into endosomes and degraded.

Adverse Effects To avoid anaphylactic reactions, most antibodies require pre-medication with anti-histamines and acetaminophen (e.g. 25–50 mg diphenhydramine, 650 mg acetaminophen orally). Recommended infusion rates are usually lower for the initial dose, with incremental increases as tolerated by individual patients. Chills during the infu-

Table 4.13 Stems for monoclonal antibody nomenclature. (Adapted from Wikipedia)

Prefix	Target substem				Source substem	Stem
	Old	New	Meaning		Meaning	
Variable	*-anibi-*	–	Angiogenesis (inhibitor)	*-a-*	Rat	*-mab*
	-ba(c)-	*-b(a)-*	Bacterium	*-e-*	Hamster	
	-ci(r)-	*-c(i)-*	Circulatory system	*-i-*	Primate	
	-fung-	*-f(u)-*	Fungus	*-o-*	Mouse	
	-ki(n)-	*-k(i)-*	Interleukin	*-u-*	Human	
	-les-	–	Inflammatory lesions	*-xi-*	Chimeric (human/foreign)	
	-li(m)-	*-l(i)-*	Immune system	*-zu-*	Humanized	
	-mul-	–	Musculoskeletal system	*-xizu-*	Chimeric/humanized hybrid rat/mouse hybrid (including *trifunctional antibody*)	
	-ne(u)(r)-	*-n(e)-*	Nervous system	*-axo-*		
	-os-	*-s(o)-*	Bone			
	-toxa-	*-tox(a)-*	Toxin			
	-co(l)-		Colonic tumor			
	-go(t)-		Testicular tumor			
	-go(v)-		Ovarian tumor			
	-ma(r)-	*-t(u)-*	Mammary tumor			
	-me(l)-		melanoma			
	-pr(o)-		prostate tumor			
	-tu(m)-		miscellaneous tumor			
	-vi(r)-	*-v(i)-*	virus			

sion may require medication (e.g. 25 mg intravenous meperidine). For patients experiencing symptoms of serious infusion related reactions, the administration needs be interrupted and prompt treatment with corticosteroids and other supportive measures needs to be initiated. Pulmonary toxicity may occur as part of the infusion related reaction or as a distinct entity. Additionally, the toxicities of monoclonal antibodies are determined by the selectivity of their target antigens.

Antibodies may suppress cancer by inducing complement mediated cytotoxicity, antibody dependent cytotoxicity, inhibition of oncogenic signaling, or activation of cell cycle arrest or apoptosis.
Administration is by infusion.
As foreign proteins, antibodies may induce anaphylactic infusion reactions. The risk is highest at the first infusion and can be reduced by pre-treatment.

4.3.1 EGFR Family Targets

The growth factor EGF (Epidermal Growth Factor) is a 53 amino acid polypeptide, constrained by 3 internal disulfide bonds. It stimulates many epithelial and mesenchymal cells. EGF binds to a transmembrane receptor, which then becomes internalized and activated as a tyrosine kinase. The EGF Receptor was first cloned as a surface molecule related to a viral oncogene in 1984. The four related genes *erbB1* (*egfr*), *erbB2* (*her-2*/*neu*), *erbB3* (*her-3*), and *erbB4* (*her-4*) encode the family of EGF Receptor tyrosine kinases. They are composed of a cysteine rich extracellular domain, a single transmembrane domain, and an intracellular catalytic domain. ERBB2 (HER-2/NEU, $P185^{HER2}$) transduces signals through Phospholipase C, phosphatidylinositol 1,4,5-trisphosphate, and diacylglycerol. Ensuing effects are the cellular influx of calcium ions from the exterior and the up-regulation of the anti-apoptotic proteins BCL-X_L and BCL-2. ERBB2 is over-expressed by various adenocarcinomata including breast adenocarcinoma.

anti-ERBB2 HER-2/NEU (ERBB2) is a member of the EGF Receptor family that is amplified in about 30% of breast cancers. Trastuzumab (anti-HER-2/NEU monoclonal antibody) <Herceptin> is a recombinant humanized monoclonal antibody directed to the extracellular domain IV of ERBB2. The drug was jointly developed by Axel Ullrich at Genentech and Dennis Slamon at the Jonsson Cancer Center at UCLA by humanizing a mouse monoclonal antibody (antibody 4D5, previously defined in 1990). Trastuzumab received U.S. FDA approval in 1998. Since 2006, the antibody has been made available through a public benefits scheme for Australian women with early stage breast cancer. As a molecularly targeted treatment, trastuzumab benefits patients whose cancer cells over-express ERBB2. Routine ERBB2 status analysis is performed on tissue biopsies by immunohistochemistry to stratify patients. Multiple mechanisms may contribute to the therapeutic effects of this antibody:

- After binding to its cognate receptor on the tumor cell surface, trastuzumab induces antibody dependent cell mediated cytotoxicity against tumor cells that over-express ERBB2.
- Cells treated with trastuzumab undergo arrest during the G_1 phase of the cell cycle. Trastuzumab induces a down-regulation of ERBB2, leading to the disruption of receptor dimerization and signaling through the downstream PI-3 Kinase cascade.
- A contribution to the unregulated growth of breast cancer cells may be the proteolytic cleavage of ERBB2 that releases the extracellular domain and results in constitutive activation of the tyrosine kinase domain with consecutive unrestricted cell cycle progression. Trastuzumab inhibits ERBB2 ectodomain cleavage by blocking the protease site on domain IV.

Based on sensitization theory, the co-administration of trastuzumab and ERBB ICD (a peptide fragment of the C-terminal and the intracellular domain of ERBB2) may result in the potentiation of an ERBB specific cytotoxic T-lymphocyte response in breast cancer patients via MHC class I antigen presentation.

- Ado-trastuzumab emtansine (T-DM1) <Kadcyla> is an antibody-drug conjugate consisting of trastuzumab linked to the cytotoxic agent mertansine (DM1). The linker is designed not to be cleaved by lysosomal proteases. The drug is administered by injection for intravenous use, as a single agent. It is indicated for the treatment of patients with ERBB2-positive, metastatic breast cancer, who previously received trastuzumab and a taxane, separately or in combination.

