Molecular Biomarkers in Cancer

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Techniques, Discoveries and Translational Applications

Edited by

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PREFACE

Molecular biomarkers of cancer are measurable molecular signals of cancer risk, prevalence or patient outcome. These markers encompass genetic variations within the germline or soma, epigenetic indicators, modifications in transcription, and proteomic signatures. These indications stem from biomolecules present in samples obtained via tumor biopsy or, more conveniently and non-invasively, from sources such as blood (including serum or plasma), saliva, buccal swabs, stool, and urine. Genetic alterations, particularly single nucleotide variants (SNVs) and small insertions and deletions (indels), exemplified by driver mutations like EGFR, KRAS, BRAF, TP53, KITK, and others, constitute the predominant genetic changes utilized as cancer biomarkers. Modifications in gene and protein expression can catalyze shifts in cellular morphology and function, collectively referred to as cellular phenotypes. Consequently, cellular responses like oxidative stress, DNA damage, or cellular apoptosis can serve as cellular biomarkers for cancer. A foundational tool in cancer diagnosis, protein biomarkers predominantly hinge on cancer-specific enzymes, hormones, antigens, and alterations in protein glycosylation patterns, hallmarks of cancer. The infiltration of immune cells into tumors or immune profiling through RNA or protein biomarkers have both shown potential as prognostic or predictive indicators for identifying immunotherapy candidates. Amidst the trove of knowledge, microRNAs (miRNAs) stand prominently. Certain miRNAs exhibit oncogenic potential and distinct expression in tumor tissues—miR-21 and miR-155 are notably overexpressed in numerous cancers. Conversely, miRNAs can exert tumor-suppressive effects, exemplified by let-7, miR-128b, miR-15, and miR-16, which experience downregulation due to methylation, deletion, or other mechanisms. The realm of clinical applications for biomarkers is extensive, offering invaluable tools across various facets of cancer management. They facilitate risk assessment, enable screening and early detection, refine diagnosis, illuminate patient prognoses, predict therapeutic responses, monitor disease progression, and inform treatment decisions within clinical practice.

The ascent of precision oncology underscores the necessity of identifying specific patient subsets poised to benefit from targeted therapies, particularly those designed around distinct genetic mutations. Biomarkers

play an indispensable role in pinpointing these subsets with precision, thereby optimizing treatment outcomes.

The selection of a potential biomarker necessitates a comprehensive evaluation of its efficacy compared to preceding markers. Rigorous scrutiny is followed by potential clinical application. The process entails scrutinizing a cohort of samples, subsequently validated by an independent sample, and further corroborated to establish the utility of the novel biomarker for clinical decision-making. These stages are classified as analytic validity, clinical validity, and clinical utility. Evolution within the sphere of cancer biomarkers is imperative to surmount the scientific challenge of developing novel biomarkers endowed with heightened sensitivity, specificity, and positive predictive value.

The present book endeavors to provide a comprehensive overview of cancer biomarkers, delving into the underlying technology and their multifarious applications.

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CHAPTER ONE

CANCER EVOLUTIONARY DEVELOPMENT IN THE LAST 100 YEARS

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Abstract

The era of cancer research begins with a model development. Chemotherapy was initiated medically in around 1940 with the use of a nitrogen mustard drug post World War II. At the same time, it was observed that green leafy vegetables improve bone marrow function, leading to the discovery of antifolate drugs. The advancement was slow but steady, with the development of thiopurines and antimetabolite groups of anti-cancer drugs. The success of the chemotherapeutic drugs encouraged scientists to go forward, resulting in the discovery of taxols. Cisplatin (a platinum-based compound) was developed for testicular cancer. The advancement in therapy started to change the clinical scenario. A combination of drugs took the place of a single drug with different regimens as the survival rate of patients improved. In targeted therapy, Imatinib mesylate brought a revolution. Adjuvant therapy came in to enhance the activity of the standard chemotherapy regimen.

