Review Article

Targeted Therapy for Cancer

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Cancer is one of the most common causes of death, taking nearly 7 million lives each year worldwide. New cancer targeted therapies that make use therapeutic antibodies or small molecules have made treatment more tumor specific and less toxic. Nevertheless, there remain several challenges to the treatment of cancer, including drug resistance, cancer stem cells, and high tumor interstitial fluid pressure. In many solid tumors, for example, increased interstitial fluid pressure makes the uptake of therapeutic agents less efficient. One of the most promising ways of meeting such challenges is ligand-targeted therapy that may be used to make targeting more specific and carry higher dosages of anti-cancer drug to tumor tissue. This article reviews and discusses recent advances in the treatment of cancer and the challenges that remain.

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Introduction

The first description of cancer is found in an Egyptian papyrus and dates back to approximately 1600 BC. It was regarded as an incurable disease until the nineteenth century, when surgical removal was made more efficient by anaesthesia, improved techniques and histological control. Before 1950, surgery was most preferred means of treatment. After 1960, radiation therapy started being used to control local disease. However, over time it was realized that neither surgery nor radiation or the two in combination could adequately control the metastastic cancer and that, for treatment to be effective, therapy needed to reach every organ of the body. Therefore, current efforts to cure cancer have been focusing on drugs, biological molecules and immune mediated therapies. The introduction of nitrogen mustard in the 1940s can be considered the origin of antineoplastic chemotherapy targeting all tumor cells [1]. To date, cancer remains one of the most life-threatening diseases. Efforts to fight this disease were intensified when the US passed the National Cancer Act in 1971 and president Nixon declared a "war on cancer" [2]. Today, more than 30 years later, although we have not improved mortality rate or prolonged survival time for metastatic cancer as much as we would had expected, we have identified the characteristics and pathways of different tumor entities. This knowledge is now used to generate specific tumor therapies either by directly targeting the proteins involved in the neoplastic process or by targeting drugs to the tumor (Figure 1).

Targeted therapy encompasses a wide variety of direct and indirect approaches (Figure 1). Direct approaches target tumor antigens to alter their signalling either by monoclonal antibodies (MoAbs[2]) or by small molecule drugs that interfere with these target proteins. Indirect approaches rely on tumor antigens expressed on the cell surface that serve as target devices for ligands containing different kinds of effector molecules. In these approaches, drugs can actively target tumors using tumor-specific MoAbs or peptide ligands binding to receptors that are present on tumor cells. In addition to active targeting, tumors can also be passively targeted by macromolecules through the "enhanced permeability and retention effects" attributed to the hyperpermeable angiogenic tumor vasculature and the lack of effective tumor lymphatic drainage. This review will focus on the target therapy found to be significantly efficacious and the novel approaches with clinical promise.

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[2]**Abbreviations:** MoAb, monoclonal antibody; ADCC, antibody-dependent cell-mediated cytotoxicity; CDC, complement-dependent cytotoxicity; NK, natural killer; NSCLC, non-small-cell lung cancer; IFP, interstitial fluid pressure; CSC, cancer stem cell; NPC, nasopharyngeal carcinoma.

Antibody-targeted therapy

In 1975, Köhler & Milstein developed techniques for producing MoAbs, making it possible to produce large quantities of identical antibodies directed against specific antigens [3]. Antibodies, which initially were viewed as "targeting missiles", have proved much more complex in their targeting and biologic properties than the field's pioneers envisioned them. MoAbs have emerged as important therapeutic agents for several different malignancies [4]; they have been found to be well-tolerated and effective for the treatment of different cancers, and were consequently approved by the FDA of the US (Table 1). In addition to their own role as anti-cancer agents, their ability to target tumors also enables them to improve the selectivity of other types of anti-cancer agents, some of which cannot be applied effectively alone. Murine antibody can be readily transformed into human or humanized formats that are not readily recognized as foreign by the human immune system. In addition, novel antibody-based structures with multiple antigen recognition sites, altered size, or effector domains have been shown to influence the targeting ability of antibodies. Coupled with the identification of appropriate cancer targets, antibody-based therapeutics are finding increasing number of applications in cancer treatment, and they can be effective alone, in conjunction with chemotherapy or radiation therapy, or when conjugated to toxic moieties such as toxins, chemotherapy agents, or radionuclides.

Generation of therapeutic antibodies

Although there has been great optimism about techniques using MoAbs to engineer a therapeutic "magic bullet",

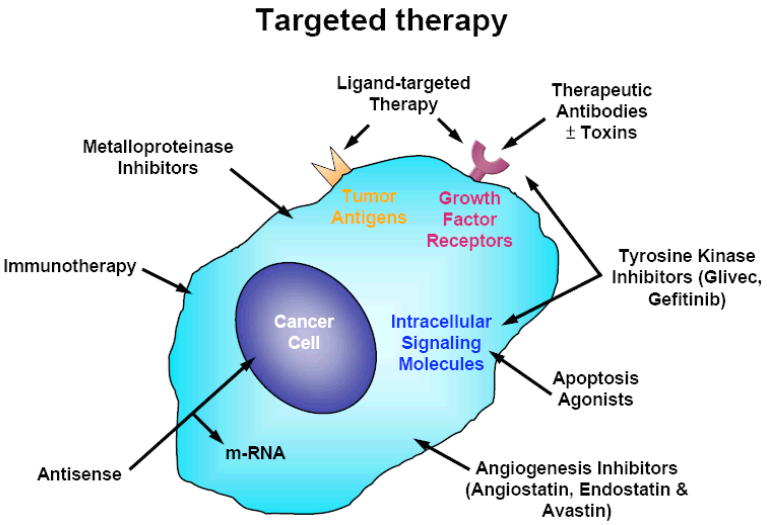

Figure 1: Targeted therapy refers to a new generation of cancer drugs designed to interfere with a specific target protein that is believed to have a critical role in tumor growth or progression. This approach contrasts with the conventional cytotoxic chemotherapeutics that have been used in major cancer therapy in past decades. The molecular identification of cancer antigens has opened new possibilities for the development of effective immunotherapies, antibodies therapy and ligand-targeted therapy for cancer patients. Ligand-targeted therapy is a successful means of improving the selective toxicity of anticancer therapeutics. It can also be applied to the targeted delivery of small molecule drugs or gene medicines such as antisense oligonucleotides. Angiogenesis inhibitors are a relatively new class of cancer drugs. The biological and biochemical characteristics of angiogenesis inhibitors, however, differ from conventional cytotoxic chemotherapy. They might be added to chemotherapy or to radiotherapy, or used in combination with immunotherapy or vaccine therapy.

Table 1: **Therapeutic antibodies approved by the US FDA for cancer treatment**

Monoclonal antibody	Target	Indication	Product	Year	Corporate sponsors	References
Rituximab (Rituxan)	CD20	Low-grade B-cell NHL	Chimeric	1997	IDEC, Genentech	[21-24]
Trastuzumab (Herceptin)	HER2/neu	Metastatic breast cancer	Humanized	1998	Genentech	[17, 19, 25]
Gemtuzumab-ozogamicin (Mylotarg)	CD33	Acute myeloid leukemia	Humanized	2000	Wyeth Laboratories	[26-28]
Alemtuzumab (Campath)	CD52	Chronic lymphocytic leukemia	Humanized	2001	Millennium and ILEX Partners	[29-31]
Ibritumomab-tiuxetan-^{90}Y (Zevalin)	CD20	Non-Hodgkin lymphoma	Mouse	2002	IDEC	[32-34]
Tositumomab/ Tositumomab-^{131}I (Bexxar)	CD20	Non-Hodgkin lymphoma	Mouse	2003	Corixa, GlaxoSmithKline	[35-37]
Cetuximab (Erbitux)	EGFR	Metastatic CRC; HNSCC	Chimeric	2004	ImClone Systems	[38-40]
Bevacizumab (Avastin)	VEGF	Metastatic CRC; NSCLC	Humanized	2004	Genentech	[41-44]

NHL, Non-Hodgkin lymphoma; CRC, Colorectal cancer; HNSCC, Head and neck squamous cell carcinoma; NSCLC, Non-small cell lung cancer.

success is still many years away. Several issues must be considered, including choice of target antigen, immunogenicity of antibodies, penetration into solid tumors, half-life of antibody, and ability of antibodies to recruit immune effector functions. Choice of target antigen plays a key role in determining the success of treatment. To ensure specificity, the antigen must be reactive with the target cell and not cross-react significantly with healthy tissue. The antigen should be present on most of the malignant cells to allow effective targeting and to prevent a subpopulation of antigen-negative cells from proliferating. Antigens that shed from the cell surface and circulate in the peripheral blood do not make the best targets. The administered antibody binds freely with circulating antigen, which prevent it from reaching the cancer cells and, therefore, higher doses of antibody are needed to clear the circulating antigen [5].

The first antibodies studied were murine, rabbit, or rat proteins purified following immunization of the animal with a target antigen. Patients often generated antibodies to these foreign antigens; these host antibodies are often referred to as HAMA (human anti-mouse antibody) or HARA (human anti-rat antibody or human anti-rabbit antibody). The host antibody reduces the effectiveness of therapy by prematurely clearing the treatment antibody and limiting the possibilities for future immunotherapy. HAMA or HARA responses can be associated with immune complex-related adverse events such as serum sickness and anaphylaxis. The problem of immunogenicity of murine and chimeric MoAbs could be solved quickly with the progress in MoAb engineering and the generation of fully human antibodies.

In solid tumors, the therapeutic agent must overcome several obstacles, including the vascular endothelium, stromal and epithelial barriers and high interstitial pressure [6]. In addition, solid tumors are quite heterogeneous and it is, therefore, difficult to target them completely. When trying to target them, smaller recombinant MoAb structures such as single-chain antibodies should be able to penetrate into the tumor with higher efficacy than the parental antibody [7]. However, this advantage is accompanied by the disadvantage that small structures such as these are more rapidly cleared from the plasma, and therefore they have shorter half-lives [8]. One promising approach to solid tumors is to target the tumor microenvironment in general and the endothelium of tumor blood vessels in particular [9], because several tumor endothelial markers are well characterized [10].

