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Commentary

Models for prevention and treatment of cancer: Problems vs promises

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ABSTRACT

Current estimates from the American Cancer Society and from the International Union Against Cancer indicate that 12 million cases of cancer were diagnosed last year, with 7 million deaths worldwide; these numbers are expected to double by 2030 (27 million cases with 17 million deaths). Despite tremendous technological developments in all areas, and President Richard Nixon's initiative in the 1974 "War against Cancer", the US cancer incidence is the highest in the world and the cancer death rate has not significantly changed in the last 50 years (193.9 per 100,000 in 1950 vs 193.4 per 100,000 in 2002). Extensive research during the same time, however, has revealed that cancer is a preventable disease that requires major changes in life style; with one third of all cancers assigned to Tobacco, one third to diet, and remaining one third to the environment. Approximately 20 billion dollars are spent annually to find a cure for cancer. We propose that our inability to find a cure to cancer lies in the models used. Whether cell culture or animal studies, no model has yet been found that can reproduce the pathogenesis of the disease in the laboratory. Mono-targeted therapies, till know in most cases, have done a little to make a difference in cancer treatment. Similarly, molecular signatures/predictors of the diagnosis of the disease and response are also lacking. This review discusses the pros and cons of current cancer models based on cancer genetics, cell culture, animal models, cancer biomarkers/signature, cancer stem cells, cancer cell signaling, targeted therapies, therapeutic targets, clinical trials, cancer prevention, personalized medicine, and off-label uses to find a cure for cancer and demonstrates an urgent need for "out of the box" approaches.

1. Introduction

Intuition says the history of cancer has to be as old as the history of man himself. Perhaps one of the earliest mentions of cancer can be found in a manuscript named Bhrigu Samhita almost 3000 B.C., that describes the origin of cancer and its treatment [1]. Books such as Ayurveda (part of Atherveda before 2500 B.C.) and Charak Samhita (1500 B.C.) also discuss the treatment of cancer quite in detail. In fact, vincristine, an alkaloid isolated from the periwinkle plant (*Vinca rosea,* also called Sadabahar) has its origin from Ayurveda and today is still used for cancer treatment. Sushruta Samhita (1500 B.C.) describes surgery for treatment of cancers that are localized. Egypt is also credited with one of the oldest description of cancer (1600 B.C.). The Greek physician Hippocrates (460–370 B.C.) used the terms cancer, carcinos and carcinoma to describe non-ulcer forming and ulcer-forming tumors based on the Greek word for crab. Hippocrates also suggested that an excess of black bile in an organ can lead to cancer, a concept that lasted for almost 2000 years until 1761 when Giovanni Morgagni (Padua) performed surgery and found no evidence of black bile. A Scottish surgeon John Hunter (1728–1793) suggested that cancers that had not invaded nearby tissue might be cured by surgery.

Today, it is well accepted that dysregulation in cell growth leads to cancer (see Fig. 1). Although Percival Pott was the first to show in 1775 that carcinogens can cause cancer (he noted the association between coal dust and scrotal cancer in chimney sweeps), the mechanism by which various carcinogens cause cancer is now well known. The "initiation" step involves interaction of carcinogens with DNA that leads to transformation of normal cells to tumor cells. This step is usually followed by the "promotion" step, in which the cancer cells proliferate and form tumors. This step is mediated through various life style factors and may take as many as 20 years to result in full-fledged cancer (Fig. 1).

Within last half a century there have been major developments in our understanding of cancer at the molecular level. Various growth factors, hormones, cytokines, oncogenes, viruses, bacteria, and carcinogens have been identified that initiate and promote cancer. Many of the subcellular mechanisms that promote hyperproliferation, invasion, angiogenesis and metastasis, have also been delineated. The structure of entire human genome consisting of almost 25,000 genes and at least some of those genes that mediate tumorigenesis, is also quite apparent now. In spite of

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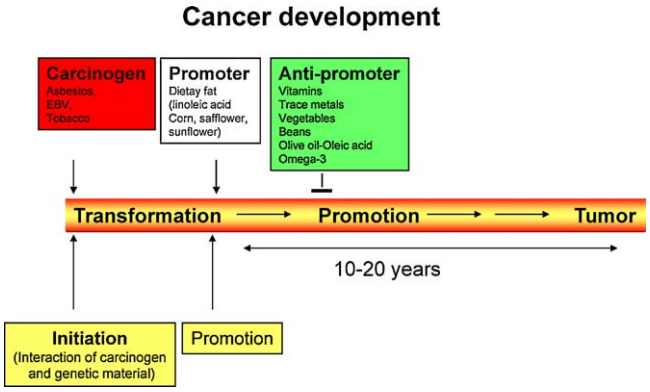

Fig. 1. Various steps in the development of cancer in humans. Transformation or initiation of cells by various carcinogens leads to promotion by various agents and this ultimately leads to tumors. During initiation, a carcinogen interacts with the DNA resulting in various somatic mutations.

this tremendous increase in knowledge about cancer, its prevention and treatment is still lacking. It was recently announced that in 2010 cancer will become number one killer and the number of cancer deaths will exceed that of cardiovascular disease in the U.S. The current estimates from the American Cancer Society and from International Union Against Cancer indicate that 12 million cases of cancer were diagnosed last year with 7 million deaths worldwide. In 2030, 27 million cases are expected to be diagnosed with this disease and 17 million deaths. In 1974, President Richard Nixon started the "War against Cancer" to make America, the wealthiest nation, into the healthiest nation in the world. This war, however, has been lost, as the US cancer incidence is the highest in the world and the cancer death rate has not changed in the last 50 years (193.9 per 100,000 in 1950 vs 193.4 per 100,000 in 2002). This is despite the fact that as many as 20 billion dollars are spent on cancer research and 200 billion dollars are spent by Americans on cancer care every year. To have a chance to reverse this trend, we must answer several questions. How the cancer problem is being currently approached? What are the promises and problems with these approaches? Since cancer is now believed to be a preventable disease, is prevention is our best choice rather than treatment? Are the model systems available adequate to the study the complex pathology of this disease? These questions are the focus of this review.

2. Cancer models

2.1. Cancer and its genetics

The gain or loss of specific chromosomes by the cell, called aneuploidy, is the hallmark of most tumors [2]. It is clear today that all cancers are caused by genetic alterations in the DNA [3]. These alterations, including deletions, inversions, amplification, and chromosomal translocations, could occur when proto-oncogenes, tumor suppressor genes, and DNA repair genes are the proximate culprits. Whether this kind of genetic instability is truly responsible for tumor development, is controversial [4]. Genetic instability has been linked with frequent failure of chemotherapy in cancer patients. These alterations, however, can be used as diagnostic markers for cancer and its therapeutics. How these alterations are induced is not fully understood, but the role of various carcinogens in inducing these alterations is well documented. More than 350 mutated genes, also called cancer genes, have been implicated in cancer development [5].

Cancer arises due to mutation in subset of genes. Large-scale sequencing has allowed us to determine the total number of mutations that occur in human cancer genome [6]. A systematic sequence of cancer genomes has revealed 1007 somatic mutations in 274 megabases of DNA corresponding to the coding exons of 518 protein kinase genes in 210 diverse human cancers. Most somatic mutations are expected to be "passengers" that do not contribute to oncogenesis, but evidence was found for "driver" mutations contributing to the development of the cancers in 119 genes. Out of 210 cancers analyzed, 169 were primary tumors, 2 were early cultures, and 39 were immortal cancer cell lines. Prevalence of somatic mutation was highest in cancer of the lung, followed by gastric, ovarian, colorectal, kidney, breast, and testis cancer, in that order. Most of the driver mutations identified have not been previously detected. Another large scale sequencing study with colorectal cancer and breast cancer identified 189 genes mutated at significantly high frequency [7].

