TIMELINE

Chemotherapy and the war on cancer

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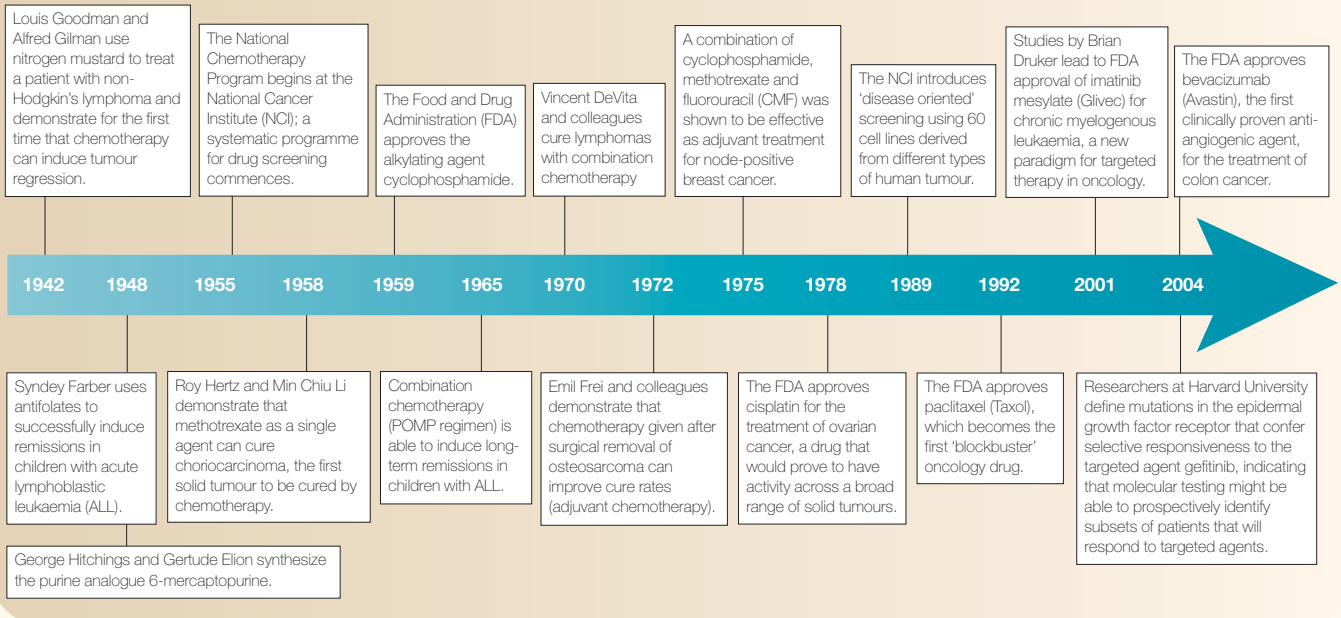
Abstract | The era of chemotherapy began in the 1940s with the first uses of nitrogen mustards and antifolate drugs. Cancer drug development since then has transformed from a low-budget, government-supported research effort to a high-stakes, multi-billion dollar industry. The targeted-therapy revolution has arrived, but the principles and limitations of chemotherapy discovered by the early researchers still apply. This article chronicles the history of modern chemotherapy and identifies remaining challenges for the next generation of researchers.

Of the many challenges of medicine, none has had a more controversial beginning and none has experienced more hard-fought progress than the treatment and cure of cancer. Although the neoplastic process has been recognized for centuries, little was known about the biological mechanisms of transformation and tumour progression until the advent of molecular medicine in the latter half of the twentieth century. Before 1950, therapy remained largely the province of the surgeon. Radiation therapy became a valuable tool for control of local and regional disease after 1960, with the invention of the linear accelerator, but, like surgery, could not eradicate metastatic cancer. Effective treatment for most patients needed to reach every organ in the body. Drugs, biological molecules and immune-mediated therapies have therefore become the focus for current efforts to cure cancer. From the first experiments with nitrogen mustard 60 years ago to current attempts to develop drugs for specific cancer-related targets, researchers from multiple disciplines have joined together in the search for more effective cancer drugs. Over time, the development of anticancer therapies, based at first on empirical observations, has become increasingly dependent on an understanding of human tumour biology.

The first efforts (1940–1950)

The beginnings of the modern era of chemotherapy can be traced directly to the discovery of nitrogen mustard as an effective treatment for cancer[1] (see TIMELINE). In 1942, Louis Goodman and Alfred Gilman and colleagues were recruited by the United

Timeline | **The history of chemotherapy**



States Department of Defense to examine the potential therapeutic value of a series of toxins developed for chemical warfare. In May 1942, Goodman and Gilman, both pharmacologists at the Yale School of Medicine, convinced their collaborator, Gustav Lindskog, a thoracic surgeon, to treat a patient with non-Hodgkin's lymphoma with nitrogen mustard[2]. They proposed that this reagent might destroy a lymphoid tumour, based on autopsy findings from soldiers dying of exposure to sulphur mustard gas during the First World War. These victims had profound lymphoid hypoplasia and myelosuppression. Reasoning that measured doses of a similar agent might cause regression of a lymphatic tumour[3], Goodman and Gilman carried out experiments in mice bearing a transplanted lymphoid tumour. When they observed a marked level of tumour regression, they convinced Lindskog to inject the closely related compound 'nitrogen mustard', a simple but highly reactive molecule, into the bloodstream of a patient with advanced non-Hodgkin's lymphoma and airway obstruction. The mediastinal and lymphatic masses of the patient regressed. This remission, however, lasted only a few weeks, and disease again progressed, but the principle was established that drugs could be administered systemically to induce tumour regression.