Murine, rabbit, and rat antibodies are not always able to recruit human immune effector functions, such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), which are needed to facilitate destruction of a malignant cell. To overcome obstacles inherent in the first-generation antibodies, DNA technology has been used to construct hybrids composed of human antibody regions linked with a murine or primate backbone [11]. These are referred to as chimeric or humanized antibodies, depending on the exact antibody structure. A chimeric antibody is a composite of antibodies from two different species. Humanized antibody is a human antibody containing the complementarity-determining region (CDR) from a non-human source. These antibodies have been successful in activating immune effector functions, thereby improving response rates in clinical trials. When these antibodies bind, the complement cascade is activated, resulting in CDC. Lysis takes place through chemical processes but also involves recruitment of phagocytic cells. In ADCC, antibody binds to the antigen on the surface of a target cell and

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is subsequently bound by Fc receptors on effector cells. Monocytes, macrophages, natural killer (NK) cells, killer cells, and granulocytes express Fc receptors and can exert cytotoxic effects. ADCC results when the bound antibody binds NK cells. The Fab portion of the antibody attaches to the malignant cell while the opposing Fc region binds to Fc receptors on NK cells. The NK cells in turn release cell-lysing molecules that destroy the target. Genetically engineered antibodies have also enhanced efficacies through longer half-life. The half-life of the chimeric anti-CD20 antibody, rituximab, is 76 h after a single infusion and 206 h after four infusions, compared with 28 h for the murine counterpart, ibritumomab [12,13].

Therapeutic antibodies for cancer

The first chimeric antibodies were generated in the late 1980s [14,15], and in 1997 the first therapeutic antibody, rituximab (Rituxan; Genentech/Biogen Idec), was approved by the US FDA for the treatment of B-cell non-Hodgkin's lymphoma [16]. Since then, MoAb-based therapies have become a major strategy in medicine. In fact, approximately a 25~30% of all biotechnology products being developed are MoAbs, and several have now been approved by the US FDA for the treatment of cancer (Table1).

Trastuzumab (Herceptin; Genentech) was approved by the US FDA for use in patients with metastatic breast cancer in 1998. It is a genetically engineered anti-HER2 monoclonal antibody that inhibits proliferation *in vitro* of tumor cells overexpressing HER2 protein [17] and specifically targets the HER2 oncoprotein. The HER2 protein is produced in excessive amounts in 25-30% of patients with breast cancer and is associated with aggressive growth of tumor cells [18]. Trastuzumab was humanized by adding the critical mouse recognition sequence to the framework of a human IgG1 to maximize immune recruitment [19]. The reduction of murine components (95% human and 5% murine) decreases the potential for immunogenicity that is seen with murine monoclonal antibody therapy and increases the potential for recruiting immune mechanisms.

Bevacizumab (Avastin; Genentech) was approved by US FDA as a first-line treatment for patients with metastatic colorectal cancer on February 2004 (Table 1). The median duration of survival was 20.3 months in the group given irinotecan, fluorouracil, and leucovorin (IFL) plus Avastin, as compared with 15.6 months in the group given IFL plus placebo (increase of 4.7 months) in a phase III trial with metastatic colorectal cancer. In 1993, it was shown that a monoclonal antibody that targeted VEGF resulted in a dramatic suppression of tumor growth *in vivo*, which led to the development of bevacizumab, a humanized variant of anti-VEGF antibody, as an anticancer agent. The approval of bevacizumab by the US FDA supports the ideas that VEGF is a key mediator of tumor angiogenesis and that blocking angiogenesis is an effective strategy for treating cancer in humans. The potential clinical utility of VEGF inhibition in oncology is not limited to solid tumors. There is growing evidence that VEGF and VEGF receptors are expressed by a variety of leukaemias and other haematological malignancies, indicating that inhibition of VEGF or VEGFR signaling might play a role in the treatment of such conditions [20]. However, in February 2006 the pharmaceutical manufacturer stopped recruiting patients for testing Avastin in late stage clinical trials after sudden deaths of four patients, especially in three younger patients.

Targeted therapy by small molecules

The growing understanding of the molecular events underlying the etiology of different cancers as well as the signaling events that are critical for the continued growth and proliferation of cancer cells has enhanced the opportunities to develop novel agents. This new type of chemotherapy has been termed targeted therapy, and the goal of this modern chemotherapy is to provide molecular levels-based agents that are more specific for cancer cells. In most current drug discovery programs, rational and empiric approaches are being used either in parallel or in combination with one another. Lead compounds are identified as inhibitors for molecular targets through molecular screening [45]. Protein phosphorylation regulates most aspects of cell life, whereas abnormal phosphorylation is a cause or consequence of disease especially in cancer biology, such as abnormal proliferation, anti-apoptosis and angiogenesis [46-48].

Many studies have also indicated that activation of protein phosphorylation-related pathways in tumors can occur through mutation or overexpression if compared to normal cells [49,50]. For these reasons, the targeted therapeutics ascribes to pharmacological agents that are as close to be mono-specific as possible to avoid the detrimental side effects that sometimes occur with traditional therapies. Therefore, small molecule inhibitors of protein kinases have emerged as indispensable for studying target therapy [51]. The protein kinases that have been targeted most intensively for drug development are plasma membrane-associated protein tyrosine kinases [52]. The first kinase inhibitors were described nearly 20 years ago and developed in the early 1980s by Hiroyoshi Hidaka. Naphthalene sulphonamides had already been developed as antagonists of the calcium-binding protein calmodulin [53,54]. There are more than 30 such agents in clinical trials now [55] and the most well-known small molecule inhibitors are glivec and gefitinib (Figure 2).

Glivec (imitanib mesylate, Gleevec, STI571; Novartis) is the first selective tyrosine kinase inhibitor to be approved for the treatment of cancer in 2001 [56]. Glivec is a 2-phenylaminopyrimidine which competitively inhibits ATP binding to the Abl kinase, thereby inhibiting the constitutively activated Bcr-Abl tyrosine kinase, which is a specific genetic change encoding abnormal protein associated with human cancer [57-59] (Figure 2A). As the tyrosine kinase activity of Bcr–Abl is crucial for its transforming activity, the enzymatic activity of this deregulated gene could plausibly be defined as an attractive drug target for addressing Bcr-Abl-related chronic myelogenous leukaemia (CML). In an *in vitro* screen against a panel of protein kinases, Glivec was found to inhibit the autophosphorylation of three kinases: Bcr–Abl, c-Kit and platelet-derived growth factor (PDGF) receptor. More recently, activity against ARG kinase has also been reported [56,60,61]. Glivec can be used to treat CML, gastrointestinal stromal cell tumors (GIST) and metastatic dermatofibrosarcoma protuberans, afflictions that are associated with the expression and activity of these kinases [62-64]. CML represents the first human cancer in which molecularly targeted therapy was reported to lead to a dramatic clinical response [65-68]. Glivec has also been used in clinical trails for other types of cancers overexpressing related proteins, but the results did not show significant clinical activity, further confirming the collective evidence that prediction of efficacy of novel therapeutic agents is based on target expression rather than on pathway activation (for example, through activating mutations)[69-74].

Gefitinib (Iressa; AstraZeneca Pharmaceuticals), a selective oral epidermal growth factor receptor tyrosine kinase inhibitor, has been found to disrupt EGFR kinase activity by binding the ATP pocket within the catalytic domain [79](Figure 2B). Gefitinib has also been reported to prevent EGFR phosphorylation, decrease mitogen-activated protein kinase activity, increase apoptosis, and also increase levels of the cyclin-dependent kinase inhibitor p27 which is believed to lead to G1 cell cycle arrest [79-81]. Following

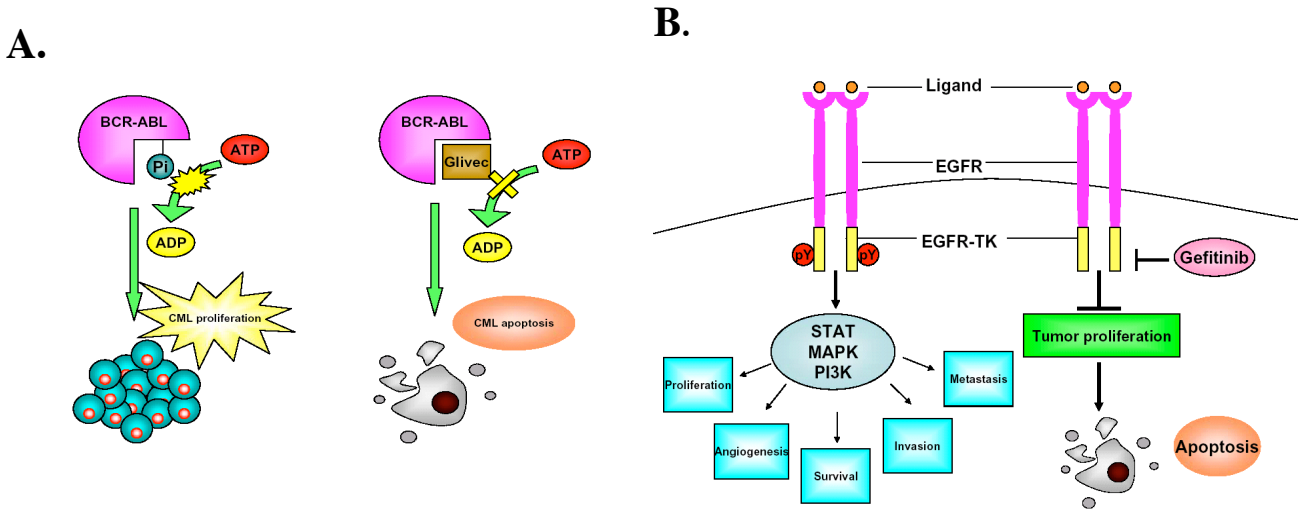

Figure 2: Mechanisms of small molecule inhibitors for tumor treatment. Most of the small molecule inhibitors are designed to specifically target overexpressed or mutated signaling pathway in tumor cells rather than normal cells. And then, the life cycle of tumor cells will be blocked and triggered to apoptosis. (A) Constitutively activated Bcr-Abl tyrosine kinase causes chronic myelogenous leukaemia (CML). The activity of Bcr-Abl is catalyzed by ATP, and the phosphorylation binding site can be inhibited by glivec. Therefore, the tumor cells' proliferation will be terminated and alternatively switch to apoptosis pathway. (B) EGFR-mediated signaling contributes to the up-regulation of many processes that are essential for tumor growth and progression. Gefitinib is a small molecule that inhibits ATP binding within the tyrosine kinase domain of EGFR, which inhibits EGFR autophosphorylation and consequently blocked signal transduction from activated EGFR. The critical mechanisms of tumor growth are inhibited as following the gefitinib treatment.

