Targeted cancer therapies: the future of cancer treatment

Manoj Kumar[1], Ravinder Nagpal[2], Hemalatha R.[1], Vinod Verma[3], Ashok Kumar[4], Satvinder Singh[5], Francesco Marotta[6], Shalini Jain[7], Hariom Yadav[7]

[1] Department of Microbiology and Immunology, National Institute of Nutrition, Hyderabad, India; [2] Division of Laboratory for Probiotic Research (Yakult), Juntendo University Graduate School of Medicine, Tokyo, Japan; [3] Research and Development Unit, National Heart Centre, Singapore; [4] Department of Zoology, M.L.K. P.G. College, Balrampur (U.P.), India; [5] Animal Biochemistry Division, National Dairy Research Institute, Karnal (Haryana), India; [6] ReGenera Research Group for Aging Intervention, Milano, Italy; [7] National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland, USA

Abstract. For decades, the hallmark of medical treatment for cancer has been intravenous cytotoxic chemotherapy, where these drugs target rapidly dividing cells, including cancer cells and certain normal tissues. As a result, many patients experience the classic toxicities of alopecia, gastrointestinal symptoms, and myelosuppression. In the past decade, however, a dramatic shift has been witnesses in the cancer therapy. Although traditional cytotoxic chemotherapy still remains the treatment of choice for many malignancies, targeted therapies are now a component of treatment for many types of cancer, including breast, colorectal, lung, and pancreatic cancers, as well as lymphoma, leukemia, and multiple myeloma. (www.actabiomedica.it)

Key words: anticancer therapy, antitumor drugs, cancer treatments, cancer prescription

Introduction

Targeted therapy is a type of medication that blocks the growth of cancer cells by interfering with specific targeted molecules needed for carcinogenesis and tumor growth (1), rather than by simply interfering with rapidly dividing cells (e.g. with traditional chemotherapy). By focusing on molecular and cellular changes that are specific to cancer, targeted cancer therapies may be more effective than other types of treatment, including chemotherapy and radiotherapy, and less harmful to normal cells.

Anticancer drugs act by a variety of mechanisms (2) including:

- DNA damage by direct (e.g. alkylating agents), protein-mediated (e.g. topoisomerase poisons) and fraudulent base pathways (e.g. nucleoside analogues).
- interference with synthesis of vital co-factors and DNA/RNA/protein precursors (e.g. antimetabolites, asparaginase).
- interference with other cellular structures and processes (e.g. anti-microtubule drugs such as docetaxel, paclitaxel and Vinca alkaloids).
- inhibition of growth/ anti-death signal (e.g. tyrosine kinase inhibitors such as imatinib mesylate, trastuzumab).

These mechanisms induce acute cell death (necrosis), programmed cell death (apoptosis), growth arrest or differentiation; and many anticancer drugs have multiple actions on the cell. Many targeted cancer therapies have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of specific types of cancer. The three main types of targeted therapy are monoclonal antibodies, small molecules and apoptosis inducers. Both antibodies and small-molecule compounds are therefore promising tools for target-protein-based cancer therapy. With their distinct mechanisms of action and toxicities, these agents have changed many aspects of the practice of oncology.

Conventional chemotherapy, although directed toward certain macromolecules or enzymes, typically

does not discriminate effectively between tumor cells and rapidly dividing normal cells (e.g., bone marrow and gastrointestinal tract), thus leading to several toxic side effects. Tumor responses from cytotoxic chemotherapy are usually partial, brief, and unpredictable. In contrast, targeted therapies interfere with molecular targets that have a role in tumor growth or progression. These targets are usually located in tumor cells, although some like the antiangiogenic agents may target other cells such as endothelial cells. Thus, targeted therapies have a high specificity toward tumor cells, providing a broader therapeutic window with less toxicity. They are also often useful in combination with cytotoxic chemotherapy or radiation to produce additive or synergistic anticancer activity because their toxicity profiles often do not overlap with traditional cytotoxic chemotherapy. Thus, targeted therapies represent a new and promising approach to cancer therapy, one that is already leading to beneficial clinical effects. There are multiple types of targeted therapies available, including monoclonal antibodies, small molecules that are inhibitors of tyrosine kinases, and antisense inhibitors of growth factor receptors.

Tyrosine kinase is an enzyme that can transfer a phosphate group from ATP to a tyrosine residue in a protein. Tyrosine kinases are a subgroup of the larger class of protein kinases. Phosphorylation of proteins by kinases is an important mechanism in signal transduction for regulation of cellular activity. Tyrosine kinases can be classified as receptor protein kinases and non-receptor protein kinases. The receptor tyrosine kinases are membrane-spanning cell surface proteins that play critical roles in the transduction of extracellular signals to the cytoplasm (3). There are approximately 60 receptor tyrosine kinases that have been identified, and they are divided into some 20 subfamilies as defined by receptor and/or ligand (3). Non-receptor tyrosine kinases, on the other hand, relay intracellular signals. Ligand binding induces dimerization of these receptor tyrosine kinases, resulting in autophosphorylation of their cytoplasmic domains and activation of tyrosine kinase activity. Multiple cytoplasmic signaling pathways, including the Ras/Raf mitogen-activated protein kinase pathway, the phosphoinositol 3-kinase/Akt pathway, the signal transducer and activator of transcription 3 pathway, the protein kinase C pathway, and scaffolding proteins may then be activated (4). Intracellular mediators in these pathways transduce signals from membrane receptors through the cytosol and into the nucleus, culminating in altered DNA synthesis and cell division as well as affecting a variety of biological processes, including cell growth, migration, differentiation, and death (5, 6).

This paper discusses the antitumor activity, mechanism of action, and adverse effects of several small molecule inhibitors of tyrosine kinases whose clinical effects have been fairly well defined. These include imatinib, which inhibits the non-receptor tyrosine kinases BCR-ABL and KIT, as well as receptor tyrosine kinase inhibitors targeting epidermal growth factor receptor (EGFR) (ErbB/HER) family members, vascular endothelial growth factor receptors (VEGFR), and platelet-derived growth factor receptors (PDGFR) (α and β).

