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# Future Cancer Therapy with Molecularly Targeted Therapeutics: Challenges and Strategies

**Mi-Sook Kim***

*Millennium Pharmaceuticals, Cambridge, MA 02139, U.S.A.*

## Abstract

A new strategy for cancer therapy has emerged during the past decade based on molecular targets that are less likely to be essential in all cells in the body, therefore confer a wider therapeutic window than traditional cytotoxic drugs which mechanism of action is to inhibit essential cellular functions. Exceptional heterogeneity and adaptability of cancer impose significant challenges in oncology drug discovery, and the concept of complex tumor biology has led the framework of developing many anticancer therapeutics. Protein kinases are the most pursued targets in oncology drug discovery. To date, 12 small molecule kinase inhibitors have been approved by US Food and Drug Administration, and many more are in clinical development. With demonstrated clinical efficacy of bortezomib, ubiquitin proteasome and ubiquitin-like protein conjugation systems are also emerging as new therapeutic targets in cancer therapy. In this review, strategies of targeted cancer therapies with inhibitors of kinases and proteasome systems are discussed. Combinational cancer therapy to overcome drug resistance and to achieve greater treatment benefit through the additive or synergistic effects of each individual agent is also discussed. Finally, the opportunities in the future cancer therapy with molecularly targeted anticancer therapeutics are addressed.

**Key Words:** Targeted cancer therapy, Small molecule kinase inhibitor, Ubiquitin proteasome system (UPS) inhibitor, Ubiquitin-like protein (UBL) conjugation system inhibitor, Combination of molecularly targeted cancer therapy, Personalized medicine

## INTRODUCTION

Cancer represents one of the most pressing challenges in current health care. One in three people in developed countries dies from the cancer, and the newly diagnosed patients often have a very short expected survival time (five-year survival for pancreatic carcinoma is <5% (Kamb, 2005)). The worldwide incidence of newly diagnosed cases of cancer is about 10 million cases per year, and it is expected to double by 2030.

Since the war on cancer initiated by the signing of the National Cancer Act of 1971 by then US President Richard Nixon, details of the biological basis of cancers have been revealed for the past four decades. Global commercial development of cancer treatments has dramatically increased over the past 20 years and the average number of therapeutic candidates entering clinical study per year more than doubled, 33 in the early 1990s to 75 in the mid 2000s (Reichert and Wenger, 2008). However, the approval rate for the cancer therapeutics is still low with only 8% during 1990-2006 in the US (Kamb *et al.*, 2007; Reichert and Wenger, 2008).

In a pharmaceutical industry in which nine out of ten attempts to bring a product to market fail, oncology is among the most challenging therapeutic area (Kola and Landis, 2004; Kamb *et al.*, 2007). In the recent article published in the *Nature Rev. Drug Discov.* (Arrowsmith, 2011), 83 Phase III and submission failures during 2007 to 2010 were divided according to therapeutic area and reason for failure. Largest numbers of failures was in the area of cancer (28%) followed by neurodegeneration (18%). The 66% of the failures across all therapeutic areas were attributed to lack of efficacy and 21% of the failures were due to safety issues. Therefore, there is a desperate need for new drugs, especially in the cancer treatment.

As a rational approach to cancer therapy in the middle of the last century, a new class of drug discoveries emerged to exploit the differential dependence of proliferating cancer cells on vital functions such as DNA metabolism, replication, chromosome segregation and cytokinesis. These efforts ultimately produced range of nucleoside analogues, DNA-modifying chemicals and natural products - the traditional chemotherapeutic (cytotoxic) agents. The mechanism of action of these

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***Corresponding Author**

E-mail: mi-sook_kim@mpi.com
Tel: +1-617-444-1242, Fax: +1-617-444-1501

drugs-inhibition of essential cellular functions-dictates a narrow therapeutic window (Kamb *et al.*, 2007). A new strategy for cancer therapy has emerged during the past decade based on molecular targets that are less likely to be essential in all cells in the body, therefore more apt to confer a wider therapeutic window than traditional cytotoxic drugs (Kamb *et al.*, 2007; Reichert and Wenger, 2008).

In this review, some of the strategies and the opportunities in the molecular targeted oncology drug discovery are discussed.

## EXCEPTIONAL HETEROGENEITY AND ADAPTABILITY OF CANCER: MAJOR CHALLENGES IN ONCOLOGY DRUG DISCOVERY

Cancer is an extraordinarily heterogeneous disease with somatic alterations arise as individual cancer develops (Greenman *et al.*, 2007; Kamb *et al.*, 2007; Harris and McCormick, 2010). This exceptional heterogeneity of cancer is reflected in observed differences in drug responses, and is the probable cause of acquired resistance. It is also presumably related to drug sensitivity and tumor aggressiveness which display a wide range of variation among malignancies.

Due to the heterogeneity both among the cells of an individual tumor and among different cancers, the efficacy predictions made from xenograft animal models often fail (Kamb, 2005), which presents significant challenges in the oncology drug discovery where advancement of compounds largely based on a pharmacological effect in the xenograft models. Even though xenograft represents significant aspects of the tumor from which it was derived, it probably captures only a fraction of the total genetic and epigenetic heterogeneity of a given tumor subtype. Low response rate in phase I oncology trials is reflective of poor predictability of clinical efficacy based on these xenograft models (Kamb *et al.*, 2007).

Hanahan and Weinberg (2000) proposed six hallmarks of cancer which comprise six biological capabilities acquired during the multistep development of human cancer (Fig. 1). Those hallmarks include self-sufficiency in growth signals, insensitivity to anti-growth signals, replicative immortality, sustained angiogenesis, evading apoptosis and tissue invasion and metastasis. Genetic instability and consequent genetic alterations is one of the underlying mechanisms of these hallmarks.

During the past decade, there have been a remarkable progress in terms of understanding the mechanistic foundation of each hallmark Hanahan and Weinberg initially proposed (Hanahan and Weinberg, 2000). In a recent article published (Hanahan and Weinberg, 2011), they proposed the capability to modify or reprogram cellular metabolism to effectively support neoplastic proliferation and the evasion of immunological destruction as two additional emerging hallmarks involved in the pathogenesis of some and perhaps all cancers (Fig. 2). This concept of the complex tumor biology has led the framework of developing anticancer therapeutics; many drugs have been approved or in clinical trials targeting each of cancer hallmark.

## TARGETED CANCER THERAPIES WITH SMALL MOLECULE KINASE INHIBITORS

During the past decade, a new strategy for cancer therapy has emerged based on well defined molecular targets that are less likely to be essential in all cells in the body, therefore

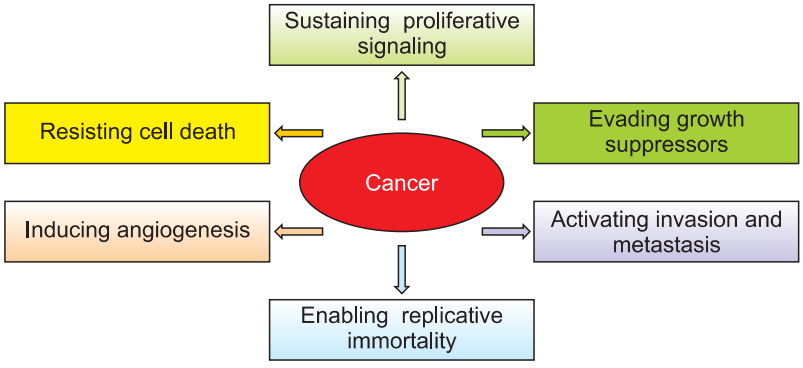


**Fig. 1.** Six Hallmarks of cancer (Adopted from Hanahan and Weinberg (2000) with permission from Elsevier).

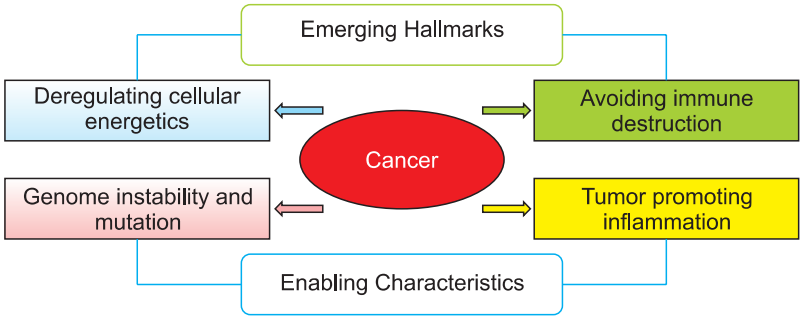


**Fig. 2.** Emerging Hallmarks and Enabling Characteristics of cancer (Adopted from Hanahan and Weinberg (2011) with permission from Elsevier).

more apt to confer a wider therapeutic window than traditional cytotoxic drugs. Among those targets, protein kinases have become one of the most extensively pursued molecular targets (Cohen, 2002; Kamb *et al.*, 2007; Reichert and Wenger, 2008; Zhang *et al.*, 2009; Baker and Reddy, 2010; Force and Kalaja, 2011). Protein kinases play a pivotal role in mediating diverse intracellular signaling, and deregulation of their activity has been emerged as a common feature in tumorigenesis (Giamas *et al.*, 2007; Lengyel *et al.*, 2007; Holden *et al.*, 2008; Giamas *et al.*, 2010; Brognard and Hunter, 2011).

Protein kinases catalyse the transfer of the terminal phosphate of ATP to substrates that usually contain a serine, threonine or tyrosine residue. They typically share a conserved arrangement of secondary structure elements in the ATP binding site, and the majority of approved kinase inhibitors and drugs in development target this ATP binding pocket, preventing ATP from binding to the kinase (Zhang *et al.*, 2009). Despite this high degree of conservation in the ATP binding site, highly selective as well as multi-targeted small molecule inhibitors have been developed (Table 1).

