

Protein Kinase Inhibitors for Treatment of Cancer

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Research and development of cancer drugs targeting kinase has seen explosive growth in recent years. Marketed kinase (or kinase pathway) inhibitors such as imatinib (Gleevec/Glivec) and Bevacizumab (Avastin) have revolutionized cancer treatment. Many kinase inhibitors are predicted to reach market in 5 years, especially in the cancer therapy.

Protein kinases play important roles in regulating cell growth, cell differentiation, cell division, cell migration, metabolism and gene expression. Analysis of human genome identifies 518 protein kinases.¹ Majority of these kinases have been characterized at least to some extent. Because the critical roles kinases play in almost every prospect of cellular functions, kinases have gained more and more significance in both academic and industrial research especially in cancer targeted therapy, rivaling the G-protein-coupled receptors (GPCR) as the most important drug target group in the foreseeable future.^{2,3} Currently, three small molecules and two monoclonal antibody kinase inhibitors have won regulatory approvals for treatment of cancer. In addition, close to 100 kinase inhibitors have entered clinical testing. In this review, the author will focus on the small molecule kinase inhibitors in mid to late stage clinical trials as anti-cancer agents and only discuss monoclonal antibody therapy briefly.

Receptor Tyrosine Kinase Inhibitors

Monoclonal antibodies:

Antibodies or antibody fragments work by binding to the extracellular domains of receptor tyrosine kinases (RTK) or its ligands. The binding prevents the receptor dimerization that is required for the activation of intracellular kinases. Thus achieving the inhibition of kinase signaling pathway. Currently, three highly successful antibodies are approved with many more in various stages of clinical trials. Trastuzumab (Herceptin), a monoclonal antibody targeting ErbB2 (HER2/neu) was approved in 1998 for breast cancer. Strong correlation was established between the response and the HER2 over-expression in metastatic breast tumors. This allows the selection of patients through molecular diagnosis, which improves the response rate and reduces overall health cost. Recently, it is found that trastuzumab also significantly improves the survival of breast cancer patients after surgery. Cetuximab (Erbiximab), an EGFR (ErbB1/HER1) targeting antibody that was approved for colorectal cancer in 2004, is currently undergoing phase 2 and 3 trials in several solid tumors. Panitumumab (ABX-EGF) is a fully humanized monoclonal antibody against EGFR in phase 3 trial for colorectal cancer. It is expected to have fewer side effects than the chimeric cetuximab. Pertuzumab (Omnitarg), a second-generation antibody against HER2 in phase 2 trials, blocks HER2 dimerization with other members of ErbB receptors. It is expected to have broader application than trastuzumab or cetuximab, which inhibits only homo-dimerization of EGFR or HER2. Bevacizumab (Avastin) that targets VEGF won approval for colorectal cancer in 2004 as a combination with 5-FU-based chemotherapy. This approval validates the anti-angiogenesis based therapy in treating human cancers. Subsequently, bevacizumab has been found to provide progression-free survival benefits for patients with non-small cell lung cancer (in combination with paclitaxel and carboplatin) and with breast

cancer (in combination with paclitaxel). More recently, monoclonal antibodies against insulin-like growth factor 1 receptor (IGF-1R) from Pfizer (CP-751871) and Imclone (A12) entered clinical phase 1 trial. It remains to be seen how effective to target signal transduction pathway of IGF-1R.

Bcr-Abl kinase inhibitors:

The clinical trial of the first small molecule RTKI imatinib (Gleevec/Glivec) for chronic myeloid leukemia (CML) was spectacularly successful, which led to its accelerated approval in 2001 after phase 2 trial.⁴ CML is a myeloproliferative disease caused by *constitutive activation* of Abl kinase, as a consequence of fusion of Bcr gene (Philadelphia chromosome translocation). In the chronic phase of CML, peripheral blood counts become normal in >90% of patients, and 50-70% have no evidence of Philadelphia chromosome translocation after treatment. In the blast phase (late-stage) of the cancer, imatinib still has impressive activity, albeit short-lived due to rapid emergence of drug resistance.⁵ Imatinib is also approved for the treatment of gastrointestinal stromal tumors (GIST) due to its activity in inhibiting c-Kit. Point mutations leading to constitutively activated c-Kit are found in a significant fraction of GIST patients. Appreciable responses to imatinib were also found in patients with chronic myelomonocytic leukemia (CMML) because imatinib inhibition of constitutively activated fusion tyrosine kinase, TEL-PDGFRb in CMML. Due to the genetic instability and heterogeneity, significant resistance to imatinib emerges in the CML patients. Currently, two small molecule kinase inhibitors, BMS354825⁶ and AMN107 with high inhibitory activity against multiple Abl kinase mutants have entered phase 2 clinical trials for CML.