Japanese approval in 2002, gefitinib was approved by the US FDA in May 2003 for the treatment of advanced non-small-cell lung cancer (NSCLC) after other treatment options had failed [82]. Despite the almost universal presence of EGFR expression in NSCLC tumors, therapeutic inhibition of EGFR has resulted in significant tumor regressions in only 10-30% of patients [83,84]. Gefitinib has induced substantial clinical responses in about 10% of patients with chemotherapy-refractory NSCLC [85-89]. An infrequent but serious side effect of gefitinib is interstitial lung disease [90] and its most often reported side effect is skin toxicy [91]. Recent clinical reports have indicated that gefitinib offers lasting tumor control with a disease progression-free survival interval between 9 and 25 months [92]. Elkind and the colleagues showed that multidrug transporter ABCG2 displayed a high-affinity interaction with several tyrosine kinase receptor inhibitors, including Gefitinib [93]. Variable expression and polymorphisms of ABCG2 may significantly modify the anti-tumor effect as well as the absorption and tissue distribution of Gefitinib [93].

Ligand-targeted therapy

Most cancer cells share many common features with the normal host cells from which they are derived. Therefore, high levels of selective toxicity cannot be achieved with anti-cancer chemotherapeutics because of the lack of unique molecular targets that would distinguish them from normal cells. This can lead to increased toxicities against normal tissues, including bone marrow, gastrointestinal tract and hair follicle tissues. Furthermore, trying to avoid the side effects that occur as a result of toxicities to normal tissues, we often give sub-optimal doses of anticancer chemotherapeutics, resulting in the eventual failure of therapy, which is often accompanied by the development of drug resistance and metastatic disease. The selective toxicity of an anti-cancer drug can be increased by either increasing the amount of the drug that reaches the cancer tissue or by decreasing the concentration of drug that reaches at the normal tissues. Therefore, ligand-targeted therapy makes possible tumor specificity and limited toxicity and shows promise in the development of novel therapies for cancer. Ligand-targeted therapy can carry higher doses of a drug to the tumor tissue and may overcome obstacles presented by cytotoxic chemotherapy. There are several obstacles in cancer therapy including drug resistance, high tumor interstitial fluid pressure (IFP), and cancer stem cells (CSCs).

Obstacles in cancer therapy

Heterogeneous cancer cells and drug resistance: Human tumors of any given histological type have great genetic diversity, as revealed by gene-expression profiling, and in most types of cancer only a subset of patients will prove responsive to any given new agent. Under the selective pressure of a toxic therapy, the genetic diversity within most human tumors leads to rapid outgrowth of drug-resistant cells. A vast array of resistance mechanisms, involving mutations or amplification of the target enzyme, overexpression of drug transporters, or mutations in cell death pathways, can defeat single agents, no matter how well designed and targeted – this was also observed by Gilman and colleagues 60 years earlier. Both targeted drugs and conventional anti-tumor agents can be affected by a common resistance mechanism involving drug efflux (the MDR transporter) or mutations in cell death pathways. The transition from cytotoxic drugs to targeted therapies represents an important advance, but the basic principles of cancer treatment and drug resistance remain the same [94]. Human malignancies are a very diverse group of diseases, even within histological classifications, and quickly display their diversity when exposed to the various forms of chemotherapy. The next decade will present the challenge of designing trials to combine targeted drugs and cytotoxic agents in a more effective manner. Molecular profiling and the study of patient selection will become more important in the development of cancer drugs [95].

High tumor IFP: High IFP of tumor is another barrier for efficient drug delivery [96]. Increased IFP contributes to a decreased transcapillary transport in tumors leads to a decreased uptake of drugs or therapeutic antibodies. Cancer cells are therefore exposed to a lower effective concentration of therapeutic agent than normal cells, lowering the therapeutic efficiency and increasing toxicity. It

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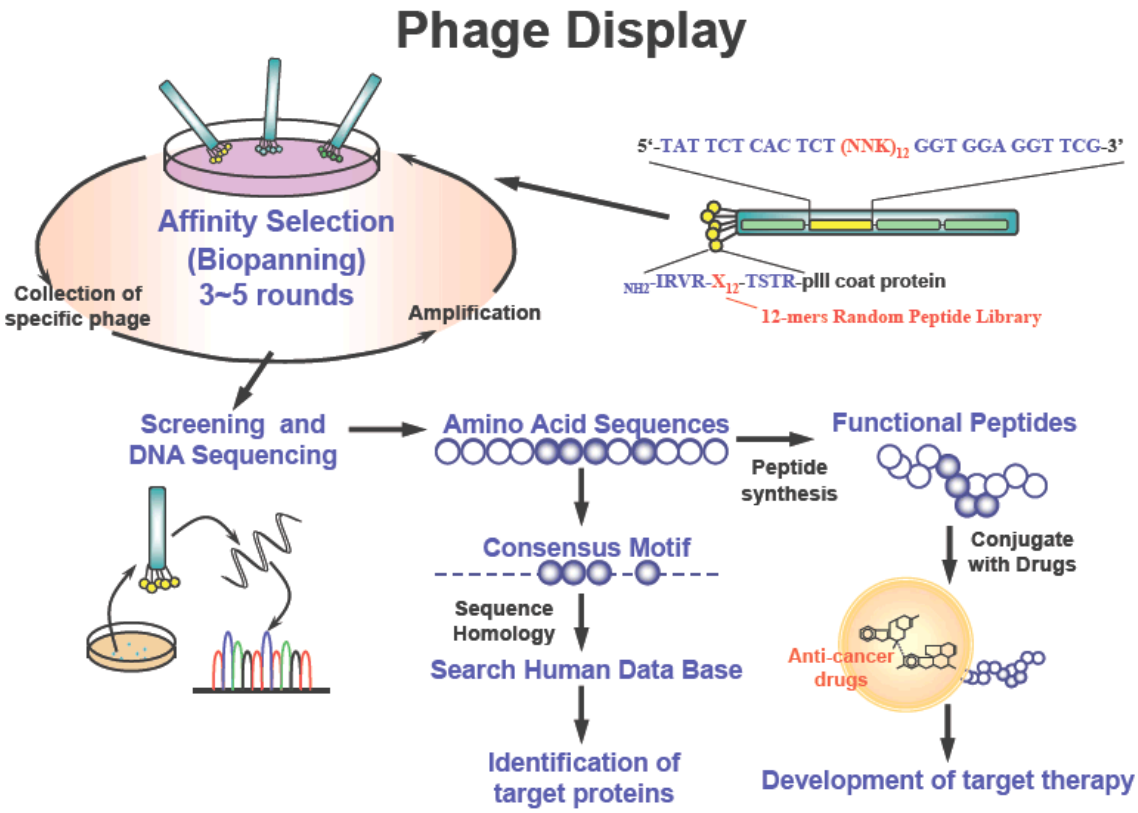

Figure 3: Identification of targeting ligands to cancer cells by phage display. Peptide or antibody libraries can be expressed as fusion proteins with a coat protein (pIII) of a bacteriophage, resulting in display of fused proteins on the surface of virion. Affinity selection (biopanning) of phage-displayed peptide libraries represents a powerful means of identifying peptide ligands for targets of interest. For screening of targeting ligands, phage-displayed peptide library was pre-cleared by normal cells and affinity selection with cancer cells. After biopanning three to five times, targeting phage clones were selected by ELISA, flow cytometry, immunofluorescence, and *in vivo* homing assays. Targeting ligands were further identified and characterized by synthetic peptide binding and competition assay. Targeting ligands can be used to identify cell surface markers and develop ligand-targeted therapy.

is now well established that the IFP of most solid tumors is increased. This has been shown in breast carcinoma [97,98], metastatic melanoma [99,100], head and neck carcinoma [101], and colorectal carcinoma [97]. Values as high as 60 mm Hg have been recorded in some tumors. The tumor IFP is uniform throughout the centre of the tumor and drops steeply in its periphery [102,103]. The mechanisms that determine the increased tumor IFP are not fully understood, but probably involve blood vessel leakiness, lymph vessel abnormalities, interstitial fibrosis, and a contraction of the interstitial space mediated by stromal fibroblasts.

Many studies have provided experimental support for the concept that a reduction in IFP is associated with an increase drug uptake and treatment efficacy [41,102-106]. In a study of patients with melanoma or lymphoma, the patients that responded best to chemotherapy showed a progressive lowering of the tumor IFP [100]. Moreover, high IFP in tumors has been correlated with a high recurrence rate and a poor prognosis for patients with cervical cancer receiving radiation therapy [107,108]. Ligand-targeted therapy, which utilizes the affinity of ligand with the receptor on plasma membrane of cancer cells to carry anti-cancer drugs to tumor tissue, may increase the accumulation of drugs in high IFP of the tumor and improve the therapeutic efficacy.

CSCs: The discovery of CSCs in solid tumors has changed our view of carcinogenesis and chemotherapy. CSCs are biologically distinct from other cancer cell types. Natural properties of CSCs are likely to increase their resistance to standard chemotherapy agents [109]. Thus, if cancer therapies do not effectively target the CSC population during initial treatment, relapse may occur as a consequence of CSC-driven tumor expansion. Therefore, in developing new cancer therapeutics, analyses of CSC-specific treatments still need to be formally established. Clearly CSC biology and target therapy will be a very exciting and active area of research in years to come.