There are advantages and disadvantages in developing strategies of selective vs. multi-targeted kinase inhibitors. It is relatively straightforward to validate a target and to understand the mechanism of action of selective kinase inhibitors; however, multi-targeted kinase inhibitors could offer better chance of overcoming molecular heterogeneity of cancer therefore better chance of success. On the other hand, multi-targeted kinase inhibitors might cause undesirable toxicity related to the additional targets which may or may not be relevant in a given tumor.

To date, 12 small kinase inhibitors have been approved by US Food and Drug Administration (FDA) (Table 1), and approximately 80 small molecule kinase inhibitors have been advanced to some stage of clinical evaluations. They are the most entered drug candidates in the clinical trials during or after 2000 (78%), and the overall success rate of these small molecule protein kinase inhibitors during 1990 and 2006 is 26%, which is considerably higher than the overall approval rate (8%) of all cancer therapeutics (Reichert and Wenger, 2008).

Despite remarkable advances have been made, there are still significant challenges remain in the development of small molecule protein kinase inhibitors for the treatment of cancer. Disease remissions after treatment with these kinase inhibitors are almost followed by eventual disease progression in patients. The mechanisms for this resistance appear to include increased drug efflux, altered drug metabolism, secondary mutations in the kinases which disrupt drug binding, increased expression of target kinase and activation of alternative signaling pathways of cell survival (Engelman and Settleman, 2008). Appropriate multi-targeted inhibitors or combination therapy can be considered as strategies to overcome or prevent this resistance (combination therapy is also addressed in this review). Unexpected toxicities including cardiotoxicity, have emerged during the preclinical and clinical stages of development of these kinase inhibitors (Force and Kolaja, 2011) and these toxicity issues also need to be adequately addressed. Several selected small molecule kinase inbibitors are discussed.

## BCR-ABL inhibitors

Chronic myeloid leukemia (CML) is a form of leukemia

**Table 1.** Kinase inhibitors in the US market

| Drug | Year of approval | Target | Indication |
|---|---|---|---|
| Sirolimus (Rapamune; Pfizer) | 2000 | mTor | Prevention of organ rejection in patients receiving transplants |
| Imatinib (Gleevec; Novartis) | 2001 | ABL, ARG, PDGFR-α/β, KIT | CML, GIST, B-ALL, CMML, CEL |
| Gefitinib (Iressa; AstraZeneca) | 2003 | EGFR | NSCLC |
| Erlotinib (Tarceva; Roche/Genentech) | 2004 | EGFR | NSCL and pancreatic carcinoma |
| Sorafenib (Nexavar; Bayer) | 2005 | B-RAF, VEGFRs, PDGFRα/β, FLT3, KIT | RCC, liver carcinoma |
| Sunitinib (Sutent; Pfizer) | 2006 | VEGFR, PDGFR, CSF1R, FLT3, KIT | RCC, GIST |
| Dasatinib (Sprycel; Bristol-Myers Squibb) | 2006 | ABL, ARG, KIT, PDGFRα/β, SRC | CML with imatinib resistance and/or intolerance |
| Temsirolimus (Torisel; Pfizer) | 2007 | mTOR | RCC |
| Nilotinib (Tasigna; Novartis) | 2007 | ABL, ARG, KIT, PDGFRα/β | CML with imatinib resistance and/or intolerance |
| Lapatinib (Tykerb; GlaxoSmithKline) | 2007 | EFGR (ERBB1 and 2) | HER2 positive breast cancer |
| Everolimus (Afinitor; Novartis) | 2009 | mTOR | RCC |
| Pazopanib (Votrient; GlaxoSmithKline) | 2009 | VEGFR, PDGFRα/β, and KIT | RCC |

ARG: ABL-related gene protein, B-ALL: B-cell acute lymphoblastic leukaemia, CEL: chronic eosinophilic leukaemia, CML: chronic myeloid leukaemia, CMML: chronic myelomonocytic leukaemia, CSF1R: colony-stimulating factor 1 receptor, EGFR: epidermal growth factor receptor, ERBB2: human epidermal growth factor receptor 2 (also known as HER2), ERBB4: human epidermal growth factorreceptor 4, FLT3: FMS-related tyrosine kinase 3, GIST: gastrointestinal stromal tumour, mTOR: mammalian target of rapamycin, NSCLC: non-small-cell lung cancer, PDGFR: platelet-derived growth factor receptor, RCC: renal cell carcinoma, VEGFR: vascular endothelial growth factor receptor (Adopted from Force and Kolaja (2011) with permission from Nature Publishing Company).

characterized by the increased and unregulated proliferation of myeloid cells (Deininger *et al.*, 2000; Kalidas *et al.*, 2001). CML accounts for 15-20% of all adult leukemias in western populations and in the US only, 3,500 to 5,000 new cases are diagnosed per year. It progresses to the more aggressive accelerated and blast phases and once reaching blast phase, the medium survival rate is less than 6 months (Kalidas *et al.*, 2001; Knight and McLellan, 2004). The underlying mechanism of causing CML is a chromosomal translocation between the Abelson (ABL) oncogene from chromosome 9 and breakpoint cluster region (BCR) on chromosome 22 resulting in the expression of the BCR-ABL fusion protein which is seen in almost all patients with CML (Deininger *et al.*, 2000).

**Imatinib (STI-571; Gleevec®; Novartis):** Imatinib is an inhibitor of Ableson kinase (ABL) which received US FDA approval in 2001 for the treatment of CML, gastrointestinal stromal tumors (GISTs) and a number of other malignancies. Imatinib is a revolutionary anticancer drug as a first targeted kinase inhibitor. Animals have limited requirement for ABL activity judged by the mouse knockout phenotype, which is one of the reasons that imatinib is highly effective in CML and is well tolerated as chronic therapy (Kamb *et al.*, 2007). The success of imatinib development was attributed to the following key factors. First, CML is a proliferative disorder with dependency on a single target that is non-essential in most of normal cell. Second, imatinib is a relatively selective kinase inhibitor. Lastly, there is a clear way to select patients who will respond to this drug (Kamb *et al.*, 2007). Majority of patients receiving imatinib respond; however, relapse occurs in a subset of patient population with chronic disease, and the patient number increases to nearly 100% in those patients with advanced stage of CML (Shah and Sawyers, 2003). Several potential mechanisms for relapse have been reported and the mutation in the BCR-ABL gene accounts for the majority of imatinib-resistant leukemias (Gambacorti-Passerini *et al.*, 2003; Deininger, 2005; Branford and Hughes, 2006; Engelman and Settleman, 2008; Baker and Reddy, 2010). The discovery of these mutations prompted the development of the second-generation of BCR-ABL inhibitors to overcome the resistance of imatinib. The recommended dose of imatinib is 400 mg/day for patients with CML and GIST, and 600 mg/day for the patients with the accelerated phase or blast crisis of CML (Hartmann *et al.*, 2009).

## Second-generation of BCR-ABL inhibitors

**Dasatinib (BMS-354825; Sprycel®; Bristol-Myers Squibb):** Dasatinib is a potent, orally bioavailable inhibitor of tyrosine kinases including BCR-ABL, SRC family and c-KIT at nanomolar concentrations (Lombardo *et al.*, 2004). It is 325-fold more potent than imatinib against cells expressing wild type BCR-ABL, and retains activity against 14 of 15 imatinib-resistant BCR-ABL mutants (Shah *et al.*, 2004; O'Hare *et al.*, 2005). Dasatinib prolongs survival of mice with BCR-ABL-driven disease and inhibits proliferation of BCR-ABL-positive bone marrow progenitor cells from patients with imatinib-sensitive and imatinib-resistant CML (Shah *et al.*, 2004). The results from the studies with crystal structure of dasatinib-bound ABL kinase suggests dasatinib might have less stringent binding requirement and the increased binding affinity of dasatinib over imatinib is at least partially due to its ability to recognize multiple states of BCR-ABL (Tokarski *et al.*, 2006). Adverse events in patients treated with dasatinib were mild to moderate in severity and were clinically manageable. Dasatinib was approved in 2006 as an oral drug for the treatment of chronic, accelerated or blast phase of CML with resistance or intolerance to prior therapy, including imatinib. Based on the results from phase III trials, the recommended clinical dose is 100 mg once daily for chronic phase CML and 70 mg twice daily for accelerated myeloid or lymphoid blast phase CML (Keam, 2008).

**Nilotinib (AMN107; Tasigna®; Novartis):** Nilotinib is an oral kinase inhibitor binds to the ATP-binding site of the BCR-ABL protein with higher affinity than imatinib. It showed greater potency than imatinib (20-30-fold with $IC_{50}$ <30 nM) against wild-type BCR-ABL. Nilotinib is also significantly active against 32/33 imatinib-resistant BCR-ABL mutants. In preclinical studies, nilotinib demonstrated activity in vitro and in vivo against wild-type and imatinib-resistant BCR ABL-expressing cells (O'Hare *et al.*, 2005; Manley *et al.*, 2005; Golemovic *et al.*, 2005; Weisberg *et al.*, 2005; Weisberg *et al.*, 2006). Phase II trials in patients with chronic phase CML, nilotinib showed high activity in imatinib-resistant or intolerant CML patients (Kantarjian *et al.*, 2007; Breccia and Alimena, 2010). Adverse events reported with nilitinib are generally mild to moderate; grade 3 or 4 neutropenia and thrombocytopenia were reported in 29% of patients. Nilotinib was approved in 2007 for the treatment of chronic and accelerated phase CML, resistant to or intolerant of prior therapy inculuding imatinib. The clinical dose is 400 mg twice daily (Plosker and Robinson, 2008).

Other than these approved BCR-ABL inhibitors, several BCR-ABL inhibitors are currently in clinical development (Table 2).

