

TIMELINE

Paul Ehrlich's magic bullet concept: 100 years of progress

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Abstract | Exceptional advances in molecular biology and genetic research have expedited cancer drug development tremendously. The declared paradigm is the development of 'personalized and tailored drugs' that precisely target the specific molecular defects of a cancer patient. It is therefore appropriate to revisit the intellectual foundations of the development of such agents, as many have shown great clinical success. One hundred years ago, Paul Ehrlich, the founder of chemotherapy, received the Nobel Prize for Physiology or Medicine. His postulate of creating 'magic bullets' for use in the fight against human diseases inspired generations of scientists to devise powerful molecular cancer therapeutics.

"Although modern chemotherapy has now assumed its role in science and practical experience, it has its origin in histological staining techniques. Hence, it did not happen by chance that the first promising chemotherapeutical experiments used dyes like methylene blue and trypan red. Thus, early chemotherapy can be looked upon as dye therapy." The foreword to his ground-breaking article, "Aus Theorie und Praxis der Chemotherapie", establishes the roots of Paul Ehrlich's (FIG. 1) scientific work in his enthusiasm for the world of colourful dyes and their capacity to interact with histological and cellular structures¹⁻³. Over several decades, chemical companies provided hundreds, maybe thousands, of new dyes for Ehrlich's research. By visualizing the differential affinities of chemical dyes for specific biological structures, Ehrlich taught us that, in the broadest sense, the biological effect of a chemical compound depends on its chemical composition and the cell on which it acts. Ehrlich connected chemistry with biology and medicine in an ingenious fashion; chemical dyes were the catalyst for this revolutionary association.

In a second period of his scientific endeavours, Ehrlich acquired his fascination for immunology from his contemporaries Louis Pasteur, Robert Koch, Emil von Behring and Shibasaburo Kitasato. His studies on the plant toxins abrin and ricin, development of high-titre anti-diphtheria serum, experiments on 'natural and acquired immunity' and, most importantly, his standardizing of sera for antibody concentration inspired his famous article on the 'side-chain theory of immunity'⁴⁻⁶ (TIMELINE). Ehrlich's

remarkable gift of abstraction facilitated his perceptions of the molecular interaction of toxins with anti-toxins that constitute the foundation of this theory. He postulated the existence of specific receptors, which may be regarded as side chains that bind antigens (for example the haptophore of a toxic molecule), and hypothesized that these receptors are either associated with cells or distributed more abundantly in the blood stream in response to antigen interaction^{7,8}. In 1900 Ehrlich replaced the term 'side chain' with 'receptor'. According to Ehrlich's receptor theory, which shifted over time from the binding of toxins and nutritive substances to the binding of drugs, various types of receptors with different structures and binding groups exist⁹⁻¹¹. His landmark immunological insights were rewarded in 1908 with the Nobel Prize for Physiology or Medicine (see URL in Further information).

These immunological achievements evolved into what became Ehrlich's 'magic bullet concept': drugs that go straight to their intended cell-structural targets. Targeted medicine should in theory efficaciously attack pathogens yet remain harmless in healthy tissues. Ehrlich based his receptor theory upon his experiences in the treatment of infectious diseases with drugs derived from the German dye industry. Targeting the receptors of the invading parasites that are not shared by the host clearly reduces the likelihood of adverse effects in patients. A therapeutic index, describing the relationship between parasitotropism and organotropism, would determine the extent of the healing process. Ehrlich, as the founder of chemotherapy, originated