The same scientists next pursued studies to define the molecular action of the mustard compound, demonstrating its formation of an alkylating intermediate, the ethyleneimmonium ring, which reacted with electron-donating sites on proteins and nucleic acids. The principle was established that tumours might be more susceptible to toxins than normal tissues, although the reasons for this were not understood. The discovery that the reagent formed a covalent bond with DNA was made through later studies that demonstrated specific sites of alkylation on purine bases, leading to crosslinking of strands and induction of apoptosis. Other improved alkylating agents, developed in the following 20 years, were chemically stabilized through electron-rich substitutions, and could be administered orally. Cyclophosphamide, chlorambucil and others became standard components of regimens used to treat patients with lymphomas, leukaemias and, to a limited extent, solid tumours. Unfortunately, Goodman and his collaborators noted in their earliest experiments that tumours quickly became resistant to these drugs — an observation that predicted the clinical experience with single-agent nitrogen mustards.

Figure 1 | **Sydney Farber working at his microscope**. Courtesy of the Dana-Farber Cancer Institute, Boston, Massachusetts, USA.

The antifolates

A second approach to drug therapy of cancer began shortly after the Second World War, when Sydney Farber (FIG. 1), a pathologist at Harvard Medical School and at the Children's Hospital in Boston, investigated the effects of folic acid on patients with leukaemia. This vitamin, which had been identified by Lucy Wills in 1937 to be the factor that was deficient in patients with megaloblastic anaemia[4], seemed to stimulate proliferation of acute lymphoblastic leukaemia (ALL) cells when administered to children with this cancer. Farber's collaboration with Harriett Kilte and the medicinal chemists at Lederle Laboratories led to the synthesis of folate analogues — first aminopterin and then amethopterin (methotrexate) — which Farber administered to children with ALL in the late 1940s (REF. 5). By blocking the function of folate-requiring enzymes, these agents became the first drugs to successfully induce remission in children with ALL. Remissions were brief, but the principle was clear — antifolates could suppress proliferation of malignant cells, and could thereby re-establish normal bone-marrow function.

As a single agent, methotrexate proved to have antitumour activity in a range of epithelial malignancies, including breast, ovarian, bladder, and head and neck cancers. However, its most remarkable effects were recognized in two uncommon tumours. In 1958, 8 years after Farber's discovery of antifolates, Roy Hertz and Min Chiu Li at the National Cancer Institute (NCI)[6] found that methotrexate treatment alone could cure choriocarcinoma[7], a germ-cell malignancy that originates in trophoblastic cells of the placenta. This was the first solid tumour to be cured by drug therapy in humans. Further usefulness of methotrexate was demonstrated 16 years later, in 1974, when Emil Frei and colleagues demonstrated that high doses of methotrexate with leucovorin prevented recurrence of osteosarcoma following surgical removal of the primary tumour, establishing the principle of adjuvant therapy[8,9]. Although this therapy was associated with bone-marrow toxicity, the toxic effects were reversible, whereas the antitumour effects cured patients of their cancer.

The basis for selective effects of these agents against tumour cells versus normal tissue was not apparent from the early laboratory or clinical experiments. It would take 10 years after the initial studies by Farber and colleagues for Michael Osborn and Frank Huennekens to discover, in 1958, that the antifolate drugs specifically inhibited dihydrofolate reductase (DHFR)[10,11]. Subsequently, Joseph Bertino (FIG. 2), David Goldman, Robert Schimke and Bruce Chabner provided further insight into the mechanisms of methotrexate[12], leading to the model for our current understanding of the pharmacological principles of cancer chemotherapy. The action of methotrexate depends on active transport into cells through the reduced-folate transporter 1 (RFT-1), its conversion to a long-lived intracellular polyglutamate, and its binding to DHFR, which leads to inhibition of the synthesis of thymidylate and purines and the induction of apoptosis (FIG. 3).

Cellular defects in any of these steps can lead to drug resistance. Mutations in RFT-1, amplification or mutation of DHFR, loss of polyglutamation, and defects in the apoptotic pathway have all been shown to lead to loss of efficacy[13,14]. Methotrexate was also the first drug for which pharmacokinetic analysis was routinely used to monitor drug clearance and identify patients at risk of severe toxicity[15]. Methotrexate is still primarily used to treat patients with ALL, as well as those with certain lymphomas, osteosarcoma and choriocarcinoma. The

Figure 2 | **Mentor, Joe Bertino, and student, Bruce Chabner, at Yale University in 1970 with Barbara Morrison**. Reproduced with permission from REF. 90 © AlphaMed Press (2001).