## Epidermal growth factor receptor (EGFR) inhibitors

EGFR is a member of the EGFR tyrosine kinase family and is involved in the regulation of cellular homeostasis. Upon ligand binding, EGFR activates downstream cell signaling cascades that stimulates cell proliferation, apoptosis, migration, survival and angiogenesis (Yarden and Sliwkowski, 2001; Casalini *et al.*, 2004). EGFR has been strongly implicated in the biology of human epithelial malignancies; aberrant expression or activity of EGFR has been identified as an important factor in human epithelial cancers, including head and neck squamous–cell carcinoma (HNSCC), non-small cell lung cancer (NSCLC), colorectal cancer, breast cancer, pancreatic cancer and brain cancer (Moscatello *et al.*, 1995; Normanno *et al.*, 2006). It has been the most comprehensively studied molecular target in oncology therapeutics over the past decade (Carter *et al.*, 2009; Vivanco and Mellinghoff, 2010; Baker and Reddy, 2010). Several small molecule EGFR inhibitors as well as monoclonal antibodies received FDA approval for the indications of colorectal cancer, head and neck cancer, lung cancer and pancreatic cancer.

**Gefinitib (ZD1839; Iressa®; Astra Zeneca):** Gefinitib, a substituted anilinoquinazoline, is a potent EGFR inhibitor with $IC_{50}$ value of 0.033 μM and selectively inhibits EGF-stimulated tumor cell growth. In studies with mice bearing a range of human tumor-derived xenografts, gefitinib inhibited tumor growth in a dose-dependent manner (Barker *et al.*, 2001; Wakeling *et al.*, 2002; Sirotnak, 2003). In a randomized, double-blind, phase II, multicenter trial, a total of 216 patients were treated with gefitinib (250 vs. 500 mg/day). Among them, 142 patients were refractory to or intolerant of a platinum and docetaxel. A partial tumor response occurred in 14% (9 of 66) of patients received gefitinib at 250 mg/day and in 8% (6 of 76) of patients

**Table 2.** Selected kinase inhibitors in clinical development

| Target | Drug | Company | Clinical trial |
|---|---|---|---|
| BCR-ABL | Bosutinib (SKI-606) | Wyeth | Phase III |
| | Saracatinib (AZD0530) | AstraZeneca | Phase II |
| | AT9238 | Astex Therapeutics | Phase II |
| | PHA-73958 | Pfizer/Nerviano Medical Sciences | Phase II |
| | Tozasertib (MK-0457; VX-680) | Merck | Phase II (discontinued) |
| | XL228 | Exelixis | Phase I |
| | INNO-046 (NS-187; CNS-9) | Innovivie/Nippon Shinyaku | Phase I |
| | LS-104 (AEG-41174) | Aegera Therapeutics | Phase I |
| | AP24534 | Ariad Pharmaceutical | Phase I |
| | SGX393 | SGX Pharmaceuticals | Phase I |
| EGFR | Vandetanib (ZD6474; Zactima®) | AstraZenica | Phase III |
| | XL647 | Exelixis | Phase II |
| | PF-00299804 | Pfizer | Phase II |
| | BIBW 2992 | Boehringer Ingelheim | Phase II |
| | Neratinib (HKI-272) | Wyeth | Phase II |
| | AV412 | AVEO | Phase II |
| | CP-724,714 | Pfizer | Phase I |
| | BMS-599626 | Bristol-Myers Squibb | Phase I |
| | BMS-690514 | Bristol-Myers Squibb | Phase I |
| | ARRY-543 | Array | Phase I |
| | ARRY-380 | Array | Phase I |
| | AZD-4769 | AstraZenica | Phase I |
| | AZD-8931 | AstraZeneca | Phase I |
| | Pelitinib (EKB-569) | Wyeth | Suspended |
| | Canertinib (CI-1033) | Pfizer | Suspended? |
| | AEE788 | Novartis | Suspended? |
| mTOR | Everolimus/RAD001 | Novartis | Phase III |
| | Deforolimus/AP23573/MK-8669 | Ariad/Merck | Phase III |
| | ABI-009 (Nab-rapamycin) | Abraxis | Phase I |
| | OSI-027 | OSI Pharmaceuticals | Phase I |
| | AZD8055 | AstraZeneca | Phase I |

Matthews and Gerritsen (2010).

received 500 mg daily dose of gefitinib. The overall objective response rate (RR) for both doses combined was 10.6% (15 of 142 patients; 95% confidence interval, 6.0-16.8%). Common adverse events associated with gefitinib treatment included diarrhea, rash, acne, dry skin, nausea, and vomiting (Cohen *et al.*, 2004). In 2003, gefitinib received accelerated approval by the US FDA as monotherapy for the treatment of patients with locally advanced or metastatic NSCLC after failure of both platinum-based and docetaxel chemotherapies.

**Erlotinib (OSI-774, CP-358,774; Tarceva®; OSI/Roche/Genentech):** Erlotinib is the same quinazoline derivative as gefitinib and selectively and reversibly inhibits the activity of EGFR tyrosine kinase activity with $EC_{50}$ values of 2 and 20 nM in enzyme and cell based assay, respectively (Moyer *et al.*, 1997). In a large randomized phase III clinical trial, erlotinib showed superior to placebo for survival, progression-free survival, and tumor response rate (Johnson *et al.*, 2005; Bareschino *et al.*, 2007; Iyer and Bharthuar, 2010). Based on these positive results, US FDA granted erlotinib regular approval in 2004 for the treatment of advanced NSCLC patients after failure of a platinum-containing chemotherapy. The maximum tolerated dose of erlotinib was 150 mg in a daily administration schedule and the most common adverse events were the rash and diarrhea (Johnson *et al.*, 2005; Bareschino *et al.*, 2007; Iyer and Bharthuar, 2010). Erlotinib received additional approval for the combination with gemcitabine chemotherapy for the treatment of advanced pancreatic cancer in 2005. The results from the various clinical trials, erlotinib has also shown the activity in head and neck tumors, in glioblastoma, and in other tumor types. The presence of a rash, epidermal growth factor receptor expression and mutation status are the predictive factors for response in patients (Johnson *et al.*, 2005; Bareschino *et al.*, 2007; Iyer and Bharthuar, 2010). On April 16, 2010, US FDA granted erlotinib approval for maintenance treatment of patients with stage IIIB/IV NSCLC whose disease had not progressed after four cycles of platinum-based first-line chemotherapy (Cohen *et al.*, 2010).

**Lapatinib (GW-0572016; Tykerb®; GlaxoSmithKline):** Lapatinib is a 4-anilinoqinazoline derivative of dual inhibitor of EGFR and human epidermal growth factor receptor 2 (HER2) with $IC_{50}$ value of about 10 nM for both tyrosine phosphorylation of EGFR and HER2 (Rusnak *et al.*, 2001) and consequently inhibits activation of downstream effectors of proliferation and cell survival, resulting in a 23-fold increase in apoptosis compared with vehicle controls (Xia *et al.*, 2002). Based on the results from a randomized phase III clinical trial where

lapatinib showed significantly longer median time to progression in combination with capecitabine than the capecitabine monotherapy (6.2 vs. 4.3 months) in patients with breast cancer or metastatic breast cancer whose tumors overexpress HER2 and who have received previous treatment including an anthracycline, a taxane, and trastuzumab (Cameron *et al.*, 2008). US FDA granted lapatinib approval in 2007. The recommended dosing regimen is 1,250 mg once daily oral administration of lapatinib with oral capecitabine 2,000 mg/m$^2$/day on days 1-14 of 21-day cycle. Most frequent adverse events were including diarrhea, nausea, rash and fatigue with low rate of cardiac adverse effects. Currently numerous clinical studies are underway with various combinations (Dhillon and Wagstaff, 2007; Cameron and Stein, 2008; Giampaglia *et al.*, 2010).

### Multi-targeted kinase inhibitors

**Sorafenib (BAY 43-9006; Nexavar; Bayer):** Sorafenib is a novel oral kinase inhibitor targets multiple tyrosine kinases including RAF, vascular endothelial growth factor receptors (VEGFR)-1, 2, and -3, platelet-derived growth factor receptor-β (PDGFR-β), fms-related tyrosine kinase 3 (FLT 3) and the stem cell factor receptor (KIT) which are implicated in tumorigenesis and tumor progression (Wilhelm *et al.*, 2004; Wilhelm *et al.*, 2006). In the large clinical trial of patients with advanced renal cell cancer in whom previous systemic therapy had failed, the patients received sorafenib showed longer median progression-free survival compared with those received placebo (5.9 vs. 2.6 months). Significantly more patients who received sorafenib experienced complete or partial responses or stable disease than those received placebo (Escudier *et al.*, 2007). Sorafenib received fast track US FDA approval in 2005 for the treatment of advanced renal cell cancer and hepatocellular cancer. The recommended dosage is 400 mg twice daily and most drug related adverse events included hand-foot skin reaction, diahhrea, and fatigue (Strumberg *et al.*, 2002; Mckeage and Wagstaff, 2007). Sorafenib has been associated with hypertention; in a total of 4,599 patients with renal cell carcinoma (RCC) or other solid tumors, the overall incidence of all grade and high-grade (3 or 4) hypertention was 23.4 and 5.7%, respectively (Wu *et al.*, 2008). Appropriate monitoring and treatment are strongly recommended to prevent any cardiovascular complications. Multiple clinical trials are currently underway to further investigate the role of sorafenib alone or in combination for the treatment of various tumor types (Iyer *et al.*, 2010).