Epigenetics is a system of gene regulation that is independent of DNA sequence. Changes such as DNA promoter methylation and chromatin remodeling or histone modification (such as phosphorylation and acetylation) are prominent epigenetic modifications that can play a prominent role in cancer. While DNA mutations have been closely linked with inherited cause of cancer in certain individuals, some evidence suggests that epigenetic alterations can also contribute to familial cancer risk [8]. Environmental factors such as diet and life style (such as tobacco smoke, alcohol, aflatoxin B1, UV, and infectious agents) have been linked with epigenetic alterations. The enzymes that are involved in these epigenetic changes are also being used as targets for drug development.

2.2. Cell culture as a model for cancer prevention/treatment

The technique of cell culture was first established by Carrel and Burrows in 1911, when they grew chick embryo cells on a glass dishes. Using this system it was possible to grow normal rodent cells that undergo spontaneous transformation but not human cells. The latter grew senescent and died. The higher ability to repair the DNA could be the reason why mouse cells but not human cells could be grown in culture. HeLa was the first continuous human cancer cell line; it was established from cervical cancer of a young woman more than 50 years ago [9]. Today cell culture is used routinely to discover new anticancer drugs. Since 1990, the National Cancer Institute (NCI) has screened more than 60,000 compounds against a panel of 60 human cancer cell lines [10–12]. Screens of the NCI's database of more than 460,000 compounds are expected to provide potential target molecules as candidate anticancer drugs. How effective this strategy is at generating new clinically active agents is still not clear even 20 years later. So far no agent with anticancer activity has yet been found using this approach. Whether an agent shows an activity in this system, it is unclear why and how? Furthermore this system has little relevance to predict the activity in rodents or patients.

Almost 75% of all publications in Cancer Research are based on the use of a total of 112 cell lines [13]. The cell culture models, although highly convenient, have numerous limitations (Fig. 2). First, inhibition of proliferation (cytostasis) or killing of tumor cells (cytotoxicity) is not a good indicator of efficacy of a compound in the patient. No direct correlation has yet been found between the efficacy of a compound in a given cell culture model to that in the patient. Second, cell lines do change when they are propagated in culture over long periods of time, which compromises its usefulness. Third, numerous cell lines have been found to be cross-contaminated as seen with human esophageal squamous carcinoma cell line [14] and HeLa cells used to find a cure to cancer. The source of contamination could be mycoplasma, cell lines derived from other organs, and even from other species [15,16]. Both interspecies and intraspecies cross contamination of HeLa cells has been reported [9,17]. For instance MDA-MB-435 cell line that was thought by most

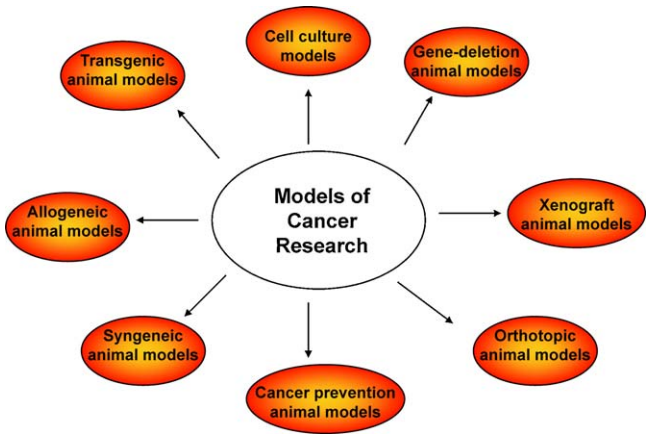

Fig. 2. Various models currently being used to prevent and treat cancer.

to be human breast cancer cell lines was later shown to be a human melanoma cell line [18]. There is also evidence that human cancer cells can transform normal rodent cells [19]. Thus too many grants, publications and claims have been made based on false cell lines. With current technology, DNA profiling of each cell line provides a simple solution to authenticate the cell lines. Even when the cell lines are authenticated, however, the question remains whether it is useful for the discovery of an anticancer agent.

2.3. Animal model for cancer prevention/treatment

Over the years numerous animal models have been discovered as incubators of primarily human tumors, including newborn Syrian hamsters, mice, and rats (Fig. 2). Perhaps the most common model is athymic nude mouse model because it is deficient in its immune system and thus unable to reject human tumors. There are xenograft (subcutaneous injection of tumor cells [20,21], orthotopic (injection of tumor cells into the organ from which it was derived), transgenic [22], gene knock-out, Zebrafish [23], and other animal models. These models are used to understand the process of tumor growth, invasion, angiogenesis, and metastasis of cancer. They are also used to investigate the role of specific genes in tumorigenesis. Perhaps most importantly, such animal models are used for cancer drug discovery [21,24].

The use of mouse models for drug discovery was started at the NCI to screen for anticancer compounds, first using mouse tumors (L1210) in 1950s and then human tumors in nude mouse in the 1970s [25,26]. As many as 200,000 studies in animals have been published in an attempt to understand the biology of cancer and to find novel therapeutics. Although such models have contributed significantly to our understanding of cancer biology, there remain serious concerns, about the value of mouse cancer models for predicting therapeutic efficacy in humans. Given that 90% of the agents eventually fail in clinical testing despite antitumor efficacy in animal models, the predictive value of these models is low [27]. Most modern targeted therapies such as gleevec, herceptin and rituximab are even more likely to fail in these subcutaneous xenograft animal models. Neither negative (exhibits efficacy in human but not in mice) nor positive (exhibits efficacy in mice but not in human) predictive value of these models is clear.

Whether genetically engineered mouse (GEN) models in which tumor formation is driven by a clinically relevant oncogene [28,29] or loss of a tumor suppressor gene (e.g., p53) [30,31] is a better predictor of cancer drug discovery is also not clear. GEN mouse models have been used to discover agents that can prevent breast cancer [31,32] and colon cancer [30,33]. Such mice exhibit asynchronous development of tumors which is major disadvantage. To overcome this problem, the use of models in which tumor formation can be induced in a conditional manner (CRE-lox), are being explored. An alternate approach involves the use of genetically defined mouse tumor cells transplanted in syngeneic naïve mice to test for novel therapeutics. The usefulness of any of these approaches in drug discovery, remains to be determined.

The use of human cell lines that have been propagated for long periods of time in culture and then implanted in mice is a serious problem, as the genetic and physiological characteristics of cell lines do change. Implanting primary tumor explants from patients directly into nude mice for testing new drugs may have a better chance of leading to drug discovery than most of the methods currently employed [34]. As invasion and metastasis play major roles in cancer-induced mortality in human, whether any of the animal models have any predictive value for metastasis is even less clear. Tumor cells implanted orthotopically are more likely to undergo metastasis than those xenotransplanted. Whether pharmacodynamic markers improve the predictive value in any of these models is also not clear. More often than not pharmacodynamic markers are not closely linked to the actual response of the tumor.

Thus currently available models are limited in predicting the modulation of target, tumor growth, invasion, angiogenesis, metastasis, and survival for any of the anticancer agents currently available [21,35]. Considering that it can cost over a billion dollars (most of the expense is in clinical trials) and may take as many as 15 years to develop a single drug entity for cancer, discordance of animal models with that in patients is a serious limitation in cancer research at present. Although there are instances where compounds were found to be effective antitumor agent in both animals and in human trials, the use of cell culture and rodent models in predicting clinical response, has been questioned [36,37]. Although over 100 anticancer compounds have been approved for human use, and most exhibit therapeutic effect in animal model, very few of them are effective in the majority of humans.

The primary goal of cancer therapy is to improve long-term survival while maintaining the quality of life of the patient. Clinical trials normally examine the time to disease progression, objective response rates, surrogate markers and quality of life. Animal models, however, are not designed to address all of these issues.