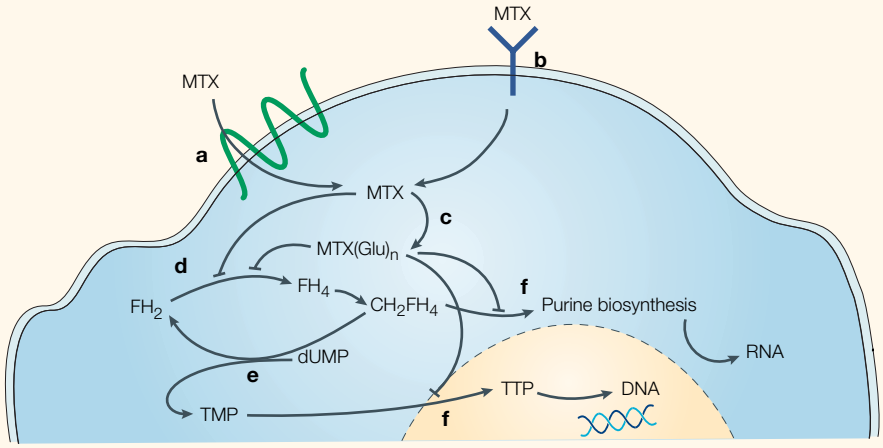


Figure 3 | **Mechanism of action of methotrexate.** Methotrexate (MTX) enters the cell through the reduced folate carrier (**a**) using an endocytic pathway activated by a folate receptor (**b**). After entering the cell, methotrexate is polyglutamated (Glu) by the the enzyme folylpolyglutamate synthase (**c**). Methotrexate and its polyglutamates inhibit the enzyme dihydrofolate reductase (**d**), thereby blocking the conversion of dihydrofolate (FH2) to tetrahydrofolate (FH4). As tetrahydrofolate stores are depleted, thymidylate (TMP) synthesis (**e**) is reduced,which ultimately inhibits DNA synthesis (**f**). Long-chain polyglutamates of MTX have the same affinity as MTX for the target enzyme dihydrofolate reductase, but have markedly increased inhibitory effects on both thymidylate synthesis (**e**) and purine biosynthesis (**f**), which is required for RNA production. Adapted from figure 1 of REF. 91.

lessons learned from studies of this drug have provided a valuable model for understanding mechanisms of resistance for other agents.

Beginnings of modern chemotherapy

Other antileukaemic drugs came to clinical trials in the early 1950s through the work of George Hitchings and Gertrude Elion, who studied purine analogues such as 6-mercaptopurine (6-MP)[16,17]. In synthesizing 6-MP, Elion and colleagues demonstrated that small changes in a compound needed by cells could inhibit the growth of tumour cells in part through the *de novo* inhibition of early steps preceding RNA and DNA synthesis. Furthermore, the Eli Lilly natural products group found that *Vinca* alkaloids, originally discovered in a screen for antidiabetic agents, blocked proliferation of tumour cells[18]. The antitumour effect of the *Vinca* alkaloids was later shown to be due to their ability to inhibit microtubule polymerization, and therefore cell division[19]. Finally, James Holland, Emil Freireich, and Emil Frei showed in 1965 that a combination of methotrexate (an antifolate), vincristine (a *Vinca* alkaloid), 6-MP and prednisone — which together was referred to as the POMP regimen — could induce long-term remissions in children with ALL[20]. In a manner similar to that of antibiotic therapy for tuberculosis and subacute bacterial endocarditis, combinations of drugs, each with a different site of action, proved to be the most effective way to prevent drug-resistant tumour cells. From the 1970s to the present, researchers at The St. Jude's Children's Research Hospital in Memphis, Tennessee, under the leadership of Donald Pinkel, Joseph Simone and their successors, developed and refined curative therapy for ALL.

A national treatment research effort

This success in treating ALL led to legislation by the United States Congress to create a National Cancer Chemotherapy Service Center (NCCSC) at the NCI in 1955, the first federal programme to promote drug discovery for cancer. As most pharmaceutical companies were not yet interested in developing anticancer drugs, the NCCSC established all the necessary components for the discovery, development, toxicological testing and clinical evaluation of candidate agents. Novel components of the NCCSC included the development of animal models of cancer, the P388 and L1210 lymphoid leukaemia cell lines, and a range of transplantable solid tumours[21–23]. Subsequently, the clinical programmes of the NCCSC were re-shaped into the Cancer Therapy Evaluation Program (see online links box), which continues to have a crucial role in the development of cancer drugs; and the preclinical efforts fell under the Developmental Therapeutics Program. Paul Carbone and Marvin Zelen of the NCI led the efforts to establish broad-based cooperative group trials for the common solid tumours.