Keywords: nitrogen mustard, antifolate drugs, antimetabolites, adjuvant therapy, imatinib mesylate

Abbreviations

NCI-National Cancer Institute
ALL-Acute lymphoblastic leukaemia
DHFR-Dihydrofolate reductase
RFT-1-Reduced-folate transporter 1
NCCSC-National Cancer Chemotherapy Service Center
WWII-World War II
5-FU-5-Fluorouracil
6MP-6-Mercaptopurine
L1210-Leukaemia1210 cells
BMS-Bristol Myers Squibb
FDA-American Food and Drug Administration
NSABP-National Surgical Adjuvant Breast Project
CML-Chronic myeloid leukaemia
PDG-FRB Platelet-derived growth factor receptor-β
GIST-Gastrointestinal Stromal Tumours
VEGF-Vascular endothelial growth factor
PDGF-Platelet-derived growth factor

Introduction

Cancer is a set of diseases, characterized by unregulated cell growth invasion and the spread of cells from the site of origin or primary site to other locations in the body. Classically, anti-cancer drugs are grouped as chemotherapy, hormonal therapy, and immunotherapy. Chemotherapy includes many families defined by their chemical structure and mechanism of action: alkylating agents, antibiotics, antimetabolites, topoisomerase I and II inhibitors, mitosis inhibitors, platinum compounds and others. However, the group ‘others’ has expanded so much that this classification is no longer useful (Espinosa et al., 2003).

Paul Ehrlich, a German chemist in the early 1900s developed drugs to treat infectious diseases. He coined the term "chemotherapy", defining it as the chemical used for treating disease. Ehrlich also had a keen interest in drugs for the treatment of cancer in which; he used aniline dyes and the first primitive alkylating agents. The available therapy to treat cancer mainly was surgery and radiotherapy until the 1960s when it became clear that the cure

rates after ever more radical local treatments had plateaued at about 33%. The reason may be the presence of heretofore-unappreciated micrometastases. Modern research indicates that combination chemotherapy could cure patients with various advanced/metastatic cancers. The latter research opened up the opportunity to combine drugs in conjugation with surgery and/or radiation treatments to treat micrometastases. Adjuvant chemotherapy was used initially in breast cancer patients; with this, the adjuvant chemotherapy field came into the picture combining treatments by tailoring the side effect of the drugs whilst maximizing their therapeutic potential for the treatment of cancer (Brested, 1930; Osler, 1912; Papac et al., 2001). The development of anti-cancer therapy is evolving along with the understanding of human tumour biology. The chronological discovery is represented in (Fig 1).

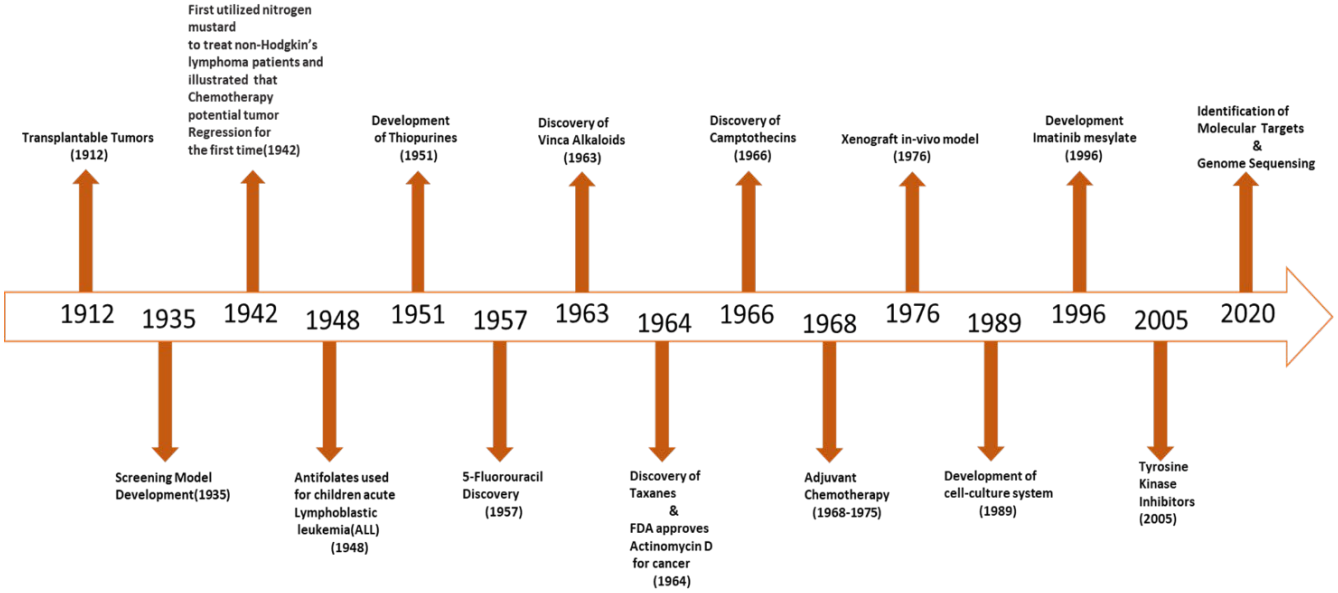

Figure 1- A brief timeline of the discovery of chemotherapy

Cancer Drug Development: Early Period

The initial forty years of the 20th century were mainly committed to developing models. The significant limitations of drug development were in twofold: first, the development of models that could efficiently reduce the vast chemicals to those of the few which might have anti-cancer activity in humans, and second, the establishment of clinical facilities to test such agents (Devita et al., 2008).