Certain animal models are better predictor of side effects of the drug that could occur in patients. Mice, rats, hamsters, dogs and monkeys have been used as predictor of toxicity. Evaluation of effects of 25 anticancer drugs in dogs and monkeys showed only 40% concordance with the results in humans for neurological and neuromuscular toxic effects [38,39] and a concordance of less than 10% for cardiotoxicity. Hepatic, renal, and hematopoietic toxicities of these drugs were similar in humans, rodents, dogs and monkeys. Most human toxicities reach plateaus within one month in animals. Overall, dog was found to be a better quantitative

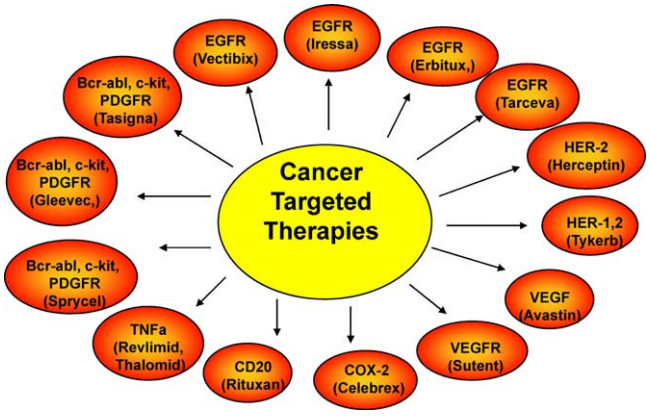

Fig. 3. Various targeted therapies discovered within last 10 years for the treatment of cancer. The commercial names of drugs, is indicated in parenthesis.

predictor of human toxicities of various anticancer drugs [40]. Interestingly, dog was found to be superior to monkeys (resistant to vomiting) in predicting gastrointestinal toxicity. Since these studies are based on chemotherapeutic agents, whether targeted therapies will exhibit similar profile remains to be determined.

2.4. Cancer biomarkers/signatures

It is estimated that as many as 30 years can lapse from the time of initiation of cancer to the time the cancer can be detected. By that time, most cancers have advanced to a difficult to treat stage. Thus early detection of cancer is one of the area of active research. An identification of a biomarker for each cancer that is an indicator of the disease itself and its progression is absolutely critical. Biomarkers are also useful in determining the sensitivity or resistance of cancer cells to therapy.

An ideal biomarker should be easy to detect, measurable across populations, and evident at an early stage. The biomarker should also assist in identification of high-risk individuals, early detection of recurrence, and allow evaluation of treatment results at an intermediate endpoint [41]. These biomarkers are usually examined in least invasive settings such as urine, plasma, or oral mucosa. Prostate-specific antigen (PSA), CA-125 and HER2/neu are some of the biomarkers that are currently being used to detect cancers of the prostate, ovary and breast respectively. Some of the biomarkers which are currently being explored for their potential assay in the urine include MMPs, ADAM-12, TMPRSS2 and sarcosine [42]. At genetic levels, the biomarkers that are being investigated include microsatellite instability, DNA hypermethylation, and single-nucleotide polymorphisms. Genomic and proteomic approaches are also being used to find molecular signatures of cancer at early stages.

The identification of biomarker for most cancers has been very difficult. Currently for most cancers, there is no biomarker available. Even for those where biomarkers have been identified, the biomarkers have very poor predictive value. For instance elevated PSA predicts prostate cancer only 20% of the time. Additionally, as tumors progress, a given biomarker becomes obsolete with time.

Recently several targeted anticancer agents have been approved for treatment of various cancers. These include herceptin, gleevac, EGFR inhibitor (gefitinib, erlotinib and cetuximab), avastin, sorafenib, and sunitinib [43]. Which kind of patients are likely respond to these drugs should be determined by specific biomarkers that are used to design the drugs. Most studies, however, have found inconsistent relationships between the expression of biomarker and the drug designed to take advantage of the marker. For instance EGFR protein expression was found to be unrelated to the efficacy of EGFR-TKI [44,45]. Similarly, contradictory reports have also been published about EGFR gene amplification status and the efficacy of EGFR-TKI [46]. Interestingly, EGFR mutation was found to be a prognostic marker of better survival rather than predictive marker of drug efficacy. Thus so far no predictive biomarkers of EGFR-TKI efficacy have been validated for clinical application. The same is true for two antibodies against EGFR, cetuximab and panitumumab.

Avastin is an antibody against VEGF that has been approved for the treatment of colon cancer and lung cancer. So far, the results of treatment of cancer patients with avastin have not correlated with VEGF levels before and after the treatment [47]. Predictive markers have also been sought for sunitinib and sorafenib, which inhibit kinases activated by VEGF, placental growth factor, and bFGF. A randomized phase III trial in renal cell carcinoma patients with sorafenib vs placebo showed a longer progression-free survival in patients with high base line plasma levels of VEGF [48].

Thus no biomarker has yet been found that predicts the efficacy of any of the EGFR or VEGF-based therapies. This conclusion, however, differs from that of HER2 or bcr-abl/c-kit expressing tumors. Numerous approaches are underway to define the biomarkers of different cancers. An integrated genomic analysis approach has been used recently to define the biomarkers for pancreatic cancers and for glioma [49,50].

2.5. Cancer stem cells

Recently it has been hypothesized that a stem cell, the mother of all cancer cells, with very unique features plays a major role in the development and progression of tumors [51,52]. A cancer stem cell (CSC), also called "tumor initiating cell" or "tumorigenic cell", is defined as a cell within a tumor that is able to self renew and to produce the heterogenous lineage of cancer cells that form the tumor. CSC has been compared with queen bees in a hive [53]. CSC was first identified in leukemia in 1994 by John Dick from the University of Toronto and in breast cancer in 2003 by Michael Clark from Stanford University. Since then they have been identified in brain and breast cancers, head and neck squamous cell carcinoma, and prostate, colon, and pancreatic cancers. The idea of CSC is more convincing in leukemia than solid tumors. CSC usually constitutes very small subset (one in 1000 to one in million) of undifferentiated cells in the tumor. Other reports, however, indicate that CSC are not as rare as initially envisioned [54]. Interestingly, it was found that melanoma CSC in immunocompromised mice, only 1 in 837,000 cells formed tumors but in more severely immunocompromised mice, 1 in 4 did.

These cells can be identified by cell surface markers. High expression of CD44 and CD133 and low expression of CD24 are the characteristic markers of CSC. CD133 is also expressed by normal tissue residents as well as hematopoietic stem cells. This antigen is also expressed by early progenitors but usually not detectable upon differentiation [55]. In tumor cells, CD133 has been used for the identification of a subpopulation of highly tumorigenic cells as demonstrated for cancers of the nervous system, colon, and pancreas. These cells are highly tumorigenic in orthotopic tumor models [56]. As CSC differentiate they lose the antigen. In contrast to "stationary" CSC, those that express CXCR4 receptors have been called "invasive" or "migrating" CSC and mediate tumor metastasis. CXCR4 and its ligand SDF1, commonly found on leukocytes, have been linked with metastasis of large number of cancers including those of the lung, liver, colon, and breast. Furthermore it is believed that stationary CSC are embedded in the epithelial tissue whereas invasive CSC must be located at the tumor–host interface. Whether CSC arises by mutation from normal stem cell or from mutated progenitor cells is less clear. It is not clear whether CSC arises from tissue-specific stem cells.

The failure of cancer treatments could be due to lack of elimination of CSC. In cases in which bulk disease is eradicated by aggressive chemotherapy, the reason for relapse is that CSC were not destroyed but instead enriched [53,57,58]. Such possibilities should be carefully explored. Whether CSC can be directly targeted for therapy is already being explored by numerous companies including Genentech, Merck, Oncomed Pharmaceuticals and Raven Biotechnologies [53,59].