The biology of stem cells and their intrinsic properties are now recognized as integral to tumor pathogenesis in several types of cancer. This observation has broad ramifications in the cancer research field and is likely to impact our understanding of the basic mechanisms of tumor formation and the strategies we use to treat cancers. One role for stem cells has been demonstrated in cancers of the hematopoietic system, breast and brain. Going forward it is likely that stem cells will also be implicated in other malignancies. However, the fact that scientists can now identify, purify and propagate cancer stem cells allows the development of new strategies for improving targeted therapies in cancer [109]. Hence, a detailed understanding of stem cells and how they mediate tumor pathogenesis will be critical. Furthermore, identification of CSC markers by phage display will lead to improved diagnostic tools to detect pre-malignant lesions and tumors, as well as targeted therapies, such as antibodies or ligand-targeted therapy, directed against tumor stem cells.

Search targeting ligands using phage display

Phage display, a selection technique in which a peptide or protein is expressed as a fusion with a coat protein of bacteriophage, results in a display of the fusion peptide or protein on the surface of the virion. Phage-displayed random peptide libraries provide opportunities to map B-cell epitopes [110-114] and protein–protein contacts [115-118], select bioactive peptides bound to receptors [119-121] or proteins [116,122-125], search for disease-specific antigen mimics [126-128], and determine cell- [129-131] and organ-specific peptides [125,132-134].

Recently, we have developed phage display methods to identify the receptors expressed specifically on cancer cells and tumor vessels. The strategy for identification of tumor-targeting ligand is shown in Figure 3. Using these technologies, we identified a 12-mer peptide (L-peptide) specifically binding to nasopharyngeal carcinoma (NPC) cells. The L-phage and synthetic L-peptide bound to the tumor cell surfaces of most NPC cell lines and biopsy specimens [131]. In SCID mice bearing NPC xenograft, the L-phages specifically bound to the tumor mass. Synthetic L-peptide has been shown to inhibit the binding of L-phage particles to the tumor mass in the competitive inhibition assay [131]. Once we have discovered the targeting ligands to the cancer cells, we can conjugate the targeting peptides with chemotherapeutic drugs and develop ligand-targeted therapies to kill cancer cells specifically. We have chosen liposomes to conjugate with targeting ligands because of the following advantages: (i) prolonged blood circulation, (ii) sufficient tumor accumulation, and (iii) controlled drug release and uptake by tumor cells with a release profile matching the pharmacodynamics of the drug.

Development of ligand-targeted therapy

Traditional chemotherapy became one of the pillars for the treatment of cancer, though the selectivity of cytotoxic agents largely relied on the premise that cells are rapidly proliferating. These drugs are prone to be systemically toxic to normal cells because they are not tumor specific. Selective targeting of ligand could home to tumor-associated or -specific proteins expressed on the cancer cell surface. When the recognized ligands are linked with cytotoxic reagents, they could bring sufficient chemical drugs to tumor mass. In this way, tumor cells can be exposed to abundant cytotoxic drugs and be killed. Ligand-targeted therapy may not only improve the therapeutic efficacy of cancer treatment, it also allows us to avoid the problem of toxicity to normal tissue. Accordingly, the phenomena of incomplete tumor response, early disease relapse, and ultimately, the development of drug resistance stemming from suboptimal doses will be reduced. Furthermore, delivery of chemotherapeutic drugs to tumor tissue by affinity of targeting ligand may overcome an obstacle in cancer therapy caused by high tumor IFP. Therefore, it is expected that ligand-targeted therapy will improve the therapeutic efficacy over conventional anti-cancer drugs [131].

Many nano-particle delivery systems for anti-cancer drugs have entered the clinic trials and have been shown to have improved anti-cancer effects because they can improve the pharmacokinetics and pharmacodynamics of their associated drugs [135]. Liposomes are the most advanced form of particulate drug carriers. The drug delivery research field has successfully constructed long circulating liposomes that will accumulate in the tumor tissue where the entrapped drugs can leak out of the liposomes by "passive diffusion". Passive targeting can result in several-fold increases of drug concentrations in solid tumors relative to those obtained with free drugs [136]. It is thought that the mechanism of action of the liposomal drugs is due to sustained release of drugs from the liposomes and diffusion of the released drugs throughout the tumor interstitial fluid, with subsequent uptake by tumor cells. The range of diameters of drug-containing liposomes is approximately 60-150 nm [137]. However, because tumor vessels lack tight junctions between adjacent vasculature endothelial cells, the size of the gaps between the cells that line tumor blood vessels has been estimated to be 100-600 nm [138,139], which is large enough to allow the extravasation of most liposomes from the vessel into the tumor interstitial space.

Passively targeted liposomal systems can be used to treat cancer, but they can be improved by the higher and more selective anti-cancer activity made possible by ligand-targeted therapy, sometimes termed "active" targeting [131,140,141]. The advantages of targeted liposomal anti-cancer drugs are both the high drug-to-carrier ratio relative to ligand-drug conjugates or free drugs and the multivalent presentation of univalent ligands such as scFv fragments, leading to increased binding avidity [135]. Targeting moieties can include: monoclonal antibodies or antibody fragments such as scFv, peptides, growth factors, carbohydrates, glycoproteins, or receptor ligands which overexpressed or selectively expressed on cancer cells [140,142-144]. Ligand-mediated targeting of liposomal anti-cancer drugs such as doxorubicin or vincristine has resulted in improved survival times in a variety of disease models relative to non-targeted liposomal therapeutics or to signaling ligands on their own [145]. Various animal models of human disease in which targeted therapeutics have resulted in significantly improved survival rates include: anti-CD19 or anti-CD20-targeted liposomal doxorubicin or vincristine in the treatment of murine models of human B lymphoma [140,145,146]; NGR-targeted liposomal doxorubicin in the treatment of murine models of human neuroblastoma (an anti-angiogenic effect)[147]; anti-GD2-targeted liposomal doxorubicin and anti-GD2-targeted liposomal anti-c-*myc* or c-*myb* antisense oligonucleotides in the treatment of neuroblastoma and melanoma [148-150]; and anti-HER-2/Neu-targeted liposomal doxorubicin in the treatment of murine models of human metastatic breast cancer [151].

Recently, we have developed methods of identifying the targeting ligand specifically bound to NPC cells. In an effort to develop ligand-targeted therapy, we used peptide-linked liposomes that carried doxorubicin to treat SCID mice bearing human cancers. The targeting liposomes were found to have an enhanced anti-tumor effect and to have significant clinical potential in a targeted drug delivery system [131]. Discovery of targeting ligands to cancer cells (including cancer stem cells) and development of ligand-targeted therapy will help us improve the therapeutic efficacy and reduce side effects. Unlike other forms of therapy, it will allow us to maintain quality of the patient's life while efficiently attacking the cancer.

Conclusion

Since the 1950s, significant advances have been made in the chemotherapeutic management of cancer. Unfortunately, more than 50% of all cancer patients either do not respond to initial therapy or experience relapse after an initial response to treatment and ultimately die from progressive metastatic disease. Even though the pharmaceutical industry has been successful in discovering many new cytotoxic drugs that can potentially be used for the treatment of cancer, this life-threatening disease still causes near 7 million deaths every year worldwide and the number is growing. Thus, the ongoing obligation to the design and discovery of new cancer therapy is urgent. In general, cancer chemotherapy is usually accompanied by severe side effects and acquired drug resistance. Therefore, we anxiously await the development of target therapy that will allow greater tu-

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mor specificity and less toxicity. Recently, some attempts have been made for this purpose including the usage of monoclonal antibodies [20,22,41] or small molecules [56,82] to inhibit the tumor growth. Despite the promising clinical results from the agents that we have highlighted, there is still significant limitation to the concept of "pathway-specific" targeted therapies. These agents are only effective in tumor types that are dependent upon the tumor antigens that are expressed or the pathways that are being inhibited. It is readily apparent that most solid tumors are the result of numerous genetic mutations, and thus inhibiting a single cellular pathway may not result in a significant therapeutic outcome. Design of agents that target a number of pathways will possibly increase the therapeutic effect, but also increase the risk of treatment-related toxicities.

Most small molecule drugs are distributed in large volumes when given intravenously [152]. The result of this treatment is often a narrow therapeutic index due to a high level of toxicity in normal tissues. Through encapsulation of drugs in a macromolecular carrier, such as liposomes, the volume of distribution is significantly reduced and the concentration of drug in the tumor is increased [153], resulting in a decrease in the amount and types of non-specific toxicities and an increase in the amount of drug that can be effectively delivered to the tumor [154,155]. Liposomes containing various lipid derivatives of polyethylene glycol (PEG) have resulted in extension of the half life [156]. However, they need a tumor targeting ligand to carry them to the tumor site. For solid malignancies, which comprise more than 90% of human cancers, antibodies recognizing tumor-specific antigens have provided only some utility for drug delivery because the immunoconjugates cannot easily penetrate the tumor tissue [157,158]. Therefore, identification of peptide ligands and development of peptide-targeting liposome is highly desirable. Ligand-targeted therapy via targeting liposome may be able to allow us to carry higher dosage of drugs to the tumor tissue and help us overcome some of the obstacles to effective cancer therapy.

