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Protein Kinase Inhibitors as a Therapeutic Modality

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ABSTRACT
Most of the signal transduction pathways are mediated by protein kinases regulating every aspect of cell function. Mutations which deregulate their expression or their function or both result in cancers. Therefore, protein kinase inhibitors has become the focus of development of new therapies for cancer. Almost all 120 protein tyrosine kinases are involved in signaling, whereas only a handful of Ser/Thr kinases are involved. Thus, most of the effort is directed toward the development of tyrosine phosphorylation inhibitors. The success of Gleevec in the treatment of chronic myeloid leukemia and of Iressa for lung cancer validates the approach.

I. Principles of Signal Transduction Therapy

Cancer cells differ from their normal counterparts in their mode of communication with their neighbors due to aberrations in their signaling network. The realization of aberrant signal transduction as the source of the transformed phenotype of cancer cells has generated mounting interest toward developing therapies targeting these aberrations.[1,2] Cancer cells develop as a result of a series of mutations in their signaling pathways: (1) cells divide inappropriately with respect to their environmental context, (2) cells develop robust anti-apoptotic signals, avoiding stresses, and (3) cells develop molecular mechanisms to escape the immune system.

These cancer-specific signaling networks can, in principle, become their "Achilles' heel". Identification of these signaling pathways, has become the basis "signal transduction therapy".[2] Rather than using cytotoxic agents, one targets selectively signaling elements of the cancer cell.[3] This approach is also applicable to other diseases in which signaling pathways drive the diseased cell.[4] In the late 1980s, the most prominent signaling elements, identified as key drivers of the cancer cells, were protein tyrosine kinases (PTKs). Therefore, the first signal transduction inhibitors were tyrosine phosphorylation inhibitors (tyrphostins).[5] Some tyrphostins were particularly effective since in certain leukemias the targeted PTKs, namely, Jak-2 or Bcr-Abl, were essential for the survival of the leukemic cell.[6−8] In early stage CML (chronic myeloid leukemia), blockade of Bcr-Abl is sufficient to induce apoptosis and therefore lead to the full remission of patients.[9,10] Similarly, the blockade of pre-B acute lymphoblastic leukemia by the Jak-2 inhibitor AG 490 clears the engrafted tumor from the experimental mouse.[8] The form of therapy aimed at tempering with the signaling pathways of cancer cells was coined as "signal transduction therapy".[2,3,14] The agents, developed as signal transduction inhibitors, are small molecules, antibodies, proteins, double stranded RNA molecules, and viral (gene) therapy.

II. Protein Tyrosine Kinase Inhibitors Vis-a-Vis SER/THR Inhibitors

The genome project identifies ∼520 protein kinases from which a few dozens are involved in signal transduction.[16] PTKs were identified in the 1980s as major players in cancer and, as a result, chosen as therapeutic targets.[17] EGFR (epidermal growth factor receptor),[15] Bcr-Abl,[7] PDGFR (platelet derived growth factor receptor),[18] VEGFR (vascular endothelial growth factor receptor),[19] and IGF-1R (insulin growth factor 1 receptor kinase)[23] were targeted having established their involvement in various malignancies. Targeting PDGFR was also motivated because of its involvement in restenosis.[20−22] A small group of serine /threonine kinases, cyclin dependent kinase (Cdks), Erks, Raf, and PKB/Akt, were identified as major players in cell proliferation, cell division, and anti-apoptotic signaling. These Ser/Thr kinases are downstream to PTKs (Figure 1). Some believe that it is reasonable to target the upstream PTK rather than the downstream Ser/Thr kinase. Shutting off the elevated activity of the overactive PTK will nullify all the elements whose activities become enhanced as a result of the enhanced activity of the PTK targeted. When other mechanisms contribute to the activation of the Ser/Thr kinase, it is reasonable to utilize a Ser/Thr kinase inhibitor. One notable example is PKB (protein kinase B). PKB is most important in the anti-apoptotic network, and its activity is highly elevated, especially in metastatic cancer. The elevated activity of PKB results from enhanced signaling of upstream PTKs combined with the deletion of PTEN (phosphatase and tensin homologue), its negative regulator (Figure 1).