In the 1960s, seminal experiments of Frank Schabel and Howard Skipper at the Southern Research Institute complemented the NCI's work, by forming an intellectual framework for analysing the kinetics of tumour growth, as well as the creation of *in vivo* assays for quantifying cytotoxicity[24,25]. They showed that each dose of anticancer drug therapy killed a fraction of tumour cells. Depending on the drug, cell killing could require exposure of cells during a particular stage in the cell cycle. Inhibitors of DNA synthesis, such as cytosine arabinoside and methotrexate, were most effective against rapidly dividing cells, whereas drugs that physically damaged DNA, such as alkylating agents, killed cells in all phases of the cell cycle. These researchers also showed that cytotoxicity was a direct function of dose, and demonstrated the effectiveness of combination therapies in preventing drug resistance. Finally, Schabel and Skipper were the first to suggest that high-dose chemotherapy might be used to cure patients with otherwise refractory tumours. Their work led directly to the current practice of using high-dose chemotherapy, along with bone-marrow transplantation, to treat patients with lymphoma and leukaemia.

Combination chemotherapy

Clinically, the move to combination chemotherapy began with the development of curative therapy for childhood ALL by Holland, Frei and Freireich. This approach was extended to the lymphomas in 1963 by Vincent DeVita, George Canellos and colleagues at the NCI (FIG. 4), who ultimately proved in the late 1960s that nitrogen mustard, vincristine, procarbazine and prednisone — known as the MOPP regimen — could cure patients with Hodgkin's lymphoma and non-Hodgkin's lymphoma[26,27]. Other promising reports of the ability of combination chemotherapy to cure diffuse large-cell lymphomas were offered by Joseph Bertino and colleagues at Yale University[28,29]. As predicted by studies in animal models, drugs were most effective when used in patients with tumours of smaller volume, and as combination therapies. Even modestly effective drugs such as 5-fluorouracil, an inhibitor of DNA synthesis, could improve survival when used as an adjuvant in treating patients with colon cancer[30]. Similarly, the landmark trials of Bernard Fisher, who chaired the National Surgical Adjuvant Breast and Bowel Project from 1967 to 1994, and of Gianni Bonadonna proved that adjuvant chemotherapy after complete surgical resection of breast tumours significantly extended survival — particularly

Figure 4 | **The 'gang of five'.** Left to right: George Canellos, Bruce Chabner, Phillip Schein, Vincent DeVita and Robert Young (1970). Photograph from the author's collection.

in those women whose cancer had spread to the axillary lymph nodes[6]. In general, combinations of drugs proved to be more effective than single agents against both metastatic cancer and in patients at high risk of relapse after primary surgical treatment.

Natural products: trials and tribulations

In 1956, C. Gordon Zubrod, who had formerly led the development of antimalarial agents for the United States Army in Aberdeen, Maryland, assumed leadership of the Division of Cancer Treatment of the NCI, and guided development of new drugs at both the experimental and, later, clinical level. In the two decades that followed, the establishment of the NCCSC, a large network of cooperative clinical trial groups evolved under the auspices of the NCI to test anticancer agents, first in children with ALL, and later in adults with solid tumours. Zubrod had a particular interest in natural products, and established a broad programme for the collecting and testing of plant and marine sources, a controversial programme that led to the discovery of taxanes (in 1964) and camptothecins (in 1966).

Both classes of drug, which were isolated and characterized by the laboratory of Monroe Wall at the Research Triangle Institute, encountered significant problems in development. Paclitaxel (Taxol), a novel antimitotic that promoted microtubule assembly, proved difficult to synthesize and could only be obtained from the bark of the Pacific Yew tree, which forced the NCI into the costly business of harvesting substantial quantities of yew trees from public lands. Furthermore, this drug was virtually insoluble and had to be formulated in a lipid emulsion that causes hypersensitivity reactions in some patients. After 4 years of clinical testing in solid tumours, it was found in 1987 (23 years after its initial discovery) to be consistently effective in the treatment of patients with ovarian cancer[31]. Although no patent position had been established, the NCI signed a collaborative research and development agreement under which it agreed to share clinical and manufacturing data exclusively with Bristol Myers Squibb in 1991. Taxol subsequently became BMS's first billion dollar per year drug, leading to acrimonious debate in Congress about industrial exploitation of a government discovery[32] and to calls for price controls.

Another drug class that had a difficult start was the camptothecins. Camptothecin, derived from a Chinese ornamental tree, inhibits topoisomerase I, an enzyme that allows DNA unwinding and strand passage. Despite showing promise in preclinical studies, the agent had little antitumour activity in early clinical trials and was toxic to kidneys. Its ineffectiveness *in vivo* was determined to be due to instability of its lactone ring at neutral pH. When it entered the urine, which has an acidic pH, the active molecule reformed, causing renal tubular damage. Not until 1996 would a stable camptothecin analogue, irinotecan, finally win Food and Drug Administration (FDA) approval for the treatment of colon cancer[33]. Later, this agent would also be used to treat lung and ovarian cancers[34,35].