Pharmacokinetics Although trastuzumab treatment is the standard of care for ERBB2-positive breast cancer, it shows only a 15% response rate as a mono-therapy and a 50% response rate in combination with paclitaxel. The antibody is administered either once a week or once every 3 weeks intravenously over 30–90 min.

Adverse Effects ERBB2 is an important developmental growth factor receptor, which is vital to normal cardiac development and myocardial trabeculation. One of the substantial complications associated with trastuzumab is cardiac dysfunction in about 5% of cases. Approximately 10% of patients are unable to tolerate this drug because of pre-existing heart problems. The risk of cardiomyopathy is increased when trastuzumab is combined with anthracycline chemotherapy, which itself has cardiac toxicity (myocardial ERBB2 may be unregulated early in response to anthracycline injury, rendering the myocardium particularly vulnerable to trastuzumab exposure).

Drug Resistance A problem of therapy with trastuzumab is the rapid development of resistance in virtually all patients. It is based on

- truncation of the receptor that leads to reduced antibody binding but retains active signaling,
- activation of downstream signaling via PTEN inactivation or increased Protein Kinase B activity, or
- compensation due to alternative signaling; one potential underlying mechanism is the lack of $P27^{KIP1}$ translocation into the nucleus, which enables CDK2 to sustain cell proliferation.

Pertuzumab (2C4) <Perjeta> is a humanized monoclonal IgG_1 antibody designed to target ERBB2 at the extracellular domain II. It blocks the formation of ERBB2 heterodimers with other members of the ERBB family and thus reduces signaling through the multiple pathways associated with ERBB2 activation (Agus 2005). Because it inhibits ERBB2/ERBB3 hetero-dimer formation, pertuzumab may be useful in suppressing ERBB3 over-expressing tumors, some of which include ovarian, breast, prostate, colon, and lung cancers. The agent is given in combination with trastuzumab (which binds a different domain of the receptor) and docetaxel. It received U.S. FDA approval in 2012.

Adverse Effects Common adverse effects in combination therapy (with trastuzumab and docetaxel) comprise diarrhea, alopecia, neutropenia, nausea, fatigue, and rash. Grade 3–4 adverse reactions may include neutropenia, leukopenia, anemia, diarrhea, or peripheral neuropathy. Other adverse reactions to pertuzumab can include left ventricular dysfunction, infusion reactions, and hypersensitivity. The antibody may be associated with embryo-fetal toxicity and birth defects.

anti-ERBB1 Cetuximab <Erbitux> is a chimeric monoclonal antibody, composed of the Fv regions of a murine anti-EGFR antibody (m225) with human IgG_1 heavy and κlight chain constant regions and has an approximate molecular weight of 152 kD. It is specific for ERBB1 (Epidermal Growth Factor Receptor, EGFR, HER1), a receptor tyrosine kinase that triggers cell division. Cetuximab inhibits EGF Receptor dependent signal transduction and leads to the inhibition of tumor growth by cancers that over-express EGF Receptors. The agent can mediate antibody dependent cellular cytotoxicity (ADCC) against certain tumor types. Cetuximab was approved by the U.S. FDA in 2004 for the second-line treatment of metastatic colorectal cancer with over-expression of ERBB1. Treatment with cetuximab is given intravenously,

- in combination with irinotecan <Camptosar> for patients whose tumor growth has progressed after receiving chemotherapy with irinotecan
- as a single agent for patients who are unable to tolerate chemotherapy with irinotecan.

Cetuximab, in combination with radiation therapy, is indicated for the treatment of locally or regionally advanced squamous cell carcinoma of the head and neck. It may also be

indicated as mono-therapy for the treatment of patients with recurrent or metastatic squamous cell carcinoma of the head and neck, for whom prior platinum based therapy has failed.

Pharmacokinetics Cetuximab is formulated in a preservative-free salt solution for injection. The half-life is approximately 110 h. In patients with colorectal cancer, female patients have a 25% lower intrinsic clearance of cetuximab than male patients. However, the gender difference in clearance does not necessitate any alteration of dosing because of comparable safety profiles.

Adverse Effects Severe allergic reactions, with symptoms including difficulty breathing, rash, itching, low blood pressure, and loss of consciousness occur in about 3% of patients treated. On rare occasions, these reactions can result in death. Severe cases require that treatment with cetuximab be stopped immediately and not started again. Additional adverse effects include serious lung disease (1%), fever (5%), infection, kidney failure, and pulmonary embolism. Heart attacks or sudden death occur in 2% of patients with head and neck cancer treated with radiation therapy and cetuximab.

Panitumumab (ABX-EGF) <Vectibix> is a fully human monoclonal IgG_2 antibody that engages the ligand-binding region of ERBB1 (EGFR, HER1) on transformed and untransformed cells. Panitumumab[23] may inhibit autocrine EGF stimulation of tumor cells that express the EGF Receptor, thereby suppressing tumor cell proliferation. The agent received U.S. FDA approval in 2006 for the treatment of ERBB1 expressing metastatic colorectal cancer with disease progression despite prior treatment.

Pharmacokinetics Following a single dose 1-h infusion of panitumumab, the area under the concentration-time curve increases in a greater than dose proportional manner, and clearance of panitumumab decreases as the dose is increased. Following the recommended dose regimen (6 mg/kg once every 2 weeks as a 1-h infusion), panitumumab concentrations reach steady state levels by the thrid infusion. The elimination half-life is approximately 7.5 days.

Adverse Effects Dermatologic toxicities (acneiform dermatitis, pruritus, erythema, rash, exfoliation, paronychia, dry skin, skin fissures) occur in 90% of patients and reach grade 3 or higher in 10% of patients. Pulmonary fibrosis or pulmonary embolism may become serious. Other common adverse events comprise hypomagnesemia, paronychia, fatigue, abdominal pain, nausea, vomiting, and diarrhea (possibly resulting in dehydration). Ocular toxicities (conjunctivitis, ocular hyperemia, increased lacrimation, eyelid irritation) affect 15% of patients. Severe infusion reactions arise in approximately 1%. The drug is Pregnancy Category C.