The Development of Drug Screening Techniques

In early 1910, a significant breakthrough in model development occurred. George Clowes, at the Roswell Park Memorial Institute (RPMI) in Buffalo, New York, was the first to develop them (Devita et al., 2008). Tumour systems transplanted in rodents allowed for the standardization of the testing models. This gave the chance to test a large number of chemicals to identify the lead molecules. Researchers also keenly focused on the development of models and searched for the ideal model for the development of cancer drug testing. This development took the research on cancer drugs forward over the next decades.

It was in 1973 when Murray Shear (Office of Cancer Investigations of the USPHS) initiated the most organized program model to enhance the screening of cancer drugs (Shear et al., 1947). In 1937 NIH Laboratory of Pharmacology became the National Cancer Institute (NCI). The program became the first to test an extensive array of chemical entities/compounds, including natural products. For the first time, over 3000 compounds were screened, and a murine S37 model was used. Many potential compounds were tested, but the majority of them failed. Only two drugs managed to enter the clinical trial, but the latter was discontinued due to intolerable toxicity; in 1953, the program was dissolved, which ascertained the need to establish an organized national effort for cancer drug screening. This failure was mainly because of two factors (i) due to strict protocol for testing drugs to treat cancer and (ii) the lack of available data and expertise in the field of testing potentially toxic chemicals (Devita et al., 2008).

The Initial Efforts (Post World War II period)

The current chemotherapy era's origin can be traced back to the accidental finding of nitrogen mustard as an efficacious treatment for cancer (Krumbhaar et al., 1919; Krumbhaar, 1919). The men who were exposed to

mustard gas were observed to have a depletion of bone marrow and lymph nodes, both markedly. In 1942, Louis Goodman and Alfred Gilman, both pharmacologists at the Yale School of Medicine, were appointed by the United States Department of Defence to evaluate the potential therapeutic value of a series of toxins developed for chemical warfare. In May 1942, Goodman and Gilman carried out some experiments on mice bearing a transplanted lymphoid tumour and treated them with nitrogen mustard. The experiment was successful, and marked regressions were observed. They wanted to check the effect on humans with non-Hodgkin's lymphoma; they convinced their collaborator, Gustav Lindskog, a thoracic surgeon, to treat a patient with non-Hodgkin's lymphoma with nitrogen mustard (Gilman, 1963). Autopsy findings suggest that soldiers died of exposure to sulfur mustard gas during the First World War, which may be due to the depletion of bone marrow and lymph nodes.

Hence the scientist proposed that this compound might have the ability to destroy a lymphoid tumour (Gilman et al., 1946). Goodman and Gilman used mice bearing a transplanted lymphoid tumour for the experiment. The result was positive, and a marked level of tumour regression was observed; they convinced Lindskog to inject the closely related compounds '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. The suspension of the progression of the disease lasted only a few weeks. Progression of the disease was again observed. The principle was established that drugs might be systemically administered to prevent tumour progression. Goodman and Gilman had carried out experiments to find the mechanism of action of the mustard compound. This group of drugs inhibits tumour growth by the formation of an alkylating intermediate, the ethyleneimmonium ring, which reacts with electron-donating sites on proteins and nucleic acids. The discovery of the molecular mechanism that the drug formed a covalent bond with DNA was studied later, which also demonstrated specific sites of alkylation on purine bases, which leads to crosslinking of strands, hence the induction of apoptosis. In the following 20 years, other improved alkylating agents were chemically stabilized through electron-rich substitutions and administered orally. Cyclophosphamide, chlorambucil and others became standard components of the regimens used to treat patients with lymphomas, leukaemias, and solid tumours to a limited extent. The tumours quickly became resistant to these drugs—an observation that predicted the clinical experience with single-agent nitrogen mustards.