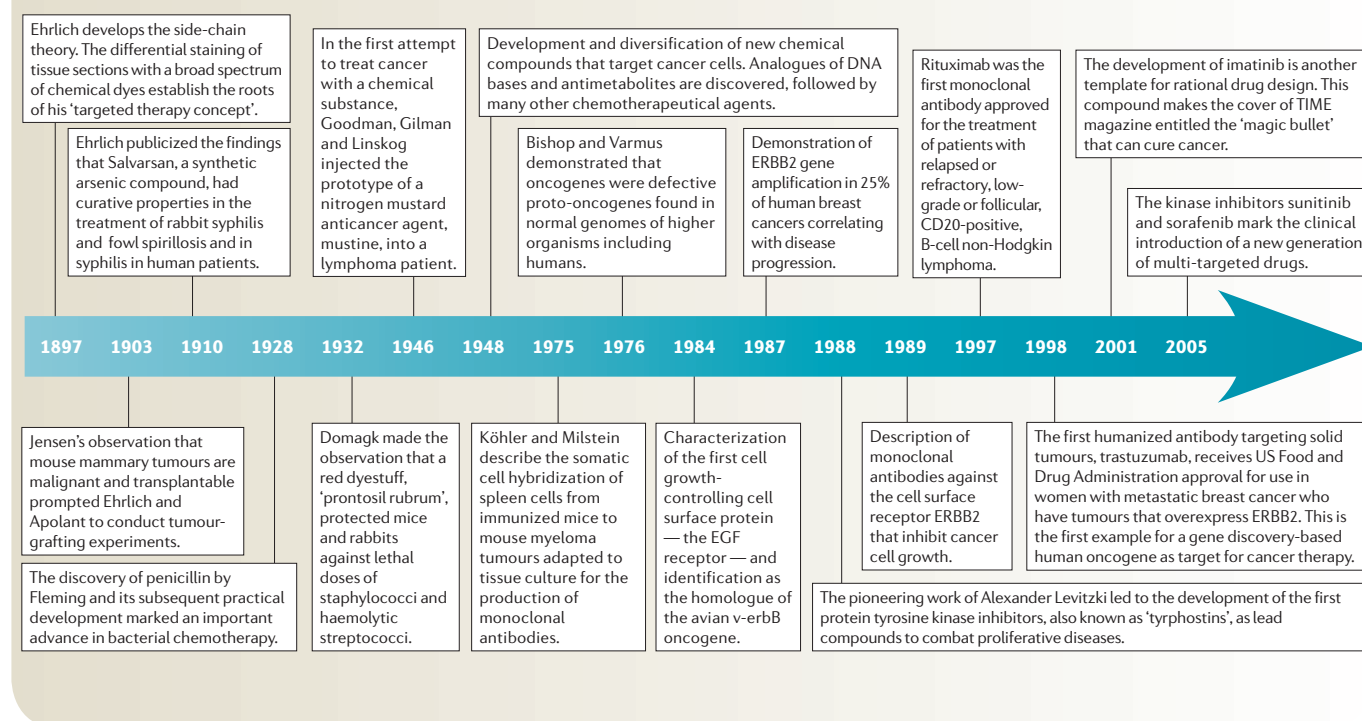
the postulate "wir müssen chemisch zielen lernen" ("we have to learn how to aim chemically"). Ehrlich surmised that the key for synthetic chemistry was to modify some starting material in various ways and evaluate the products for their ability to heal. While director of the Georg-Speyer Haus in Frankfurt am Main, he theorized that by analysing many compounds, an anti-microbial drug would eventually be discovered to treat the syphilis spirochete. In Ehrlich's laboratory in 1908, Sahachiro Hata detected the anti-syphilitic activity of arsphenamine, also known as Salvarsan, during a screening of hundreds of newly synthesized organic arsenic compounds¹². This drug discovery programme was the first to optimize the biological activity of a lead compound through systematic chemical modifications and represented the dawn of nearly all modern pharmaceutical research. In this article, we pay tribute to Paul Ehrlich's revolutionary ideas, his talented experimental work and his unique ability to bridge the divide between chemistry, biology and medicine, by discussing how his findings have influenced the search for ever more effective anticancer drugs.

Cancer therapy development

Ehrlich's genius inspired multiple ground-breaking studies that resulted in the successful treatment of human diseases. As a physician during World War I, Alexander Fleming recognized that the chemical compounds used to treat septic injuries were in fact more toxic to white blood cells than bacteria. In a search for a less toxic agent, which may well have been inspired by Ehrlich's revolutionary and spectacular ideas, Fleming identified penicillin as a powerful antibacterial drug¹³. Ehrlich's discovery that dyes such as trypan red were effective in the treatment of trypanosome infections further influenced the development of novel chemotherapeutics. In 1932, Gerhard Domagk found that the red dye 'prontosil rubrum', a derivative of sulphanilamide, protected mice and rabbits against lethal doses of staphylococci and haemolytic streptococci.