Panitumumab is not indicated for use in combination with chemotherapy. In clinical studies, the addition of panitumumab to the combination of bevacizumab and chemotherapy resulted in decreased overall survival and increased incidence of grade 3–5 adverse reactions.

Drug Resistance There is no treatment benefit in patients whose tumors have K-RAS mutations in codon 12 or 13.

Nimotuzumab <BIOMAb EGFR, TheraCIM, Theraloc, CIMAher> is a humanized monoclonal antibody that binds to and inhibits ERBB1, resulting in growth inhibition of tumor cells that over-express this receptor. In addition, this antibody may act synergistically with radiation therapy. Nimotuzumab is in testing for the treatment of non-small cell lung cancers, refractory colorectal tumors, squamous cell carcinomata of the head and neck, recurrent or refractory high grade malignant gliomata, and for intrinsic diffuse pontine glioma (an inoperable, treatment-resistant malignant brain cancer in children). Nimotuzumab is approved in India, China, Argentina, Columbia, and Cuba.

Adverse Effects Most adverse reactions are mild to moderate and are considered infusion reactions, including chills, fever, nausea and vomiting, dizziness and hypotension, dryness of the mouth, asthenia, flushing.

Hypomagnesemia and debilitating skin rashes, which may be caused by other anti-ERBB1 antibodies, do not arise in patients treated with nimotuzumab.

Zalutumumab <HuMax-EGFr> is a fully human IgG_1 that binds to the ERBB1 extracellular domain III. This locks the receptor in an inactive conformation. The antibody is designed for the treatment of squamous cell carcinoma of the head and neck, as over 90% of head and neck cancers over-express ERBB1.

Matuzumab (EMD 72000) is a humanized monoclonal antibody that binds ERBB1 with high affinity. It competitively blocks the binding of endogenous ligands and suppresses receptor mediated downstream signaling. This results in an impairment of tumor cell proliferation. Due to a lack of efficacy of the antibody in clinical trials its further development is on hold.

Ligand binding to the ERBB1 and ERBB3 receptors leads to their activation, dimerization, and downstream cell signaling. When these receptors are dysregulated in cancer, overactive signaling may lead to tumor cell survival and proliferation. The monoclonal anti-ERBB1/ERBB3 antibody MEHD7945A is a human IgG_1 with dual binding specificity. It inhibits ligand binding to both receptors. Each antigen-binding arm of the dual-action antibody engages a unique

[23] The agent was produced by immunization of transgenic mice (xenomice) that are able to generate human immunoglobulin heavy and light chains. A specific clone of B-lymphocytes secreting an antibody to ERBB1 was selected and immortalized.

epitope target. Simultaneously binding two receptors may inhibit the activity of multiple cancer signaling pathways. MEHD7945A is designed to block downstream signaling by inhibiting ligand binding and thus preventing the activation of all major ERBB pathways.

ERBB1 inhibition is efficacious in cancer therapy, but initially sensitive tumors often develop resistance, in some cases through compensatory ERBB3 signaling. In cancer cells resistant to the ERBB1 inhibitors cetuximab and erlotinib, MEHD7945A (but not single-target EGFR inhibitors) can suppress tumor growth and cell cycle progression due to ERBB1/ERBB3 signaling pathway modulation. MEHD7945A is more effective than a combination of cetuximab and anti-ERBB3. The agent may also limit the cross-resistance to radiation by cells resistant to EGFR inhibitors via modulating cell cycle progression and repair processes that control apoptotic cell death.

anti-ERBB3 MM-121 is a human monoclonal antibody that targets ERBB3 (HER3). It was the first selective ERBB3 antagonist to have entered clinical development. MM-121 prevents the phosphorylation of ERBB3 and its downstream targets.

Antibody inhibition of ERBB is suitable in cancers where the ligand or receptor is over-expressed.
Among the adverse effects are skin rashes and heart problems because of the important roles for ERBB molecules in these organs.

4.3.2 Lymphocytic Surface Targets

anti-CD20 In some forms of non-Hodgkin lymphoma, B-lymphocytes expressing the surface molecule CD20 expand excessively. Rituximab <Rituxan, Mabthera> is a recombinant chimeric murine/human antibody that selectively targets CD20. Following binding, this agent triggers a host cytotoxic immune response against CD20 bearing cells. Rituximab is indicated

- for the treatment of patients with relapsed or refractory, low grade or follicular, CD20-positive, B-cell non-Hodgkin lymphoma
- for the first-line treatment of diffuse large B-cell, CD20-positive, non-Hodgkin lymphoma in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) or other anthracycline-based chemotherapy regimens
- for the first-line treatment of follicular, CD20-positive, B-cell non-Hodgkin lymphoma in combination with CVP (cyclophosphamide, vincristine, prednisolone) chemotherapy
- for the treatment of low grade, CD20-positive, B-cell non-Hodgkin lymphoma in patients with stable disease or who achieve a partial or complete response following first-line treatment with CVP chemotherapy.

Rituximab is administered intravenously. Approved by the U.S. FDA in 1997, rituximab was the first therapeutic antibody marketed for treating cancer in the U.S. In 1998, rituximab was approved in the European Union (the antibody is sold by Genentech, one of the first biotechnology companies, founded in 1976).

Adverse Effects Serious adverse effects associated with the use of rituximab include

- potentially fatal allergic and infusion reactions: Deaths within 24 h of rituximab infusion follow an infusion reaction complex, which includes hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, or cardiogenic shock. Approximately 80 % of fatal infusion reactions occur in association with the first infusion. Prior to the rituximab infusion, patients should receive diphenhydramine and acetaminophen as premedications. Discontinuation of infusion is required in patients who develop severe infusion reactions
- tumor lysis syndrome: Acute renal failure requiring dialysis with instances of fatal outcome may arise following treatment with rituximab
- mucocutaneous reactions: Rituximab may cause skin reactions, in rare cases with fatal outcome. Toxic epidermal necrolysis is a severe manifestation
- progressive multifocal leukoencephalopathy: Patients treated with rituximab may experience JC virus activation[24], resulting in progressive multifocal leukoencephalopathy and death
- in some patients treated with rituximab, reactivation of the hepatitis B virus (HBV) may occur. Carriers of hepatitis B should be closely monitored during therapy.