2.6. Cell signaling and cancer

Cancer is caused by dysregulation of various cell signaling pathways, resulting in hyperproliferation of tumor cells. Besides cell growth, invasion, angiogenesis, metastasis, inflammation, and apoptosis play major roles in tumor development. Hundreds of growth factors, growth factor receptors, adaptor proteins, protein kinases, protein phosphatases, and proteases have been linked

with the development of cancer. These proteins constitute 537 non-redundant cell signaling pathways, each containing a different combination of growth factors, receptors, protein kinases, and others molecules (http://www.pantherdb.org/pathway). Large-scale sequencing studies suggest mutations in large number of these proteins that derive tumorigenesis. Which cell signaling pathway is critical for a given type of cancer, is not fully understood. These pathways, however, have been targeted for cancer drug discovery.

2.7. Single target vs multi-targeted therapies

At one time drugs that hit a single target were called "smart drugs" and those that hit multiple targets were called "dirty drugs". In view of the fact that most diseases are caused by dysregulation of multiple targets, this paradigm is now debatable. In addition, mono-targeted therapies by themselves have in most cases shown very little or no therapeutic effect, especially in the case of cancer. Various cell signaling network models indicate that partial inhibition of a number of targets is more effective than the complete inhibition of a single target [60]. In addition, higher therapeutic efficacy of multi-targeted drugs (e.g., salicylates, metformin, Gleevec, chemotherapeutic agents) and combination therapies also suggest that drugs directed against multiple targets are preferable. Multi-targeted drugs often exhibit low affinity for their targets, which is likewise preferable. Sorafenib and sunitinib are the two recent examples of multi-targeted kinase inhibitors. In addition to multi-targeted therapeutics, multicomponent therapeutics are also proposed [61]. The latter, although routinely used, are hard to optimize. A recent example showed that combining erbitux with avastin for treatment of colon cancer made therapy worst rather than better [62].

It is now clear that most tumors depend on more than one signaling pathway for their growth, survival, invasion and metastasis. Moreover, multiple cell signaling pathways may control a given step in tumorigenesis. Thus agent or drug that inhibits multiple pathways or their combination is needed for cancer treatment [63,64]. In addition, cells may develop adaptive resistance to a mono-targeted agent due to mutation in the target, but resistance is less likely if there are multiple targets [65,66].

In contrast to targeted therapies, most natural products that have been used for centuries are usually multicomponent and are multi-targeted; and thus are preferred. They are also relatively safe and affordable in most cases. For instance, curcumin derived from turmeric (*Curcuma longa*) [67], resveratrol from red grapes [68], guggulsterone from guggul (*Commiphora mukul*) [69] and silymarin from milk thistle plant (*Silybum marianum*) [70] are all multi-targeted and yet quite safe.

2.8. Therapeutic targets

Frequent failure rate of new drugs may be due to lack of preclinical models that accurately predict efficacy and toxicity, thus resulting in more than 10 years for drug approval. About 5% of the investigation new drug (IND) application for new drugs submitted to the FDA reach beyond the investigational phase, and more than 50% of the agents fail in phase III clinical trials. Until 1971, the only treatments available for cancer were surgery, chemotherapy and radiotherapy. Most chemotherapeutic agents target either DNA (e.g., cyclophosphamide, doxorubicin, vincristine, 5-fluorouracil), microtubules (e.g., paclitaxel) or inflammatory pathways (dexmethasone, prednisone). Most of these agents, however, are not specific for cancer cells and thus produce numerous side effects. Additionally it seems that the effect of chemotherapeutic agents is not simply through inhibition of tumor cell proliferation but also disruption of the tumor vasculature [71,72]. These activities have been shown for taxanes, cyclophosphamide, and vinbalstin. Interestingly, most chemotherapeutic agents are known to activate the transcription factor NF-κB and mediate chemoresistance [73]. Another report recently indicated chemotherapy may enrich the tumor for cancer stem cells and thus mediate chemoresistance [58,74]. Chemotherapeutic agents were recently reported to induce circulating endothelial progenitor (CEP) to home to tumor vasculature [75].

Since 1971 several therapies have been developed that target specific cell-signaling molecules [76]. Cancer cell growth is estimated to be regulated by over a hundred different key gene products. Among the agents that target these products are inhibitors of VEGF, EGFR, Bcr-Abl, PDGFR, VEGFR, HER2, COX2, proteasome, SAHA and CD20. All these inhibitors have been approved for cancer treatment. Imatinib (bcr-abl) has improved CML outcomes, trastuzumab (HER2) has done the same for breast cancer outcomes, bevacizumab (VEGF) and cetuximab (EGFR) for colon cancer,) and renal cell carcinoma with sorafenib and sunitinib (Fig. 3). Cetuximab in combination with radiation has shown some promise for head and neck cancer. Although all these inhibitors are very specific, their mechanism of action in patients is not so specific in most cases as discussed below. Moreover, preclinical models whether cell culture or animals, do not predict their efficacy in patients. Because multiple protein kinases are likely to be involved in development of different cancers, a high throughput kinase profiling has been used for drug discovery [77]. Whether the target is expressed or not by the patient also fails to predict their efficacy. Some examples are outlined below.

2.8.1. EGFR-based therapeutics

EGF was identified in 1965 [78], in 1983 its receptor was proposed as a target for cancer treatment [79], and in 2003 the first EGFR inhibitor was approved by the FDA. Most of the clinical trials with EGFR inhibitors have been negative, and in the few cases where they were not negative, any response was very modest (Table 1).

2.8.1.1. Iressa. Iressa (also called ZD 1839 or gefitinib) is a quinazoline derivative that has been found to inhibit the tyrosine kinase activity of EGFR. EGFR is overexpressed in non-small cell lung cancer (40–80%), CRC (72–82%), head and neck cancer (95–100%), breast cancer (14–91%), pancreatic cancer (57%), and kidney cancer (50–90%) [80]. It has been recently tried in lung cancer (NSCLC) patients and was found to be as effective as chemotherapy: patients receiving iressa survived 7.6 months and those on chemotherapy for 8 months; also after 1 year 32% of patients on iressa were still alive versus 34% of those on chemotherapy. This led to the approval of iressa for patients who failed to respond to platinum-containing chemotherapy. Iressa has also been approved in combination with gencitabin for the treatment of pancreatic cancer. In spite of the high level of expression of EGFR receptors, iressa did not show efficacy in all those cancers. Thus expression of receptors on cancer cells was not directly related with the response. This suggests that iressa must mediate its effects through mechanisms independent of EGFR [81] or molecular defects in downstream signaling pathways [82]. Constitutively active MAPK and AKT and loss of PTEN have also been linked with resistance to EGFR inhibitors. Skin rash, diarrhea, infection, and stomatitis are some of the major side effects of the EGFR blockers.

2.8.1.2. Erlotinib. Erlotinib (tarceva or OSI-774) is another oral EGFR-PTK inhibitor that targets the ATP binding site of the kinase. This EGFR inhibitor has been approved for patients with non-small cell lung cancer [83]. It has also been tried alone and in combination with gemcitabin for patients with advanced pan-

Table 1
A list of targeted drugs against cancer approved by the FDA.