To this day, the first clinical candidate, and one of the most straightforward members of this class, mechlorethamine, is still used in the control of non-Hodgkin lymphoma. Structural relatives abound, including cyclophosphamide (employed against leukaemia, lymphoma, breast cancer, lung cancer, prostatic cancer and ovarian cancer), chlorambucil (used for the treatment of chronic lymphocytic leukaemia), and melphalan (approved for multiple myeloma, ovarian cancer and as an adjuvant in primary breast cancer management) (Jones, 2001).

Antifolates And Methotrexate: After the Second World War, a pathologist at Harvard Medical School, and the Children's Hospital in Boston, Sydney Farber, investigated the impact of folic acid on leukaemia patients. In 1937 Lucy Wills identified this vitamin and she observed that megaloblastic anaemia patients have a deficiency of folic acid (Wills et al., 1937). When administered to children with this cancer, folic acid seemed to stimulate the proliferation of acute lymphoblastic leukaemia (ALL) cells. Farber's collaboration with Harriet Kilte and with medicinal chemists at Lederle Laboratories was conducted to synthesize folate analogues; the first was aminopterin and amethopterin (methotrexate)—which Farber administered to children with ALL in the late 1940s(Farber et al., 1948). By blocking the function of folate-requiring enzymes, these compounds became the first drugs to successfully induce remission in the children with ALL. Though the remissions were short-lasting, the principle was clear as the proliferation of malignant cells may be suppressed by antifolates, thereby restoring the normal function of bone marrow.

Methotrexate alone has antitumour activity in a wide range of epithelial malignancies, including head and neck, breast, ovarian and bladder cancers. In 1958 Scientists Roy Hertz and Min Chiu Li at the National Cancer Institute (NCI) observed that only treatment with methotrexate could cure choriocarcinoma, which was eight years after Farber discovered antifolates (Bonadonna et al., 1976; Li et al., 1958). Choriocarcinoma is a germ-cell malignancy that originates from the trophoblastic cells of the placenta. The achievement was very significant, as it was the first solid human tumour to be cured by drug therapy. High doses of methotrexate along with leucovorin inhibited osteosarcoma recurrence. They were followed by surgical removal of the primary tumour, establishing the principle of adjuvant therapy by Emil Frei and colleagues (Jaffe et al., 1974; Jaffe et al., 1981). Although this therapy was associated with bone-marrow toxicity, the toxic effects were reversible, whereas the antitumour effects cured patients of their cancer (Chabner et al., 2005).

The mechanism of action of the antifolate drugs was to inhibit specifically dihydrofolate reductase (DHFR) (Osborn et al., 1958; Osborn et al., 1958). In due course, Joseph Bertino, David Goldman, Robert Schimke and Bruce Chabner shed light on the molecular mechanism of methotrexate (Jolivet et al., 1983).The activity of methotrexate is totally dependent on the transport of the drug. Methotrexate enters the cell with the help of active transport, the reduced-folate transporter 1 (RFT-1), where it gets converted to polyglutamate (a long-lived) intercellularly, and its binding to DHFR. This process inhibits the synthesis of thymidylate and purines, leading to apoptosis initiation. Defects in any of the above pathways lead to drug resistance, such as (i) mutations in RFT-1, (ii) amplification or mutation of DHFR, (iii) loss of polyglutamation and (iv) defects in the apoptotic pathway, which lead to loss of efficacy (Curt et al., 1984). 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 (Stoller et al., 1977). Even today, methotrexate is used to treat certain lymphomas, osteosarcomas, choriocarcinomas and patients with ALL. This was the first time the principle of drug resistance was understood and established. After the successful treatment of ALL patients, the lawmakers of the United States established the National Cancer Chemotherapy Service Center (NCCSC) at National Cancer Institute (NCI) in 1955 (Chabner et al., 2005).

Penicillin: The wound healing property of Penicillin encouraged the pharmaceutical industry to conduct large-scale screening of products prepared by fermentation to isolate and produce antibiotics to treat wound infections post-WWII and, along with wound infection, to search for antibiotics having antitumour effects. Initially, it was thought that penicillin had antitumour properties but this was never established. Actinomycin D was identified in this program, with significant antitumour properties, and in the 1950s and 1960s, it had multiple uses in paediatric tumours (Pinkel, 1959). The success of this antibiotic provided interest in the research of antibiotics with antitumour activity. This effort yielded a series of active antitumour antibiotics that are in common use today.