NCI's successes in drug discovery

During the 1970s and 1980s, the NCI and others in academia, both in the United States and abroad, continued to dominate cancer drug development, primarily because there were few participants from industry. Cancer drug discovery had gained a reputation for having high risk and little chance of efficacy. Fewer than 10% of new drugs entering clinical trials in the period from 1970 to 1990 achieved FDA approval for marketing[36], and animal models seemed unreliable in predicting clinical success[37].

However, to be fair, there were successes. Cisplatin, discovered by a Michigan State University researcher, Barnett Rosenberg[38], who was working on an NCI contract, was instrumental in the cure of testicular cancer[39]. Subsequently, Eve Wiltshaw, Hillary Calvert and others at the Institute of Cancer Research in the United Kingdom extended the clinical usefulness of the platinum compounds with their development of carboplatin[40], a cisplatin derivative with broad antitumour activity and comparatively less nephrotoxicity. A second group with an NCI contract, led by John Montgomery, at the Southern Research Institute, synthesized nitrosoureas[41], which alkylated and crosslinked DNA in a novel reaction at the O6 position of guanine. This drug proved modestly effective in treating patients with malignant gliomas[42,43]. Montgomery's group also developed fludarabine phosphate, a purine analogue, which has become a mainstay in treatment of patients with chronic lymphocytic leukaemia[44]. Other effective molecules came from industry during the period of 1970 to 1990, including anthracyclines and epipodophyllotoxins — both of which inhibited the action of topoisomerase II[45], an enzyme crucial for DNA replication, transcription and repair. Additionally, several agents originally developed as anticancer drugs proved crucial in the treatment of non-neoplastic diseases, such as methotrexate in rheumatoid arthritis, cyclophosphamide in Wegener's granulomatosis, and nucleoside inhibitors in HIV/AIDS.

Throughout the clinical development of anticancer drugs, researchers repeatedly encountered significant problems because of the acute and long-term toxicities of chemotherapies, which affected virtually every organ of the body. Oncologists accepted these as the price for controlling a fatal disease. The lethality of bone-marrow suppression was significantly ameliorated by the development of platelet transfusion by Freireich and colleagues at the NCI[46,47], by aggressive use of antibiotics to prevent infections in neutropaenic patients[48], and by the later discovery of growth factors such as granulocyte colony-stimulating factor and granulocyte–monocyte colony-stimulating factor, which allowed rapid restoration of neutrophils[49,50]. Despite the advent of these supportive measures, the potential of some cytotoxic drugs to cause leukaemia[51], as well as their long-term effects on the lungs, heart and reproductive organs, remain formidable barriers, and have become increasingly important as patients are cured of their primary tumours[52].

The need to change strategies

In the early 1980s, progress in chemotherapy of cancer seemed slow, and each small success required large, long-term trials that only led to marginal gains against solid tumours. Furthermore, mouse models of leukaemia and solid tumours, which were the mainstays for drug screening at the time, were poor predictors of clinical outcome. By 1985, the NCI drug development effort had begun to produce a monotonous group of antimetabolites, alkylators, antimitotics and topoisomerase inhibitors. Analogues of these drugs, which provided marginal increases in efficacy, evoked little

Box 1 | **Screening strategies**

Systematic drug screening began in 1955 at the National Cancer Institute (NCI) with the establishment of the Cancer Chemotherapy National Service Center screening programme[81]. Throughout the 1960s and 1970s, most screening was performed *in vivo* using mouse L1210 and P388 leukaemias. Although reproducible, stable and relatively inexpensive, the use of rapidly dividing haematological mouse tumours introduced bias in the screens in favour of agents with activity against tumours with high growth fractions[82]. The inadequacy of these screening models for selecting agents active against solid tumours was implicated, at least in part, for the relatively slow progress in advancing treatments for common tumours throughout the 1960s and 1970s. In an attempt to find drugs active against solid tumours, the NCI adopted, in 1976, human tumour xenografts into its *in vivo* screening programme[83]. The human tumour xenografts were established either by direct implantation of patient biopsy material or by inoculation of continuous human tumour cell lines into immunodeficient mice. The first three human tumour xenografts included colon (CX-1), breast (MX-1) and lung (LX-1) tumours, but overall more than 300 xenografts have been established representing most main tumour types. In a parallel effort, the NCI introduced, in 1989, what was initially called 'disease-oriented' screening. This new approach used a rationally designed screening panel containing 60 cell lines derived from seven different human cancer types, including colon, brain, lung, melanoma, ovarian, renal and leukaemia. Subsequently, breast and prostate cell lines were added[84]. The use of the human cell line screening approach was increased by Kenneth Paull and colleagues, who established the COMPARE algorithm and demonstrated that the growth-inhibitory patterns of anticancer drugs against the cell lines correlated well with their mechanism of action[57]. Unfortunately, none of these screening systems has successfully predicted outcome of clinical trials[53]. More recent efforts are concentrating on using high-throughput molecular screens followed by the cell-line screens to analyse drug effects[82].

enthusiasm from clinicians, patients and Congress, who all advocated for new types of agent. The screening approaches were not yielding groundbreaking discoveries. There was strong sentiment across the board against continuing a screening system that tested random chemicals and natural products against mouse tumours.