Drug	Disease	Target	Survival (mo)	Approval	Cost/year (US$)	Adverse events[a]
Iressa (gefitinib)	NSCLC	EGFR[a]	4.3[b]	2003	~26,000	Nausea, vomiting, diarrhea, rash, acne, dry skin
Tarceva (erlotinib)	NSCLC, PC	EGFR[c]	6.7[d], 6.4[e]	2004	~43,300	Rash, diarrhea, poor appetite, fatigue, shortness of breath, cough, nausea, vomiting, infection, mouth sore, itching, dry skin, eye irritation, abdominal pain
Erbitux (cetuximab)	SCCHN, CRC	EGFR[a]	1.5	2004	~144,000	Difficulty in breathing, low blood pressure, ILD, acne-like rash, dry skin, tiredness, weakness, fever, constipation, abdominal pain.
Vectibix (panitumumab)	EGFR + CRC NSCLC	EGFR[a]	ND 2[f]	2006	~50,400	Severe skin reactions, sun sensitivity, severe infusion reactions, pulmonary fibrosis, diarrhea, loss of electrolytes, tiredness, stomach pain, nausea, constipation, vomiting, cough, swelling
Herceptin (trastuzumab)	HER2 + BC MBC	HER2[a] HER2	ND ND	2006 1998	~69,500	Cardiomyopathy, pulmonary toxicity infusion reactions, neutropenia, fever, nausea, vomiting, infusion reactions, diarrhea, infections, increased cough, headache, fatigue, dyspnea, rash, neutropenia, anemia, myalgia
Tykerb (lapatinib ditosylate)	HER2 + BC	HER1, 2[a]	ND	2007	~44,400	Diarrhea, PPE, nausea, rash, vomiting, fatigue
Sutent (sunitinib)	GIST, ARCC	VEGFR-1,2,3; PDGFR-α,β; c-kit; FLT3; RET[a]	ND	2006	~48,000	Left ventricular dysfunction, diarrhea, nausea, mouth sores, indigestion, vomiting, skin and hair changes, dryness, tiredness, high blood pressure, bleeding, swelling, mouth pain, irritation, taste changes
Nexavar (sorafenib)	HCC ARCC	VEGFR VEGFR	10.7 ND	2007 2005	~77,000	Fatigue, weight loss, rash, alopecia, diarrhea, anorexia, nausea, abdominal pain, cardiac ischemia, hypophosphatemia
Avastin (bevacizumab)	CRC, BC, NSCLC	VEGF[a]	4.7	2004	~57,600	Gastrointestinal perforation, high blood pressure, tiredness, blood clots, diarrhea, decreased white blood cells, headache, appetite loss, mouth sores.
Velcade (bortezomib)	MM, MCL	Proteasome	6	2008	~43,500	Asthenic conditions, diarrhea, nausea, constipation, peripheral neuropathy, vomiting, pyrexia, thrombocytopenia, psychiatric disorders, anorexia, neutropenia, neuralgia, leucopenia, anemia
Gleevec (imatinib)	BP-CML	Bcr-abl	6.5	2001	~61,000	Nausea, vomiting, edema, muscle cramps, skin rash, diarrhea, heartburn, headache
Sprycel (dasatinib)	Ph + ALL, CML	Bcr-abl; Src;[a] c-Kit; PDGFR-β; EphA2	ND	2006	~47,000	Low blood counts, bleeding, fluid retention, diarrhea, skin rash, headache, fatigue, nausea
Tasigna (nilotinib)	CP-CML	Bcr-abl	ND	2007	~68,400	Rash, pruritis, nausea, fatigue, headache, constipation, diarrhea, vomiting, thrombocytopenia, neutropenia, pneumonia, intracranial hemorrhage, elevated lipase, pyrexia.
Celebrex (celecoxib)	FAP	COX-2[a]	ND	1998	~3,000	Stomach ulcers, liver damage, kidney problems, fluid retention, headache, indigestion, upper respiratory tract infection, diarrhea, sinus inflammation, stomach zpain, nausea
Rituxan (rituximab)	NHL	CD20	1.5	1997	~143,000	Infection, thrombocytopenia and lung toxicity/dyspnea, severe or fatal infusion reactions, tumor lysis syndrome, severe mucocutaneous reactions, hepatitis B reactivation with fulminant hepatitis, other viral infections, hypersensitivity reactions, cardiac arrhythmias, renal toxicity, bowel obstruction and perforation.
Revlimid (lenalidomide)	MM	TNFα, IFN-γ,IL-1β, IL-6, IL-12, IL-10, IL-2, COX-2[a]	ND	2006	~97,200	Low white blood cells and platelets, Blood clots, shortness of breath, chest pain, arm or leg swelling, diarrhea, itching, rash, tiredness
Thalomid (thalidomide)	MM	TNFα	18	2006	~11–66,000	Somnolence, constipation, rash, neuropathy, venous thromboembolism (VTE)

mo, months; ND, no data; HER2 + BC, HER2 positive breast cancer; EGFR + CRC, EGFR positive colorectal cancer; VEGFR, vascular endothelial growth factor receptor; ARCC, advanced renal cell cancer; PDGFR, platelet-derived growth factor receptor; FLT, Fms-related tyrosine kinase; BP-CML, blast phase CML; HER2, human epidermal growth factor receptor 2; HER1, human epidermal growth factor receptor 1; NHL, non-Hodgkin's lymphoma; SCCHN, squamous cell carcinoma of head and neck; EGFR, epidermal growth factor receptor; MM, multiple myeloma; TNFα, tumor necrosis factor alpha; VEGF, vascular endothelial growth factor; IFN-γ, interferon gamma; IL-1β, interleukin 1 beta; PC, pancreatic cancer; NSCLC, non-small cell lung cancer; BC, breast cancer; ALL, acute lymphoblastic leukemia; IL, interleukin; Ph + ALL, Philadelphia chromosome positive acute lymphoblastic leukemia; GIST, gastrointestinal stromal tumor; MDD, myelodysplastic diseases; MCL, mantle cell lymphoma; DFSP, dermatofibrosarcoma protuberans; Bcr-abl, breakpoint cluster region Abelson; PDGFR, platelet-derived growth factor receptor; HCC, hepatocellular carcinoma; CP-CML, chronic phase CML; CML, chronic myeloid leukemia; CRC, colorectal cancer; FAP, familial adenomatous polyposis; COX-2, cyclooxygenase 2; MBC, metastases of breast cancer.

[a] U.S. Food and Drug Administration website (http://google2.fda.gov/search).
[b] Kirby 2006 Gefitinib (ZD1839, Iressa™) as palliative treatment in recurrent or metastatic head and neck cancer.
[c] Moore 2007 Erlotinib plus gemcitabine.
[d] FDA Approves New Drug for the Most Common Type of Lung Cancer; Tarceva vs. placebo: 6.7 mo vs. 4.7 mo.
[e] NCI: FDA Approval for Erlotinib Hydrochloride; 100 mg erlotinib hydrochloride/gemcitabine vs. placebo/gemcitabine groups: 6.4 mo vs. 6 mo.
[f] From ref. [124].

creatic cancer. Overall survival was found to be 5.91 months with gemcitabin alone and 6.24 months when combined with erlotinib. One-year survival was 17% vs 23% with gemcitabin vs combination [84]. Once again the responsiveness to erlotinib did not correlate with the expression of EGFR on the tumor. Downregulation of PTEN and upregulation of constitutively active AKT has been suggested as the mechanism of resistance to erlotinib [85].

2.8.1.3. Cetuximab. Cetuximab (Erbitux, C255) is an antibody against EGFR that has some efficacy against irinotecan-refractory metastatic colorectal cancer. The rate of response was 10.8% as a single agent and 22.9% when tested in combination with irinotecan; median survival was 6.9 months with cetuximab and 8.6 months with the combination [86]. There is some evidence cetuximab, which blocks the extracellular domain of EGFR, has enhanced activity against cancer when combined with gefitinib or erlotinib, which inhibit the intracellular domain of the receptor [87].

2.8.2. Trastuzumab (Herceptin)

Amplification of the HER2 gene occurs in 20–25% of human breast cancers. Trastuzumab, a monoclonal antibody targeting an epitope in the extracellular domain of the human epidermal growth factor receptor-2 (HER2/erbB-2), is active in HER2-overexpressing metastatic as well as in resected breast cancer when given postoperatively. Herceptin is the first such agent to be approved for breast cancer [88]. Phases II and III clinical trials performed in women with metastatic breast cancer that overexpresses HER2 demonstrated clinical activity when it was used as first-, second- or third-line monotherapy, and improved survival when used as first-line therapy in combination with chemotherapy. Response rates to single-agent trastuzumab ranged from 12 to 34% for metastatic breast cancer for a median duration of nine months, and significant improvements in survival rates were achieved in patients with early-stage HER2-overexpressing breast cancer in the adjuvant setting. However, the majority of cancers that initially respond to herceptin begin to progress again within 1 year, and a large subset never responds, demonstrating primary resistance [89].