EGFR family kinase inhibitors:

Gefitinib (Iressa) was approved in 2002 (Japan) and 2003 (US) as third-line treatment of advanced NSCLC after two phase 2 trials. However, the pivotal phase 3 trials of NSCLC did not meet the primary end point of increased survival. The prescription of this drug is limited to existing patients, and further development is under review by the manufacturer and US FDA. The molecular target of gefitinib, EGFR (ErbB1/HER1) tyrosine kinase is over-expressed in many cancers of epithelial origin, however the correlation between EGFR expression and clinical response has not been established. The report that NSCLC patients with EGFR mutations (most notably L858R) are sensitive to gefitinib therapy may lead to the development of diagnose of patients who may respond to the therapy and thus greatly improve the effectiveness of the drug.⁷ Surprisingly, Erlotinib (Terceva), a close analog to gefitinib (Iressa) improved overall survival of the patients who have failed 1-2 chemotherapy regimens as a single agent. Erlotinib was approved in late 2004, and the reason for the observed survival benefit difference between gefitinib and erlotinib is unknown at this moment. Lapatinib is a potent inhibitor against both EGFR and ErbB2 (HER2) in phase 3 trials

for multiple cancers. It remains to be seen whether this small molecule inhibitor would bear the combined benefits of erlotinib/cetuximab and trastuzumab. A number of other small molecules targeting either ErbB2 or multiple members of ErbBs are currently in phase 1 and 2 trials.⁸

Anti-angiogenic and multiplex kinase inhibitors:

Inhibition of angiogenesis for treatment of cancer has been a very active research area for more than a decade. The recent exciting development of Avastin further fueled the search for small molecule anti-angiogenic agents. Multiple RTKs such as VEGFRs, PDGFRs and FGFRs play important roles in tumor angiogenesis. These are good cancer targets. Over 20 small molecules targeting angiogenic kinases are currently in clinical trials. Leading the pack, Sutent (SU-11248) was found efficacious by interim phase 3 analysis of gastrointestinal stromal tumors clinical trial, and is expected to win FDA approval in late 2005 or early 2006. It has broad and potent activities against a panel of kinases (<20 nM against VEGFR2, FGFR, PDGFRb, c-Kit and FLT3). Similarly, clinically significant improvement in progression-free survival was seen in renal cancer patients treated by sorafenib (BAY 43-9006) upon interim phase 3 analysis. Though sorafenib was originally developed as a Raf kinase inhibitor, it was found to potently inhibit VEGFRs, PDGFR-beta, FGFR1, flt-3, kit. Vatalanib (PTK787) is currently in phase 3 trial for colorectal cancer and early phases for multiple cancers. It is a moderately potent pan-VEGFR inhibitor (77, 37, and 640 nM for subtype 1, 2, and 3) with weak activity against PDGFRb (580 nM) and c-Kit (730 nM).

Serine/threonine kinase inhibitors:

Though tyrosine kinase inhibitors are leading the development of kinase inhibitors for cancer, several promising serine/threonine kinase inhibitors have entered clinical trials. Constitutive Ras activation and PTEN deactivation are two most widespread mutations in human cancers. These mutations lead to constitutive activation of Raf and AKT kinases. In addition, constitutively active mutant B-Raf kinase (V600E) is found in about 40% melanoma patients. These provide very strong rationale to target kinases in Ras/Raf/MEK/ERK and PI3/AKT signaling pathways to counter those oncogenic mutations.

Recently, PD325901, a specific inhibitor against MEK kinase entered phase 1 clinical trial. The unique allosteric binding mode of PD325901 leads to its high specificity. Its good pharmacokinetic properties and *in vivo* activity should help the clinical understanding of this important signaling pathway. A number of rapamycin analogs are currently in mid to late stage of clinical trials. They inhibit mTOR (mammalian target of rapamycin) kinase. mTOR lies downstream in the PI3/AKT signaling pathway. It regulates the response of tumor cells to nutrients and growth factors and controls tumor blood supply and

angiogenesis through effects on VEGF.

Targeting kinases that regulate cell cycling is another attractive strategy in cancer therapy, though these kinase inhibitors will most likely be cytotoxic instead of cytostatic. CDKs (cyclin dependent kinases) are closely associated with cell cycle progression, while CHKs (check point kinases) help to maintain the cell cycle integrity. Other kinases, such as PLKs (polo kinases) and, aurora kinases are very important in regulating cell mitosis. A number of CDK inhibitors are in clinical testing, while one Aurora kinase inhibitor recently entered clinical trials.