In response, the NCI's Division of Cancer Treatment and its advisors adopted a screen based on testing against a panel of 60 human tumour cell lines, covering a broad range of tumour types (BOX 1)[53]. In view of the unique chemistry of compounds found in plants and marine organisms, and the important new agents previously discovered from nature, greater emphasis was placed on collecting new species worldwide and on testing their extracts in screens of human tumour cell lines. In hindsight, this screening approach has not been much more successful in identifying new anticancer drugs. Although the new cell lines used in the screen led to the identification of an increased number of anticancer agents, most of these were antimitotic agents and topoisomerase I and II inhibitors, much like those identified in the original screens. Occasionally, a new class of tumour inhibitor developed. For example, geldanomycin inhibits heat-shock protein 90 (REF. 54), which is important for regulating protein degradation, and flavopiridol inhibits a cell-cycle-dependent kinase[55]. Both drugs continue in clinical trials supported by the NCI; analogues of geldanomycin and second-generation cell-cycle inhibitors have also entered clinical trials and might hold greater promise. Perhaps the most important discoveries forthcoming from this human cell line screen were improvements in screening methodology, now widely adopted by industry, including a rapid colorimetric assay for cell viability (the MTT assay)[56], informatic techniques that can identify patterns of cytotoxic response and resistance among cell lines[57], and high-throughput automated screening.

The targeted-therapy revolution

While the attempts to improve the pace of discovery of cytotoxic agents proceeded in the late 1980s, molecular and genetic approaches to understanding cell biology uncovered entirely new signalling networks that regulate cellular activities such as proliferation and survival. Many of these networks were found to be radically altered in cancer cells. An industrial revolution unfolded, based primarily in small biotechnology firms, as researchers set out to repair these molecular defects in cancer cells, beginning the era of 'targeted therapy'. The new targets included growth factors, signalling molecules, cell-cycle proteins, modulators of apoptosis and molecules that promoted angiogenesis[58]. Cell biologists such as Stanley Cohen and Rita Levi-Montalcini identified specific growth factors. Others deciphered the network of signalling molecules that connected these receptors to the cell nucleus, creating a system for controlling cell proliferation and cell death. By the early 1990s, an explosion of drug targets transformed cancer drug development from a low-budget, government-supported research effort to a high-stakes, multi-billion dollar industry (BOX 2).

Innovations in technology increased the success of finding inhibitors of specific targets. Combinatorial chemistry provided thousands of unique structures for *in vitro* screening for inhibitors. Molecules identified in high-throughput screens could then be optimized for other properties, including greater specificity or bioavailability. The characteristics of promising anticancer drugs became clear — an agent should be metabolically stable, with a long half-life in model systems and in humans, and a slow rate of metabolism by enzymes such as the cytochrome-P450 family. Second, the candidate molecule should be well-absorbed after oral administration, which was not a typical characteristic of the chemotherapy drugs discovered in the 1970s and 1980s. Finally, it should show a favourable toxicity profile at biologically effective doses, with limited effects on bone marrow and intestinal epithelium.

One of the landmark events in the targeted revolution has been the development of imatinib mesylate (Glivec), a relatively simple structure that possesses all the desired factors of the 'ideal' targeted compound. It was derived from a natural product by chemists at Novartis. Imatanib is a moderately potent inhibitor of the kinase BCR–ABL, the fusion protein product of a chromosomal translocation that is involved in the pathogenesis of chronic myeloid leukaemia (CML). Imatinib also inhibits the KIT tyrosine kinase and platelet derived growth factor receptor-β (PDGFRβ) tyrosine kinase. These latter effects have been successfully exploited for therapy of gastrointestinal stromal tumours and the hypereosinophilic syndrome, respectively. Brian Druker showed that when imatinib is used to treat patients with chronic-phase CML, 90% achieve complete haematological remission and many lose cytogenetic evidence of the malignant clone. However, BCR–ABL translocation can still be detected by PCR analysis in cells of most patients[59–61]. In the acute leukaemic phase of CML, imatinib induces brief remissions, and treatment leads to a rapid outgrowth of drug-resistant cells that display mutations

in the catalytic kinase domain of ABL. These mutations hold the enzyme in an open configuration that binds the drug poorly but remains catalytically active[62]. In some patients in chronic phase CML, drug-resistant cells are present before drug exposure[63], a finding usually associated with rapid emergence of resistance[64]. This finding proves that cancers, through their intrinsic mutability, contain a range of drug-resistant mutant subclones, even before treatment.