How herceptin mediates its antitumor activity is not fully understood. In vitro, downregulation of the receptor correlates with inhibition of tumor growth. In contrast, the activity of herceptin in animal models depends on the engagement of Fc-receptor-expressing lymphocytes [90,91], indicating antibody-dependent cellular toxicity (ADCC) as the major mechanism of antibody action. Another study showed that herceptin may produce its antitumor activity by reducing the number of tumor blood vessels [92].

Why most patients do not respond to Herceptin in spite of amplification of HER2 gene and overexpression of HER2 both in primary and metastatic tumor is unclear [93]. It is also not clear why the majority of the patients develop resistance to herceptin after 1 year. It is possible that cellular heterogeneity in HER2 expression leads to overgrowth of HER2-negative cells, which do not respond to herceptin [94]. Additionally, it has been shown that downregulation of p27, a CDK inhibitor, may play a role in induction of resistance to herceptin [95]. The role of PI3K pathway as a major determinant of herceptin-resistance in breast cancer was identified from a large-scale RNA interference screen [96].

The mechanism by which herceptin mediates its antitumor activity in patients with HER2-positive metastatic breast cancer (MBC) was investigated [97,98]. It was found that the clinical response was not through the downregulation of HER2 expression. For instance, (a) all patients showed high levels of circulating herceptin; (b) saturating levels of HER2 were present in all of the tumors; (c) no down-modulation of HER2 was observed in any tumors; (d) no changes in vessel diameter was observed in any tumors; (e) no changes in proliferation was observed in any tumors; (f) a strong infiltration by lymphoid cells was observed in all cases. Patients with complete remission or partial remission were found to have a higher in situ infiltration of leukocytes (macrophages and granulocytes but not NK cells) and a higher capability to mediate in vitro antibody-dependent cellular cytotoxicity activity. It was concluded that the activity of herceptin in patients is not through down-modulation of HER2 but through ADCC. A decrease in serum HER2 levels, however, has been related to response to herceptin. A meta-analysis that comprised 307 patients with MBC reported that 62% of patients had a significant decline (>20%) in serum HER2/neu and 38% of patients did not [99]. The objective response rate was 57% for patients who achieved this decline in serum HER2/neu compared with 28% for patients who did not. Patients who achieved this decline in serum HER2/neu also had a significantly longer time to disease progression (320 days vs 180 days), longer duration of response (369 days vs 230 days), and longer overall survival (898 days vs 593 days).

Trastuzumab is generally well-tolerated, but cardiac toxicity (particularly when the antibody was combined with anthracyclines) was an unexpected adverse effect. Trastuzumab is currently used in both the adjuvant and neoadjuvant settings as well as in combination with other chemotherapy drugs.

2.8.3. Lapatinib sitosylate (GW572016; Tykerb)

Lapatinib is an oral receptor tyrosine kinase inhibitor, targeting both the ErbB-1 and ErbB-2 receptors. Pre-clinical in vitro and in vivo models indicate that lapatinib is active as monotherapy, synergistically in combination with trastuzumab, and in trastuzumab-resistant cell lines. When given in combination with capecitabine in women with HER2-positive trastuzumab-refractory metastatic breast cancer, lapatinib has shown activity [100]. The median time to progression was 8.4 months in the combination-therapy group as compared with 4.4 months in the monotherapy group (capecitabine alone).

2.8.4. Vorinostat (Zolinza; suberoylanilide hydroxamic acid, SAHA)

The histone deacetylase inhibitor vorinostat has been evaluated in patients with refractory cutaneous T-cell lymphoma (CTCL) [101]. Time to response (TTR), time to progressive disease (TTP), response duration (DOR) for responders were 11.9, 15.1, and 30.2 weeks, respectively. The most common drug-related toxic effects were fatigue, thrombocytopenia, diarrhea, nausea, and dehydration. In preclinical, SAHA was shown to have activity against wide variety of cancers. In the clinic, why SAHA had a significant activity against CTCL but not in other type of cancers, is not understood.

2.8.5. Sunitinib

Sunitinib is a small molecule that inhibits multiple kinases including VEGFR (FLT1 and FLK1/KDR), PDGFR (PDGFRα and PDGFRβ), SCFR (c-KIT), FLT3 and RET kinases. Because it inhibits multiple kinases, sunitinib may affect not only tumor cell growth but also angiogenesis. Although sunitinib exhibited antitumor activity in preclinical animal models of colon cancer, NSCLC, melanoma, renal carcinoma and squamous cell carcinoma, in patients it showed efficacy only in metastatic renal cell carcinoma (mRCC) and in imatinib (bcr-abl inhibitor)-refractory GIST diseases. For mRCC, sunitinib had a 37% response rate and additional 48% had stable disease, and it is now a first-line treatment. For GIST, treatment with sunitinib produced an 8% response rate and 70% stable disease rate; and so it is now second-line treatment for GIST [102]. These studies led to the approval of Sunitinib in 2006 by the FDA for treatment of kidney cancer and gastrointestinal stromal tumor.

Sunitinib's promise in animal models against variety of cancers and its limited effectiveness in patients suggests the limitation of the preclinical models.

Some of the toxicities associated with sunitinib included gastrointestinal events (diarrhea, nausea, mucositis, vomiting, dyspepsia, abdominal pain, gastroesophageal reflux, oral pain, glossodynia, and flatulence); bleeding; hypertension; dermatologic events (rash, skin discoloration, dry skin, and hair color changes); hand–foot syndrome; limb pain; decreases in cardiac ejection fraction; and peripheral edema. Iatrogenic hypothyroidism was also more common in patients receiving sunitinib. Grade 3/4 adverse events relatively common with sunitinib included hypertension, diarrhea, hand–foot syndrome, nausea, vomiting, mucositis, and bleeding. The most frequent grade 3/4 laboratory abnormalities in sunitinib-treated patients included hematologic abnormalities (neutropenia, thrombocytopenia, and leucopenia), increased lipase, increased amylase, hyponatremia, hyperuricemia, and hyperbilirubinemia.

2.8.6. Sorafenib tosylate (Nexavar)

Sorafanib is an inhibitor of several kinases including raf, PDGFR, and VEGFR. Although initially developed as raf inhibitor, the clinical activity of sorafenib does not appear to be due to raf inhibition [103]. It was approved in 2007 for the treatment of liver cancer [104] and kidney cancer. For hepatocellular carcinoma, overall survival was the primary efficacy endpoint. The trial was stopped following a pre-specified second interim analysis because survival data indicated a statistically significant advantage for sorafenib (median 10.7 vs. 7.9 months). The final analysis of time-to-tumor progression (TTP) in the sorafenib is median 5.5 vs. 2.8 months. Sorafenib is the first systemic therapy to demonstrate a survival benefit in a randomized trial for unresectable hepatocellullar carcinoma.

In 2005, the FDA approved sorafenib for the treatment of patients with advanced renal cell carcinoma who had received one prior systemic treatment. The median progression free survival (PFS) was 167 days in the sorafenib tosylate group versus 84 days in the placebo control group. Time-to-progression was similarly improved.

The most common adverse reactions considered related to sorafenib were fatigue, weight loss, rash/desquamation, hand–foot skin reaction, alopecia, diarrhea, anorexia, nausea and abdominal pain. Diarrhea was reported in 55% of sorafenib patients (grade 3 in 10%). Hand–foot syndrome (21% overall; grade 3 in 8%) and rash (19% overall; grade 3 in 1%) were the most common dermatologic adverse reactions to sorafenib. Elevated serum lipase occurred in 40% of sorafenib patients, and hypophosphatemia occurred in 35% of sorafenib patients.