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References

1. Papac RJ. Origins of cancer therapy. *Yale J Biol Med* 74: 391-398, 2001.
2. Dunn FB. National Cancer Act: leaders reflect on 30 years of progress. *J Natl Cancer Inst* 94: 8-9, 2002.
3. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256: 495-497, 1975.
4. Harris M. Monoclonal antibodies as therapeutic agents for cancer. *Lancet Oncol* 5: 292-302, 2004.
5. Parker BA, Vassos AB, Halpern SE, Miller RA, Hupf H, Amox DG, Simoni JL, Starr RJ, Green MR, Royston I. Radioimmunotherapy of human B-cell lymphoma with 90Y-conjugated antiidiotype monoclonal antibody. *Cancer Res* 50: 1022s-1028s, 1990.
6. Stohrer M, Boucher Y, Stangassinger M, Jain RK. Oncotic pressure in solid tumors is elevated. *Cancer Res* 60: 4251-4255, 2000.
7. Yokota T, Milenic DE, Whitlow M, Schlom J. Rapid tumor penetration of a single-chain Fv and comparison with other immunoglobulin forms. *Cancer Res* 52: 3402-3408, 1992.
8. Adams GP, Schier R, Marshall K, Wolf EJ, McCall AM, Marks JD, Weiner LM. Increased affinity leads to improved selective tumor delivery of single-chain Fv antibodies. *Cancer Res* 58: 485-490, 1998.
9. Hofmeister V, Vetter C, Schrama D, Brocker EB Becker JC. Tumor stroma-associated antigens for anti-cancer immunotherapy. *Cancer Immunol Immunother* 55: 481-494, 2006.
10. Neri D, Bicknell R. Tumour vascular targeting. *Nat Rev Cancer* 5: 436-446, 2005.
11. Newman R, Alberts J, Anderson D, Carner K, Heard C, Norton F, Raab R, Reff M, Shuey S, Hanna N. "Primatization" of recombinant antibodies for immunotherapy of human diseases: a macaque/human chimeric antibody against human CD4. *Biotechnology* 10: 1455-1460, 1992.
12. McLaughlin P, Grillo-Lopez AJ, Link BK., Levy R, Czuczman MS, Williams ME, Heyman MR, Bence-Bruckler I, White CA, Cabanillas F, Jain V, Ho AD, Lister J, Wey K, Shen D, Dallaire BK. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. *J Clin Oncol* 16: 2825-2833, 1998.
13. Witzig TE, White CA, Wiseman GA, Gordon LI, Emmanouilides C, Raubitschek A, Janakiraman N, Gutheil J, Schilder RJ, Spies S, Silverman DH, Parker E, Grillo-Lopez AJ. Phase I/II trial of IDEC-Y2B8 radioimmunotherapy for treatment of relapsed or refractory CD20(+) B-cell non-Hodgkin's lymphoma. *J Clin Oncol* 17: 3793-3803, 1999.
14. Liu AY, Robinson RR, Hellstrom KE, Murray ED Jr, Chang C P, Hellstrom I. Chimeric mouse-human IgG1 antibody that can mediate lysis of cancer cells. *Proc Natl Acad Sci USA* 84: 3439-3443, 1987.
15. Riechmann L, Clark M, Waldmann H, Winter G. Reshaping human antibodies for therapy. *Nature* 332: 323-327, 1988.
16. Grillo-Lopez AJ, Hedrick E, Rashford M, Benyunes M. Rituximab: ongoing and future clinical development. *Semin Oncol* 29: 105-112, 2002.
17. Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ullrich A. p185HER2 monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor. *Mol Cell Biol* 9: 1165-1172, 1989.
18. Slamon D, Pegram M. Rationale for trastuzumab (Herceptin) in adjuvant breast cancer trials. *Semin Oncol* 28: 13-19, 2001.
19. Carter P, Presta L, Gorman CM, Ridgway JB, Henner D, Wong WL, Rowland AM, Kotts C, Carver ME, Shepard HM. Humanization of an anti-p185HER2 antibody for human cancer therapy. *Proc Natl Acad Sci USA* 89: 4285-4289, 1992.
20. Gerber HP, Ferrara N. The role of VEGF in normal and neoplastic hematopoiesis. *J Mol Med* 81: 20-31, 2003.
21. Press OW, Appelbaum F, Ledbetter JA, Martin PJ, Zarling J, Kidd P, Thomas ED. Monoclonal antibody 1F5 (anti-CD20) serotherapy of human B cell lymphomas. *Blood* 69: 584-591, 1987.
22. Reff ME, Carner K, Chambers KS, Chinn PC, Leonard JE, Raab R, Newman RA, Hanna N, Anderson DR. Depletion of B cells in vivo by a chimeric mouse human monoclonal antibody to CD20. *Blood* 83: 435-445, 1994.
23. Maloney DG, Liles TM, Czerwinski DK Waldichuk C, Rosenberg J, Grillo-Lopez A, Levy R. Phase I clinical trial using escalating single-dose infusion of chimeric anti-CD20 monoclonal antibody (IDEC-C2B8) in patients with recurrent B-cell lymphoma. *Blood* 84: 2457-2466, 1994.
24. Czuczman MS, Grillo-Lopez AJ, White CA, Saleh M, Gordon L, LoBuglio AF, Jonas C, Klippenstein D, Dallaire B, Varns C. Treatment of patients with low-grade B-cell lymphoma with the combination of chimeric anti-CD20 monoclonal antibody and CHOP chemotherapy. *J Clin Oncol* 17: 268-276, 1999.
25. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire W.L. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 235: 177-182, 1987.
26. Naito K, Takeshita A, Shigeno K, Nakamura S, Fujisawa S, Shinjo K, Yoshida H, Ohnishi K, Mori M, Terakawa S, Ohno R. Calicheamicin-conjugated humanized anti-CD33 monoclonal antibody (gemtuzumab zogamicin, CMA-676) shows cytocidal effect on CD33-positive leukemia cell lines, but is inactive on P-glycoprotein-expressing sublines. *Leukemia* 14: 1436-1443, 2000.
27. Boghaert ER, Khandke K, Sridharan L, Armellino D, Dougher M, Dijoseph JF, Kunz A, Hamann PR, Sridharan A, Jones S, Discafani C, Damle NK. Tumoricidal effect of calicheamicin immuno-conjugates using a passive targeting strategy. *Int J Oncol* 28: 675-684, 2006.
28. Genentech Inc. Herceptin® (trastuzumab). in: *Investigator brochure, Sept.* San Francisco, 1998.
29. Lozanski G, Heerema NA, Flinn IW, Smith L, Harbison J, Webb J, Moran M, Lucas M, Lin T, Hackbarth ML, Proffitt JH, Lucas D, Grever MR, Byrd JC. Alemtuzumab is an effective therapy for chronic lymphocytic leukemia with p53 mutations and deletions. *Blood* 103: 3278-3281, 2004.
30. Kaufman DB, Leventhal JR, Gallon LG, Parker MA. Alemtuzumab induction and prednisone-free maintenance immunotherapy in simultaneous pancreas-kidney transplantation comparison with rabbit antithymocyte globulin induction — long-term results. *Am J Transplant* 6: 331-339, 2006.
31. Lundin J, Osterborg A, Brittinger G, Crowther D, Dombret H, Engert A, Epenetos A, Gisselbrecht C, Huhn D, Jaeger U, Thomas J, Marcus R, Nissen N, Poynton C, Rankin E, Stahel R, Uppenkamp M, Willemze R, Mellstedt H. CAMPATH-1H monoclonal antibody in therapy for previously treated low-grade non-Hodgkin's lymphomas: a phase II multicenter study. European Study Group of CAMPATH-1H Treatment in Low-Grade Non-Hodgkin's Lymphoma. *J Clin Oncol* 16: 3257-3263, 1998.
32. Chinn PC, Leonard JE, Rosenberg J, Hanna N, Anderson DR. Preclinical evaluation of 90Y-labeled anti-CD20 monoclonal antibody for treatment of non-Hodgkin's lymphoma. *Int J Oncol* 15: 1017-1025, 1999.