First efforts towards chemotherapy. The first wave of cancer drug development can be traced to the chemical warfare agent nitrogen mustard that accidentally caused robust lymphoid and myeloid suppression in humans during World War II. Initiated by the pharmacologists Louis Goodman and Alfred Gilman, the injection of mustine, the prototype nitrogen

Timeline | Cancer therapy progress since Ehrlich's side-chain theory



mustard anticancer chemotherapeutic, into a patient with non-Hodgkin lymphoma caused a dramatic anti-tumour effect^{14–17}. This approach lent weight to Ehrlich's bold assumption that small molecules (although in this case not a targeted one) might be used in the management of cancer. Elucidation of the double-helical structure of DNA by James Watson and Francis Crick in 1953 had a singular impact on strategies for cancer drug development: taking advantage of the structural aspects of DNA in the use of analogues of DNA bases (such as 5-fluorouracil or 8-azaguanine) to disrupt DNA replication^{18,19}. The finding that nitrogen mustard exerts its anti-tumour effect by alkylation of DNA initiated the identification of additional DNA-reactive drugs such as the natural products mitomycin C and bleomycin^{20–22}. Subsequently, cisplatin, which was identified in 1965 by Barnett Rosenberg, formed the basis for developing new analogues by inducing a new mode of DNA damage, but with reduced host toxicity²³.

In 1948 Sidney Farber, who observed that folic acid administered to children suffering from acute lymphoblastic leukaemia (ALL) stimulated the proliferation of cancer cells, synthesized antimetabolites — structural mimics of folic acid — to block its action. Aminopterin and methotrexate, derived

from the first rational cancer drug design, induced remission in children with ALL and became the mainstays of leukaemia chemotherapy²⁴. In 1942, George Hitchings, a pioneer of DNA antimetabolite design and synthesis, contributed significantly to the intelligent design of cancer drugs by developing 2,6-diaminopurine, which by structural refinement led to the synthesis of 6-mercaptopurine, a successful therapeutic for treating acute leukaemia²⁵. Hence, in the 1950s the identification of new drugs by the pharmaceutical industry based on the general idea that cancer cells divide at a higher rate than healthy cells was revolutionary. For the first time, compounds of known composition that interfered with mitotic structures or with DNA replication were applied to the treatment of cancer in a rational manner. Thus, in this first period of cancer drug development, several chemical compounds, often discovered accidentally, were targeted in the sense that they were more toxic to replicating cancer cells.

Further to these early findings, clinical oncologists achieved another breakthrough by using combinations of drugs, each with a different mode of action — related to a strategy described by Ehrlich in his Harben lectures²⁶ — and applying adjuvant therapy to clear any remaining tumour cells after surgical removal of the primary tumour^{27–29}.

Small molecules targeting mutated proteins. The discovery of oncogenes and tumour suppressor genes in the 1970s and 1980s, and the subsequent deciphering of the cellular signal transduction pathways, helped define the biological hallmarks of cancer^{30,31}. These revolutionary insights initiated a second wave of cancer therapeutic development: the use of 'targeted small-molecule therapeutics', that is, the development of rationally designed, low-molecular-weight compounds that target the specific mutation in molecules driving the progression of individual cancers. For example, the enthusiasm associated with the clinical success of imatinib in the treatment of patients with chronic myeloid leukaemia (CML)^{32,33} (BOX 1), and the identification of oncoproteins as key regulators of neoplasia, initiated a flurry of efforts to develop and to clinically test a growing spectrum of small-molecule inhibitors targeting crucial effectors involved in cell proliferation, invasion and metastasis, angiogenesis and apoptosis^{31,34}. The development of agents that disrupt cancer cell division by specifically targeting key components of the cell-cycle machinery such as cyclin-dependent kinases and polo-like kinase 1 (PLK1) is a major focus of recent drug discovery efforts in oncology^{35–40}. An alternative approach to the inhibition

of the enzymatic activity of oncoproteins focuses on blocking protein–protein interactions, as exemplified by tyrphostin AG538 targeting insulin-like growth factor 1 receptor (IGF1R), nutlins targeting the p53-binding pocket in MDM2 to preserve the tumour suppressor function of p53 in MDM2-overexpressing tumours, and poloxin targeting the polo-box in PLK1 (REFS 41–43).