III. Development of PTK Inhibitors

Early searches for PTK inhibitors identified natural compounds such as quercetin, genistein, erbstatin, and lavendustin. These compounds were found to be highly promiscuous, inhibiting many protein kinases, which was not surprising to the research community in those days. It was then believed that it was impossible to generate selective small molecules targeting protein kinases. Emphasis was put on the conservation of the ATP binding

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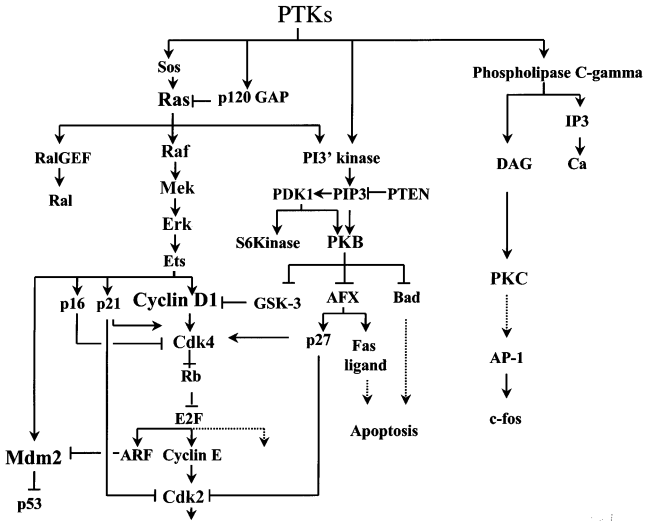


FIGURE 1. Protein kinases as signal transducers PTKs are upstream to a small number of Ser/Thr kinases which transmit their signal. The activity of some Ser/Thr kinases is enhanced by the deletion of negative regulators, like p16 and PTEN. When Ras or Raf are persistently active due to a mutation, the signaling downstream is independent of the upstream PTK.

site among the different protein kinases. The first selective PTK inhibitors discovered were the benzene malononitrile tyrphostins which block effectively the EGFR.[15,24,25] These compounds (Figure 2) were competitive with the substrate and noncompetitive with ATP, poorly blocking insulin receptor kinase and having no measurable activity against cAMP-dependent protein kinase. Many tyrphostins were found to be ATP-competitive, substrate-competitive, or competitive against both.[26] Some were found to be "mixed-competitive" against EGFR[26] and PDGFR.[27] Most cyclized tyrphostins, incorporating the nitrile nitrogen into a second ring, are ATP-competitive.[18,28,29] Since 1994 the main thrust in the development of PTK inhibitors has been toward the generation of ATP-mimics.[17] Since the degree of conservation in the ATP binding site is not absolute, one can obtain a high degree of selectivity among closely related ATP binding domains. In 1993 we demonstrated that ATP-competitive tyrphostins discriminate between the kinase domains of EGFR and its closely related Her-2/neu by 2 orders of magnitude in affinity.[30] Quinazolines were shown to selectively inhibit EGFR in the low nanomolar concentration range.[28,31] ZD 1839 (Iressa), a potent EGFR kinase inhibitor with excellent bioavailability, was chosen for clinical development.[31] Qunixaloines such as AG 1295 and AG 1296[18,32] or AGL 2043[33] (Figure 4) block PDGFR kinase with excellent inhibitory effects on the related c-Kit and Flt-3 receptors and much less efficacy against VEGFR.[34] The crystal structure of the of Hck with the Src family inhibitor PP1[35] and of Gleevec with Abl[36] revealed why these ATP analogues are highly selective. However, both PP1[37] and STI 571,[38] which are very different in structure (Figure 2), bind also to PDGFR. This finding should alert us to possible problems when novel kinase inhibitors are not tested on as many kinases as possible before they move forward in the development process.

IV. Current PTK Inhibitors—Where Do We Go Now?

The success of Gleevec/Glivec in treating early CML and gastrointestinal stromal tunors (GIST), as well as the moderate success of Iressa (ZD 1839), validate the approach. We still need to assess whether more scaffolds for the design of novel PTK inhibitors are needed. Iressa has only moderate success in treating lung cancer as a single agent and is ineffective when combined with CDDP. Thus, we need also to learn more about the role of EGFR in the various forms of lung cancer and determine which subclass of patients should be selected for the treatment. It is highly likely that signaling elements other than EGFR and Her-2 should be considered.[39]