Fluorouracil: It was not until the middle of the 1950s that there was a drug for solid tumours. Charles Heidelberger and colleagues at the University of Wisconsin developed a drug aimed the nonhematologic cancers (Heidelberger et al., 1957). They identified a unique biochemical feature in the rat hepatoma metabolism; that is, there was an enhanced uptake as well as the utilization of uracil as compared to normal tissue. Identifying this unique feature, Heidelberger ‘targeted’ this biochemical pathway. He

prepared a molecule where the uracil attaches a fluorine atom to the 5-position pyrimidine base, resulting in the synthesis of the fluoropyrimidine 5- fluorouracil (5-FU). The 5FU has excellent broad-spectrum activity against different solid tumours. Even today, 5FU is the cornerstone for colorectal cancer treatment. In retrospect, 5FU represents the very first instance of targeted therapy for biochemical pathways. These satisfactory clinical data increased the interest in the cancer drugs, which encouraged the R.B. Jackson Laboratories to become the source for transplantable tumours in mice and gave birth to many screening programs independently around the globe (Devita et al., 2008).

Mercaptopurine: The initial success of nitrogen mustard and methotrexate has developed a great interest in the synthesis of other drugs furthermore as alkylating agents and antifolate agents. In 1948, Farber demonstrated the activity of antifolate methotrexate in childhood leukaemia. The same year Hitchings and Elion isolated inhibitors of adenine metabolism. By 1951, two drugs played an essential role in treating acute leukaemia: 6-thioguanine and 6-mercaptopurine (6MP) (Elion et al., 1954, Hitchings et al., 1954). In synthesizing 6-MP, Elion and colleagues illustrated that even a small change in a compound required by cells could inhibit the growth of tumour cells in part through the de novoinhibition of early steps preceding RNA and DNA synthesis (Hitchings et al., 1954; Skipper et al., 1954). Thiopurines and other related compounds are widely used to treat acute leukaemias, and other diseases, such as gout and herpes viral infections, and are also used as immunosuppressive agents in organ transplants. The investigators of this pioneering work received the Nobel Prize in Medicine in 1988 (Devita et al., 2008) .

The concept of Cure

Howard Skipper hypothesized that for the cure of L1210 (Leukaemia1210), it is necessary to eradicate all the leukaemia cells. The extrapolations suggest that the analysis of survival after treatment indicated that even one surviving cancer cell is sufficient to kill a mouse. It is known as the "Cell Kill" hypothesis. The principle of this hypothesis is that in a given dose of the drug, it kills a constant fraction of tumour cells but not a constant number. So the curing rate of the drug would depend on the cell number present at the initiation of each treatment (Skipper, 1964). Furth and Kahn have proved that a single leukemic cell implanted was enough to cause the death of an animal; the observation is an alternative to the extant approach

of drug dosing in the clinic in favour of the more aggressive use of chemotherapy (Furth et al., 1937).

A significant breakthrough happened in the field of both leukaemia and Hodgkin's disease when plant alkaloids from Vinca rosea were discovered at the Eli Lilly Company (Johnson et al., 1963). The discovery of another constituent ibenzmethyzin, in Hodgkin's disease (renamed procarbazine) by Brunner and Young and DeVita and colleagues (Brunner et al., 1965; Devita et al., 1966). The antitumour activity of the *Vinca* alkaloids was known to be due to their ability to inhibit microtubule polymerization and, subsequently, cell division (Bensch et al., 1968; Johnson et al., 1963).

Finally, combinations of drugs, which were thought of as an offence in medicine at the time, were found to be superior to a single agents. Holland, Frei, Freireich and others were combining drugs to treat children with leukaemia, taking advantage of the recently discovered *Vinca* alkaloid, vincristine, to design the program known as "VAMP" (vincristine, amethopterin, 6-mercaptopurine and prednisone) (Dameshek et al., 1965; Devita et al., 2016; Thompson et al., 1965).

In 1965 the combination of methotrexate (an antifolate), vincristine (a *Vinca* alkaloid), 6-MP, and prednisone was known as the POMP regimen. This regimen was found to have long-term remissions in children with ALL diseases (Berd et al., 1975; Berry et al., 1980; Frei Iii et al., 1965). Combinations of drugs, where each of them has a different site of action, proved to be the most effective way to prevent drug-resistant tumour cells, such as an antibiotic combination therapy for tuberculosis and subacute bacterial endocarditis (Chabner et al., 2005).