A multiple magic bullet. Generations of chemists and pharmacologists interpreted Ehrlich's vision of the magic bullet as a compound that targets a single crucial oncoprotein in an exclusive, highly specific fashion. Our present awareness of cancer as multi-factorial disease has changed this paradigm considerably, paving the way for the development of multi-targeted inhibitors such as *sunitinib*^{44,45}, a selective inhibitor of receptor tyrosine kinases that are important for neovascularization, including vascular endothelial growth factor receptors (VEGFRs), platelet-derived growth factor receptors (PDGFRs) and others. Following binding of the ligand to the extracellular domains of receptor tyrosine kinases, the intracellular subunits undergo transautophosphorylation, which leads to receptor activation and phosphorylation of downstream substrates. Chemotherapeutic inhibitors such as *sunitinib* are ATP-competitive inhibitors that block the ATP-binding site within the kinase domain, thereby preventing phosphorylation of the receptor and its downstream targets. Our understanding of tumour angiogenesis as an essential prerequisite for the sufficient supply of oxygen and nutrients, and thereby for the growth of all solid tumours, makes pro-angiogenic receptor tyrosine kinases attractive therapeutic targets^{46,47}, and results indicate that multi-kinase inhibitors are suitable targeted drugs that, surprisingly, are in accord with Ehrlich's concept⁴⁸. The use of a substrate-competitive drug such as AG 538, which mimics side-chains of tyrosine, is an attractive alternative strategy to disrupting the activity of receptor tyrosine kinases⁴¹.

Furthermore, cancer drug developers have also considered the inhibition of histone deacetylases (HDACs), which have pleiotropic effects upon the development and progression of cancer by influencing chromatin remodelling, transcription, mitosis, and DNA replication and repair. However, the molecular mechanisms underlying the effects of HDAC inhibitors (HDACi) are not yet fully understood and, although HDACi



Figure 1 | **Paul Ehrlich in his office.** Reproduced, with permission, from the Paul-Ehrlich-Institute (Langen, Germany).

have demonstrated remarkable anti-neoplastic responses in clinical trials, toxicities have also been recorded^{49–52}.

HSP90 (also known as *HSP90AA*) can also be considered to be a pleiotropic effector. HSP90 is a member of the chaperone family with over 100 identified client proteins that are involved in signalling and chromatin remodelling pathways and are often disrupted or overexpressed in cancer, thus making it a particularly promising target for anticancer drugs^{53–55}. The development of HSP90 inhibitors has raised concerns, however, as targeting this essential chaperone could impair multiple normal cell functions. Nevertheless, the administration of the geldanamycin derivative, 17-allylamino-17-demethoxygeldanamycin (17AAG), in phase I clinical trials was associated with a favourable toxicity profile, thus stimulating the search for novel chemical compounds targeting HSP90 (REFS 56–58).

In summary, improving our knowledge of the genetic events that promote tumorigenesis in a specific epigenetic context will facilitate the tailoring of mono-specific and multi-targeted drugs for oncogene inactivation and the induction of tumour cell differentiation and death.

Development of monoclonal antibodies and antibody–drug conjugates. Ehrlich had suggested in his work that the immune system can prevent tumours⁵⁹. Nevertheless, Ehrlich and his contemporaries searched in vain for immunogenic determinants on tumour cells such as specific receptors, and as a result cancer research became less of a priority for him. However, those who followed in his footsteps succeeded in identifying specific receptors on

the surface of tumours cells. One key strategy for realizing Ehrlich's vision that “antibodies are in a way magic bullets that identify their target themselves without harming the organism” was the development of the hybridoma technology for the production of monoclonal antibodies (mAbs) by Georges Köhler and César Milstein⁶⁰. During their initial use in the 1980s, therapeutic success with murine mAbs was limited by their immunogenicity and restricted ability to induce immune effector mechanisms. This prompted the development of chimeric and humanized antibodies that use a plethora of mechanisms to attack cancer cells, such as antibody-dependent cellular toxicity, complement-dependent cytotoxicity, modulation of signal transduction and immunomodulation^{61,62}.