**Sunitinib (SU11248; Sutent; Pfizer):** Sunitinib is a small molecule multi-kinase inhibitor approved for use in treating advanced RCC and imatinib-resistant/-intolerant GIST in 2006. It inhibits VEGFR-2, PDGFR-α and β, FLT-3, KIT, colony-stimulating factor (CSF 1) and rearranged during ransfection (RET) kinase at nanomolar concentration (Chow and Eckhardt, 2007). Sunitinib exhibited broad and potent antitumor activity causing regression, growth arrest, or substantially reduced growth of various established xenografts derived from human or rat tumor cell lines (Mendel *et al.*, 2003; O'Farrell *et al.*, 2003; Chow and Eckhardt, 2007). Sunitinib demonstrated superior efficacy to interferon-α for the first-line treatment of metastatic RCC in a phase III trial with 750 patients who had not received prior treatment. Sunitinib doubled progression-free survival compared with interferon-α; furthermore, median overall survival in patients treated with sunitinib was greater than 2 years (Motzer *et al.*, 2007; Motzer *et al.*, 2009). In a clinical trial of 312 patients with GIST, sunitinib-treated patients showed significantly longer time to tumor progression compared with placebo (27.3 vs. 6.4 weeks) (Demitrib *et al.*, 2006). The recommended dose of sunitinib is 50 mg/day for 4 weeks followed by 2 weeks off-treatment. Skin and hair discoloration is a common adverse effect as well as diarrhea and nausea. Hypertention was observed in more than 25% of patients with RCC and in 15% of patients with GIST (Demitrib *et al.*, 2006; Motzer *et al.*, 2007). Blood pressure monitoring is mandatory and appropriate treatment with antihypertensive drugs is recommended. Currently, sunitinib is being further evaluated for the treatment of various other solid tumors including pancreatic neurocrine tumors, advanced NSCLC, and as second line treatment for prostate cancer, after failure of docetaxel treatment (Oudard *et al.*, 2011).

**Pazopanib (GW786034; Votrient; GlaxoSmithKline):** Pazopanib is a second-generation multi-targeted tyrosine kinase and inhibits VEGFR-1,-2, and -3, PDGFR-α and β, and KIT (Sloan and Scheinfeld, 2008; Castaneda and Gomez, 2009; Hamberg *et al.*, 2010). In a multinational phase III clinical trial in patients with locally advanced or metastatic RCC, the patients treated with pazopanib 800 mg once daily showed significantly longer median progression-free survival than that of placebo recipients (9.2 vs. 4.2 months) (Stenberg *et al.*, 2010). The most common adverse events were diarrhea, hypertention and hair color changes. Severe hepatic toxicity (≥ grade 3) was also seen in some patients (Hurwitz *et al.*, 2009; Bible *et al.*, 2010; Bukowski, 2010; Stenberg *et al.*, 2010; Sanford and Keating, 2010). In 2009, the US FDA granted pazopanib approval for treatment of RCC based on these positive results, and pazopanib is being further evaluated in a variety of malignancies. An international phase III trial of pazopanib versus sunitib (COMPARZ trial) in patients with treatment-naïve metastatic RCC is also ongoing with a total of 876 patients (Bukowski, 2010; Pal and Figlin, 2010; Sanford and Keating, 2010).

## PROTEASOME SYSTEMS AS DRUG TARGETS IN CANCER THERAPY

The systemic regulation of protein homeostasis is essential for normal cellular processes. The ubiquitin-proteasome system (UPS) mediates much of the regulated protein degradation in the cell by the 26S proteosome complex (Hershko and Ciechanover, 1998; Voges *et al.*, 1999; Goldberg, 2003; Adams, 2003; Hershko, 2005; Goldberg, 2007). The 26S proteosome consists of one 20S core and two 19S regulatory subunits (Fig. 3). Specific proteins are targeted for degradation *via* the attachment of ubiquitin and the ubiquitination is a highly regulated process with the interplay of ubiquitin-activating enzyme (E1), ubiquitin-conjugating enzyme (E2) and ubiquitin-protein ligase (E3). First, E1 binds ATP and ubiquitin to form a ternary complex consisting of E1-ubuquitin thioester with ubiquitin-AMP bound. The thioester-bound ubiquitin is then passed to one of several E2 conjugating enzymes through a transthiolation reaction. Ubiquitin-charged E2 then forms a complex with an E3 ligase and a protein substrate to transfer ubiquitin to a lysine residue on the substrate. Following the ubiquitination of the substrate, 19S chaperones unfold ubiquitin-tagged protein substrates and feed them through

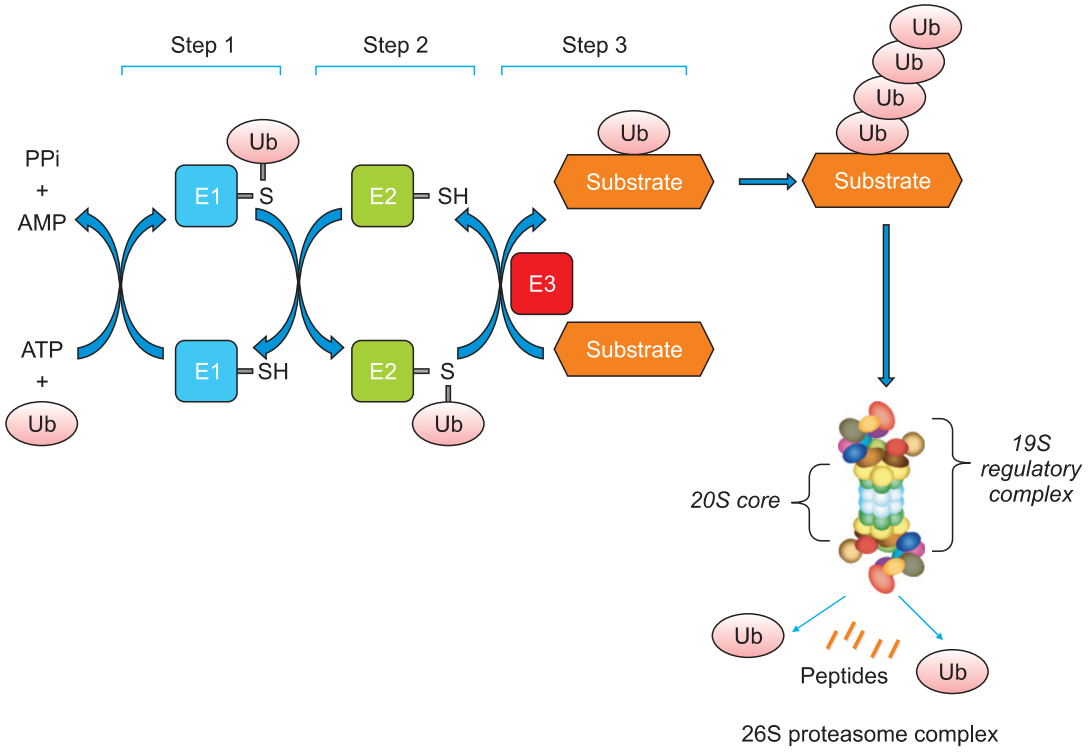


**Fig. 3.** The Ubiquitin-proteasome pathway of protein degradation.

**Table 3.** Selected proteasome inhibitors

| Drug | Development stage | Chemical structure | Binding kinetics |
|---|---|---|---|
| Bortezomib | Approved | Peptide boronic acid analogue | Slowly reversible (Half-life: 110 min) |
| Carfilzomib | Phase III | Peptide epoxyketone | Irreversible |
| MLN9708 | Phase I | Peptide boronic acid | Rapidly reversible (Half-life: 18 min) |
| CEP18770 | Phase I | P2 threonine boronic acid | Slowly reversible |
| NPI-0052 | Phase I | Non-peptide bicyclic γ-lactam β-lactone | Irreversible |
| ONX0912 (formerly PR047) | Preclinical | Peptide epoxyketone | Irreversible |
| PR957 | Preclinical | Peptide epoxyketone | Irreversible |
| IPSI | Preclinical | Peptidyl aldehyde | Not reported |

Adopted from Bedford *et al.* (2011) with permission from Nature Publishing Company.

the cylinder-shaped 20S core. Eukaryotic 20S proteosomes harbor seven different β-subunits in their two-fold symmetrical stacked complexes, with only three proteolytic active sites including subunits β1 (caspase-like), β2 (trypsin-like) and β5 (chymotrypsin like). Substrates of proteasome include misfold proteins and highly regulated proteins involved in critical signaling cascade such as growth control, cell cycle regulation and apotosis (Adams, 2003; Hershko, 2005). Inhibition one or more catalytic β-subunit of 20S proteosomes with small molecules emerged as an important therapeutic opportunities for a number of diseases such as cancer and inflammation (Schenkein, 2002; Kane *et al.*, 2003; Kane *et al.*, 2007; Nalepa *et al.*, 2006; Zavrski *et al.*, 2007; de Bettignies and Coux, 2010).