V. EGFR Family Kinase Inhibitors

The role of EGFR in many cancers has been appreciated early on and, therefore, was one of the first targets identified. Indeed, Iressa (ZD 1839)[31,40] (Figure 3) is in the clinic for the treatment of nonsmall cell lung carcinoma, and the quinazoline AG 1478[29] is in clinical development for the treatment of glioblastoma multiforme (GBM) in which the persistently active Δ (2−7) EGFR is overexpressed.[41,42] AG 1478 will be used in combination with CDDP, with which it synergies to induce apoptosis in GBM in vitro and in vivo.[42] Since heterodimer combinations of the four members of the Her family play a role in the oncogenic phenotype of many cancers, there have been attempts to generate inhibitors of both Her-1 and Her-2 like GW 2016 (Figure 3), which blocks both EGFR and Her-2 12nM.[43]

VI. Irreversible EGFR Kinase Inhibitors

The covalent attachment of a selective inhibitor to the EGFR kinase domain abolishes completely the catalytic activity and, therefore, is believed to possess better clinical potential. Indeed, CI-1033 (Figure 5)[44] is effective in preclinical in vivo experiments. CI-1033, however, is, quite toxic (unpublished), most probably due to the high chemical reactivity of the acryloyl group. To improve the profile of such compounds, one may have to reduce the chemical reactivity of the labeling group. In doing so, the reaction of the inhibitor with nonspecific targets should be reduced. Recently, this has been successfully achieved by enhancing the affinity of the quinazoline but in parallel reducing the chemical reactivity of the labeling group. Enhanced affinity is due to diminished k_{off}, which keeps the compound at the active long enough to allow interaction with cysteine 773, even with a chemical group which is less reactive than the acryloyl moiety. The diminished chemical reactivity has a chance to reduce the toxicity of the compound, compared with CI-1033.

VII. From Tyrphostins to Gleevec

In the early 1990s, the Bcr-Abl kinase inhibitors, tyrphostin AG 1112, AG 1318,[6] and AG 957[7,45] (Figure 6), were shown

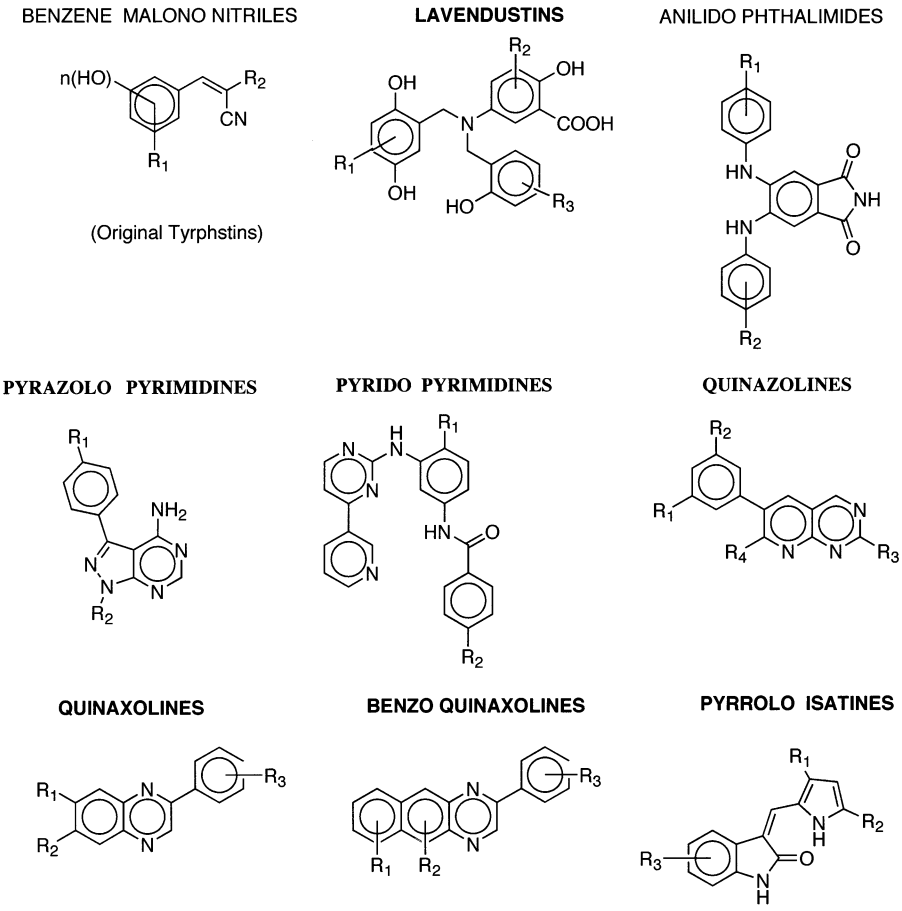


FIGURE 2. Protein tyrosine kinase inhibitors dDifferent core structures used by investigators in the field are shown.