Drug Resistance Patient populations likely to respond to rituximab have the drug target, CD20, expressed on the transformed cells. In addition, specific polymorphisms can predict the response rate. Antibody dependent cytotoxicity is associated with the FcγRIIIA (CD16) genotype, determined by a SNP at residue 158 (either phenylalanine, F, or a valine, V). Patients with the 158VV genotype show higher affinity for human IgG_1 and better clinical responses to rituximab than 158F carriers. Similarly, FcγRIIA (CD32) residue 131

[24] The JC virus is a polyomavirus that resides in latent form in up to 80 % of healthy adults. It usually remains latent, typically only causing disease in immunocompromised patients.

affects the rituximab response rate, which is higher in histidine/histidine carriers (131HH) than in arginine carriers (131R). C1q is a complement neutralizing molecule that functions in the clearance of apoptotic bodies. Polymorphisms in C1q also affect the response rate to rituximab.

Ofatumumab (HuMax-CD20) <Arzerra> is a human IgG_1 antibody directed to CD20. It binds in proximity to the membrane to the small and large loops of CD20, which may allow for effective complement deposition and subsequent B-cell killing. Ofatumumab is indicated for chronic lymphocytic leukemia that is refractory to fludarabine and alemtuzumab. It is also under study for follicular non-Hodgkin lymphoma, diffuse large B-cell lymphoma, and refractory chronic lymphocytic leukemia.

Ofatumumab is administered in 12 doses, with dose 1 of 300 mg, followed 1 week later by 2000 mg weekly for 7 doses (doses 2–8), followed 4 weeks later by 2000 mg every 4 weeks for 4 doses (doses 9–12).

Pharmacokinetics Ofatumumab is subject to dose dependent clearance. The antibody is eliminated through a target independent route and a B-lymphocyte mediated route. Due to the depletion of B-cells, the clearance decreases substantially after subsequent infusions compared to the first infusion. Renal impairment does not have a clinically important effect on ofatumumab pharmacokinetics.

Adverse Effects Serious adverse reactions may include infusion reactions (the risk can be reduced by premedication with acetaminophen, an anti-histamine, and a corticosteroid), cytopenias (prolonged severe neutropenia and thrombocytopenia), progressive multifocal leukoencephalopathy, Hepatitis B reactivation (possibly fulminant and fatal), and intestinal obstruction. The most common adverse reactions are neutropenia, anemia, fatigue, pyrexia, rash, diarrhea, nausea, cough, dyspnea, pneumonia, bronchitis, and upper respiratory tract infections. The drug is Pregnancy Category C.

Tositumomab <Bexxar> is a murine IgG_2 monoclonal antibody directed to the cell membrane antigen CD20 on the surface of B-lymphocytes. Upon receptor binding, the antibody induces apoptosis, and it may stimulate anti-tumor cell mediated or antibody dependent cytotoxicity. Tositumomab is administered as a radio-immunotherapeutic, conjugated to ^{131}iodine (see Sect. 13.2.). It is a drug for the treatment of follicular lymphoma.

Adverse Effects The most serious adverse reactions are prolonged cytopenias, the sequelae of cytopenias which included infections (sepsis) and hemorrhage in thrombocytopenic patients, allergic reactions (bronchospasm and angioedema), secondary leukemia, and myelodysplasia.

Drug Interactions Drugs or supplements that interfere with blood clotting (vitamin E, non-steroidal anti-inflammatory drugs, warfarin, ticlopidine, clopidogrel) can raise the risk of bleeding during treatment with tositumomab.

Ibritumomab tiuxetan <Zevalin> is a monoclonal antibody radio-immunotherapy treatment for some forms of B-cell non-Hodgkin lymphoma. The drug combines a mouse monoclonal $IgG_1\kappa$ that recognizes CD20 with the chelator tiuxetan, to which a radioactive isotope (either ^{90}yttrium or ^{111}indium) is added (see Sect. 13.2.). Tiuxetan is a modified version of diethylene triamine pentaacetic acid (DTPA), the carbon backbone of which contains an isothiocyanatobenzyl and a methyl group.

Others Epratuzumab (AMG 412) <LymphoCide> is a recombinant, humanized monoclonal antibody (LL2) directed to CD22, a cell surface glycoprotein present on mature B-lymphocytes and on many types of malignant B-cells. After binding to CD22, the predominant anti-tumor activity of epratuzumab may be mediated through antibody dependent cellular cytotoxicity, but not by complement dependent cytotoxicity. The drug may be radio-conjugated (see Sect. 13.2.). It is under study for follicular lymphoma and acute lymphoblastic leukemia (ALL).

Pharmacokinetics The half-life of the drug increases over repeated infusions and may reach 25 days.

Adverse Effects Epratuzumab is well tolerated when infused over 30 min. Headache, nausea, dizziness, and upper respiratory tract infections are the most common adverse events.

CD33 is a cell surface receptor, the expression of which is restricted to cells of the myelomonocytic lineage, mostly to progenitor cells. CD33 is a member of the SIGLEC (Sialic Acid Binding Immunoglobulin-Like Lectin) family of receptors, which contains cytoplasmic immune receptor based tyrosine signaling motifs typical of inhibitory receptors of the immune system. Engagement of the receptor by specific antibodies results in a dose dependent induction of apoptotic cell death. Because of its almost universal presence on over 90% of acute myeloid leukemia (AML) blast cells and lack of expression in most non-hematopoietic tissues, antibodies to CD33 have been developed to treat patients with acute myeloid leukemia.

Lintuzumab (HuM195, SGN-33) <Zamyl> is a humanized recombinant monoclonal IgG_1 antibody directed to CD33. Lintuzumab stimulates antibody dependent cell mediated cytotoxicity against tumor cells that express the antigen. This results in a decrease in tumor burden. The U.S. FDA and the European EMA had granted lintuzumab orphan drug status for the treatment of acute myeloid leukemia and myelodysplastic syndromes. However, while this agent was safe in clinical studies, its efficacy is low and it was pulled from further development.