Imatinib mesylate (Gleevec, also known as STI–571)

Imatinib mesylate is approved for chronic myelogenous leukemia, gastrointestinal stromal tumor and some other types of cancer. Early clinical trials indicate that imatinib may be effective in treatment of dermatofibrosarcoma protuberans. Imatinib is a 2-phenylaminopyrimidine derivative that functions as a specific inhibitor of a number of tyrosine kinase enzymes. It occupies the *TK* active site, leading to a decrease in activity. There are a large number of *TK* enzymes in the body, including the insulin receptor. Imatinib is specific for the *TK* domain in *abl* (the Abelson proto-oncogene), c-kit and PDGF-R (platelet-derived growth factor receptor). In chronic myelogenous leukemia, the Philadelphia chromosome leads to a fusion protein of *abl* with *bcr* (*breakpoint cluster region*), termed *bcr–abl*. Since, this is now a constitutively active tyrosine kinase, imatinib is used to decrease *bcr–abl* activity. Each active site of tyrosine kinases has a binding site for ATP. The enzymatic activity catalyzed by a tyrosine kinase is the transfer of the terminal phosphate from ATP to tyrosine residues on its substrates, a process known as protein tyrosine phosphorylation. Imatinib works by binding close to the ATP binding site of *bcr–abl*, locking it in a closed

or self-inhibited conformation, and therefore inhibiting the enzyme activity of the protein semi-competitively (7). This fact explains why many BCR-ABL mutations can cause resistance to imatinib by shifting its equilibrium toward the open or active conformation (8). Imatinib is quite selective for *bcr-abl* – it does also inhibit other targets mentioned above (c-kit and PDGF-R), but no other known tyrosine kinases. Imatinib also inhibits the *abl* protein of non-cancer cells but cells normally have additional redundant tyrosine kinases which allow them to continue to function even if *abl* tyrosine kinase is inhibited. Some tumor cells, however, have a dependence on *bcr-abl* (9) Inhibition of the *bcr-abl* tyrosine kinase also stimulates its entry in to the nucleus, where it is unable to perform any of its normal anti-apoptopic functions (10).

Gefitinib (Iressa, also known as ZD1839)

Gefitinib targets the epidermal growth factor receptor (EGFR) tyrosine kinase and is approved in the U.S. for non small cell lung cancer. Gefitinib is the first selective inhibitor of EGFR's tyrosine kinase domain. Thus gefitinib is an EGFR inhibitor. The target protein (EGFR) is also sometimes referred to as Her1 or ErbB-1 depending on the literature source. EGFR is overexpressed in the cells of certain types of human carcinomas, for example in lung and breast cancers. This leads to inappropriate activation of the anti-apoptotic Ras signalling cascade, eventually leading to uncontrolled cell proliferation. Research on gefitinib-sensitive non-small cell lung cancers has shown that a mutation in the EGFR tyrosine kinase domain is responsible for activating anti-apoptotic pathways (11, 12) These mutations tend to confer increased sensitivity to tyrosine kinase inhibitors such as gefitinib and erlotinib. Of the types of non-small cell lung cancer histologies, adenocarcinoma is the type that most often harbors these mutations. These mutations are more commonly seen in Asians, women, and non-smokers (who also tend to more often have adenocarcinoma). Gefitinib inhibits EGFR tyrosine kinase by binding to the ATP-binding site of the enzyme. Thus the function of the EGFR tyrosine kinase in activating the Ras signal transduction cascade is inhibited, and malignant cells are inhibited (7).

Erlotinib (marketed as Tarceva)

Erlotinib inhibits EGFR and works through a similar mechanism as gefitinib. Erlotinib has been shown to increase survival in metastatic non small cell lung cancer when used as second line therapy. Because of this finding, erlotinib has replaced gefitinib in this setting. The drug follows Iressa gefitinib, which was the first drug of this type. Erlotinib specifically targets the EGFR tyrosine kinase, which is highly expressed and occasionally mutated in various forms of cancer. It binds in a reversible fashion to the ATP binding site of the receptor (13). For the signal to be transmitted, two members of the EGFR family need to come together to form a homodimer. These then use the molecule of ATP to autophosphorylate each other, which causes a conformational change in their intracellular structure, exposing a further binding site for binding proteins that cause a signal cascade to the nucleus. By inhibiting the ATP, autophosphorylation is not possible and the signal is stopped.

Bortezomib (Velcade)

Bortezomib is an apoptosis-inducing drug that causes cancer cells to undergo cell death by interfering with proteins. It is approved in the U.S. to treat multiple myeloma that has not responded to other treatments. The boron atom in bortezomib binds the catalytic site of the 26S proteasome (14) with high affinity and specificity. In normal cells, the proteasome regulates protein expression and function by degradation of ubiquitinylated proteins, and also cleanses the cell of abnormal or misfolded proteins. Clinical and preclinical data support a role in maintaining the immortal phenotype of myeloma cells, and cell-culture and xenograft data support a similar function in solid tumor cancers. While multiple mechanisms are likely to be involved, proteasome inhibition may prevent degradation of pro-apoptotic factors, permitting activation of programmed cell death in neoplastic cells dependent upon suppression of pro-apoptotic pathways.

Tamoxifen

Tamoxifen is currently used for the treatment of

both early and advanced ER+ (estrogen receptor positive) breast cancer in pre- and post-menopausal women (15). Additionally, it is the most common hormone treatment for male breast cancer (16). It is also approved by the FDA for the prevention of breast cancer in women at high risk of developing the disease (17). It has been further approved for the reduction of contralateral (in the opposite breast) cancer. It competitively binds to estrogen receptors on tumors and other tissue targets, producing a nuclear complex that decreases DNA synthesis and inhibits estrogen effects. It is a non-steroidal agent with potent anti-estrogenic properties which compete with estrogen for binding sites in breast and other tissues. Tamoxifen causes cells to remain in the G0 and G1 phases of the cell cycle. Because it prevents (pre)cancerous cells from dividing but does not cause cell death, tamoxifen is cytostatic rather than cytocidal.

Tamoxifen itself is a prodrug, having relatively little affinity for its target protein, the estrogen receptor. It is metabolized in the liver by the cytochrome P450 isoform CYP2D6 and CYP3A4 into active metabolites such as 4-hydroxytamoxifen and N-desmethyl-4-hydroxytamoxifen (endoxifen) (18) which have 30-100 times more affinity with the estrogen receptor than tamoxifen itself. These active metabolites compete with estrogen in the body for binding to the estrogen receptor. In breast tissue, 4-hydroxytamoxifen acts as an estrogen receptor antagonist so that transcription of estrogen-responsive genes is inhibited (19).

Tamoxifen binds to estrogen receptor (ER) which in turn interacts with DNA. The ER/tamoxifen complex recruits other proteins known as co-repressors to stop genes being switched on by estrogen. Some of these proteins include NCoR and SMRT (20). Tamoxifen function can be regulated by a number of different variables including growth factors (21). Tamoxifen needs to block growth factor proteins such as ErbB2/HER2 (22) because high levels of ErbB2 have been shown to occur in tamoxifen resistant cancers (23). Tamoxifen seems to require a protein PAX2 for its full anticancer effect (22, 24). In the presence of high PAX2 expression, the tamoxifen/estrogen receptor complex is able to suppress the expression of the pro-proliferative ERBB2 protein. In contrast, when AIB-1 expression is higher than PAX2, tamoxifen/estrogen receptor complex upregulates the expression of ERBB2 resulting in stimulation of breast cancer growth (22, 25).