Tripeptide aldehydes such as the calpain inhibitor I and actinomycete natural product leupeptin were the first class of inhibitors of proteasomes (Vinitsky *et al.*, 1992). More potent and selective peptide boronates which are aldehyde surrogates with subnanomolar potency were synthesized. The dipeptide boronic acid bortezomib (Velcade®; Millennium Phamaceuticals Inc.), a reversible inhibitor of the β5-subunit of 20S proteosomes, was approved by the US FDA in 2003 as the first in class proteasome inhibitor for the treatment of relapsed multiple myeloma (Kane *et al.*, 2003). Several proteosome inhibitors are currently in preclinical and clinical development (Table 3). In addition to the UPS, there are nine classes of the ubiquitin-like protein (UBL) and eight E1 activating enzymes which

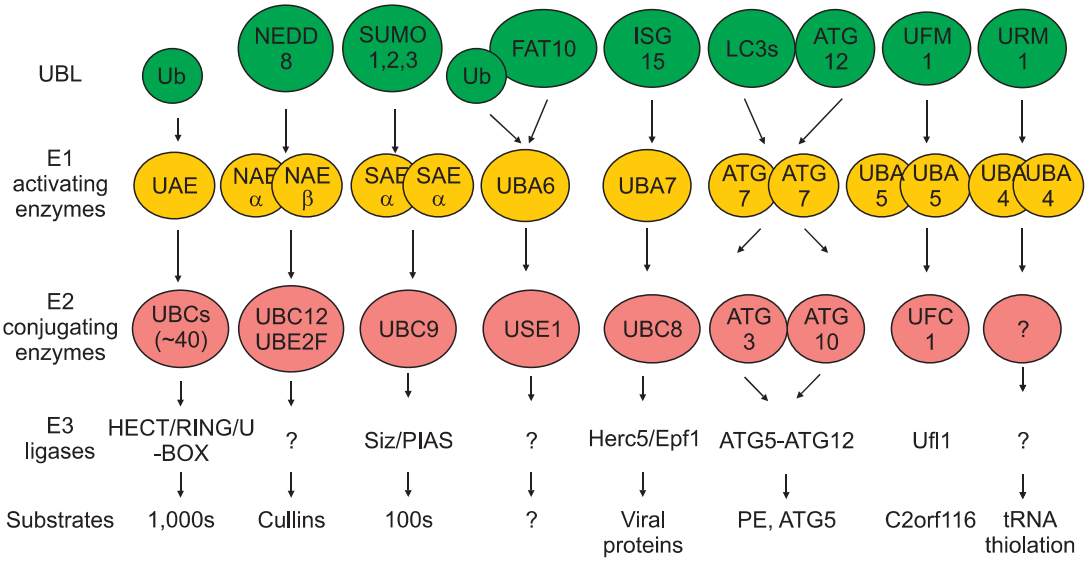


**Fig. 4.** Ubiquitin-like protein (UBL) conjugation and the ubiqutin proteasome system (UPS) (Adopted from Bedford *et al.* (2011) with permission from Nature Publishing Company).

are involved in diverse biological pathways (Fig. 4) (Bedford *et al.*, 2011). The UBL conjugation pathway is also emerging as a new therapeutic target in cancer therapy. Selected inhibitors of UPS and UBL conjugation system are discussed.

**Bortezomib (PS-341; Velcade®; Millennium Pharmaceuticals Inc.):** Bortezomib, a modified dipeptidyl boronic acid derived from leucine and phenylalanine, is a reversible inhibitor of 26S proteasome β5 subunit (chymotrypsin like) with a $IC_{50}$ value of 2.4-7.9 nM (Demo *et al.*, 2007; Chauhan *et al.*, 2005; Kupperman *et al.*, 2010). In vitro and in vivo studies demonstrated bortezomib is active against various lymphoid tumors as a single agent and showed additive or synergistic effects in combination with other drugs used in the typical standard chemotherapy, which prompted evaluation of clinical efficacy for the treatment of multiple myeloma (Adams *et al.*, 1999; Hideshima *et al.*, 2001; Mitsiades *et al.*, 2003; Ma *et al.*, 2003).

Multiple myeloma is a disorder where malignant plasma cells are accumulated in the bone marrow (Kyle and Rajkumar, 2004). This accumulation of plasma cells in the bone marrow leads to the skeletal destruction, bone marrow failure, suppression of normal immunoglobulin production, anemia, increased susceptibility to infections and renal failure (Kyle and Rajkumar, 2004). Projected 5-year survival rates for patients diagnosed in 2006-10 is 36% in the US (Brenner *et al.*, 2009). Multiple myeloma can be treated with a variety chemotherapeutic agents; however, the responses are not durable (International Myeloma Foundation, 2008/2009).

Two phase II clinical trials were conducted with administrations of bortezomib with a twice-weekly i.v. dosing regimen for the first 2 weeks of each 3-week cycle at 1.0 or 1.3 mg/m$^2$ (Kane *et al.*, 2003). In a randomized study with 54 patients of progressive myeloma, bortezomib showed responses at both dose levels (23% vs. 35%) including one complete response (Kane *et al.*, 2003). In the other phase II study where 202 heavily pre-treated myeloma patients participated, 3% of patients showed complete responses and partial responses occurred in 25% of patients at 1.3 mg/m$^2$ with a twice-weekly i.v. dosing regimen. The median duration of response was 365 days. The most clinically relevant adverse events were including asthenic conditions (malaise-fatigue), nausea, vomiting diarrhea, anorexia, thrombocytopenia, and a peripheral neuropathy (Kane *et al.*, 2003). In 2003, US FDA granted bortezomib accelerated approval for the treatment of more refractory multiple myeloma based on the adequate response rate and the duration (Kane *et al.*, 2003).

Bortezomib received additional approvals in 2005 and 2008 for the treatment of progressive multiple myeloma after one prior therapy and for the treatment of multiple myeloma as a front-line therapy, respectively (Kane *et al.*, 2006; Morabito *et al.*, 2010).

In 2006, US FDA granted bortezomib a marketing approval for the treatment of patients with relapsed or refractory mantle cell lymphoma (MCL) (Leonard *et al.*, 2006). MCL is an aggressive subtype of non-hodgkin's lymphoma (NHL). In the US only, NHL is the fifth most common cancer with more than 54,000 new cases diagnosed each year (Chiu and Weisenburger, 2003). MCL counts for about 5% of all cases NHL in the US and Europe with a median survival of 3-4 years and is generally considered incurable. With the first-line chemotherapeutic regimens including cyclophosphamide, vincristine, doxorubicin and dexamethasone alternating with methotrexate, 30-95% of initial overall response rate can be achieved; however, remission durations are short and overall survival remains limited (Hiddemann and Dreyling, 2003; Lenz *et al.*, 2004; Lenz *et al.*, 2005).

In the clinical trial with 155 patients with progressive mantle cell lymphoma after at least one prior therapy, bortezomib showed 31% of overall response including complete response and partial response; median response duration was 9.3 months. Adverse events were similar to those reported previously (Kane *et al.*, 2003; Leonard *et al.*, 2006; Kane *et al.*, 2007).

Overall, bortezomib demonstrated a highly statistically significant improvement in all efficacy measures compared to ex-

isting standard therapies for the treatment of for the treatment of multiple myeloma and mantle cell lymphoma. Current recommended dosing regimen of bortezomib is 1.3 mg/m$^2$ administered as a bolus injection twice weekly for 2 weeks (Days 1, 4, 8 and 11) every 21 days. For extended therapy of more than 8 cycles, bortezomib may be administered on the standard schedule or once weekly for 4 weeks (Days 1, 8 15 and 22) every 35 days (Millennium Pharmaceutical Inc., 2010).

## Second-generation proteasome inhibitors

Even though bortezomib has shown clinical efficacy in the treatment of multiple myeloma and MCL, there are some limitations with bortezomib therapy. Bortezomib hasn't showed strong efficacy in solid tumors (Orlowski and Kuhn, 2008) and prolonged treatment can be associated with reversible peripheral neuropathy (Richardson *et al.*, 2009). Currently, several structurally diverse potent proteasome inhibitors are in the clinical development (Table 3). These inhibitors show different enzyme binding kinetics, which might have impact on their pharmacololgy, efficacy and safety profiles. Selected second-generation proteasome inhibitors are discussed.

**MLN9708 (Millennium Pharmaceuticals Inc.):** MLN9708 is a second-generation small molecule proteasome inhibitor being developed by Millennium Pharmaceuticals and is currently in phase I clinical trials for the treatment of a broad range of cancers. MLN9708 is a citric ester of MLN2238 and is immediately hydrolyzed to pharmacologically active MLN2238 upon exposure to aqueous solutions or plasma (Kupperman *et al.*, 2010). MLN2238 showed comparable selectivity and potency as bortezomib with $IC_{50}$ value of 3.4 nM toward 20S proteasome β5 proteolytic site. However, the proteasome binding kinetics of MLN2238 is different from bortezomib with a shorter 20S proteasome dissociation life than velcade (18 vs.110 min) (Kupperman *et al.*, 2010). MLN2238 could offer improved tissue distribution over bortezomib with the shorter 20S proteasome dissociation life. Following an intravenous administration to mouse, MLN2238 showed larger volume of distribution at steady state (Vdss, b) than bortezomib (20.2 and 4.3 L/kg), supporting the better tissue distribution of MLN2238 than bortezomib. MLN2238 also showed greater pharmacodynamic effects in xonograft tumors including CWR22 (human prostate tumor) and WSU_DLCL2 (human lymphoma tumors) (Kupperman *et al.*, 2010). In current on-going phase I clinical trials, safety, maximum tolerated dose and pharmacokinetics of MLN9708 are being assessed as well as the extent of whole blood 20S proteasome inhibition and tumor response in patients with hematologic malignancies and solid tumors, following intravenous and oral administration. So far, MLN9708 (measured as MLN2238) shows approximately linear PK over the range of doses tested (1 to 2.34 mg/m$^2$), and MLN9708 is rapidly absorbed and is substantially bioavailable following oral administration (Gupta *et al.*, 2010).

**Carfilzomib (PR-171, Proteolix Inc.):** Carfilzomib is a novel proteasome inhibitor of the epoxyketone class which is selective and structurally distinct from bortezomib. Proteasome inhibition by carfilzomib is mechanistically irreversible (Demo *et al.*, 2007). Its irreversible binding mechanism to the *N*-terminal threonine catalytic sites of proteasome has been postulated to overcome resistance to bortezomib (Marblestone, 2009). In various tumor cell lines including bortezomib-resistant, carfilzomib showed cytotoxicity activity (Demo *et al.*, 2007; Kuhn *et al.*, 2007). In a phase I trial where the safety and efficacy of carfilzomib were investigated in relapsed or refractory hematologic malignancies, patients received 5 consecutive days of intravenous administration of carfilzomib at doses of 1.2, 2.4, 4, 6, 8.4, 11, 15, and 20 mg/m$^2$ within 14-day cycles. Nonhematologic toxicities included fatigue, nausea, and diarrhea in more than one third of patients-mostly grade 1 or 2 in severity. At 20 mg/m$^2$, grade 3 febrile neutropenia and grade 4 thrombocytopenia were reported, and 15 mg/m$^2$ was established as the maximum tolerated dose (O'Connor *et al.*, 2009). Phase II trials are currently ongoing in patients with relapsed and refractory multiple myeloma as well as a phase Ib combination study and a phase Ib/II study in patients with solid tumors (Dick and Fleming, 2010).