Brentuximab vendotin (SGN-35, cAC10-vcMMAE) <Adcetris> consists of the chimeric monoclonal antibody brentuximab, which targets CD30, connected via a protease cleavable linker to 3–5 units of the anti-mitotic (microtubule disrupting) agent monomethyl auristatin E (MMAE). Approved by the U.S. FDA in 2011, the agent is used for Hodgkin lymphoma and anaplastic large cell lymphoma. The antibody is a second line of defense after failure of autologous stem cell transplant or of two prior multi-agent chemotherapy regimens. The recommended dose is 1.8 mg/kg administered as an intravenous infusion over 30 min every 3 weeks, continued over a maximum of 16 cycles, or until disease progression or unacceptable toxicity occurs.

Pharmacokinetics Brentuximab vendotin exposure is increased in patients with hepatic impairment or severe renal impairment.

Adverse Effects Adverse effects include peripheral neuropathy, neutropenia, upper respiratory tract infection, fatigue, nausea, and diarrhea. Brentuximab vendotin is associated with progressive multifocal leukoencephalopathy due to JC virus reactivation. The drug is Pregnancy Category D.

CD2 (Sheep Red Blood Cell Receptor) is a specific receptor on T-lymphocytes and natural killer cells. It is expressed by most peripheral T-cell lymphomata, cutaneous T-cell tumors, and T-cell prolymphocytic leukemia. Further, CD2 is also expressed by the malignant cells of large granular lymphocytic leukemia, extranodal T/NK cell lymphoma, nasal type and blastic or aggressive NK cell leukemia.

Siplizumab (MEDI-507) is a monoclonal IgG_1 κ antibody that binds to CD2 and causes the depletion of T-lymphocytes. Receptor engagement by the antibody triggers a host immune response that results in cell lysis, selective suppression of the immune system, and control of activated T-cell growth.

Pharmacokinetics Siplizumab pharmacokinetics follows a 1-compartment model with linear FcRn mediated[25] and nonlinear target mediated clearance.

Adverse Effects The antibody is safe and well tolerated in patients with $CD2^+$ T-cell lymphomata and leukemias. The most common adverse effects comprise lymphopenia, chills, headaches, pyrexia, and infusion reactions.

CD52 is a differentiation antigen typically expressed at high levels on thymocytes, lymphocytes, monocytes, and macrophages. The molecule is also present on the epithelial cells lining the male reproductive tract; it is shed into seminal plasma and acquired by spermatozoa. CD52 is expressed at variable levels on the majority of lymphoid malignancies.

Alemtuzumab <Campath, Campath-1H, MabCampath> is a humanized IgG_1 to CD52 that can activate complement and antibody dependent cell mediated cytotoxicity. It is used as a second-line therapy to treat B-cell chronic lymphocytic leukemia. The agent was approved by the U.S. FDA in 2001. Alemtuzumab is administered by dose escalation over 3–7 days. Once the recommended dose is achieved, the treatment is given 3 times a week for 4–12 weeks.

Adverse Effects Adverse effects comprise infusion related events. They include syncope, pulmonary infiltrates, adult respiratory distress syndrome (ARDS), respiratory arrest, cardiac arrhythmias, myocardial infarction, and cardiac arrest. A complication of therapy with alemtuzumab is that it increases the risk for opportunistic infections, in particular the reactivation of cytomegalovirus (CMV). Active vaccinations should be avoided during treatment with the antibody.

Alemtuzumab is contraindicated for patients with active systemic infections, underlying immunodeficiency, or known type I hypersensitivity or anaphylactic reactions to Campath or to any of its components.

Lymphocytic surface molecules are highly accessible targets for lymphoma and leukemia treatment.
Antibodies that recognize lymphocytic surface receptors may cause the depletion of white blood cells.

4.3.3 Various Targets

Antibodies to HGF and MET Hepatocyte Growth Factor (HGF, Scatter Factor, SF) and its receptor MET are involved in the initiation, progression, and metastasis of lung cancer, colorectal cancer, renal cancer, and glioma. Blockage of the HGF-MET interaction with an anti-HGF monoclonal antibody has therapeutic potential through abrogation of the excessive growth signal. As there is interdependence between the HGF/MET pathway and the ERBB1 (EGFR) pathway in colorectal and lung cancers, anti-HGF antibody may be beneficial if used in combination with blockers of EGFR. Similarly, combination with SHH blockers in medulloblastoma may enhance therapeutic efficacy. However, targeting the HGF/MET pathway may not be effective if downstream signaling is activated by a K-RAS mutation.

DN-30 is a monoclonal anti-MET antibody that recognizes the extracellular portion of the receptor. It induces the proteolytic cleavage of the MET extracellular region (receptor shedding), thus decreasing the number of cell surface receptors and generating a decoy effect for HGF binding. DN30 also induces cleavage of the intracellular domain, which is successively degraded by the proteasome. However, DN-30 binding to MET also results in a partial activation of the kinase due to antibody-mediated receptor homo-dimer-

[25] The neonatal Fc receptor (FcRn) plays a critical role in regulating IgG homeostasis.

ization. To dissociate shedding activity from agonistic activity, the use of a DN-30 Fab fragment has been suggested. The antibody inhibits tumor growth and prevents metastasis.

OA-5D5 is a one-armed monovalent anti-MET Fab antibody fragment that prevents HGF binding. OA-5D5, composed of murine variable domains for the heavy and light chains with human IgG_1 constant domains, is produced as a recombinant protein in Escherichia coli. The agent is in clinical development. It has an intracerebral tumor suppressive effect when given topically.

Rilotumumab (AMG102) is a fully humanized monoclonal antibody (IgG_2) that binds to and neutralizes HGF, preventing its binding to the receptor c-MET. The inhibition of c-MET mediated signal transduction may result in the induction of apoptosis. Rilotumumab is in clinical trials for the treatment of glioblastoma, renal cell carcinoma, and metastatic colorectal cancer.

The antibody L2G7 is a neutralizing anti-HGF antibody that may have efficacy against lung cancer. It crosses the blood-brain barrier, potentially causing intracranial tumor regression.