33. Cheson BD. Radioimmunotherapy of non-Hodgkin lymphomas. *Blood* 101: 391-398, 2003.
34. Nademanee A, Forman S, Molina A, Fung H, Smith D, Dagis A, Kwok C, Yamauchi D, Anderson AL, Falk P, Krishnan A. Kirschbaum M, Kogut N, Nakamura R, O'Donnell, , Parker P, Popplewell L, Pullarkat V, Rodriguez R, Sahebi F, Smith E, Snyder D, Stein A, Spielberger R, Zain J, White C, Raubitschek A. A phase 1/2 trial of high-dose yttrium-90-ibritumomab tiuxetan in combination with high-dose etoposide and cyclophosphamide followed by autologous stem cell transplantation in patients with poor-risk or relapsed non-Hodgkin lymphoma. *Blood* 106: 2896-2902, 2005.
35. Kaminski MS, Zasadny KR, Francis IR, Fenner MC, Ross CW, Milik AW, Estes J, Tuck M, Regan D, Fisher S, Glenn SD, Wahl RL. Iodine-131-anti-B1 radioimmunotherapy for B-cell lymphoma. *J Clin Oncol* 14: 1974-1981, 1996.
36. Vose JM, Wahl RL, Saleh M, Rohatiner AZ, Knox SJ, Radford JA, Zelenetz AD, Tidmarsh GF, Stagg RJ, Kaminski MS. Multicenter phase II study of iodine-131 tositumomab for chemotherapy-relapsed/refractory low-grade and transformed low-grade B-cell non-Hodgkin's lymphomas. *J Clin Oncol* 18: 1316-1323, 2000.
37. Liu SY, Eary JF, Petersdorf SH, Martin PJ, Maloney DG, Appelbaum FR, Matthews DC, Bush SA, Durack LD, Fisher DR, Gooley TA, Bernstein ID, Press OW. Follow-up of relapsed B-cell lymphoma patients treated with iodine-131-labeled anti-CD20 antibody and autologous stem-cell rescue. *J Clin Oncol* 16: 3270-3278, 1998.
38. Huang SM, Bock JM, Harari PM. Epidermal growth factor receptor blockade with C225 modulates proliferation, apoptosis, and radiosensitivity in squamous cell carcinomas of the head and neck. *Cancer Res* 59: 1935-1940, 1999.
39. Baselga J, Pfister D, Cooper MR, Cohen R, Burtness B, Bos M, D'Andrea G, Seidman A, Norton L, Gunnett K, Falcey J, Anderson V, Waksal H, Mendelsohn J. Phase I studies of anti-epidermal growth factor receptor chimeric antibody C225 alone and in combination with cisplatin. *J Clin Oncol* 18: 904-914, 2000.
40. Jimeno A, Rubio-Viqueira B, Amador ML, Oppenheimer D, Bouraoud N, Kulesza P, Sebastiani V, Maitra A, Hidalgo M. Epidermal growth factor receptor dynamics influences response to epidermal growth factor receptor targeted agents. *Cancer Res* 65: 3003-3010, 2005.
41. Willett CG, Boucher Y, di Tomaso E, Duda DG, Munn LL, Tong RT, Chung DC, Sahani DV, Kalva SP, Kozin SV, Mino M, Cohen KS, Scadden DT, Hartford AC, Fischman AJ, Clark JW, Ryan DP, Zhu AX, Blaszkowsky LS, Chen HX, Shellito PC, Lauwers GY, Jain RK. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med* 10: 145-147, 2004.
42. Ferrara N, Hillan KJ, Gerber HP, Novotny W. Discovery and development of bevacizumab, an anti-VEGF antibody for treating cancer. *Nat Rev Drug Discov* 3: 391-400, 2004.
43. Sonpavde G. Bevacizumab in colorectal cancer. *N Engl J Med* 351: 1690-1691, 2004.
44. Kabbinavar FF, Schulz J, McCleod M, Patel T, Hamm JT, Hecht JR, Mass R, Perrou B, Nelson B, Novotny WF. Addition of bevacizumab to bolus fluorouracil and leucovorin in first-line metastatic colorectal cancer: results of a randomized phase II trial. *J Clin Oncol* 23: 3697-3705, 2005.
45. Chu E. *Drug development.* in: Vincent T, DeVita J, Hellman S, Rosenberg SA, ed. Cancer: Principles and Practice of Oncology, Philadelphia: Lippincott Williams and Wilkin 2005.
46. Cicenas J, Urban P, Kung W, Vuaroqueaux V, Labuhn M, Wight E, Eppenberger U, Eppenberger-Castori S. Phosphorylation of tyrosine 1248-ERBB2 measured by chemiluminescence-linked immunoassay is an independent predictor of poor prognosis in primary breast cancer patients. *Eur J Cancer* 42: 636-645, 2006.
47. Maatta JA, Sundvall M, Junttila TT, Peri L, Laine VJ, Isola J, Egeblad M, Elenius K. Proteolytic cleavage and phosphorylation of a tumor-associated ErbB4 isoform promote ligand-independent survival and cancer cell growth. *Mol Biol Cell* 17: 67-79, 2006.
48. Troussard AA, McDonald PC, Wederell ED, Mawji NM, Filipenko NR, Gelmon KA, Kucab JE, Dunn SE, Emerman JT, Bally MB, Dedhar S. Preferential dependence of breast cancer cells versus normal cells on integrin-linked kinase for protein kinase B/Akt activation and cell survival. *Cancer Res* 66: 393-403, 2006.
49. Freedman NJ, Kim LK, Murray JP, Exum ST, Brian L, Wu JH, Peppel K. Phosphorylation of the platelet-derived growth factor receptor-beta and epidermal growth factor receptor by G protein-coupled receptor kinase-2. Mechanisms for selectivity of desensitization. *J Biol Chem* 277: 48261-48269, 2002.
50. Lammering G, Hewit TH, Hawkins WT, Contessa JN, Reardon DB, Lin PS, Valerie K, Dent P, Mikkelsen RB, Schmidt-Ullrich RK. Epidermal growth factor receptor as a genetic therapy target for carcinoma cell radiosensitization. *J Natl Cancer Inst* 93: 921-929, 2001.
51. Knight ZA, Shokat KM. Features of selective kinase inhibitors. *Chem Biol* 12: 621-637, 2005.
52. Cohen P. The development and therapeutic potential of protein kinase inhibitors. *Curr Opin Chem Biol* 3: 459-465, 1999.
53. Hidaka H, Inagaki M, Kawamoto S, Sasaki Y. Isoquinolinesulfonamides, novel and potent inhibitors of cyclic nucleotide dependent protein kinase and protein kinase C. *Biochemistry* 23: 5036-5041, 1984.
54. Cohen P. Protein kinases--the major drug targets of the twenty-first century? *Nat Rev Drug Discov* 1: 309-315, 2002.
55. Dancey J, Sausville EA. Issues and progress with protein kinase inhibitors for cancer treatment. *Nat Rev Drug Discov* 2: 296-313, 2003.
56. Capdeville R, Buchdunger E, Zimmermann J, Matter A. Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nat Rev Drug Discov* 1: 493-502, 2002.
57. Clark J, Cools J, Gilliland DG. EGFR inhibition in non-small cell lung cancer: resistance, once again, rears its ugly head. *PLoS Med* 2: e75, 2005.
58. Druker BJ, Sawyers CL, Kantarjian H, Resta DJ, Reese SF, Ford JM, Capdeville R, Talpaz M. Activity of a specific inhibitor of the BCR-ABL tyrosine kinase in the blast crisis of chronic myeloid leukemia and acute lymphoblastic leukemia with the Philadelphia chromosome. *N Engl J Med* 344: 1038-1042, 2001.
59. Sawyers CL, Hochhaus A, Feldman E, Goldman JM, Miller CB, Ottmann OG, Schiffer CA, Talpaz M, Guilhot F, Deininger MW, Fischer T, O'Brien SG, Stone RM, Gambacorti-Passerini CB, Russell NH, Reiffers JJ, Shea TC, Chapuis B, Coutre S, Tura S, Morra E, Larson RA, Saven A, Peschel C, Gratwohl A, Mandelli F, Ben-Am M, Gathmann I, Capdeville R, Paquette RL, Druker BJ. Imatinib induces hematologic and cytogenetic responses in patients with chronic myelogenous leukemia in myeloid blast crisis: results of a phase II study. *Blood* 99: 3530-3539, 2002.
60. Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. *Science* 247: 1079-1082, 1990.
61. Okuda K, Weisberg E, Gilliland DG, Griffin JD. ARG tyrosine kinase activity is inhibited by STI571. *Blood* 97: 2440-2448, 2001.
62. Druker BJ. Inhibition of the Bcr-Abl tyrosine kinase as a therapeutic strategy for CML. *Oncogene* 21: 8541-8546, 2002.
63. Joensuu H, Fletcher C, Dimitrijevic S, Silberman S, Roberts P, Emetri G. Management of malignant gastrointestinal stromal tumours. *Lancet Oncol* 3: 655-664, 2002.
64. Rubin BP, Schuetze SM, Eary JF, Norwood TH, Mirza S, Conrad EU,Bruckner JD. Molecular targeting of platelet-derived growth factor B by imatinib mesylate in a patient with metastatic dermatofibrosarcoma protuberans. *J Clin Oncol* 20: 3586-3591, 2002.
65. Druker BJ, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J,Lydon NB. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* 2: 561-566, 1996.
66. Siwak DR, Tari AM,Lopez-Berestein G. The potential of drug-carrying immunoliposomes as anticancer agents. Commentary re: J. W. Park et al., Anti-HER2 immunoliposomes: enhanced efficacy due to targeted delivery. *Clin Cancer Res* 8: 955-956, 2002.
67. Holtz MS, Slovak ML, Zhang F, Sawyers CL, Forman SJ,Bhatia R. Imatinib mesylate (STI571) inhibits growth of primitive malignant progenitors in chronic myelogenous leukemia through reversal of abnormally increased proliferation. *Blood* 99: 3792-3800, 2002.
68. Hughes TP, Kaeda J, Branford S, Rudzki Z, Hochhaus A, Hensley ML, Gathmann I, Bolton AE, van Hoomissen IC, Goldman JM, Radich JP. Frequency of major molecular responses to imatinib or interferon alfa plus cytarabine in newly diagnosed chronic myeloid leukemia. *N Engl J Med* 349: 1423-1432, 2003.
69. Einhorn LH, Brames MJ, Heinrich MC, Corless CL, Madani A. Phase II study of imatinib mesylate in chemotherapy refractory germ cell tumors expressing KIT. *Am J Clin Oncol* 29: 12-13, 2006.
70. Vuky J, Isacson C, Fotoohi M, dela Cruz J, Otero H, Picozzi V, Malpass T, Aboulafia D, Jacobs A. Phase II trial of imatinib (Gleevec) in patients with metastatic renal cell carcinoma. *Invest New Drugs* 24; 85-88, 2006.
71. Dy GK, Miller AA, Mandrekar SJ, Aubry MC, Langdon RM Jr, Morton RF, Schild SE, Jett JR, Adjei AA. A phase II trial of imatinib (ST1571) in patients with c-kit expressing relapsed small-cell lung cancer: a CALGB and NCCTG study. *Ann Oncol* 16: 1811-1816, 2005.
72. Dispenzieri A, Gertz MA, Lacy MQ, Geyer SM, Greipp PR, Rajkumar SV, Kimlinger T, Lust JA, Fonseca R, Allred J, Witzig TE. A phase II trial of imatinib in patients with refractory/relapsed myeloma. *Leuk Lymphoma* 47: 39-42, 2006.
73. Rao K, Goodin S, Levitt MJ, Dave N, Shih WJ, Lin Y, Capanna T, Doyle-Lindrud S, Juvidian P, DiPaola RS. A phase II trial of imatinib mesylate in patients with prostate specific antigen progression after local therapy for prostate cancer. *Prostate* 62: 115-122, 2005.
74. Modi S, Seidman AD, Dickler M, Moasser M, D'Andrea G, Moynahan ME, Menell J, Panageas KS, Tan LK, Norton L, Hudis CA. A phase II trial of imatinib mesylate monotherapy in patients with metastatic breast cancer. *Breast Cancer Res Treat* 90: 157-163, 2005.