to induce the terminal differentiation of K562 cells, the purging of Ph+ cells, and synergies with the anti-Fas receptor antibody in order to induce their demise.[46] These compounds were not developed for the clinic since it was not believed at that time that they have a commercial future because of the rarity of CML compared to other cancers. Druker and Lydon followed up these studies, utilizing the Ciba-Geigy (than Novartis) compound CGP 57148 (Figure 6). This compound,[47] renamed STI 571/Gleevec/Glivec, became the first signal transduction inhibitor to show excellent efficacy in the clinic[9,10] for early CML. The absence of severe side effects in these patients was unexpected in view of the strong inhibitory activity of the compounds toward c-Abl, PDGFR, and c-Kit. This is probably due to the ability of normal cells to sustain high levels of inhibition of these elements, whereas the CML cells' survival depends on Bcr-Abl. Thus, the principle of the enhanced sensitivity of the cancer cell to an inhibitor, which targets the element whose signaling is enhanced,[48] is validated in the clinic. Gleevec is highly active on a subpopulation of gastrointestinal stromal tumor (GIST) patients.[49] The patients who respond best to STI 571 express Kit receptors, which carry mutations in exon 11, converting the receptor to a persistently active kinase essential for the survival of the cancer cell.

VIII. ATP Mimics and Substrate Mimics

Currently, all PTK inhibitors in clinical development or heading toward the clinic are competitive inhibitors of ATP and noncompetitive vis-à-vis the substrate. Although substrate competitive inhibitors never made it to the clinic, they are likely to be less toxic than ATP-mimics, since they bind to domains at the kinase site that are less conserved than the ATP binding site and are therefore less likely to hit many other targets. Indeed, tyrphostins such as AG 490, which blocks Jak-2,[8] and AG 556, which possesses antiinflammatory properties, have been shown to be highly nontoxic in vivo.[50−53] A major concern with these compounds, voiced in the literature, is that they possess hydroxyl groups, which are metabolically unstable. Although DOPA, a catechol, is a success story, the current feeling is than one should aim at tyrphostins with diminished numbers of hydroxyl groups. This has been partially accomplished by developing substrate mimics in which the hydroxyls are replaced by "bioisosteres".[54] AG 538, which is a competitive inhibitor of the IGF1-R,[55] was converted quite successfully to "bioisosteres", retaining the substrate competitive nature of the compound[54] (Figure 7), but loosing some efficacy. When one examines the efficacy of the ATP-competitive inhibitors in cellular assays, it is observed that these nanomolar compounds act on cells in the micromolar concentration range, most

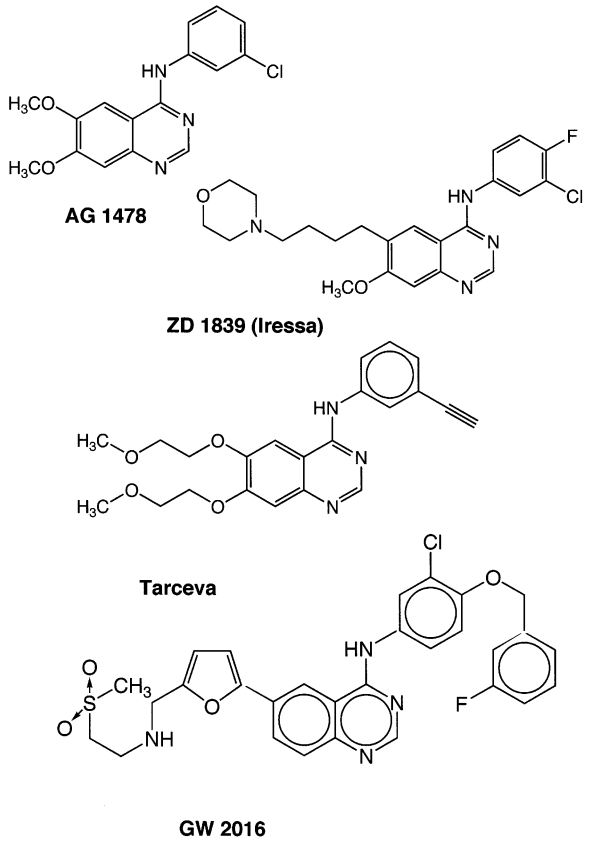


FIGURE 3. Quinazoline EGFR kinase family inhibitors.