Monoclonal Antibodies

Antibodies or immunoglobulins are a crucial component of the immune system, circulating in the blood and lymphatic system, and binding to foreign antigens expressed on cells. Once bound, the foreign cells are marked for destruction by macrophages and complement. In the context of cancer immunotherapy, monoclonal antibodies have brought to light a wide array of human tumor antigens (26). In addition to targeting cancer cells, antibodies can be designed to act on other cell types and molecules necessary for tumor growth. For example, antibodies can neutralize growth factors and thereby inhibit tumor expansion. Antibodies and immune-conjugates are gaining a significant and expanding role in the therapy of cancer. Because patients generally tolerate antibody treatments with minimal side effects, compared with many other cancer treatment modalities, immunotherapy with antibodies represents an exciting opportunity for combining with standard modalities, such as chemotherapy, as well as combinations between diverse biological agents.

Monoclonal antibodies achieve their therapeutic effect through various mechanisms (27). They can have direct effects in producing apoptosis or programmed cell death. They can block growth factor receptors, effectively arresting proliferation of tumor cells. In cells that express monoclonal antibodies, they can bring about anti-idiotype antibody formation. Indirect effects include recruiting cells that have cytotoxicity, such as monocytes and macrophages. This type of antibody-mediated cell kill is called antibody-dependent cell mediated cytotoxicity (ADCC). Monoclonal antibodies also bind complement, leading to direct cell toxicity, known as complement dependent cytotoxicity (CDC).

Monoclonal antibodies directed against unique tumor antigens have some efficacy against neoplastic tissue. Trastuzumab, an antibody directed against a protein called Her-2 or Erb-B2, plus chemotherapy

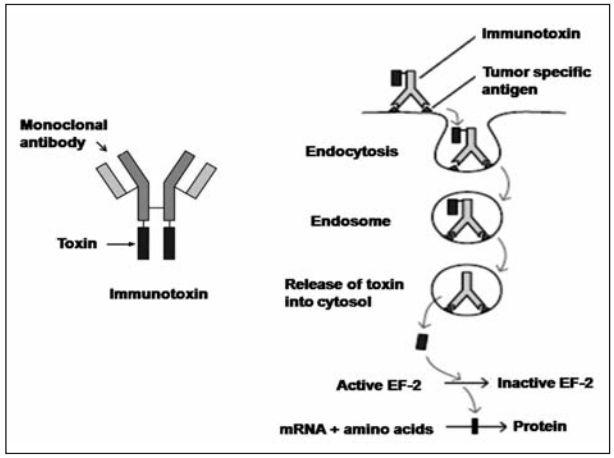

Figure 1. One of the proposed actions of immunotoxin on cancer cells (EF: Elongation factor).

has shown benefit in metastatic breast cancer. Antibodies against CD antigens expressed on neoplastic cells, such as CD20 and CD33, are used to treat patients with non-Hodgkin lymphoma (rituximab, anti-CD20 antibody) and acute myelocytic leukemia (gemtuzumab, an antibody linked to a potent toxin) (Fig. 1). The effectiveness of monoclonal antibodies may be increased by linking them to radioactive nuclide. One such drug, ibritumomab, is used to treat non-Hodgkin lymphoma.

Campath® (alemtuzumab)

Alemtuzumab (marketed as Campath, MabCampath or Campath-1H) is a monoclonal antibody used in the treatment of chronic lymphocytic leukemia (CLL), cutaneous T-cell lymphoma (CTCL) and T-cell lymphoma. It targets CD52, a protein present on the surface of mature lymphocytes, but not on the stem cells from which these lymphocytes are derived. It is used as second-line therapy for CLL. It was approved by the Food and Drug Administration for CLL patients who have been treated with alkylating agents and who have failed fludarabine therapy. A significant complication of therapy with alemtuzumab is that it significantly increases the risk for opportunistic infections, in particular, reactivation of cytomegalovirus. Alemtuzumab is also used in some conditioning regimens for bone marrow transplantation and kidney transplantation. It is also used under clinical trial protocols for treatment of some autoimmune diseases, such as multiple sclerosis, in which it shows promise.

Erbitux® (cetuximab)

Cetuximab (IMC-C225—marketed under the name Erbitux) is a chimeric (mouse/human) monoclonal antibody, an epidermal growth factor receptor (EGFR) inhibitor, given by intravenous infusion for treatment of metastatic colorectal cancer and head and neck cancer. It is indicated for the treatment of patients with EGFR expressing KRAS wild-type metastatic colorectal cancer in combination with chemotherapy or as a single agent in patients who have failed in oxaliplatin- or irinotecan- base therapy and who are intolerant to irinotecan. While there remains some scientific controversy on this, assessment for EGFR expression is required for use in colorectal cancer, but not in head & neck cancer. It is best to refer to updated Prescription Information.

Many clinical trials have been conducted to investigate the efficacy of cetuximab (Erbitux) in metastatic colorectal cancer (mCRC) and there is increasing evidence to support the use of biomarkers, such as KRAS, to predict tumor response to anti-EGFR therapies. Two large clinical trials of cetuximab, OPUS (28) and CRYSTAL (29, have recently been published, and have provided further evidence that cetuximab significantly improves response rates and disease free survival rates in mCRC patients with KRAS wild-type tumors. A study by Schrag (30) found that Erbitux failed to benefit patients with less advanced (non-metastasized) stages of colorectal cancer with no improvement in survival rates. Adding Erbitux instead increased the side effects of chemotherapy.

Rituxan® (rituximab)

Rituximab, sold under the trade names Rituxan and MabThera, is a chimeric monoclonal antibody against the protein CD20, which is primarily found on the surface of B cells. It can therefore destroy B cells. Rituximab is used in the treatment of many lymphomas, leukemias, transplant rejection and

some autoimmune disorders. The antibody binds to the cluster of differentiation 20 (CD20). CD20 is widely expressed on B cells, from early pre-B cells to later in differentiation, but it is absent on terminally differentiated plasma cells. CD20 does not shed, modulate or internalise. Although the function of CD20 is unknown, it may play a role in Ca2+ influx across plasma membranes, maintaining intracellular Ca2+ concentration and allowing activation of B cells.

The exact mode of action of rituximab is unclear, but the following effects have been proposed (31):

- The Fc portion of rituximab mediates antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).
- Rituximab has a general regulatory effect on the cell cycle.
- It increases MHC II and adhesion molecules LFA-1 and LFA-3 (lymphocyte function-associated antigen).
- It elicits shedding of CD23.
- It downregulates the B cell receptor.
- It induces apoptosis of CD20+ cells.

The combined effect results in the elimination of B cells (including the cancerous ones) from the body, allowing a new population of healthy B cells to develop from lymphoid stem cells. Rituximab binds to amino acids 170-173 and 182-185 on CD20, which are physically close to each other as a result of a disulfide bond between amino acids 167 and 183 (32).