Print ISSN 1816-0735; Online ISSN 1817-4256

75. Seget S. *Orphan Drugs to 2008 - Understanding regulation and market opportunity in Europe*. London: Urch Publishing 2005.
76. Bhatia R, Holtz M, Niu N, Gray R, Snyder DS, Sawyers CL, Arber DA, Slovak ML, Forman SJ. Persistence of malignant hematopoietic progenitors in chronic myelogenous leukemia patients in complete cytogenetic remission following imatinib mesylate treatment. *Blood* 101: 4701-4707, 2003.
77. Chu S, Xu H, Shah NP, Snyder DS, Forman SJ, Sawyers CL, Bhatia R. Detection of BCR-ABL kinase mutations in CD34+ cells from chronic myelogenous leukemia patients in complete cytogenetic remission on imatinib mesylate treatment. *Blood* 105: 2093-2098, 2005.
78. Michor F, Hughes TP, Iwasa Y, Branford S, Shah NP, Sawyers CL, Nowak MA. Dynamics of chronic myeloid leukaemia. *Nature* 435: 1267-1270, 2005.
79. Wakeling AE, Guy SP, Woodburn JR, Ashton SE, Curry BJ, Barker AJ, Gibson KH. ZD1839 (Iressa): an orally active inhibitor of epidermal growth factor signaling with potential for cancer therapy. *Cancer Res* 62: 5749-5754, 2002.
80. Albanell J, Rojo F, Averbuch S, Feyereislova A, Mascaro JM, Herbst R, LoRusso P, Rischin D, Sauleda S, Gee J, Nicholson RI, Baselga J. Pharmacodynamic studies of the epidermal growth factor receptor inhibitor ZD1839 in skin from cancer patients: histopathologic and molecular consequences of receptor inhibition. *J Clin Oncol* 20: 110-124, 2002.
81. Herbst RS, Maddox AM, Rothenberg ML, Small EJ, Rubin EH, Baselga J, Rojo F, Hong WK, Swaisland H, Averbuch SD, Ochs J, LoRusso PM. Selective oral epidermal growth factor receptor tyrosine kinase inhibitor ZD1839 is generally well-tolerated and has activity in non-small-cell lung cancer and other solid tumors: results of a phase I trial. *J Clin Oncol* 20: 3815-3825, 2002.
82. Muhsin M, Graham J, Kirkpatrick P. Gefitinib. *Nat Rev Drug Discov* 2: 515-516, 2003.
83. Janne PA, Engelman JA, Johnson BE. Epidermal growth factor receptor mutations in non-small-cell lung cancer: implications for treatment and tumor biology. *J Clin Oncol* 23: 3227-3234, 2005.
84. Chen YM. Clinical application of epidermal growth factor receptor-tyrosine kinase inhibitors against non-small cell lung cancer. *J Cancer Mol* 1: 83-91, 2005.
85. Baselga J. Targeting the epidermal growth factor receptor with tyrosine kinase inhibitors: small molecules, big hopes. *J Clin Oncol* 20: 2217-2219, 2002.
86. Baselga J, Rischin D, Ranson M, Calvert H, Raymond E, Kieback DG, Kaye SB, Gianni L, Harris A, Bjork T, Averbuch SD, Feyereislova A, Swaisland H, Rojo F, Albanell J. Phase I safety, pharmacokinetic, and pharmacodynamic trial of ZD1839, a selective oral epidermal growth factor receptor tyrosine kinase inhibitor, in patients with five selected solid tumor types. *J Clin Oncol* 20: 4292-4302, 2002.
87. Fukuoka M, Yano S, Giaccone G, Tamura T, Nakagawa K, Douillard, JY, Nishiwaki Y, Vansteenkiste J, Kudoh S, Rischin D, Eek R, Horai T, Noda K, Takata I, Smit E, Averbuch S, Macleod A, Feyereislova A, Dong RP, Baselga J. Multi-institutional randomized phase II trial of gefitinib for previously treated patients with advanced non-small-cell lung cancer (The IDEAL 1 Trial) [corrected]. *J Clin Oncol* 21: 2237-2246, 2003.
88. Giaccone G, Herbst RS, Manegold C, Scagliotti G, Rosell R, Miller V, Natale RB, Schiller JH, Von Pawel J, Pluzanska A, Gatzemeier U, Grous J, Ochs JS, Averbuch SD, Wolf MK, Rennie P, Fandi A, Johnson DH. Gefitinib in combination with gemcitabine and cisplatin in advanced non-small-cell lung cancer: a phase III trial--INTACT 1. *J Clin Oncol* 22: 777-784, 2004.
89. Sordella R, Bell DW, Haber DA, Settleman J. Gefitinib-sensitizing EGFR mutations in lung cancer activate anti-apoptotic pathways. *Science* 305: 1163-1167, 2004.
90. Birnbaum A, Ready N. Gefitinib therapy for non-small cell lung cancer. *Curr Treat Options Oncol* 6: 75-81, 2005.
91. Laux I, Jain A, Singh S, Agus DB. Epidermal growth factor receptor dimerization status determines skin toxicity to HER-kinase targeted therapies. *Br J Cancer* 94: 85-92, 2006.
92. Reck M, Gatzemeier U. Benefit in lung function improvement and side-effect profile of long-term responders: an analysis of 14 NSCLC patients treated for at least 9 months with gefitinib. *Lung Cancer* 50: 107-114, 2005.
93. Elkind NB, Szentpetery Z, Apati A, Ozvegy-Laczka C, Varady G, Ujhelly O, Szabo K, Homolya L, Varadi A, Buday L, Keri G, Nemet K, Sarkadi B. Multidrug transporter ABCG2 prevents tumor cell death induced by the epidermal growth factor receptor inhibitor Iressa (ZD1839, Gefitinib). *Cancer Res* 65: 1770-1777, 2005.
94. Chabner BA, Roberts TG Jr. Timeline: Chemotherapy and the war on cancer. *Nat Rev Cancer* 5: 65-72, 2005.
95. Roberts TG Jr, Chabner BA. Beyond fast track for drug approvals. *N Engl J Med* 351: 501-505, 2004.
96. Jain RK. Transport of molecules in the tumor interstitium: a review. *Cancer Res* 47: 3039-3051, 1987.
97. Less JR, Posner MC, Boucher Y, Borochovitz D, Wolmark N, Jain RK. Interstitial hypertension in human breast and colorectal tumors. *Cancer Res* 52: 6371-6374, 1992.
98. Nathanson SD, Nelson L. Interstitial fluid pressure in breast cancer, benign breast conditions, and breast parenchyma. *Ann Surg Oncol* 1: 333-338, 1994.
99. Boucher Y, Kirkwood JM, Opacic D, Desantis M, Jain RK. Interstitial hypertension in superficial metastatic melanomas in humans. *Cancer Res* 51: 6691-6694, 1991.
100. Curti BD, Urba WJ, Alvord WG, Janik JE, Smith JW 2nd, Madara K, Longo DL. Interstitial pressure of subcutaneous nodules in melanoma and lymphoma patients: changes during treatment. *Cancer Res* 53: 2204-2207, 1993.
101. Gutmann R, Leunig M, Feyh J, Goetz AE, Messmer K, Kastenbauer E, Jain RK. Interstitial hypertension in head and neck tumors in patients: correlation with tumor size. *Cancer Res* 52: 1993-1995, 1992.
102. Boucher Y, Baxter LT, Jain RK. Interstitial pressure gradients in tissue-isolated and subcutaneous tumors: implications for therapy. *Cancer Res* 50: 4478-4484, 1990.
103. DiResta GR, Lee J, Larson SM, Arbit E. Characterization of neuroblastoma xenograft in rat flank. I. Growth, interstitial fluid pressure, and interstitial fluid velocity distribution profiles. *Microvasc Res* 46: 158-177, 1993.
104. Pietras K, Rubin K, Sjoblom T, Buchdunger E, Sjoquist M, Heldin CH Ostman A. Inhibition of PDGF receptor signaling in tumor stroma enhances antitumor effect of chemotherapy. *Cancer Res* 62: 5476-5484, 2002.
105. Pietras K, Stumm M, Hubert M, Buchdunger E, Rubin K, Heldin CH, McSheehy P, Wartmann M, Ostman A. STI571 enhances the therapeutic index of epothilone B by a tumor-selective increase of drug uptake. *Clin Cancer Res* 9: 3779-3787, 2003.
106. Wildiers H, Guetens G, De Boeck G, Verbeken E, Landuyt B, Landuyt W, de Bruijn EA, van Oosterom AT. Effect of antivascular endothelial growth factor treatment on the intratumoral uptake of CPT-11. *Br J Cancer* 88: 1979-1986, 2003.
107. Milosevic M, Fyles A, Hedley D, Pintilie M, Levin W, Manchul L, Hill R. Interstitial fluid pressure predicts survival in patients with cervix cancer independent of clinical prognostic factors and tumor oxygen measurements. *Cancer Res* 61: 6400-6405, 2001.
108. Roh HD, Boucher Y, Kalnicki S, Buchsbaum R, Bloomer WD, Jain RK. Interstitial hypertension in carcinoma of uterine cervix in patients: possible correlation with tumor oxygenation and radiation response. *Cancer Res* 51: 6695-6698, 1991.
109. Dean M, Fojo T, Bates S. Tumour stem cells and drug resistance. *Nat Rev Cancer* 5: 275-284, 2005.
110. D'Mello F, Partidos CD, Steward MW, Howard CR. Definition of the primary structure of hepatitis B virus (HBV) pre-S hepatocyte binding domain using random peptide libraries. *Virology* 237: 319-326, 1997.
111. Fu Y, Shearing LN, Haynes S, Crewther P, Tilley L, Anders RF, Foley M. Isolation from phage display libraries of single chain variable fragment antibodies that recognize conformational epitopes in the malaria vaccine candidate, apical membrane antigen-1. *J Biol Chem* 272: 25678-25684, 1997.
112. Scott JK, Smith GP. Searching for peptide ligands with an epitope library. *Science* 249: 386-390, 1990.
113. Wu HC, Huang YL, Chao TT, Jan JT, Huang JL, Chiang HY, King CC, Shaio MF. Identification of B-cell epitope of dengue virus type 1 and its application in diagnosis of patients. *J Clin Microbiol* 39: 977-982, 2001.
114. Wu HC, Jung MY, Chiu CY, Chao TT, Lai SC, Jan JT, Shaio MF. Identification of a dengue virus type 2 (DEN-2) serotype-specific B-cell epitope and detection of DEN-2-immunized animal serum samples using an epitope-based peptide antigen. *J Gen Virol* 84: 2771-2779, 2003.
115. Atwell S, Ultsch M, De Vos AM, Wells JA. Structural plasticity in a remodeled protein-protein interface. *Science* 278: 1125-1128, 1997.
116. Bottger V, Bottger A, Howard SF, Picksley SM, Chene P, Garcia-Echeverria C, Hochkeppel HK, Lane DP. Identification of novel mdm2 binding peptides by phage display. *Oncogene* 13: 2141-2147, 1996.
117. Nord K, Gunneriusson E, Ringdahl J, Stahl S, Uhlen M, Nygren PA. Binding proteins selected from combinatorial libraries of an alpha-helical bacterial receptor domain. *Nat Biotechnol* 15: 772-777, 1997.
118. Smith WC, McDowell JH, Dugger DR, Miller R, Arendt A, Popp MP, Hargrave PA. Identification of regions of arrestin that bind to rhodopsin. *Biochemistry* 38: 2752-2761, 1999.
119. Li B, Tom JY, Oare D, Yen R, Fairbrother WJ, Wells JA, Cunningham BC. Minimization of a polypeptide hormone. *Science* 270: 1657-1660, 1995.
120. Wrighton NC, Farrell FX, Chang R, Kashyap AK, Barbone FP, Mulcahy LS, Johnson DL, Barrett RW, Jolliffe LK, Dower WJ. Small peptides as potent mimetics of the protein hormone erythropoietin. *Science* 273: 458-464 1996.