The remarkable finding that overexpression of *ERBB2* (also known as *HER2*) has a crucial role in the pathogenesis of breast and ovarian cancer prompted the development of *trastuzumab*^{63,64} (BOX 2). The development of highly specific mAbs targeting *ERBB2* opened the door to novel strategies for targeted cancer therapy, and was an impressive step towards the realization of the magic bullet concept. The clinical efficacy of *trastuzumab* in patients with breast cancer demonstrated impressively for the first time that a therapeutic agent targeted against a human oncoprotein is a powerful agent in the fight against cancer. Furthermore, over the past 10 years, the introduction of *rituximab*, a genetically engineered chimeric mouse–human antibody that binds to the transmembrane antigen *CD20*, has revolutionized the treatment of B-cell non-Hodgkin lymphoma^{65–68}. Clinical success with *rituximab* and *trastuzumab* energized the development and clinical

assessment of many novel antibodies that target membrane proteins in lymphomas, such as CD40, CD80 and CD52 (alemtuzumab), and membrane proteins in solid tumours, such as epidermal growth factor receptor (EGFR; cetuximab), epithelial cell adhesion molecule (EpCAM, also known as TACSTD1), carcinoembryonic antigen (CEA, also known as CEACAM5), tumour necrosis factor (TNF) family receptors (including TRAILR1 (also known as TNFRSF10A), TRAILR2 (also known as TNFRSF10B) and lymphotoxin β receptor) and many others that impinge on signal transduction for apoptosis induction in cancer cells^{65,69–79}. Beyond targeting membrane proteins in malignant cells, the identification of molecular targets in the microenvironment associated with tumours, such as ligands that trigger signalling events or target the tumour stroma, has led to new ‘rational’ strategies, as shown, for instance, in the case of the anti-VEGF mAb bevacizumab that targets tumour angiogenesis. Encouraging data from a combinatorial trial using bevacizumab plus 5-fluorouracil and leucovorin for colorectal cancer led to US Food and Drug Administration approval of this antibody in 2004 (REF. 80).

Ehrlich also envisioned attaching toxins to antibodies to improve their therapeutic specificity. Accordingly, in recent years, antibodies conjugated with toxins, cytokines or radionuclides have proved a promising approach for treating cancer patients^{81–85}. Current antibody conjugates predominately target lymphohaematopoietic diseases owing to accessibility, as illustrated by gemtuzumab ozogamicin and BL22 (REFS 86–88). For the treatment of solid tumours, the association of geldanamycin with the therapeutic mAb trastuzumab showed augmented efficacy in a murine xenograft tumour model compared with trastuzumab alone⁸⁹.

Although the impact on cancer patient care with therapeutic mAbs has been profound, mechanism-dependent toxicity

may also arise. For example, cardiotoxicity induced by trastuzumab results from the binding of this antibody to a receptor in heart tissue, and rituximab is associated with toxicity due to its binding normal B cells expressing CD20 (REFS 90–92).

Scientific and technological advances

Drug discovery: from lead generation to lead optimization. Ehrlich postulated “to use synthetic chemistry to modify the starting material chemically in various ways and analyse the resulting products for their quality to heal”. Although synthetic organic chemistry had a cardinal role in Ehrlich’s work and in cancer drug development during the past century, its contributions varied over time. Random screening of libraries containing synthetic compounds and natural products dominated drug discovery programmes between 1960 and 1985 (REF. 93). In the early years of small-molecule development, when few targets other than DNA were available, structural and synthetic chemistry had to meet severe requirements. Many screens yielded natural compounds of high structural complexity (for example, maytansine and vincristine) that could not be synthesized or modified in an economically feasible way^{94,95}. Although the random screening of natural products led to the discovery of important lead compounds (for example, anthracyclines, vinca alkaloids, epipodophyllotoxins and taxanes), the increasing power of modern synthetic chemistry was helpful in producing desirable pharmacological properties (for example, solubility, distribution, pharmacokinetics and resistance to metabolism) for new leads^{96–102}. A novel instrument, the structure–activity relationship, relates drug structure to biological activity and supported the evaluation of novel compounds. For instance, for tubulin binding, paclitaxel and epothilone analogues share a common pharmacophore, a term intellectually

founded on Ehrlich’s theory that a “molecular scaffold carries (phoros) the essential features responsible for a drug’s (pharmaco-) biological activity”^{103,104}. Additionally, the identification of less complex synthetic compounds, and the application of new methods in physical organic chemistry, facilitated the synthesis of a range of analogues with quantifiable parameters (for example, charge distribution and electronic, steric and hydrophobic effects). This delineated the use of quantitative structure–activity relationship as a standard tool for the development of cancer therapeutics^{105,106}.