Prospective

Constitutively active kinases:

Certain types of tumors may depend on particular kinases to proliferate and survive, therefore identifying and targeting these molecular targets could have drastic effect on cancer treatment as seen in the success of imatinib (Gleevec) and trastumab (Herceptin). Constitutively active kinase in the cancer would be the best indicator for this kind of dependence. Besides BCR-ABL, c-Kit, PDGFB and AKT mentioned earlier in this article, other constitutively active kinases have been discovered so far: Flt3 in acute myeloid leukemia (AML) and acute lymphoid leukemia, EGFR in glioblastoma, and B-Raf in melanoma.

Drug resistant mutant kinases:

Because of the heterogeneity and genetic instability in cancer, drug resistant mutant kinases have emerged from patients treated with imatinib and gefitinib. Mutations rendering the inhibitors ineffective may also emerge from other kinases, especially the constitutively active kinases that cancer depends on. This provides the challenge and opportunity to develop follow-on generations of kinase inhibitors that preferably inhibit both the wild type and mutant kinases. For examples, even though the second generation Bcr Abl inhibitors (BMS354825, AMN107) overcame most of imatinib resistant mutations, both are inactive against one of the major mutants, T351I. Furthermore, mutations to these second-generation drugs may also emerge.

Combination therapy and multi-targeting in cancer:

Though the approach to inhibit constitutively active kinases is very attractive, it is often difficult to identify the constitutively active kinases in most cancers. In addition, because of the complexity and heterogeneity, it is conceivable that the progression of majority of cancers may not be dependent on single kinases. Combination therapies may overcome these challenges. Currently, combination of kinase inhibitor of parallel pathways; and combination of kinase inhibitors with traditional cytotoxic agents are being tried both clinically and preclinically. Synergy has been demonstrated in animal models and shown in late stage clinical trials. It is predicted that combination therapy involving kinase inhibitors will be the norm instead of exception in a decade.

Alternative to the combination of selective kinase inhibitors, multiplex kinase inhibitor is gathering popularity.⁹ Designing selective ATP-competitive inhibitors is technically challenging, but it may not be necessary or even not desirable.^{10,11} Superior clinical anticancer efficacy has been observed for the less selective inhibitor Sunitinib and sorafenib. Approaches to develop inhibitors with different spectrum of kinase inhibition could offer broader efficacy with manageable side effect in treatment of cancer through inhibition of multiple kinases based on their involvement in cancer. However, less-selective inhibitors could potentially result in more and severe side effects. It has also been highly empirical in developing kinase inhibitors against multiple targets. Structure based design, frequent profiling of inhibitors against a panel of kinases, and cell pathway profiling have been used to select inhibitors with desirable selectivity profiles.

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Table 1. Some kinase inhibitors in clinical trials and proved drug for cancer

Inhibitor	Targets	Indication^a	Status	Company
Monoclonal antibodies				
Cetuximab (Erbix)	EGFR	Colorectal HN, Pancreatic, NSCLC	Launched Phase 3	Imclone, BMS
Panitumumab	EGFR	Colorectal NSCLC, Prostate, renal	Phase 3 Phase 2	Abgenix, Amgen
Trastuzumab (Hercetpin)	ErbB2 (HER2/neu)	Breast	Launched	Genentech, Roche
Pertuzumab	ErbB2	NSCLC, Breast, Prostate, Ovary	All Phase 2	Genentech
CP-751871	IGF1R	Cancer	Phase 1	Pfizer
A-12	IGF1R	Cancer	Phase 1	Imclone
Tyrosine Kinase Inhibitors				
Imatinib	Abl, Kit, PDGFR	CML, GIST	Launched	Novartis
AMN107	Abl	CML	Phase 2	Novartis
BMS-354825	Abl, Src, Kit	CML	Phase 2	BMS
AZD-0530	Abl, Src	Solid tumor	Phase 1	AstraZeneca
Gefitinib (Iressa)	EGFR	NSCLC	On-hold	AstraZeneca
Erlotinib (Tarceva)	EGFR	NSCLC Pancreatic Colorectal	Launched Registered Phase 2	OSI, Genentech, Roche
BMS-599626	EGFR, ErbB2,4	Cancer	Phase 1	BMS
XL647	EGFR, ErbB2, EphB	Solid tumor	Phase 1	Exelixis
Lapatinib	EGFR, ErbB2	NSCLC, Breast, GIST, Bladder, HN, Colorectal	All Phase 3	GSK
Canertinib	EGFR, ErbB2,4	Cancer	Phase 2	Pfizer
HKI-272	EGFR, ErbB2	Cancer	Phase 1	Wyeth
TAK-165	ErbB2	Breast cancer	Phase 1	Takeda
Pelitinib	EGFR, ErbB2	NSCLC, Breast, Colorectal, HN, Renal	All Phase 2	Wyeth
CP-724714	ErbB2	Cancer	Phase 1	Pfizer, OSI
Sutent	VEGFR2-4, Kit, PDGFRb, Flt3	GIST Renal Various cancer	Registered Phase 3 Phase 2	Pfizer
Tandutinib (MLN-518)	Flt3, Kit, PDGFR	AML CML	Phase 2 Phase 1	Millennium
XL999	VEGFR, Flt3, FGFR, PDGFR	Cancers	Phase 1	Exelixis
Midostaurin	FLT-3, PKC	AML, CLL, NHL, Melanoma	All Phase 2	Novartis
XL880	Met, Tie2, VEGFR2, Kit, PDGFR, Flt3	Solid tumor	Phase 1	Exelixis
Sorafenib, Bay43- 9006	RAF, VEGFR2, Flt3, Kit, PDGFRb	Renal	Pre- Registration	Onyx, Bayer
Lestaurtinib	TrkA, Flt3, VEGFR2	AML, prostate	Phase 2	Cephalon
CEP-5214	VEGFR	Solid tumor	Phase 1	Cephalon
Vatalanib, (PTK787)	VEGFR1-3, PDGFRb	Colorectal Renal	Phase 3 Phase 2	Novartis, Schering