Antibodies to IGF-1R Insulin, Insulin Like Growth Factors -1 and -2, and Relaxin stimulate DNA synthesis and cell growth. Insulin Like Growth Factors are expressed in two types, IGF-1 (Somatomedin C) and IGF-2 (Somatomedin A, Multiplication-Stimulating Factor, MSF). They are bound to carrier proteins and are maintained at relatively steady blood levels. While IGF-2 is a primary growth factor required for early development, IGF-1 expression mostly occurs in later life. The biological actions of the Insulin Like Growth Factors are predominantly exerted through the type 1 Insulin Like Growth Factor Receptor (IGF-1R), which binds IGF-1 and IGF-2 with high affinity. Activation of the hetero-tetrameric tyrosine kinase receptor IGF-1R occurs following IGF-1 binding to the receptor σ ubunit ⟨, and leads to auto-phosphorylation of the subunit β IGF-1R is linked to the RAS→RAF→MAPK and PI 3-K→PKB signal transduction cascades, which are involved in mitogenesis and cell survival. The IGF-1 Receptor displays potent mitogenic, anti-apoptotic, and transforming activities, which may be a prerequisite for oncogenesis. IGF-1R is expressed on many tumor cells, where it may contribute to proliferation and progression.

Several anti-IGF-1R antibodies have not succeeded in clinical trials.

Figitumumab (CP-751,871) is a human monoclonal antibody directed to IGF-1R. Figitumumab selectively binds to IGF-1R, thus preventing IGF-1 from engaging the receptor and inducing receptor auto-phosphorylation. Inhibition of IGF-1R auto-phosphorylation can result in a reduction of the anti-apoptotic effect of IGF and cause an inhibition of tumor growth. The antibody is under investigation for the treatment of adrenocortical carcinoma and non-small cell lung cancer. Responses may be achieved with combination therapy of figitumumab plus paclitaxel and carboplatin.

Adverse Effects The most common adverse events are hyperglycemia, fatigue, neutropenia, and neuropathy.

Ganitumab (AMG479) blocks IGF-1R and consecutively inhibits the PI3K→AKT signaling pathway downstream of the receptor. It is under study in pancreatic cancer.

The monoclonal antibody cixutumumab (IMC-A12) is a fully human IgG_1 monoclonal antibody directed to IGF-1R. It prevents the binding of the natural ligand IGF-1 and the subsequent activation of PI 3-K→PKB signaling pathway. The effects may result in the induction of cancer cell apoptosis and may decrease cancer cell proliferation. cixutumumab has not progressed to phase III clinical trials.

R1507 is a human monoclonal antibody that blocks the action of IGF-1R. Its development was abandoned.

Others Apolizumab <Remitogen> is a humanized monoclonal antibody directed to 1D10, a polymorphic determinant on the HLA-DR β chain that is expressed on normal and neoplastic B-lymphocytes. Apolizumab induces complement mediated cytotoxicity, antibody dependent cell mediated cytotoxicity, and apoptosis of 1D10 expressing B-lymphocytes. It is under investigation for treating low grade or follicular B-cell non-Hodgkin lymphomata and chronic lymphocytic leukemia (CLL).

The ganglioside GD2 is over-expressed in malignant melanoma, neuroblastoma, osteosarcoma, and small cell carcinoma of the lung. 14G2A is a murine monoclonal antibody directed to GD2. Binding of this antibody to its cognate antigen induces antibody dependent cell mediated cytotoxicity and complement dependent cytotoxicity against GD2 expressing tumor cells.

Pharmacokinetics The peak serum levels and elimination are dose dependent. The decay from the plasma is biphasic with the longer half-life plateauing at doses of 250 mg/m^2. There likely exists a saturable mechanism for antibody elimination.

The human/mouse chimeric antibody ch14.18 recognizes GD2. It triggers tumor cell lysis via antibody dependent cellular cytotoxicity and complement dependent cytotoxicity. In clinical trials against melanoma, neuroblastoma, and osteosarcoma ch14.18 has displayed an acceptable safety profile.

The effects may be enhanced by administering the antibody in combination with systemic cytokines (GM-CSF or IL-2).

For most patients with advanced ovarian cancer, the front-line treatments, surgery and chemotherapy, do not suffice to control the high recurrence rate. CA125 is a Mucin-like glycoprotein that is expressed on the surface of more than 95% of all non-mucinous stage III/IV epithelial ovarian cancers, which serves as a clinical marker.

Oregovomab (MAb B43.13) <OvaRex> is a murine monoclonal antibody (B43.13) that attaches to the tumor associated carbohydrate antigen CA125. Vaccination with this antibody may stimulate a host cytotoxic immune response against tumor cells that express CA125. Patients who mount a T-cell response to CA125 show significantly improved survival compared to patients who do not.

MUC-1 is a heavily glycosylated transmembrane glycoprotein that is over-expressed in many carcinomata. MUC-1 consists of 3 domains, a large extracellular motif, a transmembrane motif, and a cytoplasmic tail. The molecule mediates signal transduction events that stimulate the motility, invasion, and metastasis of cancer cells. In cancer patients, humoral and cellular responses to MUC-1 may arise, implicating MUC-1 as a potential target for immunotherapy.

A murine monoclonal antibody to Human Milk Fat Globule 1 (muHMFG1) recognizes an epitope localized in the extracellular MUC-1 domain. A humanized variant of muHMFG1, AS1402, is in clinical trials. It could represent a potential treatment agent for patients with ovarian cancer, possibly as a radiotherapeutic after labeling with ^{90}yttrium.

Phosphatidylserine is a highly immunosuppressive molecule that is usually located inside cells. It becomes exposed on the outer membrane of cells that line tumor blood vessels, creating a specific target for treatment.

Treatment of B-cell Lymphocytic Leukemia

1. **B-cell acute lymphocytic leukemia**

The standard treatment of acute lymphocytic leukemia (ALL) is generally divided into three phases,

- induction chemotherapy for the rapid restoration of bone marrow function
- central nervous system prophylaxis or treatment
- post-remission therapies to eliminate minimal residual disease.