In the current scenario, the majority of children diagnosed with acute lymphocytic leukaemia are cured with the help of an aggressive combination chemotherapy regimen (George et al., 1968; Pinkel et al., 1971). In the early 1960s, advanced Hodgkin's disease was fatal. When treated with a single alkylating agent the remissions were in around 25% of patients. As with acute childhood leukaemia, they were brief and usually incomplete. DeVita, Moxley and Frei developed a combination regimen with the advantage of the *Vinca* alkaloids and the NCI data on procarbazine in Hodgkin's disease to establish first the MOMP program (Moxley et al., 1967). MOMP contains a combination of nitrogen mustard with vincristine, methotrexate and prednisone. The MOPP program replaced methotrexate with procarbazine to test the principle of combination chemotherapy in advanced for untreated Hodgkin's disease (Devita Jr et al., 1970; Longo et

al., 1986). The MOMP and MOPP protocols developed extreme resistance (Devita et al., 2008; Devita et al., 2016).

By 1970, advanced Hodgkin's disease was also considered curable with the help of drugs. It became the first example of advanced cancer of a central organ system in adults to be cured by chemotherapy. Currently, Hodgkin's disease is treatable in 90% of cases. A combination of chemotherapy is incorporated with radiotherapy for early-stage disease. In 1975, NCI investigators discovered that diffuse large B-cell lymphoma patients were also cured by the combination regimen referred to as C-MOPP, substituting nitrogen mustard with cyclophosphamide (Berd et al., 1975; Devita Jr et al., 1972; Levitt et al., 1972). The efficacy of the MOPP program was confirmed for leukaemia. In 1984 in the United States, the national death rates from childhood leukaemia and Hodgkin's disease were reduced by 65%, with new therapies being quickly taken into practice. By the end of the 1960s, the chemotherapy program became better with the continuous effort to find the missing links, and it gave an expectation that anti-cancer drugs could cure cancer (Devita Jr et al., 1975).

Zubrod was interested in natural products, and he established a program for the collecting and testing of plant and marine sources. This program led to the discovery of taxanes (in 1964) and camptothecins (in 1966). Paclitaxel (Taxol) is a novel antimitotic extracted from Pacific Yew tree bark. The mechanism of action to arrest cell growth promoted a microtubule assembly. The drug has the drawback that it is insoluble in water; hence the lipid emulsion formulation was prepared, and some patients developed hypersensitivity reactions. Going through a long clinical trial (four years) for efficacy in solid tumours in 1987, after 23 years of discovery, it showed consistent efficacy in patients with ovarian cancer(Mcguire et al., 1989). Bristol Myers Squibb's (BMS) first billion-dollar drug/year was Taxol (Goodman et al., 2001). Another drug, Camptothecin, obtained from a Chinese ornamental tree, inhibits the enzyme that helps in DNA unwinding and strand passage, known as topoisomerase I. Despite exhibiting encouraging preclinical data, the early clinical trials suggested very little antitumour activity and were also toxic to the kidneys. The loss of activity in vivowas due to the instability of its lactone ring at pH 7. The molecule becomes active when it enters the urine, with acidic pH, causing renal tubular damage. In 1996 a camptothecin analogue, Irinotecan, which was stable, finally got approved by the Food and Drug Administration (FDA) to treat colon cancer (Saltz et al., 2000). Later, this drug was allowed to be used to treat lung and ovarian cancers (Bodurka et al., 2003; Noda et al., 2002) .

Around 60 cell lines from different cancers, including the brain, colon, melanoma, ovarian, lung, leukaemia and renal, were derived from human cell lines. In 1976, considerable development occurred with the success of xenografts in in-vivo models, which were established from patient biopsy material or human cancer cells (Kelland, 2004) . Breast (MX-1), colon (CX-1), and lung (LX-1) were the first human tumour xenograft models (Teicher, 2013) .

Age of Adjuvant chemotherapy

The use of chemotherapy as an adjunct to surgery or radiotherapy received mixed opinions. The use of adjuvant therapy programs in patients with the same tumour type and advanced stages are preferable advantageous. The combination therapy gave confidence that chemotherapy might have the capacity to cure patients with micrometastases while not being excessively toxic.

In the late 1960s clinicians started to use a combination of chemotherapy in cases of advanced breast cancer and found some encouraging results. Despite the exciting results of the new chemotherapy, surgeons in the United States were not willing to test its use postoperatively in clinical trials. The first courageous surgeon Bernard Fisher and his group the National Surgical Adjuvant Breast Project (NSABP) conducted the early adjuvant study. Thiotepa, an alkylating agent, was used postoperatively to kill cancer cells dislodged during surgery to check the efficacy of the drug in cancer patient samples (Fisher et al., 1968).