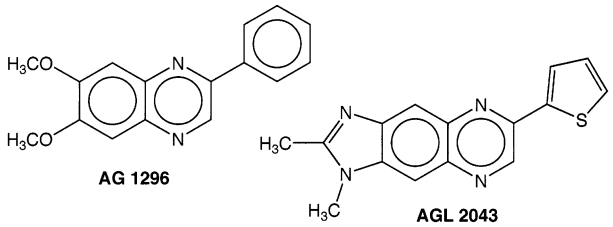


FIGURE 4. Quinoxaline PDGFR kinase inhibitors.

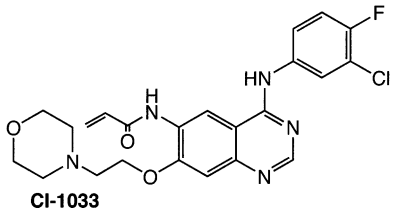


FIGURE 5. Irreversible EGFR kinase inhibitor.

probably because they need to compete against milllimolar concentrations of ATP. For example, quinazolines, which bind to the EGFR with Ki of a few nanomolar,[28] inhibit EGFR autophosphorylation in intact cells at the micromolar concentrations.[29,56] Similarly, PP1, which inhibits Src family kinases at 20−170 nM in cell-free assays,[57] inhibit Src activity in cells in the range of 5−40 μM.[56,58] Another issue is that the selectivities of newly developed compounds are tested only against a limited number of PTKs and Ser/Thr kinases. Many selective inhibitors are soon discovered to hit other kinases: PP1 is equipotent as a PDGFR kinase inhibitor,[37] and Gleevec is as potent

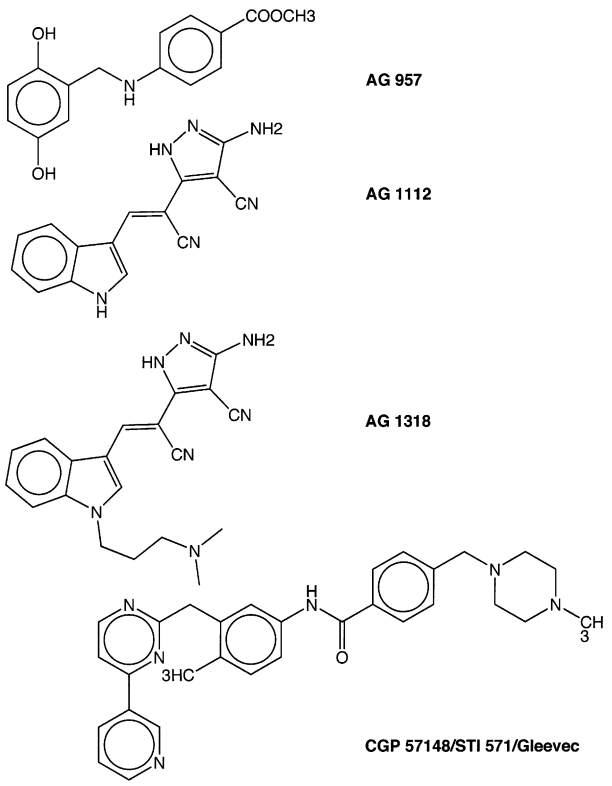


FIGURE 6. Bcr-Abl kinase inhibitors.

