Left. Dr Jonathan Hartwell (right) and his assistant Sylvy R Levy Kornberg conduct some of the earliest chemotherapy tests at the National Cancer Institute, about 1950.

WAR ON CANCER PT.2

LANDMARKS IN THE DEVELOPMENT OF CHEMOTHERAPY

The second article of two, which briefly reviews the historical developments and use of chemotherapy since 1948 to treat different cancers.

The early years of the 1950s were a watershed in the history of chemotherapy, it would appear that cautious optimism was countered by serious, but well intended reservations on the clinical benefits of chemotherapy and quality of life issues. This was due, in part, to the transitory nature of remissions and often severe side effects of treatments. However, this was about to change dramatically with a range of clinical successes, combined with a number of significant advances in understanding the biology of the cell and genetics of cancer leading to a more structured, scientific and targeted approach to the development of more effective chemotherapeutic agents. Future cancer drugs would attempt to counter the proliferation of cancer cells targeting relevant cellular processes, notably mitosis and DNA synthesis. Surprisingly perhaps, many important agents of this period were derived from plant sources, often after long, complex and laborious experimentation and sometimes with much good fortune.

The antimetabolites

Methotrexate

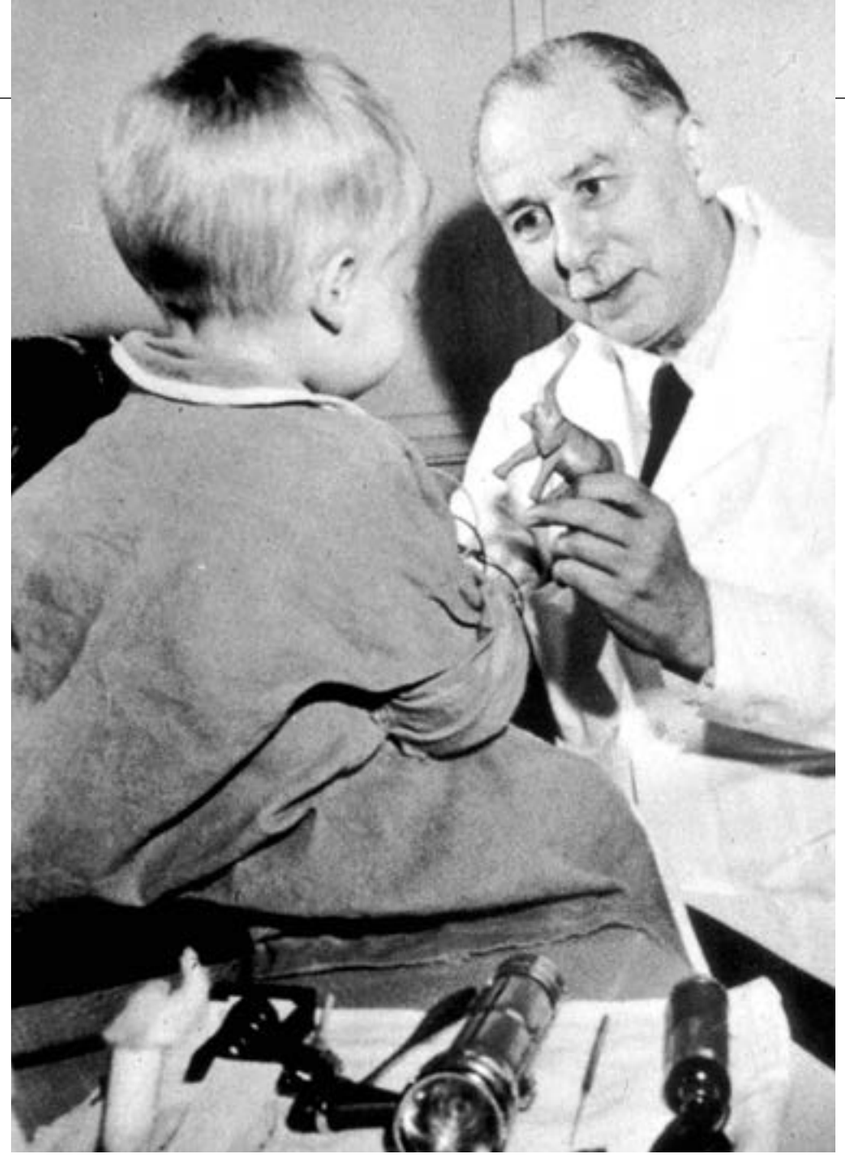
In 1948, Sidney Farber showed that chemical agents antagonistic to folic acid could achieve short term clinical remission in childhood acute lymphoblastic leukaemia (ALL) with aminopterin. Other antagonists were developed and amethopterin (methotrexate) was shown to be the most effective therapeutically. Methotrexate (MTX) has a number of structural similarities to folic acid and is now known to act as a competitive inhibitor of dihydrofolate reductase, which catalyses the conversion of inactive dihydrofolate to active tetrahydrofolate, which is required for the synthesis of nucleotide bases