2.8.7. Avastin (Bevacizumab)

Avastin is a recombinant human monoclonal antibody against human VEGF that was approved by the FDA as first-line therapy in 2004 for use in combination with fluorouracil for metastatic colorectal cancer [105,106]. Median overall survival was increased from 13.8 months to 16.6 months by avastin. VEGF is expressed in nearly 50% of colorectal carcinomas, and increased expression has been correlated with decreased survival. Interestingly, however, surrogate markers of disease progression or treatment response to avastin, such as pretreatment plasma VEGF levels, showed no significant correlations with any outcome measure [107]. When tested in advanced pancreatic cancer patients, again the response of the patient was found to have no correlation with pretreatment plasma VEGF levels [108]. In patients with metastatic breast cancer, avastin combined with capectabine increased the response rate but not overall survival [72]. The observed toxicities included hypertension, proteinuria, mild to moderate hemorrhage, wound healing complications [109], and thromboembolic events. Thus how avastin is manifesting its effect in the patients remains unclear. Since tumor vasculature is needed for supply of chemotherapeutic agents, the possibility exists that by disrupting blood vessels avastin interferes with a supply of these agents [74]. Whether this decrease in the supply of the drug is compensated for by tumor starvation is an interesting question.

2.8.8. Bortezomib

Bortezomib (also called Velcade or PS-341) is a dipeptidyl boronic acid that reversibly inhibits the chymotrypsin-like activity of the 26S proteasome. It is estimated that more than 80% of all intracellular proteins are degraded by the ubiquitin-proteasome pathway (UPP) system. MG132, lactacystin, NPI-0052 and epoxomicin are some of the other proteasome inhibitors with similar mechanism of action and are thus potential therapeutic candidates. In cell culture and in animal models, velcade was found to have activity against a wide variety of tumors, but in the clinic it was found to have activity only against multiple myeloma. The overall response rate was 27% and median overall survival was 16 months [110]. When velcade was compared with dexamethasone, the overall response rate was 38% vs 18%; 1-year survival was 88% vs 66% and median time to progression was 6.2 vs 3.5 months, respectively. Why velcade has activity in multiple myeloma but not in patients with renal cell carcinoma, neuroendocrine tumors, colon cancer, lung carcinoma, prostate cancer, hepatocellular carcinoma, sarcomas, acute leukemias or melanoma is not understood [111]. Velcade was originally designed as an inhibitor of NF-κB activation. In various cell line models, velcade has been shown to suppress NF-κB activation. However, the inhibition of NF-κB, does not explain its antitumor activities. Whether the activity of velcade in MM patients is due to inhibition of NF-κB, is unclear. All evidence indicates that NF-κB inhibition has little or no role in the activity of velcade. Thus the real target of velcade in multiple myeloma is not known. Activation of JNK, mitochondrial cytochrome C release, induction of expression of p53 and MDM2, and downregulation of gp130 (an IL-6 receptor associated protein) are some of the other mechanisms for velcade that have been described [110].

Some of the major toxicities associated with velcade treatment include thrombocytopenia, hyponatremia, hypokalemia, nausea, diarrhea, fatigue, malaise and peripheral neuropathy. Almost 35% of the patients have peripheral neuropathy.

2.8.9. Gleevac (Imatinib mesylate or STI-571)

Imatinib mesylate is an inhibitor of bcr-abl that was approved in 2001 for the treatment of chronic myeloid leukemia (CML), which is normally treated with interferon (IFN)-α. Treatment with IFN-α may produce remission, restoring a normal blood count in up to 70% of patients with the chronic phase of the disease. If interferon alpha is ineffective or patients stop responding to the drug, the prognosis is generally bleak. FDA approved the drug Gleevac for treating patients with three stages of CML; CML, myeloid blast crisis; CML accelerated phase; or CML in chronic phase after failure of interferon treatment. Gleevec restored normal blood counts in 53 out of 54 interferon-resistant CML patients, a response rate rarely seen in cancer with a single agent. Fifty-one of these patients were still doing well after a year on the medicine, and most reported few minor side effects.

In 2008, FDA approved imatinib to prevent postoperative recurrence of gastrointestinal stromal tumor (GIST). GIST is a rarely occurring cancer originating in the interstitial cells of Cajal, which are autonomic nervous system cells lining and regulating the movement of food and liquid through the stomach and intestines. Incidence of GIST in the United States is approximately 5000–6000 new diagnoses each year. Although surgical resection is the first line of treatment, GIST frequently recurs. Imatinib is indicated

postoperatively to help prevent the recurrence of GIST. A clinical trial showed that treatment with imatinib for 1 year after surgical removal of GIST was more effective than placebo in preventing tumor recurrence. However, optimal treatment duration is still unknown.

Adverse reactions most frequently reported with imatinib were diarrhea, fatigue, nausea, pedal edema, decreased red blood cell counts, rash, vomiting, and abdominal pain.

2.8.10. Celebrex (also called celecoxib)

Celebrex is an inhibitor of the enzyme cyclooxygenase-2. In 1999, FDA today approved it for treatment aimed at reducing the number of intestinal polyps in patients with a rare genetic disorder called familial adenomatous polyposis (FAP). Patients with FAP develop large numbers of intestinal polyps and, as a consequence, have a greatly increased risk of developing colon and rectal cancer at an early age. Its new indication provides a treatment for reducing the number of polyps in patients with FAP. The only "surrogate marker" for FAP is the number of polyps; reduced numbers appear likely to be beneficial to FAP patients, but this has not yet been proven. The approval of Celebrex for FAP was based on a 6-month placebo-controlled trial in 83 patients. In the study, there was a 28% reduction in the number of polyps in patients receiving 400 mg of celebrex compared to 5% for those receiving placebo. The effect of celebrex on the development of cancer has not yet been established.

2.8.11. Rituximab (also called Rituxane or anti-CD20 antibody)

This was the first antitumor antibody approved by the FDA in 1997 for the treatment of follicular and low-grade NHL. Its use, however, expanded for other B cell malignancies when combined with chemotherapeutic agents (cyclophosphamide, doxorubicin, vincristine, prednisone; also called CHOP). The rate of complete response in patients with diffuse Large B cell lymphoma was 63% with CHOP alone but 76% when CHOP was combined with rituximab [112].

In 2006, rituximab was approved as the first-line treatment for patients with low-grade or follicular B-cell, CD20-positive non-Hodgkin's lymphoma. Patients receiving rituximab plus chemotherapy vs chemotherapy alone had progression-free survival (PFS) of 2.4 vs. 1.4 years. For diffuse large B-cell lymphoma, the survival advantage persisted with estimated 5-year survival rates of 58% vs. 46% for R-CHOP and CHOP, respectively.

Adverse events associated with rituximab were generally consistent with the labeled adverse reactions described for single-agent rituximab. Grade 3/4 adverse events occurring with $\geq$2% excess in the rituximab arm in at least one study included infection, thrombocytopenia, and lung toxicity/dyspnea. The serious adverse reactions, some with fatal outcomes, have been reported in patients receiving rituximab: severe or fatal infusion reactions, tumor lysis syndrome, severe mucocutaneous reactions, hepatitis B reactivation with fulminant hepatitis, other viral infections, hypersensitivity reactions, cardiac arrhythmias, renal (kidney) toxicity, and bowel obstruction and perforation.