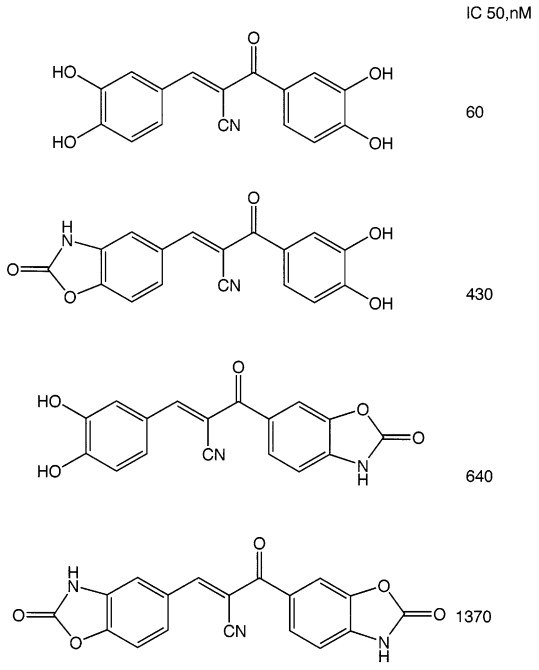
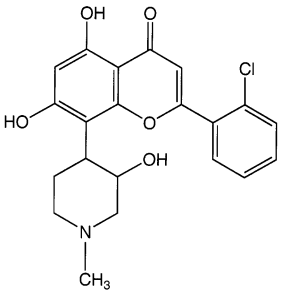
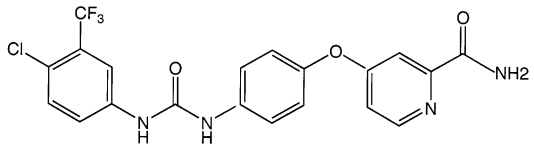


FIGURE 7. Bio-isostere substrate competitive inhibitors. AG 538 was found to be a potent substrate competitive inhibitor of IGF-1 receptor. The replacement of one catechol moiety did not result in a large reduction in affinity, but the replacement of both resulted in a severe loss of activity. All, however, retain their substrate competitive kinetics.

against PDGFR kinase and c-Kit.[49] Substrate competitive inhibitors have the potential to be more selective since the domain outside the ATP binding site is less conserved and may offer more opportunities to design a selective



Flavopiridol



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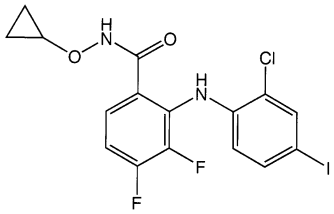

PD 184352

FIGURE 8. Ser/Thr kinase inhibitors.

inhibitor. Bi-substrate inhibitors, which compete for both ATP and substrate simultaneously may be inferior, in principle, to substrate mimics, since they still have to compete against high intracellular ATP levels.

IX. PTK Inhibitors Synergize with Pro-Apoptotic Agents

Transformed cells are more sensitive to stress signaling pathways and apoptotic signals as compared to their parental nonmalignant cells.[48,60] Therefore, transformed cells are more sensitive to cytotoxic agents such as CDDP, other pro-apoptotic cytotoxic agents, and the pro-apoptotic ligand FasL (see[48] for review). However, as the cancer progresses to a more advanced state, cells acquire an anti-apoptotic shield, masking the potentiated state of sensitivity.[48] An interesting example is advanced GBM, where the truncated EGFR, Δ (2−7) EGFR, is responsible for its resistance to chemotherapy and radiation therapy. Blockade of the Δ (2−7) EGFR by the EGFR kinase inhibitor AG 1478 sensitizes the tumor to CDDP and enhances the survival of tumor bearing nude mice treated with the two agents.[41,42]

X. PTK Inhibitors for the Treatment Restenosis, Psoriasis, and Papilloma

Restenosis, following balloon angioplasty, is largely driven by the stimulation of the PDGFR in the media of the manipulated blood vessel. Indeed, local application of the PDGFR kinase inhibitors AG 1295[22] and AGL 2043[33,34] (Banai et al., unpublished experiments), during balloon angioplasty, inhibits effectively restenosis. Enhanced activity of EGFR observed in psoriasis and Papilloma is due to the autocrinic stimulation of an EGFR, which is overexpressed. EGFR kinase inhibitors inhibit the growth of both psoriatic keratinocytes[61−64] and keratinocytes, immortalized with HPV 16,[65,66] and therefore can be considered as therapeutic agents.

XI. EGFR Kinase Inhibitors for Imaging

In principle, one can utilize radioactively labeled PTK inhibitors in order to image tumors, which overexpress the kinase and determine whether targeting a particular kinase is reasonable. A case in point is the decision whether to treat lung cancer with Iressa since not every lung cancer expresses these receptors.[39] Reversible and irreversible 11C and 18F labeled EGFR kinase inhibitors were recently developed for imaging EGFR overexpressing tumors. It was recently shown that the irreversible inhibitors[67] are actually superior to the reversible ones.[68]

XII. SER/THR Kinase Inhibitors

A handful of Ser/Thr kinases have been shown to be to be involved in signal transduction as we know it today (Figure 1). These transmit signals of upstream PTKs, and their activity is essential for cell proliferation and the onset of anti-apoptotic signaling (Figure 1). Their