During two other programs developed for field tests at the Clinical Center of the NCI, L-phenylalanine mustard (L-PAM) with CMF regimen, a combination of cyclophosphamide, methotrexate, and 5-fluorouracil, specifically designed for use as adjuvant chemotherapy had an overall response rate over 50%, and about 20% of the patients attained complete remission (Canellos et al., 1974).

In patients with colon cancer, there is evidence of survival being improved with the use of the drug 5-Fluorouracil when used as adjuvant therapy. Studies conducted by the chairman of the National Surgical Adjuvant Breast and Bowel Project, Bernard Fisher (1967 to 1994), proved that survival in patients with breast tumours after adjuvant chemotherapy following complete surgical resection was significantly extended—particularly in those with axillary nodal disease (Bonadonna et al., 1976).

A cisplatin derivative with better anti-cancer properties along with fewer side effects on the kidney (nephrotoxicity), carboplatin,was developed by Eve Wiltshaw and Hillary Calvert (Evans et al., 1983). Nitrosourea, an alkylated agent that crosslinks with DNA, was developed by a novel reaction at the O6 position of guanine synthesized by John Montgomery and his group had activity against malignant gliomas (Montgomery, 1976; Walker et al., 1970; Wilson et al., 1970).

Montgomery's group developed a purine analogue, fludarabine phosphate, which has become a mainstay in treating patients with chronic lymphocytic leukaemia (Rai et al., 2000). From the years 1970 to 1990, some essential molecules were developed in the industry, which includes anthracyclines and epipodophyllotoxins. They both have a similar mechanism of action. They inhibit the action of topoisomerase II, which is an essential enzyme for DNA replication, translocation to the cytoplasm and repairing damaged DNA (Minocha et al., 1984).

The toxicity of the anti-cancer drugs was a major problem as it affected the entire body. Researchers started to encounter these problems one by one. Oncologists accepted these as the bargain for controlling a fatal disease. Development of platelet transfusion advocated by Freireich and colleagues at the NCI through aggressive use of antibiotics ameliorated the lethality of bone-marrow suppression significantly. The aggressive use of antibiotics in neutropenic patients to prevent infections and the rapid restoration of neutrophilsby the later discovery of growth factors such as granulocyte colony-stimulating factor and granulocyte–monocyte colony-stimulating factor were major achievements (Freireich et al., 1959; Gaydos et al., 1962; Lieschke et al., 1992; Mcguire et al., 1989; Pizzo, 1984). Despite the discovery of all these supportive measures, the potential of some cytotoxic drugs to cause leukaemia and their long-term effects on cardiopulmonary and reproductive organs remains a challenge and a concern even though the patients are cured of their primary tumours (Burstein et al., 2000; Curtis et al., 1992).

The Targeted Therapy Revolution

In the 1980s, attempts to improve the pace of the discovery of cytotoxic drugs proceeded. The contemporarily genetic and molecular approaches for cell biology were uncovered, which brought to the fore new signalling networks regulating cellular activity. The proliferation and survival networks are known to be damaged in cancer cells. In an attempt to repair

these molecular defects in cancer cells, the industrial revolution unfolded, primarily based on small biotechnology firms; this was the beginning of the targeted therapy era. The new targets which were discovered in this era were growth factors, signalling molecules, proteins involved in cell-cycle, modulators of apoptosis and the molecules that promote angiogenesis (Hanahan et al., 2000). Specific growth factors were identified by cell biologists Stanley Cohen and Rita Levi-Montalcini. Other eminent workers found the network of signalling molecules that played a role in connecting these receptors to the cell's nucleus, thus creating a controlling system for cell proliferation and death.

The cancer drug development program was transformed due to the potential development of the drug targets during the early 1990s when it got transformed from a low-budget government-supported effort of research to a high-stakes, exciting and rewarding multi-billion industry. The success of finding inhibitors of specific targets was subsequently increased due to technological innovation. High throughput screening brought the screening of molecules fast. The technique also helped to find molecules that could be optimized for properties like greater specificity and the bioavailability of potent anti-cancer drugs. This process should, first, be metabolically stable with a long half-life expressed in the model system as well as in humans; second, have a slow metabolic degradation by enzymes like the cytochrome P450 family. Furthermore, the candidate molecule should have good oral bioavailability, which was not a property of chemotherapeutics drugs developed in the 1970s-1980s. Finally, the agent should exhibit an affordable toxicity profile at a biologically efficient dose with the fewest side effects on body tissue like the bone marrow and gastrointestinal epithelium.