Herceptin® (trastuzumab)

Trastuzumab (INN; trade name Herceptin) is a monoclonal antibody that interferes with the HER2/neu receptor. The HER receptors are proteins that are embedded in the cell membrane and communicate molecular signals from outside the cell to inside the cell, and turn genes on and off. The HER proteins regulate cell growth, survival, adhesion, migration, and differentiation - functions that are amplified or weakened in cancer cells. In some cancers, notably some breast cancers, HER2 is stuck in the "on" position, and causes breast cells to reproduce uncontrollably, causing breast cancer (33).

The HER2 gene (also known as HER2/neu and ErbB2 gene) is amplified in 20-30% of early-stage breast cancers, which makes it over-expressed (34). Also, in cancer, HER2 may send signals without mitogens arriving and binding to any receptor, making it overactive. It extends through the cell membrane, and carries signals from outside the cell to the inside. In healthy people, signaling compounds called mitogens arrive at the cell membrane, and bind to the outside part of other members of the HER family of receptors. Those bound receptors then link (dimerize) with HER2, activating it. HER2 then sends a signal to the inside of the cell. The signal passes through different biochemical pathways. This includes the PI3K/Akt pathway and the MAPK pathway. These signals promote invasion, survival and growth of blood vessels (angiogenesis) of cells (35).

Normally, when cells divide, they go through a mitosis cycle, with checkpoint proteins that keep cell division under control. Some of the proteins that control this cycle are called cdk2 (CDKs). These CDKs are inhibited by other proteins. One of those proteins is the inhibitor p27Kip1. Normally, p27Kip1 moves from the cytoplasm to the nucleus, to keep the cycle under control. When HER2 always sends signals, p27Kip1 doesn't move to the nucleus, but accumulates in the cytoplasm instead (36). This is caused by phosphorylation by Akt. Trastuzumab is a humanized monoclonal antibody that binds to the domain IV of the (37) extracellular segment of the HER2/neu receptor. Cells treated with trastuzumab undergo arrest during the G1 phase of the cell cycle so there is reduced proliferation. It has been suggested that trastuzumab induces some of its effect by downregulation of HER2/neu leading to disruption of receptor dimerization and signaling through the downstream PI3K cascade. P27Kip1 is then not phosphorylated and is able to enter the nucleus and inhibit cdk2 activity, causing cell cycle arrest (36). Also, trastuzumab suppresses angiogenesis by both induction of antiangiogenic factors and repression of proangiogenic factors. It is thought that a contribution to the unregulated growth observed in cancer could be due to proteolytic cleavage of HER2/neu that results in the release of the extracellular domain. Trastuzumab has been shown to inhibit HER2/neu ectodomain cleav-

age in breast cancer cells (38). Experiments in laboratory animals indicate that antibodies, including trastuzumab, when bound to a cell, induce immune cells to kill that cell, and that such antibody-dependent cell-mediated cytotoxicity is an important mechanism of action (39).

Avastin® (bevacizumab)

Bevacizumab (trade name Avastin, Genentech/Roche) is a humanized monoclonal antibody that recognizes and blocks vascular endothelial growth factor A (VEGF-A) (1), a chemical signal that stimulates the growth of new blood vessels (angiogenesis). Blood vessels grow uncontrollably in cancer, retinal proliferation of diabetes in the eye, and other diseases. Bevacizumab can block VEGF-A from creating new blood vessels. It was the first clinically available angiogenesis inhibitor in the United States.

Bevacizumab is currently approved by the U.S. Food and Drug Administration (FDA) for cancers that are metastatic (have spread to other parts of the body). It received its first approval in 2004 for combination use with standard chemotherapy for metastatic colon cancer and non-small cell lung cancer (40). In 2008, it was approved by the FDA for use in metastatic breast cancer, a decision that generated some controversy as it went against the recommendation of its advisory panel (41) who objected because it only slowed tumor growth but failed to extend survival. In the US, Members of a Food and Drug Administration panel have now said they do not see enough of a benefit from Avastin in advanced breast cancer to justify its serious risks, although the drug is still approved for use in Australia. Clinical studies are underway in non-metastatic breast cancer, renal cell carcinoma, glioblastoma multiforme, ovarian cancer, castrate-resistant (formally called hormone refractory) prostate cancer, non-metastatic unresectable liver cancer and metastatic or unresectable locally advanced pancreatic cancer. A study released in April 2009 found that bevacizumab is not effective at preventing recurrences of non-metastatic colon cancer following surgery (42). In May 2009, it received FDA approval for treatment of recurring glioblastoma multiforme, while treatment for initial growth is still in phase III clinical trial.

Raltitrexed

Colorectal cancer has long been considered as moderately resistant to chemotherapy. Previously, 5-fluorouracil was the only proven treatment for this indication, but it has been slowly replaced by other drugs. Natural folates are vital co-factors for many cellular enzymes, specifically those that catalyse one carbon transfer reactions. Thymidylate synthase is a critical enzyme in the synthesis of the thymidine nucleotides required for DNA synthesis. This enzyme requires a reduced folate co-factor, 5–10-methylene tetrahydrofolate, to act as a carbon donor for the synthesis of thymidylate from deoxyuridylate. Raltitrexed has been specifically developed as a potent mimic of 5–10-methylene tetrahydrofolate and therefore inhibits thymidylate synthase (2). Many antifolate drugs are polyglutamated within cells. These polyglutamate forms are retained in the cells and, in the case of raltitrexed, this increases its potency and selectivity against thymidylate synthase.

Cytokines and cancer therapy

Cytokines are the messengers of the immune system (43). Cytokines are substances, either proteins or glycoproteins, secreted by immune cells that regulate the innate immune system: natural killer (NK) cells, macrophages, and neutrophils. They also regulate the adaptive immune system, the T and B cell immune responses. In the immune system, cytokines function in cascades. Thus clinical trials of individual cytokines are rarely useful, since cytokines tend not to work individually. Some of the individual cytokines that have been tested and found ineffective for cancer treatment include interleukin 1 beta (IL-1 beta), although it may be useful because it helps to mediate the severe toxicity of interleukin 2 (IL-2). Tumor necrosis factor (TNF) certainly sounded promising, but in fact caused severe hypotension when used systemically. Interleukin 4 (IL-4) have been shown to minimize anticancer activity and be toxic. Interleukin 6 (IL-6) too have shown some activity against cancer cells, but turned out to be a growth factor for myeloma cells. Granulocyte-macrophage colony-stimulating factor (GM-CSF), used primarily in stem cell transplant to

reconstitute the myeloid series, has been studied for melanoma with controversial results.