121. Koivunen E, Arap W, Rajotte D, Lahdenranta J, Pasqualini R. Identification of receptor ligands with phage display peptide libraries. *J Nucl Med* 40: 883-888, 1999.
122. Castano AR, Tangri S, Miller JE, Holcombe HR, Jackson MR, Huse WD, Kronenberg M, Peterson PA. Peptide binding and presentation by mouse CD1. *Science* 269: 223-226, 1995.
123. DeLeo FR, Yu L, Burritt JB, Loetterle LR, Bond CW, Jesaitis AJ, Quinn MT. Mapping sites of interaction of p47-phox and flavocytochrome b with random-sequence peptide phage display libraries. *Proc Natl Acad Sci U S A* 92: 7110-7114, 1995.
124. Kraft S, Diefenbach B, Mehta R, Jonczyk A, Luckenbach GA, Goodman SL. Definition of an unexpected ligand recognition motif for alphav beta6 integrin. *J Biol Chem* 274: 1979-1985, 1999.
125. Pasqualini R, Koivunen E, Ruoslahti E. A peptide isolated from phage display libraries is a structural and functional mimic of an RGD-binding site on integrins. *J Cell Biol* 130: 1189-1196, 1995.
126. Folgori A, Tafi R, Meola A, Felici F, Galfre G, Cortese R, Monaci P, Nicosia A. A general strategy to identify mimotopes of pathological antigens using only random peptide libraries and human sera. *EMBO J* 13: 2236-2243, 1994.
127. Prezzi C, Nuzzo M, Meola A, Delmastro P, Galfre G, Cortese R, Nicosia A, Monaci P. Selection of antigenic and immunogenic mimics of hepatitis C virus using sera from patients. *J Immunol* 156: 4504-4513, 1996.
128. Liu IJ, Hsueh PR, Lin CT, Chiu CY, Kao CL, Liao MY, Wu HC. Disease-specific B Cell epitopes for serum antibodies from patients with severe acute respiratory syndrome (SARS) and serologic detection of SARS antibodies by epitope-based peptide antigens. *J Infect Dis* 190: 797-809, 2004.
129. Barry MA, Dower WJ, Johnston SA. Toward cell-targeting gene therapy vectors: selection of cell-binding peptides from random peptide-presenting phage libraries. *Nat Med* 2: 299-305, 1996.
130. Mazzucchelli L, Burritt JB, Jesaitis AJ, Nusrat A, Liang TW, Gewirtz AT, Schnell FJ, Parkos CA. Cell-specific peptide binding by human neutrophils. *Blood* 93: 1738-1748, 1999.
131. Lee TY, Wu HC, Tseng YL, Lin CT. A novel peptide specifically binding to nasopharyngeal carcinoma for targeted drug delivery. *Cancer Res* 64: 8002-8008, 2004.
132. Arap W, Pasqualini R, Ruoslahti E. Cancer treatment by targeted drug delivery to tumor vasculature in a mouse model. *Science* 279: 377-380, 1998.
133. Essler M, Ruoslahti E. Molecular specialization of breast vasculature: a breast-homing phage-displayed peptide binds to aminopeptidase P in breast vasculature. *Proc Natl Acad Sci U S A* 99: 2252-2257, 2002.
134. Pasqualini R, Ruoslahti E. Organ targeting in vivo using phage display peptide libraries. *Nature* 380: 364-366, 1996.
135. Allen TM. Ligand-targeted therapeutics in anticancer therapy. *Nat Rev Cancer* 2: 750-763, 2002.
136. Northfelt DW, Martin FJ, Working P, Volberding PA, Russell J, Newman M, Amantea MA, Kaplan LD. Doxorubicin encapsulated in liposomes containing surface-bound polyethylene glycol: pharmacokinetics, tumor localization, and safety in patients with AIDS-related Kaposi's sarcoma. *J Clin Pharmacol* 36: 55-63, 1996.
137. Yuan F, Leunig M, Huang SK, Berk DA, Papahadjopoulos D, Jain RK. Microvascular permeability and interstitial penetration of sterically stabilized (stealth) liposomes in a human tumor xenograft. *Cancer Res* 54: 3352-3356, 1994.
138. Hashizume H, Baluk P, Morikawa S, McLean JW, Thurston G, Roberge S, Jain RK, McDonald DM. Openings between defective endothelial cells explain tumor vessel leakiness. *Am J Pathol* 156: 1363-1380, 2000.
139. Ruoslahti E, Specialization of tumour vasculature. *Nat Rev Cancer* 2: 83-90, 2002.
140. Sapra P, Allen TM. Improved outcome when B-cell lymphoma is treated with combinations of immunoliposomal anticancer drugs targeted to both the CD19 and CD20 epitopes. *Clin Cancer Res* 10: 2530-2537, 2004.
141. Lopes de Menezes DE, Kirchmeier MJ, Gagne J-F, Pilarski LM, Allen TM. Cellular Trafficking and Cytotoxicity of Anti-CD19-Targeted Liposomal Doxorubicin in B Lymphoma Cells. *J. Liposome Res.* 9: 199-228, 1999.
142. Gabizon A, Shmeeda H, Horowitz AT, Zalipsky S. Tumor cell targeting of liposome-entrapped drugs with phospholipid-anchored folic acid-PEG conjugates. *Adv Drug Deliv Rev* 56: 1177-1192, 2004.
143. Medina OP, Zhu Y, Kairemo K. Targeted liposomal drug delivery in cancer. *Curr Pharm Des* 10: 2981-2989, 2004.
144. Noble CO, Kirpotin DB, Hayes ME, Mamot C, Hong K, Park JW, Benz CC, Marks JD, Drummond DC. Development of ligand-targeted liposomes for cancer therapy. *Expert Opin Ther Targets* 8: 335-353, 2004.
145. Sapra P, Moase EH, Ma J, Allen, TM. Improved therapeutic responses in a xenograft model of human B lymphoma (Namalwa) for liposomal vincristine versus liposomal doxorubicin targeted via anti-CD19 IgG2a or Fab' fragments. *Clin Cancer Res* 10: 1100-1111, 2004.
146. Sapra P, Allen TM. Internalizing antibodies are necessary for improved therapeutic efficacy of antibody-targeted liposomal drugs. *Cancer Res* 62: 7190-7194, 2002.
147. Pastorino F, Brignole C, Marimpietri D, Cilli M, Gambini C, Ribatti D, Longhi R, Allen TM, Corti A, Ponzoni M. Vascular damage and anti-angiogenic effects of tumor vessel-targeted liposomal chemotherapy. *Cancer Res* 63: 7400-7409, 2003.
148. Pastorino F, Brignole C, Marimpietri D, Pagnan G, Morando A, Ribatti D, Semple SC, Gambini C, Allen TM, Ponzoni M. Targeted liposomal c-myc antisense oligodeoxynucleotides induce apoptosis and inhibit tumor growth and metastases in human melanoma models. *Clin Cancer Res* 9: 4595-4605, 2003.
149. Pastorino F, Brignole C, Marimpietri D, Sapra P, Moase EH, Allen TM, Ponzoni M. Doxorubicin-loaded Fab' fragments of anti-disialoganglioside immunoliposomes selectively inhibit the growth and dissemination of human neuroblastoma in nude mice. *Cancer Res* 63: 86-92, 2003.
150. Pagnan G, Stuart DD, Pastorino F, Raffaghello L, Montaldo PG, Allen TM, Calabretta B, Ponzoni M. Delivery of c-myb antisense oligodeoxynucleotides to human neuroblastoma cells via disialoganglioside GD(2)-targeted immunoliposomes: antitumor effects. *J Natl Cancer Inst* 92: 253-261, 2000.
151. Park JW, Hong K, Kirpotin DB, Colbern G, Shalaby R, Baselga J, Shao Y, Nielsen UB, Marks JD, Moore D, Papahadjopoulos D, Benz CC. Anti-HER2 immunoliposomes: enhanced efficacy attributable to targeted delivery. *Clin Cancer Res* 8: 1172-1181, 2002.
152. Speth PA, van Hoesel QG, Haanen C. Clinical pharmacokinetics of doxorubicin. *Clin Pharmacokinet* 15: 15-31, 1988.
153. Drummond DC, Meyer O, Hong K, Kirpotin DB, Papahadjopoulos D. Optimizing liposomes for delivery of chemotherapeutic agents to solid tumors. *Pharmacol Rev* 51: 691-743, 1999.
154. Gabizon A, Martin F. Polyethylene glycol-coated (pegylated) liposomal doxorubicin. Rationale for use in solid tumours. *Drugs* 54 Suppl 4: 15-21, 1997.
155. Martin FJ. Clinical pharmacology and antitumor efficacy of DOXIL (pegylated liposomal doxorubicin). *in Medical Applications of Liposomes* 635-688 (Elsevier Science BV, New York, 1998).
156. Papahadjopoulos D, Allen TM, Gabizon A, Mayhew E, Matthay K, Huang SK, Lee KD, Woodle MC, Lasic DD, Redemann C, Martin FJ. Sterically stabilized liposomes: improvements in pharmacokinetics and antitumor therapeutic efficacy. *Proc Natl Acad Sci USA* 88: 11460-11464, 1991.
157. Dvorak HF, Nagy JA, Dvorak AM. Structure of solid tumors and their vasculature: implications for therapy with monoclonal antibodies. *Cancer Cell* 3: 77-85, 1991.
158. Shockley TR, Lin K, Nagy JA, Tompkins RG, Dvorak HF, Yarmush ML. Penetration of tumor tissue by antibodies and other immunoproteins. *Ann N Y Acad Sci* 618: 367-382, 1991.

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