A second class of compounds, those that inhibit the epidermal growth factor receptor (EGFR), has won FDA approval, but with less marked evidence of antitumour activity. Gefitinib (Iressa), a competitive inhibitor of the ATP-binding function of the tyrosine-kinase active site of EGFR, causes partial remissions in 10–15% of patients with non-small-cell lung cancer (NSCLC)[65], but failed to increase activity of chemotherapy in large randomized trials[66,67]. A monoclonal antibody against the extracellular domain of EGFR, cetuximab (Erbitux), also won FDA approval in 2003, in this case in combination with chemotherapy for colon cancer[68]. It is not clear that the two agents, which target the same receptor in quite different ways, produce responses in the same subset of patients or the same diseases. Subsequently, researchers at Harvard have identified molecular changes in EGFR, either mutations or in-frame deletions, that are associated with response to therapy in patients with lung cancer[69,70]. These receptor mutations and deletions confer a more robust response to the ligand and a corresponding increase in sensitivity to receptor inhibition by gefitinib. Alterations in EGFR are found in 10% of patients with NSCLC in the United States, but, interestingly, in 25% or more of patients with the same disease in Japan, where the response rate to gefitinib is correspondingly higher. Most responders to therapy are women and non-smokers, and their tumours often contain elements of bronchoalveolar carcinoma, indicating that these patients constitute a distinct subset within the larger histological classification of NSCLC. It is possible that the mutation is a transforming event in these patients, and that the tumour is in essence 'addicted' to the receptor's activation.

The discovery of the mutations that confer sensitivity to EGFR inhibitors represents an important advance in the strategy of cancer drug development. Most agents in current use, including cytotoxics and the newer targeted drugs, have relatively low response rates in patients with any given histological class of solid tumours. The use of molecular or genomic tests, or proteomics, to identify reliable markers for drug sensitivity will undoubtedly become one of the key objectives early in the clinical development of new drugs[71]. Otherwise we will continue the financially, scientifically and ethically unacceptable practice of treating many patients to benefit a few.

Along with the successes, there have also been disappointments in the targeted-therapy revolution. A series of farnesyl transferase inhibitors (FTIs), developed and tested against HRAS, entered the clinic in the late 1990s but failed to cause regression of human solid tumours[72,73]. Some FTIs, however, remain in clinical trails against acute myeloid leukaemia and myelodysplasia. The reason for their lack of efficacy against solid tumours is not obvious. The FTIs clearly affect proteins that are subject to farnesylation, but they are not consistently effective against KRAS-transformed cells in culture. Alternative pathways for KRAS lipid modulation might circumvent the block.

A second high-profile failure added to the initial gloom of the new age of biotechnology, but has more recently been vindicated by successful clinical trials. In the early 1970s, Judah Folkman demonstrated for the first time the central role of angiogenesis in allowing tumour proliferation and metastasis[74]. Folkman, Harold Dvorak and others found that tumour cells secreted angiogenic molecules such as PDGF and VEGF, which stimulated new vessel formation in their environs and made available a supply of nutrients that allowed further expansion of the tumour. Despite the clinical failure of the anti-angiogenic peptide endostatin, several small molecules (SU-11248, Bayer 43-9006) that inhibit the VEGF receptor 2, and an anti-VEGF antibody, bevacizumab (Avastin), have clear antitumour activity in patients with renal-cell carcinoma, a tumour that arises through mutation or loss of a functional *VHL* gene[75]. VHL suppresses the function of hypoxia-inducible factor-1α, the transcription factor that responds to hypoxia and stimulates angiogenesis. Bevacizumab also increases the activity of standard cytotoxic drugs against colon cancer and achieved FDA approval in 2004 for treating patients with this cancer[76]. SU-11248, which also inhibits KIT, is also active in patients with gastrointestinal stromal tumours, including those refractory to imatinib.

The past as prologue

From these experiments it became clear that the path to success for targeted molecules would be no less arduous than the trials for cytotoxic drugs. Certain principles emerged from the cytotoxic era, and are likely to be applied to the new chemotherapies. First, animal models, while instructive, are unreliable predictors for success against human disease.

Box 2 | **The growth of chemotherapy as an industry**

The drugs and biological agents that oncologists use to treat cancer now amount to a multi-billion dollar industry. In 2003, the total worldwide market for oncology-related products was approximately US $36.8 billion. Today, expenditures on oncology-related products represent almost 10% of the total $430 billion worldwide market for pharmaceuticals, and the oncology market is projected to exceed $60 billion by 2008 (REF. 85). Cancer-related chemotherapy drugs and biological treatments comprise approximately 48% and 15% of the total market size, respectively, whereas haematological growth factors, used mainly to support the use of chemotherapy, account for the remaining 37% of the market. A combination of factors has driven the growth in the oncology market, including expanded therapeutic options, greater willingness of oncologists to treat older patients, and relatively high prices of many of the newer anticancer agents. The cost of drugs to treat colon cancer is illustrative. Before 1996, 5-fluorouracil (5-FU) was the principle agent used to treat advanced colon cancer. The drug cost for 8 weeks of treatment with a regimen based on 5-FU was under $100. By 2004, the Food and Drug Administration had approved 5 additional agents for the treatment of colon cancer, including irinotecan, capecitabine, oxaliplatin, cetuximab and bevacizumab. The drug cost for the same 8 weeks of treatment can now exceed $30,000, as the newer drugs have been added onto, rather than replaced, existing agents[86]. The size and scope of the enterprise focused on cancer drug development has also grown. Cancer drug development has transformed from a small, mostly public effort focused in the United States to become a major international industrial effort. At present, more than 1,300 small biotech companies have formed in the United States alone to develop products based on molecular targets[87], and more than half are focusing on treating cancer. According to a recent industry-wide survey, there are now at least 395 agents for the treatment of cancer in clinical trials[88], more than in any other therapeutic class of medicine[89]. The pharmacoeconomic challenges of paying for all of these new agents will almost certainly become an area of increased attention as the oncology field moves forward.