IL-2 and interferon-alfa 2b are two cytokines approved by the FDA for treatment of cancer. IL-2 has demonstrated activity against renal cell, melanoma, lymphoma, and leukemia. Interferon has activity in the same histologies but also in Kaposi's sarcoma, chronic myelogenous leukemia, and hairy cell leukemia. Overall, cytokines are substances that appear to have application in the treatment of hematologic malignancies or immunogenic tumors. The major cytokines currently in use or under evaluation for cancer therapy are: interferon alfa, IL-2, GM-CSF, and interleukin-12 (IL-12). Interferon alfa is actually a family of molecules rather than a single molecule. They are encoded by closely related genes on chromosome 9, encoding proteins that are variably glycosylated. These are comprised of about 150 amino acids, and they bind to certain receptors on the surface of immune cells. They are known to have profound and diverse effects on gene expression. Interferon alfa has many roles. It upregulates genes like MHC class I, tumor antigens, and adhesion molecules. It is also an anti-angiogenic agent. It is extremely active in the immune system, promoting B and T cell activity. Interferon alfa can stimulate macrophages, even dendritic cells, and upregulates Fc receptors. Interferon was isolated in 1970 by investigators looking for antiviral substances. The substance we now know as interferon was actually isolated from white cells, and called interferon because it interfered with viral infection. It turns out type 1 and 2 interferons do have some antitumor activity, but not the hoped-for level. Interferon's activity in cancer has been well documented. However, in kidney cancer, we have seen small but consistent response rates in a number of studies. In a randomized controlled study of interferon alfa versus megestrol acetate, investigators found a minimal advantage to interferon in terms of survival (8.5 months vs. 6 months). Interferon has a long history in metastatic melanoma. A series of small phase 2 trials with high-dose interferon (up to 100 million units IV daily) reported response rates of 20% to 22% (44, 45). Interleukin-2 (IL-2) is a T cell growth factor that binds to a specific tripartite receptor on T cells. In dose escalation studies, patients treated with high doses of IL-2 showed clinical responses, although severe toxicity was seen. Response rates were as high as 24% at the highest IL-2 dose in patients with renal cell carcinoma. However, the toxicities of treatment were limiting. These toxicities stem from what is known as a capillary leak syndrome. Giving IL-2 in high doses is comparable to inducing a controlled state of septic shock. Low blood pressure, low systemic vascular resistance, high cardiac output, grade 3/4 hematologic toxicity, hepatic toxicity, renal toxicity, and pulmonary edema have all been documented. Toxicity is nearly always reversible. Low-dose IL-2 has shown activity in renal cell cancer as well (46, 47). Objective response rates of 18% to 23% were reported, without the toxicity of high dose IL-2. IL-2 is indicated for use in renal cell carcinoma and melanoma on the basis of duration of response, rather than achievement of response. A substantial number of patients with renal cancer who had an objective response to treatment are still alive, and stable or progression-free, 1 year post-treatment. Another important determinant of response was the amount of IL-2 given during the first cycle. The immunologic effect is manifested by the height of rebound leukocytosis. Lymphocyte levels plunge during initial treatment with IL-2, and rebound when treatment stops. This rebound is an indication of the response to IL-2. IL-2 has shown activity in non-Hodgkin's lymphoma and leukemia and lymphoma post-stem cell transplant. Low-dose IL-2 was also evaluated in combination with histamine, but no differences in response were observed compared with IL-2 alone. An interesting modification of the IL-2 molecule, known as BAY 50-4798, has shown potential in animal models. This agent is the same as IL-2 with two modified amino acids. It appears to have the therapeutic effect of IL-2 without the toxicity. Interleukin-12 (IL-12) is a very exciting cytokine. It is a heterodimeric protein that promotes NK and T cell activity and is a growth factor for B cells. It has demonstrated antitumor activity in mouse models. Alone, IL-12 shows minimal potential for therapeutic effect (48). However, IL-12 may have value as a vaccine adjuvant. When IL-12 was paired with peptide vaccines in patients with resected stage 3 and 4 melanoma, IL-12 appeared to boost the response to the vaccine. (49) GM-CSF is also being evaluated as an adjuvant for vaccine therapy.

Cytokines such as IL-2 have modest antitumor activity in metastatic renal cell carcinoma and melanoma. The effect is remarkable for its duration rather than strength, and its application may have greatest significance for long-term survival. IL-2 at high doses is toxic. Low doses may have promising activity post-transplant as an immune restorative agent, and that effect is under evaluation in large randomized cooperative group studies. IL-12 and GM-CSF are unlikely to be stand-alone agents for any histology, but show promise as adjuvant therapy and in combination with other cytokines

Vaccine against cancer therapy

The antibody-based therapies generally discussed are a form of passive immunotherapy, i.e., the molecules or substances are introduced into the body, rather than the body creating its own immune response. Vaccines, on the other hand, are considered active immunotherapy because they generate an intrinsic immune response. They are also considered a form of specific immunotherapy because they attempt to stimulate an immune response that can directly target the tumor antigens, in contrast to non-specific approaches such as cytokines that broadly stimulate the immune system. Efforts to treat cancer with vaccines date back to the origins of immunology (26). Patients have been injected with autologous and allogeneic malignant cells, usually irradiated to prevent further growth. However, measuring immune response was problematic. Now researchers have identified several tumor antigens and the immune response they provoke and have made progress in developing cancer vaccines.

Peptide, autologous and viral vector vaccines

Numerous approaches to stimulate the immune system to recognize tumors have been tried over the years. Vaccines consisting of peptide or protein administered with an adjuvant have been the most frequently used. These adjuvants might be compounds such as bacterial cell wall components that incite an inflammatory response or cytokines such as IL-12 or GM-CSF. Monocytes, neutrophils, eosinophils, and T cells are all recruited to the site of the inflammation where adjuvant is used, but it is believed that *in situ* dendritic cells ultimately take up the proteins and peptides, process them if necessary, and present them on the cell surface as peptides capable of binding to the MHC molecules. Dendritic cells can then stimulate T cells that have the receptors to recognize those particular peptides.

Table 1 summarizes some peptide or protein vaccines. These vaccines are primarily used in melanoma. An interesting approach to detecting a clinical response was used in lymphoma patients, in whom circulating tumor cells could be detected by polymerase chain reaction (PCR) prior to immunization. PCR enables us to actually search for lymphoma cells by identifying the particular chromosomal translocation abnormality they are known to possess. In this study, tumor cells could not be detected in peripheral blood