GSK-786034	VEGFR2	Solid tumor	Phase 2	GSK
Chir-258	VEGFR1-3, Flt3, PDGFRb, FGFR, Kit	AML, Solid tumor	Phase 1	Chiron
Vandetanib	VEGFR2	Lung, Breast, Myeloma	Phase 2	AstraZeneca
CP547632	VEGFR2	NSCLC, Ovary	Phase 2	Pfizer, OSI
AMG706	VEGFR1-3, Kit, PDGFR	Solid tumor	Phase 1	Amgen
AZD-2171	VEGFR1-3	solid tumor	Phase 1	AstraZeneca
CEP-7055	VEGFR1-2	Solid tumor	Phase 1	Cephalon
AG013736	VEGFR1-3, Flt3, PDGFRb, FGFR, Kit	Solid tumor	Phase 1/2	Pfizer
OSI-930	VEGFR2, Kit, PDGFR	Cancer	Phase 1	OSI
KRN-951	VEGFR1-3, PDGFR, Kit	Glioma, Solid tumor	Phase 1	Kirin
BIBF-1120	VEGFR2, PDGFR, FGFR, Kit	Solid tumor	Phase 1	Bohringer Ingleheim
Bay-57-9352	VEGFR2, Kit, PDGFR	Solid tumor	Phase 1	Bayer
AEE-788	VEGFR2, EGFR, ErbB2, Flt-1	Cancer	Phase 1	Novartis
Serine/Threonine Kinase Inhibitors				
RX-0201	AKT	Solid tumor	Phase 1	Rexahn
VX680	Aurora A/B/C	Solid tumor	Phase 1	Vertex, Merck
AZD5438	CDK	Cancer	Phase 1	AstraZeneca
AG-024322	CDK (pan)	Cancer	Phase 1	Pfizer
BMS-387032	CDK2	Renal, NSCLC	Phase 1	BMS, Sunesis
seliciclib	CDK2	Breast, NSCLC	Phase 2	Cyclacel
ZK-CDK	CDK1-2, VEGFR2, PDGFRb	Cancer	Phase 1	Schering AG
PD-332991	CDK4/6	Cancer	Phase 1	Pfizer, Onyxx
AZD6244	MEK	Solid tumor	Phase 1	Array, AstraZeneca
PD325901	MEK1-2	Solid tumor	Phase 1	Pfizer
AP23573	mTOR	Solid tumor	Phase 2	Ariad
Everolimus	mTOR	Cancer	Phase 2	Novartis
temsirolimus	mTOR	Renal Breast, Prostate, Sarcoma	Phase 3 Phase 2	Wyeth
Enzastaurin	PKC-beta (C11beta)	NHL, Brain, BCL	Phase 2	Lilly

^aAbbreviations: NSCLC, non-small cell lung cancer; AML, acute myeloid lymphoma; CLL, chronic lymphoma; BCL, B cell lymphoma; GIST, gastrointestinal stromal tumor; HN, Head and Neck; NHL, non-Hodgins lymphoma.



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