Imatinib mesylate (Glivec) development has been a landmark event in the targeted revolution. Imatinib, a simple structural entity, exhibits all the favourable factors of an ideal targeted compound. It was initially derived from a naturally occurring product by a chemist working in the laboratory of Novartis. Imatinib moderately inhibits the enzyme kinase BCR-ABL, a fusion protein resulting from a translocation of the chromosome implicated in the pathogenesis of CML. Imatinib inhibits two other enzymes also, namely the KIT-tyrosine kinase and PDGFRB (platelet-derived growth factor receptor-β). These properties have been successfully used respectively for the treatment of GIST (gastrointestinal stromal tumour) and hypereosinophilic syndrome.

During a research study carried out by Brian Draker in which imatinib was used in the chronic phase of CML patients, 90% achieved complete haematological remission and an associated loss of cytogenetic evidence of malignant clone. BCR-ABL translocation, however, could still be detected in most patients (Druker et al., 2001; Hughes et al., 2003; Kantarjian et al., 2002). On the other hand, in patients with an acute leukemic phase of CML. Imatinib was found to induce a brief remission, and on treatment, the rapid growth of drug-resistant cells was found, which showed ABL mutations in its catalytic kinase domain, which renders the enzyme to be in an open configuration, thus making it bind to drug poorly and still be catalytically active (Shah et al., 2002).

In some chronic phases, CML patients' drug-resistant cells were found even before drug exposure, a feature which comes with the rapid growth of resistance (Branford et al., 2003). It proves that cancer expresses many resistant mutant subclones even before the start of treatment (Roche-Lestienne et al., 2002).

Gefitinib (Iressa) the inhibitor of EGFR was approved by the FDA as having marked anti-cancer properties due to an inhibition of the ATP-binding site of tyrosine kinase on EGFR. The drug was used in non-small cell lung cancer (NSCLC), and gave a 10-15% partial cure, but failed in a randomized large clinical trial (Giaccone et al., 2004; Herbst et al., 2004; Kris et al., 2003). Later Cetuximab (Erbitux), a monoclonal antibody, was approved by the FDA and is active against the extracellular domain of EGFR. It was used as a combination therapy for colon cancer (Cunningham et al., 2004). However, the two drugs work on the same receptor differently but produce similar responses. Researchers at Harvard discovered the mutation of EGFR in cancer patients, which was the cause of the low success rate with the drugs in lung cancer (Lynch et al., 2004; Paez et al., 2004). This discovery suggested for the molecular/genomic test to identify the reliable markers to find the sensitivity of the drug which became an era-changing development in the field of chemotherapy (Roberts Jr et al., 2004).

In 1970, Judah Flokman illustrated the role of angiogenesis which in turn allows the cancer cells to cause metastasis, and also found that angiogenic molecules (PDGF, VEGF) are secreted from the cancer cells (Folkman, 1971). Though there was an initial failure of the endostatin, an antiangiogenic peptide, and the small molecules (SU-11248, Bayer 43-9006) inhibit VEGF receptor 2. Bevacizumab(Avastin) an anti-VEGF antibody, has an evident anti-cancer property on renal carcinoma with VHLgene mutation (Gnarra et al., 1994). This drug increases the activity of

standard chemotherapy and was approved by the FDA for treating a cancer patient with this drug as an adjuvant (Hurwitz et al., 2004).

Conclusion

The development and discovery of cancer therapy have gone through a cycle with better drugs. The knowledge of the disease has improved. The initial observations of Goodman, Farber and Gilman were accurate and are unchanged even today. The evolution of cytotoxic drugs to targeted therapy constitutes an essential development. The basic principle developed for treatment as well as for drug resistance mechanisms remains the same. The combination of targeted therapy improved the survival of patients and made it curable too. Clinicians managed the toxicity of the chemotherapeutics well. The molecular and genetic assay identified the patient subset with specific gene modification, resulting in an improved response to certain drugs avoiding toxicity and ineffectiveness. Adjuvant chemotherapy improves the anti-cancer property of standard drugs. The next decade will have a scientific challenge for exploiting cancer cells' mutability.

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