Table 1. Vaccines designed for cancer therapy

Peptide and protein vaccine	Tumor cell vaccine	Viral vector and plasmid vaccine
gp100,tyrosine +IL-12	Melanoma Transduced with Ad-GM-CSF	ALVAC-CEA B7.1
gp100+tetanus tox+Montanide ISA-51 or QS-21	Allogenic panc Tumor secreting GM-CSF	VacciniaCEA/avipox-CEA + GM-CSF
gp100, IL-2, IL-12	Cancer vax (Melanoma)	Vaccinia CEA
MAGE-3, PADRE, IFA	Autologous colon CA + BCG	PSMA/CD86 plasmid
HER2/neu helper peptides	Autologous GBM + New castle virus	
Idiotype + GM – CSF (lymphoma)		

of some patients following immunization. This method of measuring clinical responses is becoming increasingly important, particularly for diseases that cannot be monitored by a CT scan. Tumor cell-based vaccines are another vital area of research. Some studies have used autologous tumor, in which tumor cells are extracted from surgical resection or biopsy specimens. Allogeneic cell lines have also been developed for tumors such as melanoma that likely encompass many of the tumor-associated antigens expressed by the melanomas of most affected individuals. Tumor cells can also be modified to make them more immunogenic. To accomplish that, tumor cells may be infected with various types of viruses so that viral proteins are expressed on the surface, and transduced with genes expressing cytokines such as IL-2 and GM-CSF, or genes for HLA molecules or co-stimulatory molecules. The idea is irradiate the cells so they can no longer proliferate, then inject the tumor cells back into the patient. What we hope to see is the immune system activated by either the tumor cell or the inflammatory response that includes recruitment of dendritic cells. As the injected tumor cells undergo apoptosis or are destroyed by the inflammatory reaction, antigens are picked up by the dendritic cells and represented to the T cells. One of the most popular is to transduce with a vector containing GM-CSF, so that the tumor secretes GM-CSF and sets up an inflammatory response 50). In animal studies, this strategy has been the most promising in inducing a protective immune response. Newcastle virus is used to infect the cells in another study (51) to some effect, as shown by median survival of 46 weeks. Use of Bacille Calmette-Guerin (BCG) as an inflammation inducing adjuvant along with autologous colorectal cancer cells (52) showed increased DTH but no survival benefit. In fact, most of these tumor cell approaches show an immune response, but again limited clinical response. Nonetheless, CancerVax (53), which has been heavily tested in melanoma, has been suggested in nonrandomized studies to provide a survival benefit.

Viral vectors and indeed, naked DNA in the form of plasmids encoding tumor antigens, can be used to immunize people. Initially these vaccines were administered to muscle cells, but it is likely that dendritic cells were ultimately the targets infected by the virus, or were picking up the antigen released by apoptotic muscle cells (54, 55, 56). Poxviruses are another popular way to apply this approach, and a considerable amount of work has been done with vaccinia and avian and fowl pox vectors. One of the most interesting strategies is the prime boost approach. An example of this is when a patient is first immunized with vaccinia virus encoding the gene for CEA. Vaccinia is very immunogenic, so it can only be used once or twice – after the first injection patients develop high neutralizing antibody titers. In subsequent immunizations the antibody immediately binds to the virus, making it difficult to get true immunization against the encoded tumor antigen. However if you follow with an avian vector expressing CEA, we see better immunologic responses (55). Clinical responses have remained limited, but various modifications of these strategies continue to be explored.

Anti-cancer agents from plants

Plant-derived compounds have played an important role in the development of several clinically useful anti-cancer agents. These include vinblastine, vincristine, the camptothecin derivatives, topotecan and irinotecan, etoposide, derived from epipodophyllotoxin, and paclitaxel (57, 58). Several promising new agents are in clinical development based on selective activity against cancer-related molecular targets, including flavopiridol and combretastin A4 phosphate, and some agents which failed in earlier clinical studies are stimulating renewed interest. Other plant-derived agents in clinical use are homoharringtonine, isolated from the Chinese tree, *Cephalotaxus harringtonia* var. *drupacea* (Cephalotaxaceae), and elliptinium, a derivative of ellipticine, isolated from species of several genera of the Apocynaceae family, including *Bleekeria vitensis* A. C. Sm., a Fijian medicinal plant with reputed anti-cancer properties (57, 58). Other important examples are the camptothecin derivatives, topotecan and irinotecan, which exert their cytotoxic action through inhibition of topoisomerase I, a fundamental enzyme complex involved in DNA winding and unwinding. In spite of significant efforts on the part of many research groups, few structural classes of compounds have demonstrated topoisomerase I in-

hibitory activity. Significant new classes of topoisomerase I inhibitors in preclinical development are the 2-aryl-quinoline derivatives (indenoquinolines), 3-aryl-isoquinoline derivatives (indeno-isoquinolines), and the naphthyridines which can be traced to the protoberberine alkaloids, such as nitidine, isolated from *Zanthoxylum* and *Fagara* species (Rutaceae).

With the identification of an increasing number of molecular targets associated with particular cancers, anti-cancer drug discovery is now based on high throughput screening of compounds against a range of such targets. Cyclin-dependent kinases (Cdks), together with their cyclin partners, play a key role in the regulation of cell cycle progression, and inhibition of their activity delays or arrests progression at specific stages of the cell cycle. There are over 2000 kinases so far identified from genomic studies and all have a common site, the position where the ATP, i.e. the source of the phosphate that is donated, is bound (57, 58). An early example of a natural product compound class that ultimately led to Cdk inhibitors, was the moderately anti-tumor active flavonoid, quercetin. This flavanoid can be thought of as an ATP-mimic where the planar bicyclic chromone ring system is an isostere of adenine. Quercetin was shown to exert its anti-tumor effect through blocking cell cycle progression at the G0/G1 interface, consistent with Cdk inhibition; however, a close analogue, myricetin, shows an IC50 close to 10 M versus Cdk2. Flavopiridol showed about a 100 fold more selectivity for Cdks compared to its activity for tyrosine kinases, and was the first compound identified by the NCI as a potential anti-tumor agent that subsequently was proved to be a relatively specific Cdk inhibitor. The ability to attach agents to carrier molecules directed to specific tumors holds promise for the effective targeting of highly cytotoxic natural products to the tumors while avoiding their toxic side effects on normal healthy tissues. With the rapid identification of new proteins having significant regulatory effects on tumor cell cycle progression, and their conversion into targets for high throughput screening, molecules isolated from plants and other natural organisms are proving to be an important source of novel inhibitors of the action of these key proteins, and have the potential for development into selective anti-cancer agents.

Nanotechnology-based cancer treatment

Nanotechnology has large potential in detection as well as treatment of cancer in its budding stage. Nanotechnology can be used to create therapeutic agents that target specific cells and deliver toxin to kill them. The nanoparticles can be designed in such a way that they can circulate through the body, detect cancer-associated molecular changes, assist with imaging, release a therapeutic agent and then monitor the effectiveness of the intervention (59). The potential arises due to the ability of nanoparticles entering inside the cells and access to the chromosomes/DNA molecules. Certain nanoparticles can be designed to absorb preferentially certain wave length of radiation and gets heated. Such a nanoparticle if enters in the cancerous cell will burn it if irradiated by suitable wavelength radiation, which could be a kind of the analogue of radiation therapy. Certain nano-structures like nanocantilevers, nanopores, nanotubes, nanoshells and quantum dotes are prospective structures that would help in detecting and treatment of cancers (59). Still, there are many challenges such as toxicity of the nanoparticles, and legislative and regulatory means that are to be resolved before use of nanotechnology becomes a reality.

The foundation and validation for anti-cancer therapies

The biggest challenge before the clinicians now is the management of the rising incidence of cancer in developing countries, with little prospect of more resources becoming available to fight the disease. The death rate from cancer in the developing countries is set to rise at least 3-fold by the year 2025 largely due to the increased life expectancy, containment of infectious diseases and changing lifestyles. It is estimated that about 50% of cancers are curable if they are detected early and treated appropriately. Screening has a major role in early diagnosis. However, in the developing world, around 80% of cancer patients have late stage incurable disease when they are diagnosed (60).