purines and pyrimidines, which are structural components of DNA and RNA, which regulate the synthesis of proteins to drive mitosis. In 1951, Jane C Wright, a US pioneer researcher in cancer chemotherapy at the Harlem Hospital Cancer Centre in New York, successfully used MTX in the treatment of solid tumours with remissions in breast cancer and later MTX was used in combined chemotherapy to improve effectiveness and minimise side effects. Wright and colleagues are also particularly noted for their use of tissue culture techniques to assess drugs on cultured cancer cells and improved cancer drug delivery methods. In 1958, Min Chiu Li with Roy Hertz at the National Institute, Maryland, used MTX for the effective treatment of gestational choriocarcinoma (GC) in landmark research studies.

Currently, MTX can be used alone or in combination chemotherapy for GC, ALL, breast, epidermoid cancers of head and neck, T-cell lymphoma, lung cancer and osteosarcoma. Unfortunately, side effects are common and affect normal proliferating cells, so there may be bone marrow suppression, oral, skin, gastrointestinal tract or bladder inflammation, and lung fibrosis.

Purine and pyrimidine analogues – 6 mercaptopurine & 5 fluorouracil

US biochemist George Hitchings developed a special interest in DNA bases, notably purines, at Harvard and joined the Wellcome Research Laboratory in New York in 1942 and employed young chemist, Gertrude Elion, as his assistant in 1944. This proved to be a most fruitful working collaboration in pioneering the development of a range of effective drugs and in 1948 they isolated a competitive inhibitor of adenine metabolism in *L. casei*, which interrupted cell growth and was later shown to be an immunosuppressant in rabbit studies. By 1951 numerous purine analogues had been assessed and 6 mercaptopurine (6MP) was chosen for trials using mice at the Sloan Kettering Cancer Centre, New York, and later with some success in human childhood leukaemia there and was quickly approved by the FDA in 1953 alone or combined with methotrexate. 6MP continues to be used in the chemotherapy of ALL and lymphoblastic lymphoma. Elion and the research term developed other clinically effective drugs, such as Daraprim (malaria), allopurinol (gout), azathioprine, an immunosuppressant used in organ transplants, and acyclovir for herpes infection. For these achievements, Hitchings and Elion were awarded the Nobel Prize in Physiology or Medicine in 1988, along with Sir James Black the Scottish physician and pharmacologist who developed the beta blocker propranolol and cimetidine for the treatment of peptic ulcers.



Left. Sydney Farber

IMAGES: NATIONAL CANCER INSTITUTE

"Farber conducted a clinical trial with radiation treatment in children"

5 Fluorouracil

Working at the McCardle Laboratory at the University of Wisconsin, Charles Heidelberger and colleagues pioneered cancer research from the late 1940s. They used isotopes to study metabolic processes in cancer cells and the malignant transformation of mammalian cells in culture. They turned their attention to the development of anti-cancer drugs following a report of an increased uptake of the pyrimidine base uracil in rat hepatoma issues. They found that a fluorine substituted uracil, and 5 fluorouracil (5FU) inhibited the biochemical pathway to thymidylic acid and so interfered with DNA/RNA synthesis to limit tumour growth.

A clinical trial of purified 5FU performed in 1958 with 103 patients with a variety of solid tumours, leukaemias and lymphomas showed encouraging results and paved the way for a long-term future for 5FU alone or in combination in the treatment of cancers of breast, gastrointestinal tract and with folinic acid in advanced colorectal cancer.