Acute lymphoblastic leukemia in pediatric patients and young adolescents is highly responsive to conventional chemotherapy. In older patients, clinical remission is more difficult to obtain and early relapse is more common. Corticosteroid therapy, in combination with other anti-leukemic drugs or in cyclic combinations including methotrexate, has produced rapid and effective responses. When used for induction, methotrexate in doses of 3.3 mg/m^2 in combination with 60 mg/m^2 of prednisone, given daily, produces remissions in 50% of patients treated, usually within a period of 4–6 weeks. When remission is achieved and supportive care has produced general clinical improvement, maintenance therapy is considered essential. Methotrexate is administered two times weekly either by mouth or intramuscularly in total weekly doses of 30 mg/m^2. It can also be given in doses of 2.5 mg/kg intravenously every 14 days. Alternatively, the usual daily maintenance dose of 6-mercaptopurine is 1.5–2.5 mg/kg/day as a single dose. It may also be combined with methotrexate. If or when relapse does occur, the re-induction of remission can usually be obtained by repeating the initial induction regimen.

Daunorubicin hydrochloride <Cerubidine> for infusion is indicated in combination chemotherapy for remission induction in acute lymphocytic leukemia in children and adults.

- A representative regimen in pediatric patients comprises daunorubicin 25 mg/m^2 (1 mg/kg in children less than 2 years of age) by infusion on day 1 every week, vincristine 1.5 mg/m^2 intravenously on day 1 every week, oral prednisone 40 mg/m^2 daily. If after 4 weeks remission is incomplete one or two additional courses may be given.
- In adult patients the dose schedule may combine daunorubicin 45 mg/m^2/day by infusion on days 1, 2, and 3 with intravenous vincristine 2 mg on days 1, 8, and 15 and oral prednisone 40 mg/m^2/day on days 1 through 22, then tapered between days 22 to 29, and intravenous L-Asparaginase 500 IU/kg/day for 10 days on days 22–32.

Cyclophosphamide <Cytoxan, Neosar> shows activity against acute leukemias. It may be combined with other agents (in CALGB 8811 with daunorubicin, vincristine, prednisone, and Pegaspargase). However, cyclophosphamide treatment may induce secondary leukemais.

Hyper-CVAD chemotherapy consists of two courses of drug combinations administered in an alternating fashion. The acronym stands for hyper-fractionated CVAD (cyclophosphamide, vincristine, doxorubicin <Adriamycin>, and dexamethasone). Compared to conventional protocols, it is given in smaller doses and

more frequently, to minimize the adverse effects. The protocol was originally developed to treat leukemia in young patients with good overall health, due to its intensity, but has since been used more widely.

- Course A comprises intravenous cyclophosphamide <Cytoxan> (at 300 mg/m^2 every 12 h over 3 h on days 1, 2, and 3), vincristine <Oncovin>(at 2 mg on days 4 and 11), doxorubicin <Rubex>(50 mg/m^2 on day 4), dexamethasone <Decadron> (40 mg/day on days 1–4 and 11–14; it may be given orally), intrathecal cytarabine <Cytosar> (70 mg on day 7), methotrexate (12 mg on day 2), and infusion of mesna <Uromitexan> (to reduce the incidence of hemorrhagic cystitis associated with cyclophosphamide).
- Course B entails intravenous methotrexate (1000 mg/m^2 over 24 h on day 1), cytarabine (3000 mg/m^2 over 2 h every 12 h on days 2 and 3), leucovorin (25 mg/m^2, six doses over 6 h each, 24 h after starting methotrexate), oral sodium bicarbonate (600 mg starting a day before methotrexate, continued over 4 days).

The dosages are individualized, based on body weight, body surface area, and the overall health of the patient. Each course is given up to four times, over up to eight cycles that are 2–3 weeks apart. While the aim is to administer the largest number of cycles in the shortest time period possible, adjustments may be required based on patient recovery.

Post-remission therapy for adult acute lymphocytic leukemia includes intensive short-term chemotherapy followed allogeneic bone marrow transplant. The transplant of matched, unrelated bone marrow results in a lower incidence of leukemic relapse, when compared to syngeneic or autologous bone marrow transplant. This may be caused by an immunologic graft-versus-leukemia effect. Yet, the improvement in disease-free survival is partially offset by the treatment-induced morbidity and mortality from graft-versus-host disease, veno-occlusive disease of the liver, and interstitial pneumonitis. Therefore, allogeneic bone marrow transplantation is reserved for patients in second remission or beyond. As an alternative, hematopoietic stem cell transplant may be considered.

2. **B-cell chronic lymphocytic leukemia**

Chronic lymphocytic leukemia (CLL) is the most common type of leukemia in North America and Europe. It mainly affects the elderly, and has a highly variable course, with survival ranging from months to decades. Most patients with chronic lymphocytic leukemia are identified through blood tests performed for unrelated reasons rather than because of symptoms of the disease. Approximately 1/3 of patients never require treatment. Standard management of most patients has therefore traditionally involved a period of observation to assess disease progression. For those patients with chronic lymphocytic leukemia who are treated, therapy is generally palliative. Need-to-treat criteria include hematopoietic insufficiency, B-symptoms[26], rapidly progressive disease, or risk of complications from bulky lymphadenopathy. Regimens based on DNA alkylating agents, such as chlorambucil and cyclophosphamide, have been used for more than 30 years. In the 1980s, purine analogs (such as fludarabine) achieved more complete remissions than alkylator-based regimens. Since then, considerable efforts have focused on developing combination therapy protocols that are built on fludarabine. In a third phase of development, combination protocols have included targeted monoclonal antibodies. Throughout, the identification of agents with activity against resistant or treatment-refractory chronic lymphocytic leukemia has remained a high priority (Keating 2008).

Drug Resistance Expression of the efflux transporter ABCC1 (MRP1) occurs on the transformed cells in chronic lymphocytic leukemia. It may contribute to anti-cancer drug resistance.