In the early genomics era during the late 1980s and the 1990s, the characterization of the precise molecular pathology of different cancers became the central issue in drug discovery, manifested as the identification of genes that initiate and support carcinogenesis. The search for new leads among targeted small-molecule modulators required the implementation of highly sophisticated technical strategies, including high-throughput or focused screening of synthetic and natural compound libraries. The refinement of combinatorial chemistry (involving the simultaneous preparation of large numbers of chemical compounds) also accelerated drug discovery¹⁰⁷. During the recent genomics and proteomics era, high-resolution, genome-wide approaches (including resequencing and expression profiling and high-throughput RNA interference) have also revealed multiple promising new drug targets^{108–112}. To identify smart chemical compounds, reflecting the three-dimensional structure of the targeted protein, it was essential to improve the biochemical high-throughput screen for low-affinity compounds using sensitive biophysical techniques such as NMR, X-ray diffraction and protein–ligand and co-crystallography^{113–116}. The resulting physicochemical data also facilitated virtual screening of library structures for their three-dimensional fit with pharmacophores^{117,118}. Alternatively, imaging-based high-content screens can be applied to directly correlate chemical scaffolds with cancer cell phenotypes¹¹⁹. As Ehrlich considered that the spatial diversity of a specific target was crucial for identifying high-affinity drugs, the introduction of multiple high-throughput approaches for lead discovery and a sophisticated structure-based design, in combination with modern technologies for high-throughput genome-wide screening for novel targets and biomarkers, have added new dimensions to Ehrlich’s concept.

Box 1 | Targeted therapy for chronic myeloid leukaemia

As one of the first highly selective protein kinase inhibitors developed, the success of imatinib (Gleevec) in clinical trials for chronic myeloid leukaemia (CML) was a milestone in the development of molecular-targeted small molecules in oncology³². The principal target of imatinib is BCR–ABL, a fusion protein between part of the breakpoint cluster region (BCR) protein and the tyrosine kinase ABL³³. The search for a specific BCR–ABL inhibitor began with the analysis of the 2-phenylaminopyrimidine family, selective for the tyrosine kinases ABL and platelet-derived growth factor receptors (PDGFRs)^{157,158}. The initial lead compound, discovered by testing large compound libraries, was a relatively weak inhibitor of protein kinase C α and the PDGFRs¹⁵⁹. The activity of the 2-phenylaminopyrimidine series was optimized for PDGFR inhibition by synthesizing a series of chemically related small molecules and analysing the relationships between their structure and activity. The most potent molecules were dual inhibitors of the ABL and PDGFR kinases. Imatinib emerged as the lead compound for preclinical development.

Translation

Following its discovery in 1909, Ehrlich improved Salvarsan through repetitive cycles of synthetic chemistry and biological evaluation until Neosalvarsan appeared in the markets in 1912. It was less toxic and easier to produce and handle owing to its improved solubility, thus demonstrating that lead optimization and feedback from clinical trials is one of the keys to discovering Ehrlich's magic bullet. As discussed above, the increased knowledge of the cancer genome and the introduction of multiple new technologies has significantly improved the drug discovery process over the years, leading to key successes in targeted cancer therapeutics, including trastuzumab and bevacizumab, and the multi-targeted kinase inhibitors imatinib, sunitinib, *sorafenib* and *lapatinib*^{120–125}. These landmark achievements have proved that the targeting of oncogenic aberrations for specific types of cancer or the tumour microenvironment results in a major clinical benefit. Successful proof-of-concept clinical trials of small-molecule inhibitors (such as those that target HDACs or HSP90) have also stimulated the search for small molecules in which protein structure information guides the improvement of the drug candidate ADMET (adsorption, distribution, metabolism, excretion and toxicity) profile^{126–129}. However, despite the remarkable achievements so far, the clinical use of inhibitors also reveals their limitations, such as off-target toxicities. As with the low frequency of congestive heart failure related to imatinib and sunitinib, drug exposure requires the careful definition of an appropriate therapeutic window^{130–132}.