Cytotoxic antibiotics

Dactinomycin

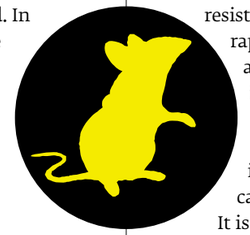
During the mid-1950s, antibiotics as growth inhibitors were considered potential candidates as anti-cancer agents. A decade earlier, a soil microflora research team led by US microbiologist Selman Waksman at Rutgers University, New Jersey USA, isolated actinomycin D from *Actinomyces antibioticus*. Actinomycin D was shown to inhibit certain transplantable tumours in mice and in 1955 Sidney Farber conducted a clinical trial of actinomycin D combined with radiation treatment in children with a variety of tumours, notably Wilms' kidney tumour. He found in many cases it prevented local recurrence and achieved clearance of metastases in the lungs with a significant improvement in their two-year survival rate. Further trials in the following two years confirmed these findings, including that led by Donald Pinkel at Roswell Park, New York. Side effects include bone marrow suppression and a risk of liver toxicity. Now known as dactinomycin, it was approved by the FDA in 1964 and is currently used in the chemotherapy of children with Wilms' tumour, rhabdomyosarcoma and in women with gestational trophoblastic disease. It is now known that dactinomycin acts by insertion into the structure of guanine and cytosine base pairs to interfere with DNA transcription at high dosage, while blocking RNA synthesis at lower doses. Other cytotoxic antibiotics used in cancer chemotherapy include bleomycin, mitomycin C and doxorubicin.

Mitotic spindle poisons

Vinca alkaloids – Vinblastine, Vincristine and Vinorelbine

During the early 1950s there was an intensive drive to investigate natural plant and marine sources for their medicinal properties including chemotherapy. In 1954 a research team led by Robert Noble at the Collip Laboratory at the University of Western Ontario, fortuitously found that extracts from the leaves of the Madagascar periwinkle plant *Vinca rosea* had a mild anti-hyperglycaemic property and, more significantly, a profound effect on the bone marrow and lowered white blood cell counts in the rats studied. In 1958 Noble with his colleague Charles Beer isolated and characterised vinblastine, a potent alkaloid extract and with the support of Eli Lilly Pharmaceuticals the first successful clinical trial of children with acute lymphoblastic leukaemia at Princess Margaret hospital in Toronto commenced in 1959. Research was continued by Eli Lilly in Indianapolis, Indiana, and in 1963 Irving Johnson led a team to perform intensive chemical and biological studies of over 30 alkaloids from *Vinca rosea*, notably vinblastine (VB) and vincristine (VC) and reviewed their activity against a wide range of cancers, notably Hodgkin's, lymphomas and leukaemias. Vinorelbine was synthesised much later during the 1980s by the late French pharmacologist Pierre Potier and has been approved for non-small cell lung cancer, and stage 4 breast cancer. Side effects include nausea, vomiting and peripheral neuropathy.

It is now known that the vinca alkaloids act as mitotic spindle poisons, binding to tubulin, unit components of the microtubules, thus inhibiting mitosis of actively dividing cells. VB has been selected for therapy in testicular cancer, Hodgkin's and non-Hodgkin's lymphoma, breast and germ cell tumours, but may cause nausea, vomiting, fever and chest pain and lower the white cell count. VC has been approved for acute leukaemia, neuroblastoma, rhabdomyosarcoma, Wilms' tumour and Hodgkin's lymphoma, but may suppress the bone marrow and cause peripheral neuropathy.



Combination chemotherapy (CC)

CC was pioneered by Emil Frei III, Emil Freireich and James Holland in 1965 for the US National Cancer Institute divisions in Maryland and Texas. Used in ALL, combining MTX, M6P, VC and prednisone, which showed improved remission rates, whereas a single agent may encounter resistance in patients and a more rapid relapse. As anti-cancer agents act on cancer cells in different ways and at different points in the cell cycle, using CC potentially increases the chance that all cancer cells will be destroyed. It is particularly useful in solid tumours, such as lung cancer, to reduce the risk of recurrence, improve survival and response to treatment. As expected there are possible disadvantages with a danger of drug interactions and more side effects, which may be more manageable now with the use of anti-emetics and Neulasta, a cytokine, which can boost white blood cell count.