Table 1. Protein Kinase Inhibitors in the Clinic and in Development

company	agent	indication	target	status
		PTK Inhibitors		
Novartis	STI571/Gleevec	CML*	Bcr-Abl	marketed
Novartis	STI571/Gleevec	GIST	C-Kit	marketed
AstraZeneca	ZD 1839/Iressa	solid tumors	EGFR,Her-2	marketed
Pharmacia/Sugen	SU 6668	solid tumors	VEGFR/PDGFR/FGFR	phase 3
OSI Pharmaceuticals	Tarceva (OSI 774)	solid tumors	EGFR	phase 2
Cephalon/Lundbeck	CEP-701	prostate cancer	NGFR	phase 2
Ludwig Institute For Cancer Research/ Algen Biopharmaceuticals	AG 1478/CDDP	glioblastoma multiforme	EGFR	phase 1
		Ser/Thr Kinase Inhibitors		
Bayer/Onyx	BAY 43−9006	colon cancer	Raf	phase 1
Falvopiridol			Cdk4/1	phase 1
Eli Lilly	LY 333531	diabetic retinopathy	protein kinase C	phase 3
Cephalon/Lundbeck	CEP-1347	Parkinson's	mixed lineage kinase	phase 1

abnormal enhanced activities are enhanced by the deletion of negative regulators, such as protein inhibitors of cyclin-dependent kinases (Cdks), like p16, and the deletion of the tumor suppressor PTEN (Figure 1). Thus, the enhanced activities of these kinases are driven by the synergistic action of the upstream PTKs, combined with the inactivation of the downstream negative regulators.

XIII. RAF and MEK Inhibitors

Inhibitors of the Ras-Raf-Mek-Erk pathway are of great potential since the activation of elements of this pathway is the hallmark of many cancers. For example, activating mutations in B-Raf occur in ~66% of human melanoma,[69] suggesting that Raf kinase inhibitors such as BAY 43−9006[70] (Figure 8) and Mek inhibitors such as PD184352[71] (Figure 8) may be useful. PD 184352 inhibits tumor growth in mice with colon carcinomas of both mouse and human origin.[71] Raf, Mek, and Erk inhibitors can be extremely useful for the treatment of many cancers since their activities are highly enhanced due to the widespread oncogenic mutations in *ras*. Once activating mutations occur in the Ras-Raf-Mek pathway, the utilization of Ser-Thr inhibitors will be essential since the pathway will be independent of PTK activation.

XIV. Cyclin Dependent Kinase Inhibitors

Inhibitors of Cdks, especially Cdk2 and Cdk 4, are in clinical development for cancer.[72−77]

Falvopiridol, for example, (Figure 8) inhibits Cdk4/cyclin D and Cdk1/CyclinB1[78] and is currently in clinical trials.[79]

XV. PKB/AKT Inhibitors

Progress toward the generation of PKB inhibitors (Aktstatins) has been recently reported. The PKA inhibitor H-89 was modified to reduce its affinity toward PKA and improve its affinity toward PKB/Akt.[80]

XVI. PKC Inhibitors

Although PKC isozymes have been known for a long time, little progress has been made in the utilization of PKC inhibitors, but some PKC inhibitors are in development as anticancer agents[73,81−83] and vascular retinopathy.[84]

XVII. Rapamycin

Rapamycin, the inhibitor of mTor, is effective as an inhibitor of angiogenesis and therefore is a potential anticancer drug.[85] Rapamycin is also effective in the inhibition of restenosis, when applied on coated stents.

Table 1 summarizes the current state of protein kinase inhibitors in the clinic.

XIII. Concluding Remarks

The success of Gleevec, in treating patients suffering from chronic myeloid leukemia, and of Iressa, in the treatment of a sub-population of lung cancer patients, demonstrates the paradigm shift taking place in cancer therapy. Since protein kinases, especially protein tyrosine kinases, play a role in every aspect of cellular function, their malfunction results in disease, often cancer. Thus, protein tyrosine phosphorylation inhibitors will occupy an increasing fraction of anticancer agents of the future. It is quite clear that a few dozens of such inhibitors, covering most of the kinases involved in the various cancers, will make a huge difference in disease outcome in the coming future. Table 1 summarizes the current protein kinase inhibitors in the clinic and in development.

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