The emergence of resistant mutants after treatment with novel drugs also reminds us of Ehrlich's idea of using combinations of drugs to prevent resistance¹⁰⁴. Alternatively, the development of second-generation inhibitors is a powerful strategy to overcome resistance¹³³. For instance, the frequency of mutations within the kinase domain of *BCR-ABL* ranges from 40 to 90% depending on the CML phase^{134,135}. A point mutation like T315I, frequently found in CML patients, prevents ABL from switching to the inactive conformation to which imatinib binds^{135–137}. The urgent need to overcome resistance to clinically approved inhibitors requires a detailed knowledge of the structural biology of the targeted kinase. Whereas type I inhibitors block the ATP binding site within the bilobe structure of the kinase domain, type II

inhibitors bind and preferentially stabilize the inactive conformation¹³⁸. Detailed crystallographic analysis has revealed that type II inhibitors, like imatinib, interact with the ATP binding site and the adjacent hydrophobic pocket. This pocket is less conserved than the surrounding ATP domain, highlighting the inactive conformation as an ideal design site for selective kinase inhibitors^{137,139}. Major advances in the structural biology of ABL accelerated the rational design of second-generation inhibitory compounds such as *nilotinib* and *dasatinib*^{140–142}. By contrast, most patients with non-small-cell lung cancer (NSCLC) do not respond to the EGFR inhibitor *gefitinib*. However, a subgroup of patients with NSCLC have specific mutations in *EGFR* that lead to increased growth factor signalling and confer susceptibility to *gefitinib*¹⁴³. Hence, screening for mutations in *EGFR* and other target genes may identify patients who will have a better response to certain inhibitors.

In view of the tremendous achievements in drug discovery during the past century, it is still imperative to accelerate the progression from gene to drug and to improve the success rate for bringing cancer drugs to the market, which is currently only about 5% (REF. 144). The main reasons for the attrition of candidate agents include inadequate activity and toxicity, which often occur in late stages of clinical development. Appropriate animal tumour models, (as originally studied by Ehrlich and Apolant), *in vitro* models for mechanism-based toxicity and molecularly defined targeted disruption of cancer genes in animal models are important tools for a better prediction of side-effects^{59,145–147}. Furthermore, using biomarkers in preclinical drug discovery and development facilitates the optimization of pharmacokinetics, pharmacodynamic and therapeutic parameters so that the best compound is selected for clinical evaluation¹⁴⁸.

Future perspectives

A scientific and technological revolution in the war against cancer has taken place in the past century. Several groundbreaking achievements have drawn their intellectual momentum from the discoveries of Paul Ehrlich. His dogma of a rational, targeted strategy against invading microbes or malignant cells has outlived numerous scientific trends; it is still a paradigm for modern cancer research as well as a valuable guideline for disease management. Ehrlich's ideal of "aiming precisely" using drugs with high efficacy dominates modern drug discovery. Despite these advances, cancer still threatens numerous lives owing to its multi-dimensional complexity. The maximal therapeutic effect of imatinib with CML can be attributed to the forceful neutralization of a single genetic aberration in a protein kinase. Other neoplasias can also be ascribed to single genetic alterations like *RET* mutations for multiple endocrine neoplasia or ALK-positive *anaplastic large-cell lymphoma*^{149,150}.

Nevertheless, most cancers have numerous molecular drivers, raising the question of the requirements for a magic bullet in a genetically complex situation. Increasing evidence from clinical trials suggests that chemical compounds targeting multiple crucial protein kinases offer an alternative approach and this challenges the use of mono-specific drugs to conquer multifactorial diseases. High-throughput screening accompanied by medicinal and combinatorial chemistry (targeted pharmacology) led to the development of *sorafenib*, which has a pharmacological profile targeting several protein kinases^{151,152}. Other promising multi-targeted drugs include inhibitors of HDACs and HSP90, or the multi-kinase inhibitor sunitinib for the treatment of gastrointestinal stromal tumours and *renal cell carcinoma*⁴⁸.

With an increase in the number of functions compromised by a particular compound, the risk of adverse effects increases. Off-target effects are likely to occur with

Box 2 | Targeted therapy for breast cancer

The first therapeutic antibody, trastuzumab, aimed at ERBB2 (also known as HER2) on solid tumours was introduced as a ERBB2-specific humanized monoclonal antibody⁷⁷. Investigation into ERBB2 was initiated by the cloning and characterization of the primary structure of the epidermal growth factor receptor. *ERBB2* is overexpressed in 20–30% of the invasive breast cancers associated with reduced patient survival and time to relapse^{63,160,161}. Since its US Food and Drug Administration approval in 1998, trastuzumab has significantly influenced the treatment of ERBB2-positive breast cancer patients. Multiple randomized trials demonstrated the efficacy of this antibody in adjuvant and neo-adjuvant clinical settings. The success associated with trastuzumab as an antibody-based magic bullet highlights the importance of developing targets with functional significance for the malignant phenotype and choosing patients whose neoplasias are driven by these targeted molecular aberrations.