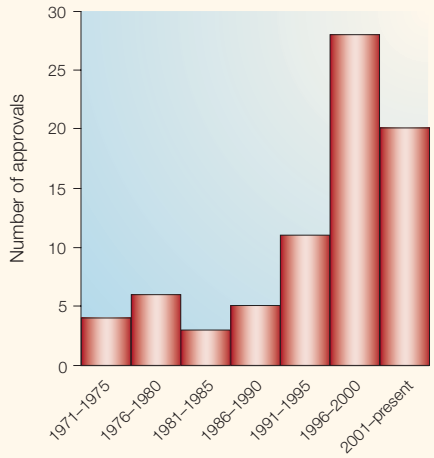


Figure 5 | **Number of approved new molecules for the treatment of cancer by the Food and Drug Administration.** The number drugs approved for the treatment of cancer by the United States Food and Drug Administration (FDA) per 5-year period was relatively constant through 1990, and has increased substantially since then. Data are updated through August 2004 and are available from the FDA web site (see online links box).

Human tumours 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. Animals imperfectly reflect the pharmacokinetics of drugs in humans, because of more rapid metabolism, greater tolerance for side effects and differences in protein binding. Even specific organ toxicities cannot be extrapolated from mice to humans. As pointed out recently by Douglas Hanahan and Robert Weinberg[58], the basic biology of murine cells, and the process of transformation, can differ significantly from that of human cells. Attempts to produce genetically engineered mouse models of human cancer in fact lead to models of the specific molecular changes in a mouse cell, and have uncertain relevance to a human counterpart.

A second enduring principle has been that human tumours, when they do respond, contain subclones that become drug resistant. Under the selective pressure of a toxic therapy, the genetic diversity within most human tumours 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. Ironically, the scenario of evolution of drug resistance has been most elegantly studied for two successful agents, methotrexate (the cytotoxic folate analogue) and imatinib (the highly effective inhibitor of the BCR–ABL tyrosine kinase). In some cases, targeted drugs and conventional antitumour agents can both be affected by a common resistance mechanism involving drug efflux (the MDR transporter)[77], or mutations in cell-death pathways. Because of resistance to single agents, combination therapy is essential for tumour eradication and cure. As we have reviewed, even the most successful of the targeted molecules, imatinib, does not fully eradicate the malignant clone. Fortunately, clinical trials have demonstrated potent synergy between targeted molecules, particularly monoclonal antibodies such as rituximab (Rituxan)[78], bevacizumab[76] and trastuzumab (Herceptin)[79], and traditional chemotherapy.

It has become increasingly clear that understanding the molecular profile of human tumours is essential to the effective use of cancer drugs. For 60 years, clinicians have depended on histological classification of tumours to dictate therapeutic choices, despite the fact that most patients with a solid tumour do not respond to any given drug. Patient selection, based on molecular features of the tumour, confers enormous advantage in trial design, allows for more efficient and cost-effective drug development, and ultimately will reduce the burgeoning cost of new technology. Molecular profiling and the study of patient selection must become a central aim of cancer drug development[71].

The discovery and development of cancer chemotherapy has therefore come full circle. We have more and better drugs (FIG. 5) and improved knowledge of cancer as a disease, but the early observations of Goodman, Gilman and Farber remain accurate and, indeed, immutable. The transition from cytotoxic drugs to targeted therapies represents an important advance, but the basic principles of cancer treatment and drug resistance, as developed in the period from 1950 to 1980, remain the same. Human malignancies are a very diverse group of diseases, even within histological classifications, and quickly display their diversity when exposed to all forms of chemotherapy. The next decade will present the challenge of designing trials to combine targeted drugs and cytotoxics in a more effective manner. These trials will be aided by the use of genomic and molecular assays to identify subsets of patients that are most likely to respond to certain drugs, thereby avoiding the needless cost and toxicity of ineffective treatment. Additional major scientific challenges for the next generation will involve exploiting the mutability of cancer cells and reversing their resistance to apoptosis[80]. Will medical treatments provide cures for most common cancers over the next 60 years? They will probably do so for subsets of the main tumour types, and will undoubtedly extend survival and improve quality of life for many others.

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Competing interests statement

The authors declare competing financial interests: see Web version for details.

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