**CEP18770 (Cephalon Inc.):** As a reversible boronic acid proteasome inhibitor, CEP18770 caused similar proteasome and apoptotic profiles in multiple myeloma xenograft models following intravenous or oral administration (Piva, 2008; Marblestone, 2009). CEP-18770 also induces synergistic inhibition of multiple myeloma cell viability with combination with melphalan or bortezomib. In multiple myeloma xenograft models, co-administration of CEP-18770 with melphalan completely inhibited the growth of both melphalan-sensitive and melphalan-resistant tumors. The combination of CEP-18770 and bortezomib also induced complete regression of bortezomib-sensitive tumors and markedly delayed progression of bortezomib-resistant tumors compared to treatment with either agent alone (Sanchez *et al.*, 2010). Phase I clinical trials for the patients with multiple myeloma, NHL and solid tumors are currently underway (Marblestone, 2009; Dick and Fleming, 2010).

**Marizomib (NPI-0052, Nereus Pharmaceuticals):** NPI-0052 is an orally active, nonpeptide β-latone derived from naturally occurring marine bacteria, *Salinispora tropica*. Also known as salinosporamide A, NPI-0052, distinct from bortezomib in its chemical structure, inhibits the chymotrypsin-like, caspase-like and trypsin-like acivities of purified human erythrocyte 20S proteasomes by irreversibly binding to the 20S proteasomes (Corey and Li, 1999). In studies in various tumor xenograft models, NPI-0052 is well tolerated and prolongs survival, with significantly reduced tumor recurrence (Chauhan *et al.*, 2005; Chauhan *et al.*, 2006; Fenical *et al.*, 2009). NPI 0052 also induces synergistic anti-multiple myeloma activity in combination with bortezomib or lenalidomide (Revlimid) (Chauhan *et al.*, 2005; Chauhan *et al.*, 2010). NPI-0052 is in currently in phase I clinical development in patients with various hematologic malignances and solid tumors (Dick and Fleming, 2010).

## Inhibitor of UBL conjugation system

**MLN4924 (Millennium Pharmaceuticlas Inc.):** With demonstrated clinical efficacy of bortezomib, inhibitors of UBL conjugation systems are also emerging as new potential therapeutic targets in cancer therapy. To date, nine classes of the UBL and eight E1 activating enzymes which are involved in diverse biological pathways have been identified (Schulman and Harper, 2009; Hochstrasser, 2009; Bedford *et al.*, 2011) (Fig. 4). Ubiquitin and UBL share similar mechanisms for the conjugation with their target protein with a cascade of enzymatic reaction involving E1, E2 and E3 enzymes.

MLN4924 is a potent inhibitor of NEDD8-activating enzyme (NAE) withan $IC_{50}$ value of 0.004 μM, and shows selectivity against closely related enzymes such as ubiquitin-activating enzyme (UAE), sumo activating enzyme (SAE), UBA6 and

ATG7 ($IC_{50}$=1.5, 8.2, 1.8 and >10 μM, respectively) (Soucy *et al.*, 2009). NAE is an essential component of the NEDD8 conjugation pathway which controls the degradation of many proteins with important roles in cell-cycle progression, DNA damage, and stress responses (Soucy *et al.*, 2010). By inhibiting NEDD8 conjugation pathway, MLN4924 controls the activity of cullin proteins which function as part of the catalytic core of cullin-RING ubiquitin ligases (CRLs). Substrates of CRLs have important roles in cellular processes associated with cancer cell growth and survival pathways including cell cycle progression (Chiba and Tanaka, 2004; Pan *et al.*, 2004; Petroski and Deshaies, 2005). MLN4924 disrupts CRL mediated protein turnover leading to the increases of the known CRL substrates including CDT1 (Nishitani *et al.*, 2006; Hu *et al.*, 2004), NRF-2 (Kobayashi *et al.*, 2004) and phosphorylated IκBα (Winston *et al.*, 1999). Unlike bortezomib, which substantially block intracellur ptrotein turnover by inhibiting 20S proteasome, MLN4924 only affect the degradation of proteins which ubiquitinylation is mediated by CRL. When HCT-116 cells were treated with bortezomib or MLN4924, bortezomib was found to inhibit protein turnover by ≈50% whereas, MLN4924 inhibited overall protein turnover only by ≈9%, indicating approximately 20% of protein turnover is mediated by CRL-ubiquitinylation in HCT-116 cells (Soucy *et al.*, 2009). These results indicate the selectivity of MLN4924 in controlling cancer cell protein homeostasis than the inhibition of proteasome activity, which might results in the different efficacy and safety profiles from bortezomib. NAE pathway inhibition by MLN4924 appeared to activate apoptosis as results of cell cycle-dependent DNA re-replication due to the inability of the cell to degrade the CRL substrate CDT1 (Lin *et al.*, 2010). Cells in S phase were most susceptible, suggesting that MLN4924 will be most toxic on highly proliferating cancers (Lin *et al.*, 2010). MLN4924 inhibited tumor growth in various xenograft models and these preclinical observations prompted the clinical evaluation of the MLN4924 (Soucy *et al.*, 2009).

MLN4924, the first-in-class small molecule NAE inhibitor, is currently in phase I clinical development with patients of acute lymphoblastic leukemia (AML) and other forms of cancer. Pharmackinetics, maximum tolerated dose, and pharmacodymamics are being evaluated in patients with treatment of MLN4924 using an IV infusion with various dosing schedules (Shah *et al.*, 2009; Shah *et al.*, 2010). The results from the pharmacodynamic analysis indicate the evidence of inhibition of NAE activity by MLN4924 in blood and skin, supporting continued investigation of MLN4924 in patients (Shah *et al.*, 2009; Shah *et al.*, 2010).

### Inhibitors of deubiquitinating enzymes (DUBs)

Protein homeostasis is largely regulated by UPS with specific proteins that are targeted for degradation via the attachment of ubiquitin. With demonstrated clinical efficacy and US FDA approval of bortezomib, UPS became a valid target for cancer treatment.

DUBs remove ubiquitin from specific protein substrates and allow protein salvage from degradation by proteasome. DUBs consist of six subclasses including ubiqitin-specific proteases (USPs), ubiquitin *C*-terminal hydrolases (UCHs), Machado-Joseph domain protease (MJD), ovarian tumor domain–containing protease (OUT), herpes virus tegument USPs (htUSPs) and JAB1/MPN/MOV34 metalloenzyme (JAMM). Among them USP and UCH are the best characterized subclasses of DUBs (Daviet and Colland, 2008; Colland, 2010).

The implications of several DUBs in various diseases including cancer have been reported (Daviet and Colland, 2008). Overexpression of USP7 in prostate cancer and its direct link with tumor aggressiveness have been reported (Song *et al.*, 2008). In nude mouse, the absence of USP7 resulted in significantly smaller tumor volumes indicating the role of USP7 in cancer cell proliferation (Becker *et al.*, 2008). In addition to USP7, several other USPs are considered to be involved in the cellular signaling pathways and putative oncogenic processes (Daviet and Colland, 2008; Colland *et al.*, 2009). Therefore, USPs are emerging as a promising target as an alternative to inhibit the proteasome by targeting the upstream, ubiquitin conjugation and deconjugation system, which might confer more specificity and less toxicity.

Several small molecule USPs inhibitors have been synthesized and currently being evaluated (Daviet and Colland, 2008; Marblestone, 2009; Colland *et al.*, 2009; Colland, 2010; Nicholson and Kumar, 2011; Wrigley *et al.*, 2011).

## COMBINATION STRATEGIES OF MOLECULARLY TARGETED CANCER THERAPY

There is a great deal of interest not only in the development of anti cancer drugs individually but in the evaluation of cancer drug candidates in combination with standard treatments or with other molecularly targeted agents. The rationales behind the combinational cancer therapy are to overcome the drug resistance and to achieve greater treatment benefit through the additive/synergistic effects of each individual agent. Given the nature of exceptional heterogeneity and adaptability of cancer, it is very unlikely that treatment focusing on a single target would offer long-lasting tumor control in most patients. Currently, there are numerous on-going clinical trials for combinations of novel targeted agents (Table 4) and for targeted agents combined with standard cancer treatments (Dancey and Chen, 2006; Kummar *et al.*, 2010). Combination strategies of targeted agents in cancer therapy can be divided into three categories: First, combinations of drugs with the same target to maximize the inhibition of a specific target; Second, combinations of drugs to maximize inhibition of a pathway by targeting multiple components; Third, combinations to expand inhibition of multiple cellular meachanisms (Table 4, Fig. 5) (Dancey and Chen, 2006; Kummar *et al.*, 2010).

With the unlimited numbers of possible drug combinations, and the results from recent clinical trials clearly indicating the poor predictability of success or failure of combination clinical trials based on the preclinical data (Dancey and Chen, 2006), good strategies are essential for successful combinations.

When specific combinations are considered for clinical development, important questions should be addressed include what type of preclinical and clinical data are needed and what types of patient populations should be considered (Dancey and Chen, 2006; Kummar *et al.*, 2010). Demonstrated synergy in multiple human tumor models or subset of tumors which are relevant to human cancers is essential information to help the prioritization of combination. Understanding of the molecular target of each agent is also pivotal knowledge to select the patient population who might have the most benefit from a particular combination therapy (Dancey and Chen, 2006; Kummar *et al.*, 2010).