Conventional chemotherapy With the possible exception of stem cell transplants, there is no curative treatment currently available but standard chemotherapy regimens can prolong survival. Induction chemotherapy may be started with an alkylating agent. Continuous or intermittent treatment with chlorambucil or cyclophosphamide reduces total lymphocyte mass and may prevent bone marrow failure until the disease becomes refractory. Chlorambucil can be given as first-line, single-agent treatment. Cyclophosphamide is often part of the COP (cyclophosphamide, vincristine <Oncovin>, prednisolone) or CHOP (cyclophosphamide, hydroxy-daunorubicin, vincristine <Oncovin>, prednisolone) regimens.

Fludarabine phosphate, a purine analog, is a mainstay in the treatment of patients with chronic lymphocytic leukemia. It was developed by John Montgomery. In

[26] B-symptoms refer to fever (greater than 38°C), night sweats, and weight loss (more than 10% of normal body weight over 6 months or less). Their presence or absence has prognostic significance for leukemias or lymphomata.

1989, the drug was found to be very effective in patients resistant to alkylating agents, and it was able to achieve complete remissions, leading to its approval for refractory chronic lymphocytic leukemia (Keating 1989). Combinations of purines and alkylators have been demonstrated to be superior to single agents, and combinations of rituximab with fludarabine or fludarabine and cyclophosphamide have led to a dramatic improvement in complete responses and progression-free survival.

Flavopiridol (alvocidib) (HMR 1275, L86-8275), a flavonoid derived from an indigenous plant from India, specifically inhibits Cyclin Dependent Kinases (CDKs 1, 2, 4, 7, and 9) with resulting blocks in cell cycle progression at the G_1/S and G_2/M boundaries. It generates responses in about one half of patients with advanced chronic lymphocytic leukemia. The drug effects are limited by binding to plasma proteins, leaving an ineffective level of free drug in circulation. This needs to be accounted for with a suitable dosing schedule.

Antibody therapy The monoclonal antibody alemtuzumab <Campath> binds to the cell surface molecule CD52. It was initially approved against chronic lymphocytic leukemia for the treatment of fludarabine-refractory patients, where there was no other available option. Alemtuzumab has demonstrated a higher response rate and longer progression-free survival than chlorambucil. It is now approved by the U.S. FDA as a single agent for first-line therapy.

Rituximab <Rituxan>, the first therapeutic antibody approved for cancer treatment, is a chimeric anti-CD20 antibody with efficacy against chronic lymphocytic leukemia, both as a single agent and in combination with traditional chemotherapies. In relapsed chronic lymphocytic leukemia, higher doses or more dose-intensive strategies can yield positive results.

There are a variety of combination regimens of rituximab with chemotherapy. FCR combines fludarabine, cyclophosphamide, and rituximab every 28 days in conjunction with infectious disease prophylaxis.

- Cycle 1 entails on day 1 intravenous rituximab 375 mg/m^2 at 50 mg/h (adjusted every 30 min by 50 mg/h until the goal of 400 mg/h is reached), on days 1–3 intravenous cyclophosphamide at 250 mg/m^2 over 10–30 min plus fludarabine at 25 mg/m^2 over 20–30 min.
- Cycles 2–6 comprise intravenous rituximab 500 mg/m^2 at 50 mg/h (adjusted every 30 min by 50 mg/h until the goal of 400 mg/h is reached), on days 1–3 intravenous cyclophosphamide at 250 mg/m^2 over 10–30 min plus fludarabine 25 mg/m^2 over 20–30 min.

Treatment of hairy cell leukemia Hairy cell leukemia is treated with the purine analog deoxycoformycin (pentostatin) <Nipent>, Interferon-α, and cladribine (2-chlorodeoxyadenosine, 2-CDA) <Leukostatin, Litak, Movectro>.

- Interferon-α improves the blood counts in about 80% of patients treated, however, only 10% of patients enter complete remission and relapse if the treatment is discontinued.
- Deoxycoformycin is indicated for patients who are refractory to Interferon-α treatment. Although it is an inhibitor of the enzyme ADA, deoxycoformycin has proved to be exceptionally active in hairy cell leukemia, a B-cell neoplasm with low intracellular concentrations of ADA.
- Cladribine is an adenosine analog. Because the agent is resistant to Adenosine Deaminase (ADA), an enzyme that inactivates some anti-neoplastic agents, it is selectively toxic to lymphocytes and monocytes, which exhibit little Deoxynucleotide Deaminase activity. Cladribine is mostly used for the treatment of hairy cell leukemia. The drug is administered by injection or infusion. The recommended dose and schedule for active hairy cell leukemia is as a single course given by continuous infusion for 7 consecutive days at 0.09 mg/kg/day.

35–40% of patients on standard therapy relapse. Second line therapy may be implemented with LMB-2, an immunotoxin that targets CD25.

Bavituximab <Peregrine> is a monoclonal antibody to phosphatidylserine. It binds to various aminophospholipids in a manner that is dependent on its interaction with the plasma protein β_2-Glycoprotein I. Bavituximab induces the immune cell mediated destruction of cells with exposed phosphatidylserine and may reactivate the immune response to cancer by blocking phosphatidylserine induced immunosuppression. Binding of bavituximab to the phosphatidylserines in the cell membrane helps mobilize the immune system to attack tumor associated blood vessels. Additionally, as chemotherapy increases the exposure of phosphatidylserine on tumor blood vessels, bavituximab combined with conventional chemotherapy, specifically carboplatin and paclitaxel, may hold potential for synergistic therapeutic effects. The antibody is in clinical trials to treat cancers of the lungs, liver, breast, and pancreas.

Farletuzumab is a humanized monoclonal antibody with high affinity for Folate Receptor α. This receptor, while almost absent from normal tissue, is over-expressed in most ovarian cancers. Farletuzumab mediates antibody dependent cellular cytotoxicity and complement dependent cytotoxicity. The antibody has shown clinical efficacy in early phase trials as single agent and combination therapy with minimal drug specific toxicity. It displays a safe toxicology profile.

4.3.4 Antibody Mimetics

Antibody mimetics are organic compounds that, like antibodies, can specifically bind antigens, but are structurally not related to antibodies. They are usually synthetic peptides or proteins with a molecular mass of about 3–20 kD. Nucleic acids and small molecules may sometimes act as antibody mimetics as well.

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