small molecules that target conserved structures within a protein family. Chemical proteomic analyses of widely used inhibitors reveal that a lack of selectivity seems to be inherent in many 'specific' inhibitors of protein kinases¹⁵³. By contrast, biologicals like mAbs are characterized by a reduced likelihood of unknown off-target effects. Low efficacy and on-target toxicity are the more likely drawbacks of some biologicals. 'Network-targeted' combination therapy is also a promising new anti-neoplastic strategy, whereby the therapeutic index might be improved by targeting multiple steps in a cell's signalling network, rather than a single pathway. A detailed analysis of protein networks in cancer cells will be essential for realizing the aim of patient-tailored molecular therapy^{154–156}. Carcinogenesis results from a deregulation in the network of intracellular and extracellular signalling cascades. Distinct crucial signalling proteins regulating the hallmarks of cancer cells are the ultimate targets of a molecular therapy³¹.

Thus, in addition to the option of treating patients with a combination of smart drugs, the careful use of multi-target inhibitors of several essential signal transduction pathways simultaneously could be one challenging interpretation of Ehrlich's magic bullet concept in the 21st century. His philosophy of high chemical selectivity remains valid, although it might be extended to a selection of crucial targets. This modern view of Ehrlich's vision has its foundation in our improved understanding of signal transduction in mammalian cells as a complex network of pathways functioning sometimes hierarchically, but mostly in parallel. Thus, to antagonize malignant cell behaviour, the flow of information through redundant signalling pathways has to be blocked. In Ehrlich's sense, the synthesis of tailor-made designer chemicals that aim at multiple yet distinct crucial targets will place more demands on genomics, molecular biology and chemical biology. However, these fields hold significant promise for fulfilling Ehrlich's demands. We foresee the design of magic bullets developing into a logical science, where the experimental and clinical complexity of cancer can be reduced to a limited number of underlying principles and crucial targets. Still, Ehrlich's maxim that scientific success requires the four big Gs: "Geduld, Geschick, Glück und Geld" (patience, ability, luck and money) will remain essential for the future development of more efficacious magic bullets against cancer.

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Acknowledgements

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DATABASES

Entrez Gene: <http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>
 ABL | BCR | CD20 | CD40 | CD52 | CD80 | CEACAM5 | EGFR | ERBB2 | HSP90AA1 | IGF1R | lymphotoxin β receptor | MDM2 | p53 | PLK1 | RET | TACSTD1

National Cancer Institute: http://www.cancer.gov/acute_lymphoblastic_leukaemia | [anaplastic large-cell lymphoma](http://www.cancer.gov/anaplastic_large_cell_lymphoma) | [breast cancer](http://www.cancer.gov/breast_cancer) | [chronic myeloid leukaemia](http://www.cancer.gov/chronic_myeloid_leukaemia) | [colorectal cancer](http://www.cancer.gov/colorectal_cancer) | [non-Hodgkin lymphoma](http://www.cancer.gov/non-Hodgkin_lymphoma) | [NSCLC](http://www.cancer.gov/NSCLC) | [ovarian cancer](http://www.cancer.gov/ovarian_cancer) | [renal cell carcinoma](http://www.cancer.gov/renal_cell_carcinoma)

National Cancer Institute Drug Dictionary:

<http://www.cancer.gov/drugdictionary/>
 5-fluorouracil | 6-mercaptopurine | alemtuzumab | aminopterin | bevacizumab | BL22 | bleomycin | cetuximab | cisplatin | dasatinib | gefitinib | gemtuzumab | ozogamicin | imatinib | lapatinib | leucovorin | maytansine | methotrexate | mitomycin C | nilotinib | rituximab | sorafenib | sunitinib | trastuzumab | vincristine

FURTHER INFORMATION

K. Strebhardt's homepage: <http://www.molgyn.kgu.de>
 The Nobel Prize in Physiology or Medicine 1908: http://nobelprize.org/nobel_prizes/medicine/laureates/1908/

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