Despite the fact that cancer research have generated a rich complex body of knowledge, and more additions are likely in the post genomic era, but the road from gene to therapy and prevention is slow and un-

predictable (61). Ideally, the detection of a gene whose mutations contribute to cancer should lead to the development of a drug or procedure that cures or prevents that cancer. The recent remarkable progress in identifying molecular alteration in human tumor cells has unfortunately not been paralleled in the field of anticancer drug discovery. The shortage of effective anticancer drugs is due in part to the fundamental difficulties associated with the development of any safe effective drug. It remains a formidable task to design small molecules that alter the function of macromolecules with both sensitivity and specificity (for example, an enzyme with a small active site). It is even more difficult to inhibit protein-protein interaction mediated over a large surface, or to restore function to a defective protein. Many of the genetic alteration frequently found in tumors are loss-of-function, mutation in tumor suppressor genes and thus do not constitute ideal drug targets, because it is difficult to develop drugs that restore the function of a missing or altered protein (62).

There are also many difficulties specific to anticancer drug discovery. An effective chemotherapeutic agent must selectively kill tumor cells. In the post genomic era cancer treatment contemplated to be more effective and specific for tumor cells compared to the normal. High-throughput screening platforms based on microarray and DNA chips are poised to significantly impact drug discovery process. However, it is to be remembered that most deaths from cancer are due to metastases that are resistant to conventional therapies (63). So the real challenge will be to design drugs that would be effective in long-term basis, target specific and easily available to all.

Acknowledgements

The manuscript has been compiled using the information available on various websites on the internet.

References

1. Los M, Roodhart JM, Voest EE. Target practice: lessons from phase III trials with bevacizumab and vatalanib in the treatment of advanced colorectal cancer. *The Oncologist* 2007; 12: 443–50.
2. Rivory LP. New drugs for colorectal cancer: mechanisms of action. *Australian Prescriber* 2002; 25: 108-110.
3. Pawson T. Regulation and targets of receptor tyrosine kinases. *Eur J Cancer* 2002; 38: S3–S10.
4. Schlessinger J. Cell signaling by receptor tyrosine kinases. *Cell* 2000; 103: 211–225.
5. Carpenter G, Cohen S. Epidermal growth factor. *J Biol Chem* 1990; 265: 7709–7712.
6. Blume-Jensen P, Hunter T. Oncogenic kinase signaling. *Nature* 2001; 411: 355–365.
7. Takimoto CH, Calvo E. Principles of Oncologic Pharmacotherapy. In: Pazdur R, Wagman LD, Camphausen KA, Hoskins WJ (Eds) Cancer Management: A Multidisciplinary Approach. (11th ed.) 2008. London: Cmp United Business Media; Chapter 3, pp 1-9.
8. Gambacorti-Passerini C, Gunby R, Piazza R, Galietta A, Rostagno R, Scapozza L. Molecular mechanisms of resistance to imatinib in Ph-positive leukemias. *Lancet Oncology* 2003; 4: 75-85.
9. Deininger M, Druker BJ. Specific targeted therapy of chronic myelogenous leukemia with Imatinib. *Pharmacol Rev* 2003; 55: 401-23.
10. Vigneri P, Wang JY. Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. *Nat Med* 2001; 7: 228-34.
11. Pao W, Miller V, Zakowski M. EGF receptor gene mutations are common in lung cancers from "never smokers" and are associated with sensitivity of tumors to gefitinib and erlotinib. Proc Natl Acad Sci U S A. 2004 ; 101 (36): 13306-11
12. Sordella R, Bell DW, Haber DA, Settleman J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* 2004*;* 305: 1163-7.
13. Raymond E, Faivre S, Armand J. Epidermal growth factor receptor tyrosine kinase as a target for anticancer therapy. *Drugs* 2000; 60: 15-23.
14. Bonvini P, Zorzi E, Basso G, Rosolen A. Bortezomib-mediated 26S proteasome inhibition causes cell-cycle arrest and induces apoptosis in CD-30+ anaplastic large cell lymphoma. *Leukemia* 2007; 21: 838-42.
15. Jordan VC. Fourteenth Gaddum Memorial Lecture. A current view of tamoxifen for the treatment and prevention of breast cancer. *Br J Pharmacol* 1993; 110: 507-17.
16. Cancer Research UK. "Breast cancer in men". *CancerHelp UK*. 2007-09-28. http://www.cancerhelp.org.uk
17. Center for Drug Evaluation and Research (03/08/2005). Tamoxifen Information: reducing the incidence of breast cancer in women at high risk. U.S. Food and Drug Administration. http://www.fda.gov/cder/news/tamoxifen/
18. Desta Z, Ward BA, Soukhova NV, Flockhart DA. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system in vitro: prominent roles for CYP3A and CYP2D6. *J Pharmacol Exp Ther* 2004; 310: 1062-75.
19. Wang DY, Fulthorpe R, Liss SN, Edwards EA. Identification of estrogen-responsive genes by complementary deoxyribonucleic acid microarray and characterization of a