**Table 4.** Selected clinical trials for combinations of novel agents

| Targets | Combination of drugs | Types of tumor |
|---|---|---|
| **Maximize inhibition of a single target** | | |
| VEGF–VEGFR | Bevacizumab–sorafenib | RCC, ovarian cancer, CRC |
| VEGF–VEGFR | Bevacizumab–cediranib | various (Phase I) |
| EGFR (MAB–TKI) | Cetuximab–erlotinib | Colon cancer, NSCLC* |
| HER2 (mAb–TKI) | Trastuzumab–lapatinib* | HER2-amplified breast cancer |
| **Inhibit signal transduction pathways** | | |
| VEGF–mTOR | Bevacizumab–temsirolimus | RCC, neuroendocrine tumor, HCC, ovarian cancer, endometrial cancer |
| VEGF–mTOR | Bevacizumab–everolimus | RCC, neuroendocrine tumor |
| VEGF–mTOR | Sorafenib–CCI-779 | Melanoma, GBM, RCC |
| HER2–mTOR | Trastuzumab–everolimus* | Breast cancer |
| EGFR–mTOR | erlotinib–temsirolimus | NSCLC, GBM |
| IGF1R–mTOR | IMC-A12–CCI-779 | Breast cancer, sarcoma, prostate cancer, paediatric tumors (Phase I) |
| IGF1R–MEK | IMC-A12–AZD6244 | Various (Phase I) |
| EGFR–MEK | Erlotinib–AZD6244 | NSCLC |
| **Inhibit parallel pathways and compensatory pathways** | | |
| VEGFR–EGFR | Bevacizumab–cetuximab | Colon cancer, pancreatic cancer |
| VEGFR–EGFR | Bevacizumab–erlotinib | NSCLC, RCC, breast cancer |
| VEGF–PDGFR | Bevacizumab–imatinib; Bevacizumab–dasatinib | Melanoma, ovarian cancer |
| EGFR–IGF1R | IMC-A12–erlotinib | NSCLC |
| HER2–EGFR | Trastuzumab–gefitinib | Breast cancer |
| mTOR–MEK | AZD6244–deforolimus* | Various (Phase I) |
| **Other** | | |
| HDAC–VEGF | SAHA–bevacizumab | RCC |
| HDAC–proteasome | SAHA–bortezomib | Pancreatic cancer, sarcoma |
| HDAC–methylation | SAHA–azacytidine | MDS, multiple myeloma |

CRC: colorectal cancer, EGFR: epidermal growth factor receptor, GBM: glioblastoma multiforme, HCC: hepatocellular carcinoma, HDAC: histone deacetylase, HER2: human epidermal growth factor 2, IGF1r: insulin-like growth factor receptor 1, mAb: monoclonal antibody, MDS: myelodysplastic syndrome, MEK: MAP-ERK kinase, mTOR: mammalian target of rapamycin, NSCLC: non-small cell lung cancer, PDGFR: platelet-derived growth factor receptor, RCC: renal cell carcinoma, SAHA: suberoylanilide hydroxamicacid (also known as vorinostat), TKI: tyrosine kinase inhibitor, VEGF: vascular endothelial cell growth factor, VEGFR: VEGF receptor. *Clinical trial not sponsored by the US National Cancer Institute. Source: ClinicalTrials.gov. (Adopted from Kummar *et al*. (2010) with permission from Nature Publishing Company).

In addition to these scientific questions, there are additional challenges in dealing with intellectual property issues given many of the target agents are investigational or being developed by different pharmaceutical companies as well as the potential regulatory issues in the commercialization of targeted combinations. To overcome intellectual property and regulatory barriers, the Division of Cancer Treatment and Diagnosis (DCTD) of the National Cancer Institute (NCI) of the US has developed data-sharing language and patent rights language over the past 5 years. Based on the master agreement language, each collaborator would have the right to access and to use the data from the combination trials and would receive non-exclusive royalty-free licenses to the combination intellectual property for all purposes including commercial use. This language essentially helped more than 100 clinical trials combining investigational agents move forward (Kummar *et al.*, 2010).

NCI also took a new initiative to screen combination drug in vitro to provide the cancer research community with publicably available data set of combination anticancer agent therapeutics. Approximately 100 approved small molecule anti cancer drugs are being tested in the NCI-60 tumor cell line panel in combination against each of them, and the results from this screening will guide the selection of specific combination for further testing in human tumor xenografts. As of June 2010, a total of 31 drug combinations out of possible 5,000 unique combinations have been tested, and the early results indicate the feasibility and the potential impact of this initiative (Kummar *et al.*, 2010).

Recently, US FDA released draft guidance to assist sponsors in the co-development of two or more novel (not previously marketed) drugs to be used in combination to treat a disease or condition (US FDA, 2010). The existing developmental and regulatory paradigm focuses primarily on assessment of the effectiveness and safety of a single new investigational drug acting alone, or in combination with an approved drug. And the new guidance provides recommendations and advice on how to address certain scientific and regulatory issues that will arise during co-development. Based on this new guideline, co development should be considered for situations

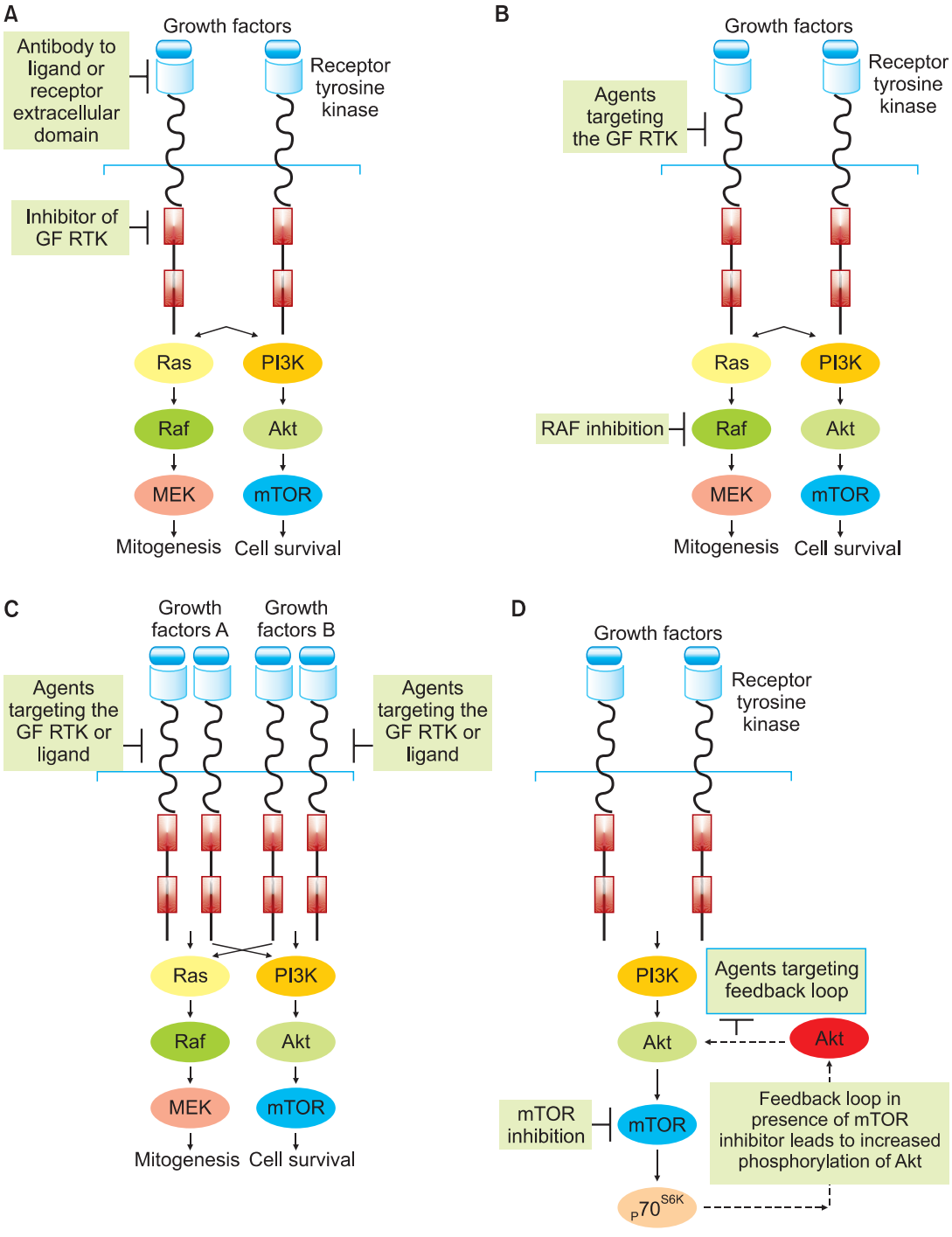


**Fig. 5.** Combination strategies of molecularly targeted cancer therapy. (A) Maximize inhibition of a target such as a growth factor receptor by inhibiting both receptor–ligand binding and tyrosine kinaseactivity. (B) Maximize inhibition of a pathway by inhibiting a series of signaling components within the pathway. (C) Inhibit parallel pathways by inhibiting two growth factor receptors or inhibiting downstream components in parallel pathways. (D) Inhibit a target and the feedback loop that results in resistance. GF: growth factor, IRS1: insulin receptor substrate 1, MEK: mitogen-activated protein kinase, mTOR: mammalian target of rapamycin, PI3K: phosphatidylinositol 3-kinase, RTK: receptor tyrosine kinase (Adopted from Dancey and Chen (2006) with permission from Nature Publishing Company).

that meet the following criteria: 1. The combination is intended to treat a serious disease or condition. 2. There is a compelling biological rationale for use of the combination (e.g., the agents inhibit distinct targets in the same molecular pathway, provide inhibition of both a primary and compensatory pathway, or inhibit the same target at different binding sites to decrease resistance or allow use of lower doses to minimize toxicity). 3. A preclinical model (*in vivo* or *in vitro*) or short-term

clinical study on an established biomarker suggests that the combination has substantial activity and provides greater than additive activity or a more durable response (e.g., delayed resistance) compared to the individual agents alone. 4. There should be a compelling reason for why the agents cannot be developed individually (e.g., monotherapy for the disease of interest leads to resistance and/or one or both of the agents would be expected to have very limited activity when used as monotherapy). US FDA also recommends that sponsors consult with them on the appropriateness of codevelopment before initiation of clinical development of the combination. This new FDA guidance reflects the increasing need for synergistic combinations in disease areas such as oncology. This new FDA guidance will be very helpful to investigators and sponsors with regard to how to move these critically important studies forward in a more expeditious fashion. Some of the examples of combination therapy in the treatment of cancer are discussed.