2.9. Clinical trials

Although drugs that are rationally designed and remarkably effective in cell culture and even in animal models, they most are not effective in patients. If they are effective, it may be effective only in very small percentage of patients. For instance the best treatment available for pancreatic cancer is gemcitabine, which is effective in less than 10% of the patients. Why these 10% patients respond and not others is not clear. Generally the effectiveness of given drug may mean partial regression of tumors. However, this may not translate into overall increase in survival of the patient. If there is some increase in survival, it is usually by a few months but at the cost of an increased drug-related toxicity. All these points indicate the complexity of the human system, the mysteries of human diseases, and the empirical nature of clinical trials. Invariably these kinds of situations are dealt with by using combination therapy, which are not always rationally designed. Thus, though most therapies are rationally designed at the molecular level, their use becomes irrational and normally driven by the need of the patient. Combination therapy has become the norm of cancer treatment. For instance agents such cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) in combination make up the standard regimen for patients with B cell lymphoma. Dozens of agents are used in combination for a patient. These combinations, however, can be potentially harmful as was recently found when Avastin was combined with Erbitux to treat metastatic colorectal cancer [113]. The addition of Erbitux to Avastin resulted in significantly shorter progression-free survival and inferior quality of life.

2.10. Cancer prevention

Extensive research during last few years has revealed that cancer is a preventable disease that requires major changes in life style; with one third of all cancers assigned to Tobacco, one third to diet, and remaining one third to the environment. As many as 95% of all cancers are caused by life style and may take as long as 20–30 years to develop. Most of this data is based to observational studies. Additional support comes from experimental studies carried out in cell culture and from animal models. To prove these studies through clinical trials, however, has been highly expansive and challenging. All placebo-controlled clinical trials, whether carried out with retinoids, vitamins, carotenoids (component of carrot), lycopenes (component of tomato), green tea, selenium or other agents, the results have been either negative or mixed. A recent example is a vitamin E and selenium trial (SELECT) to prevent prostate cancer carried out with 35,000 men age 50 and older [114]. This was a phase III randomized, placebo-controlled study that is designed to determine whether selenium and vitamin E, alone or in combination, decrease the risk of prostate cancer in healthy men who received selenium (200 μg/day of l-selenomethionine) and/or vitamin E (400 IU/day of all-rac-alpha-tocopheryl acetate) supplementation for a minimum of 7 years (maximum of 12 years). This US$ 143 million trial had to be stopped because vitamin E users alone had slightly higher incidence of prostate cancer and selenium only users were getting slightly more diabetes than the control; whereas no increase was noted in the combination group. This indicates the complexity to prove prevention by any agent experimentally. Thus models are currently available which can be used to determine the efficacy of any of the chemopreventive agents.

2.11. Personalized medicine

The reason for new wave of "personalized medicine (PM)" is based on the concept that a "good therapeutics follows good diagnostics" and has the potential to increase efficacy and decrease toxicity [115]. PM is based among others on the followings. First, the current approach to treatment of cancer patient is highly empirical, so some patients with aggressive disease may be under-treated and others with indolent disease may be over-treated. Second, those who get treated, only a very small fraction derive clinical benefit. Third, patients who are treated may not respond to the therapy but will suffer from the severe toxic side-effects. Fourth, there have been major recent developments in the area of gene sequencing, pharmacogenomics, targeted therapies and molecular diagnostic. Fifth, catalogue of the molecular signatures of various type of cancers are becoming reality. These are some of

reasons that are driving the cancer treatment from the traditional "trial-and-error" approach to a "personalized medicine" approach that involves giving the right drug at the right dose to the right patient. The latter, however, requires the following: first, availability of prognostic markers that can distinguish patients with indolent disease vs aggressive disease; second, markers that can predict the response or resistance to specific therapy; and third, markers that can identify the patients who are likely to develop toxic side effects [116].

At present, PM is a paradigm that exists more in conceptual terms than in reality. Although the rationale for PM and its benefits are clear, considering our current state of knowledge, it may not be feasible. Perhaps all the excitement is based on breast cancer where there is little more known than more cancers. For instance it is known that women with mutation in *BRCA1 and BRCA2* genes are at high risk to develop breast cancer, women with HER2 positive can be treated with Herceptin, women with ER-positive tumors can be treated with ER antagonists, or women could have breast cancer with diagnosis as triple negative (ER-, PR- and HER2-), and pharmacogenomic tests although not validated [117]. In spite of this knowledge, there are numerous uncertainties even about breast cancer. For instance it is not clear why most women do not respond to therapy such as Herceptin even though they are HER2 positive; or why most women develop resistance to Herceptin within a year. Neither the signatures of the disease nor the signatures of the response for any cancer are available at present. Thus PM could fit the aphorism "garbage in garbage out" [118,119].

2.12. Off-label uses

The approved use of a drug for a given diseases is called "on label" whereas use of a drug outside the terms of its official labeling is referred to as "off-label" prescription. One study showed almost 31 out of 100 most common uses of marketed medicines were prescribed off label [120]. Almost 21% of the prescriptions (150 millions) written in 2001 were "off label". Findings of prospective studies undertaken between 1990 and 2002 showed proportions of off-label drug use in children and adults of 6.7–33.2%. Off-label use of a drug is not regulated by the FDA. Although there is no scientific support, the off-label use is common for life-threatening diseases such as cancer. Many categories of use exist because labeling of anticancer agents is very precise in terms of type or subtype of tumor, association, line, and duration of treatment. Off-label prescription of anticancer drugs is thought to be frequent. Most off-label prescription was reported in patients treated with palliative intent [121]. For instance, avastin which is approved as a first line therapy for metastatic colon cancer in 2004, is being used off label for number of cancers including metastatic breast, kidney and lung cancers; and many develop brain metastasis [122]. This kind of "off-label" use may have benefits as it speeds drug discovery through new use for old drug, it lowers the cost for new treatment, allows experimentation and serendipitous discoveries. For drug Zometra (zoledronic acid), which is commonly used to prevent bone loss, was found to reduce the incidence breast cancer recurrence (54 cases with drug vs 83 without drug out of 1803) in pre-menopausal women [123]. Some other best known examples include Viagra, thalidomide and monoxidil, which are now being used for initially unanticipated indications. Thus although preclinical results do not always predict the outcome in the patient, the off-label use of a drug could provide a good alternate to pursue systematically for new uses.

3. Conclusions

Overall, it is clear that unlike cardiovascular or other chronic diseases, cancer is highly complex multigenic disease. It is estimated that as many as 95% of all cancers are preventable. Its treatment, however, is much more difficult. In this review, we present the pros and cons of different aspects of cancer diagnosis and treatment. While cell lines in culture and animal models are being used to examine the pathogenesis of cancer and to develop novel therapeutics, research indicates that these models are highly incomplete and are not predictive of the cancer's response in humans. Similarly, although the importance of biomarker-based diagnosis of the disease and its value in predicting the response to the therapy is clear, no reliable biomarkers are yet available. In addition, neither cell signaling pathway-based and nor targeted therapies provide adequate approach to cancer treatment. Although numerous targeted drugs have been discovered, their role in cancer treatment by themselves is very limited in spite of very great expense. The signatures for the diagnosis of the diseases and for the prediction of the response are lacking. Lack of response by most patients to the therapy and then development of resistance are major roadblocks. Whether personalized medicine is the answer to some of these problems is also not clear. Too many eggs are being placed into the basket of "genomics, proteomics, microarray, high throughput screening, combinatorial chemistry, etc.". At present we do not know why works or why not a treatment works? However the results are not as forthcoming as expected. In 2007, Americans paid $286.5 billion in pharmaceutical costs (with major portion for cancer care), that constitutes almost 50% of the total cost of the entire world for total of 5% population of the World. In spite of this, while US death rate due to cancer within last 50 years is the highest in the World and has not changed significantly (193.9 per 100,000 in 1950 vs 193.4 per 100,000 in 2002), let us hope the future 50 years are better than the past 50 years. Because cancer is a preventable disease, more focus is needed on prevention. This requires a major change in life style. Unlike most chronic diseases such as diabetes, cancer is a mixture of diseases, is complex and multigenic. Thus, we must explore more traditional methods of cancer treatment as they provide multi-targeted approach.

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