novel early estrogen-induced gene: EEIG1. *Mol Endocrinol* 2004; 18: 402-11.
20. Shang Y, Hu X, DiRenzo J, Lazar MA, Brown M. Cofactor dynamics and sufficiency in estrogen receptor-regulated transcription. *Cell* 2000; 103: 843-52.
21. Massarweh S, Osborne CK, Creighton CJ, et al. Tamoxifen resistance in breast tumors is driven by growth factor receptor signaling with repression of classic estrogen receptor genomic function. *Cancer Res* 2008; 68: 826-33.
22. Hurtado A, Holmes KA, Geistlinger TR, et al. Regulation of ERBB2 by oestrogen receptor-PAX2 determines response to tamoxifen. *Nature* 2008; 456: 663-6.
23. Osborne CK, Bardou V, Hopp TA, Chamness GC, et al. Role of the estrogen receptor coactivator AIB1 (SRC-3) and HER-2/neu in tamoxifen resistance in breast cancer. *J Natl Cancer Inst* 2003; 95: 353-61.
24. www.modernmedicine.com (2008). New Mechanism Predicts Tamoxifen Response: PAX2 gene implicated in tamoxifen-induced inhibition of ERBB2/HER2-mediated tumor growth.
25. CORDIS News. 2008-11-14. Study sheds new light on tamoxifen resistance. http://cordis.europa.eu
26. Old LJ. Immunotherapy for cancer. *Sci Am* 1996; 275: 136-43.
27. Green MC, Murray JL, Hortobagyi GN. Monoclonal antibody therapy for solid tumors. *Cancer Treat Rev* 2000; 26: 269-86.
28. Bokemeyer C, Bondarenko I, Makhson A, et al. Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. *J. Clin. Oncol.* 2009; 27: 663-71.
29. Van Cutsem E, Köhne CH, Hitre E, et al. Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. *N. Engl. J. Med.* 2009; 360: 1408-17.
30. Schrag, D. The price tag on progress: chemotherapy for colorectal cancer. *New England J Med* 2004; 351: 317-9.
31. Shaw T, Quan J, Totoritis M. B cell therapy for rheumatoid arthritis: the rituximab (anti-CD20) experience. *Ann Rheum Dis* 2003; 62: ii55-ii59.
32. Binder M, Otto F, Mertelsmann R, Veelken H, Trepel M. The epitope recognized by rituximab. *Blood* 2006; 108: 1975-8.
33. Hudis, CA. Trastuzumab—mechanism of action and use in clinical practice. *N Engl J Med* 2007; 357: 39-51.
34. Bange, J, Zwick E, Ullrich A. Molecular targets for breast cancer therapy and prevention. *Nature Medicine* 2001;7: 548-52.
35. Menard, S; Pupa SM, Campiglio M, Tagliabue E . Biologic and therapeutic role of HER2 in cancer. *Oncogene* 2003; 22: 6570-8.
36. Kute T, Lack CM, Willingham M, et al. Development of herceptin resistance in breast cancer cells. *Cytometry* 2004, 57A: 86-93.
37. www.nature.com/nature/journal/v421/n6924/full/nature01392.html
38. Albanell J, Codony J, Rovira A, Mellado B, Gascon P. "Mechanism of action of anti-HER2 monoclonal antibodies: scientific update on trastuzumab and 2C4". Advances in Experimental Medicine and Biology2003; 532: 253-68.
39. Clynes RA, Towers TL, Presta LG, Ravetch JV. Inhibitory Fc receptors modulate in vivo cytoxicity against tumor targets. *Nat Med* 2000; 6: 443-6.
40. Avastin Prescribing Information, Genentech Inc., October 2006. www.clinicaltrials.gov
41. F.D.A. Approves Drug's Use for Breast Cancer". *The New York Times*. February 22, 2008. http://www.nytimes.com/2008/02/22/business/apee-drug.html.
42. Reed K. Roche drug Avastin fails cancer study, shares fall. *Reuters* 2009 (April, 22), Zurich.
43. Berkow R, Beers MH. Cancer and the immune system. In: The Merck Manual of Medical Information. Whitehouse Station, NJ; Merck Research Laboratories 1997: 792-4.
44. Dorval T, Palangie T, Jouve M, Garcia-Giralt E, Falcoff E, Schwab D. Treatment of metastatic melanoma with recombinant interferon alfa-2b. *Invest New Drugs* 1987; 5: S61-S63.
45. Sertoli MR, Bernengo MG, Ardizzoni A, et al. Phase II trial of recombinant alfa-2b interferon in the treatment of metastatic skin melanoma. *Oncology* 1989; 46: 96-8.
46. Tourani JM, Lucas V, Mayeur D, et al. Subcutaneous recombinant interleukin-2 (rIL-2) in outpatients with metastatic renal cell carcinoma. Results of a multicenter SCAPP1 trial. *Ann Oncol* 1996; 7: 525-8.
47. Lissoni P, Barni S, Tancini G, et al. Clinical response and survival in metastatic renal carcinoma during subcutaneous administration of interleukin-2 alone. *Arch Ital Urol Androl* 1997; 69: 41-7.
48. Atkins MB, Roberston MJ, Gordon M, et al. Phase I evaluation of intravenous recombinant human interleukin-12 in patients with advanced malignancies. *Clin Cancer Res* 1997; 3: 409-17.
49. Lee P, Wang F, Kuniyoshi J, et al. Effects of interleukin-12 on the immune response to a multipeptide vaccine for resected metastatic melanoma. *J Clin Oncol* 2001; 19: 3836-47.
50. Jaffee EM, Hruban RH, Biedrzycki B, et al. Novel allogeneic granulocyte-macrophage colony-stimulating factor-secreting tumor vaccine for pancreatic cancer: a phase I trial of safety and immune activation. *J Clin Oncol* 2001; 19: 145-56.
51. Schneider T, Gerhards R, Kirches E, Firsching R. Preliminary results of active specific immunization with modified tumor cell vaccine in gliobastoma multiforme. *J Neurooncol* 2001; 53: 39-46.
52. Hanna MG Jr, Hoover HC Jr, Vermorken JB, Harris JE, Pinedo HM. Adjuvant active specific immunotherapy of stage II and stage III colon cancer with an autologous tumor cell vaccine: first randomized phase III trials show promise. *Vaccine* 2001; 19: 2576-82.
53. Hsueh EC, Famatiga E, Gupta RK, Qi K, Morton DL. Enhancement of complement-dependent cytotoxicity by polyvalent melanoma cell vaccine (CancerVax): correlation with survival. *Ann Surg Oncol* 1998; 5: 595-602.
54. Conry RM, Khazaeli MB, Saleh MN. Phase I trial of a recombinant vaccinia virus encoding carcinoembryonic anti-

gen in metastatic adenocarcinomas: comparison of intradermal versus subcutaneous administration. *Clin Cancer Res* 1999; 5: 2330-7.
55. Marshall JL, Hoyer RJ, Toomey MA, et al. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. *J Clin Oncol* 2000; 18: 3964-73.
56. von Mehren M, Arlen P, Gulley J, et al. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. *Clin Cancer Res* 2001; 7: 1181-91.
57. Newman DJ, Cragg GM, Holbeck S, Sausville EA. Natural products as leads to cell cycle pathway targets in cancer chemotherapy, *Current Cancer Drug Targets* 2002; 2: 279-308.
58. Cragg GM, Kingston DGI, Newman DJ. Anticancer Agents from Natural Products. Boca Raton, Florida 2005: Taylor and Francis.
59. Singh OP, Nehru RM. Nanotechnology and cancer treatment. *Asian J Exp Sci* 2008; 22: 45-50.
60. Pal SK, Mittal B. Fight against cancer in countries with limited resources: The Post-genomic era scenario. *Asian Pacific J Cancer Prev* 2004; 5: 328-33.
61. De la Chapelle A. Has gene detection benefited the cancer patient? Proc. 10th International Congress Human Genetics 2001, Austria, 63.
62. Hartwell LH, Szankasi P, Roberts CJ, Murray AW, Friend SH. Integrating genetic approaches into the discovery of anticancer drugs. *Science* 1997; 278: 1064-8.
63. Fidler IJ, Ellis LE. Chemotherapeutic drugs-more really is not better. *Nature Med* 2000; 6: 500-2.

Accepted: 6th December 2012
Correspondence: Hariom Yadav,
Diabetes, Endocrinology and Obesity Branch,
5W5872, Bld 10, NIDDK, National Institutes of Health,
Bethesda, MD 20892, USA
E-mail: yadavh@mail.nih.gov