**Combination of mammalian target of rapamycin (mTOR) inhibitor with a Phosphatidylinositol-3 Kinase (PI3K) or Akt inhibitor:** mTOR is a downstream effector of the (PI3K)/Akt signaling pathway and plays a central role in regulating cell growth, proliferation, and survival, in part by regulation of translation initiation (Sawyers, 2003; Bjornsti and Houghton, 2004; Hay and Sonenberg, 2004) and has emerged as an important cancer therapeutic target. So far three mTOR inhibitors have been approved by the US FDA and many more are now being actively evaluated in clinical trials. However, many cancer cells are resistant to rapamycin and its derivatives. Inhibition of mTOR by rapamycin has been reported to increase the phosphorylation of both Akt and eukaryotic translation initiation factor 4E (eIF4E), which seem to attenuate rapamycin's growth-inhibitory effects, serving as a negative feedback mechanism (Sun *et al.*, 2005).

When human NSCLC cells were treated with rapamycin combined with a PI3K inhibitor, LY294002, the effects on the growth inhibiton were greater than those caused by each single agent alone (Sun *et al.*, 2005). Takeuchi *et al.* (2005) also reported synergistic augmentation of rapamycin-induced autophagy in malignant glioma cells by PI3K and Akt inhibitor which provides a mechanistic basis for enhancing mTOR-targeted cancer therapy by combining an mTOR inhibitor with a PI3K or Akt inhibitor.

**Combination therapy of proteasome inhibitors with histone deacetylase inhibitors (HDACis):** In an effort to identify proteasome inhibitor-based combinations that produce greater clinical activity, combinations of proteaome inhibitors and HDACis showed the most potent synergistic cytotoxicity in preclinical multiple myeloma models (Mitsiades *et al.*, 2004; Pei *et al.*, 2004) and in a variety of other human solid and hematologic cancer cell lines and xenografts (McConkey and Zhu, 2008).

HDACi are a class of cancer therapeutic agents that regulate gene expression by globally increasing histone acetylation (Rashheed *et al.*, 2007). The antitumor activity of HDACi involves multiple mechanisms, including transcriptional upregulation of genes involved in apoptosis, cell cycle control, DNA repair, and differentiation (Carew *et al.*, 2008). HDACi also induces acetylation of nonhistone proteins, which may contribute to antitumor activity (Yoshida *et al.*, 2003). Vorinostat has been approved by the FDA as the first HDACi for the treatment of cutaneous T cell lymphoma (Mann *et al.*, 2007) and a structurally diverse group of compounds with varying specificity against the spectrum of histone deacetylases has been also identified. Two phase I clinical trials are currently underway to evaluate the effects of combination therapy of bortezomib with vorinostat (also known as SAHA, a pan HDACi) in refractory multiple myeloma. Preliminary results from these trials indicate the overall response rates of 50% in both trials, suggesting that there will be benefit from combining proteasome inhibitors and HDACis in patients (Mitsiades *et al.*, 2009). In additional on-going clinical trials, the combination of bortezomib with other HDACis including belinostat, panobinostat, and romidepsin is also being evaluated (Wright, 2010).

One of the possible mechanisms for this synergistic effect of combination of vortezomib with HDACi is a disruption of aggresome. Inhibition of protesome cause the accumulation of damaged and misfolded proteins that are prone to aggregation, and it is this protein aggregation that serves as the primary cytotoxic stress, causing downstream reactive oxygen species (ROS) accumulation, JNK activation, and ER caspase activation (McConkey and Zhu, 2008; McConkey, 2010). HDACis promote this proteotoxic stress by blocking HDAC6, which is required for “aggresome” formation and the transfer of protein aggregates to lysosomes via autophagy which will release cells from cytotoxic stress (McConkey, 2010; Wright, 2010).

## FUTURE CANCER THERAPY: PERSONALIZED MEDICINE

In the past decades, the paradigm of anticancer drug discovery changed significantly. With remarkable advancement in understanding tumor biology, numerous molecularly targeted drugs have been approved and hundreds more are currently in clinical development. However, overall low response rates and intrinsic resistance or acquired resistance in patients following the treatment with these drugs still pose enormous challenges in cancer treatment.

In particular, the lessons learned from the development of first generation EGFR inhibitors clearly indicate the opportunities for the cancer treatment in the next decade: *the development of personalized cancer medicines.*

Gefitinib and erlotinib were approved in 2003 and 2004, respectively, for the treatment of NSCLC; however, only small population of NSCLC patients responded to the treatment of these drugs. Even though the population of patients who responded was small, these patients showed complete remission and prolonged tumor free survival with these drugs (Shepherd *et al.*, 2005), which prompted the retrospective analysis of biomarkers in these patients (Lynch *et al.*, 2004; Paez *et al.*, 2004; Pao *et al.*, 2004).

The results from the numerous studies revealed that the response rate to the treatment of tyrosine kinase inhibitors in EGFR-mutant patients was 82%, whereas, the wild type patients showed 11.5% response (Uramoto and Mitsudomi, 2007). These studies also showed significantly higher overall survival rate in EGFR-mutant patients compared to wild type patients (>2 years vs. 8 months) (Uramoto and Mitsudomi, 2007). And among the lung carcinoma patients, Asian ethnic group showed EGFR mutation frequencies of 22-67% followed by patients in South Europe (10-24%), and patients in North America (3-25%) (Marchetti *et al.*, 2005; Lynch, *et*

*al.*, 2004; Paez *et al.*, 2004; Pao *et al.*, 2004; Uramoto and Mitsudomi, 2007; Cortes-Funes *et al.*, 2005; Eberhard *et al.*, 2005; Sequist *et al.*, 2006). These results provided valuable insights how to screen patients who would respond well to the treatment and paved the way to the future strategies of cancer treatment based on the predictive biomarkers of patients and not simply based on the tumor types.

Personalized medicine is being increasingly recognized as a new paradigm for future health care. Recently, the US congress passed a bill on personalized medicine (The Genomics and Personalized Medicine Act, 2008), and by the definition made by the US congress, personalized medicine is "the application of genomic and molecular data to better target the delivery of health care, facilitate the discovery and clinical testing of new products, and help determine a person's predisposition to a particular disease or condition". As the US congress defined, the idea of developing personalized medicine is to identify the patients at risk of illness based on their genomic profile and to provide the right drug with right dose at the right time. In September 2008, the US President's Council of Advisors on Science and Technology (PCAST) published report "Priorities for Personalized Medicine" based on the input from industry, physicians, patients, government agencies and academic scientists (President's Council of Advisors on Science and Technology, 2008). One of the recommendations in this report is to develop a strategic, long-term plan to shape public and private research efforts into personalized medicine.

Personalized approaches are relatively well reported in the treatment of non-small cell lung cancer. The activated EGFR mutation is the key genotypic biomarker to select patients for a first-line therapy with EGFR tyrosine kinase inhibitor such as gefitinib (McDermott U and Settleman, 2009; Mok *et al.*, 2010; Roberts *et al.*, 2010).

Besides, EGFR mutation, there are oncology predictive markers currently used in clinic to help select the patients who are most likely to benefit from or be resistant to treatment. Those markers include estrogen and progesterone receptors and HER-2 for the patients with breast cancer and *K-RAS* mutations for patients with advanced colorectal cancer (Duffy *et al.*, 2011). Even though further validation is needed before putting into clinical use, there are also several new emerging biomarkers for predicting response, resistance or outcome following specific cancer therapies (Kulasingam and Diamandis, 2008; Pena *et al.*, 2010; Duffy *et al.*, 2011).

The use of biomarkers in selecting patients has significant implications in the cost of cancer therapy as well as the outcome of the specific treatment. The cost of health care can be significantly saved by limiting the drug use in patients who are most likely not responsive of the treatment.

The path to personalized medicine is making a progress. Close collaboration between the pharmaceutical industry, academia, government agencies, physicians and patients can be a critical factor for a successful delivery of personalized medicine.

## CONCLUSION

During the past decade, a new strategy for cancer therapy has emerged based on well defined molecular targets. Among those targets, protein kinases have become one of the most extensively pursued molecular targets. To date, 12 small kinase inhibitors have been approved by the US FDA, and approximately 80 small molecule kinase inhibitors have been advanced to some stage of clinical evaluations. With demonstrated clinical efficacy of bortezomib, proteasome systems are also emerging as new potential therapeutic targets in cancer therapy. Even though, there has been a remarkable advancement in the development of anti cancer drugs during the past decade, low response rates and intrinsic resistance or acquired resistance in patients following the treatment with these drugs still pose enormous challenges in cancer treatment. In order to overcome drug resistance and to achieve greater treatment benefit through the additive/synergistic effects of each individual agent, there is an increasing need for combination therapy in oncology. Demonstrated synergy in multiple human tumor models or subset of tumors which are relevant to human cancers is essential information to help the prioritization of combination. Understanding of the molecular target of each agent is also pivotal knowledge to select the patient population who might have the most benefit from a particular combination therapy. Development of personalized medicine is being increasingly recognized as a new paradigm for future health care to identify the patients at risk of illness based on their genomic profile and to provide the right drug with right dose at the right time. Close collaboration between the pharmaceutical industry, academia, government agencies, physicians and patients is essential for a successful delivery of new therapeutic options for many cancer patients.

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