Left. Howard Skipper, from Southern Research Institute in Birmingham, Alabama

Taxoids – Taxol and Taxotere
Taxoids are a series of complex polycyclic compounds with oxygen containing functional groups derived from the bark and needles of the Pacific Yew *Taxus breviflora* or European Yew tree, *Taxus baccata*. During the pursuit of natural plant components in cancer chemotherapy, bark samples were submitted in 1962 to the Research Triangle Institute in North Carolina for investigation led by Monroe Wall and Mansukh Wani. Extracts were found to be cytotoxic and the active component Taxol was isolated and characterised by this research team in 1967. Taxol was subsequently classified as a mitotic spindle poison, binding to cellular microtubules preventing cell division in rapidly dividing cells. Clinical trials of patients with ovarian and breast cancer were conducted at a number of US specialist cancer centres and Taxol therapy found to significantly reduce tumour size. However, there were serious side effects with severe allergic reactions and one fatality. Working with Bristol Myers Squibb Pharmaceuticals in 1991, it was renamed paclitaxel, but with limited stocks and for sound ecological reasons active research by over 30 groups were undertaken in order to synthesise Taxol. In 1994 total synthesis was achieved at the Scripps Research Institute in California by a team led by KC Nicolaou. Alternatively taxotere, a semi-synthetic derivative of taxol, was prepared by a French research team in 1992 and given the commercial name Docetaxel and later found to be active against a wide range of cancer tumours, including breast, prostate, stomach, head and neck, and non-small cell lung cancer. NICE currently recommends its use with adjuvant treatment of breast cancer and in combination regimes for other cancers, or those resistant to previous chemotherapy. NICE recommends conditional use of paclitaxel for ovarian, breast, pancreatic and non-small cell lung cancer in combination, resistant or appropriate chemotherapy.

Topoisomerase inhibitors – camptothecin, irinotecan, etoposide

Topoisomerases (TIM 1 and 2)
TIM 1 was first described in 1971 by James Wang, a Chinese born US biochemist and shown to alter the 3D structure of DNA by separation, unwinding and recombination reactions during replication and transcription, which limit cell mitosis. TIM 1 catalyses these actions in single-stranded DNA, while TIM 2 acts on double-stranded DNA. Camptothecin (CPT) was another anti-cancer agent discovered by Wall and Wani in 1966 at the Research Triangle Institute from the bark and stem of a Chinese ornamental tree, *Camptotheca acuminate* (Happy tree) and was found to be a potent anti-tumour agent in early clinical trials in 1988, and acts by binding to TIM 1-DNA preventing DNA replication. But as for Taxol, difficulties were encountered, due to its low solubility but also there were serious risks to renal function. As a consequence, research groups investigated synthesis procedures for CPT and analogues but it was not until 1996 when a suitable and stable analogue, irinotecan, was prepared. With the collaboration of the Christus Stenlin Foundation, another analogue 9 NitroCPT was developed with low toxicity and used in a clinical trial in patients with advanced pancreatic cancer and showed a modest improvement in survival time. Irinotecan has been used to treat colon cancer and small cell lung cancer in combination with fluorouracil and cisplatin, respectively. And is also recommended in the treatment of metastatic adenocarcinoma of the pancreas. The most severe side effects are diarrhoea and suppression of the immune system.

EXAMPLES OF COMBINATION CHEMOTHERAPY

Combination	Cancer types
5 Fluorouracil/Methotrexate	Colorectal cancer
Cyclophosphamide/5 Fluorouracil/Methotrexate	Breast cancer
Cisplatin/Vinorelbine	Small cell lung cancer
Doxorubicin/Bleomycin/Vinblastine/Dacarbazine	Hodgkin's lymphoma
Doxorubicin/Cyclophosphamide	Breast cancer
Bleomycin/Etoposide/Cisplatin	Testicular cancer
Cyclophosphamide/Doxorubicin/Vincristine	Small cell lung cancer
Cyclophosphamide/Doxorubicin/Vincristine/Prednisone	Non-Hodgkin's lymphoma
Cisplatin/5 Fluorouracil	Oesophageal & Head and Neck
5 Fluorouracil/Folinic acid/Oxaliplatin/Taxotere	Oesophageal, Gastric
Irinotecan/5 Fluorouracil/Folinic acid	Colorectal cancer
Melphalan/Predisone/Thalidomide	Myeloma
Taxol/Ifosfamide/Cisplatin	Testicular & Germ cell cancers
Carboplatin/Vincristine/Etoposide	Retinoblastoma
5 Fluorouracil/Methotrexate/Doxorubicin	Gastric
5 Fluorouracil/ Folinic acid	Colorectal cancer

HOWARD EARLE SKIPPER (1915–2006)

Howard Skipper was a US oncologist who, after qualification at the University of Florida, served in the US Army Chemical Warfare Service 1941-45 and gained some experience in anti-cancer agents with nitrogen mustard. In 1946, he joined the Southern Research Institute, Birmingham, Alabama and with chemists Lee Bennett Jr, John Montgomery and virologist Frank Schabel, established a dynamic cancer research programme. The main focus was to develop quantitative animal models to screen potential anti-cancer drugs, notably for the treatment of leukaemia and lymphomas, using murine leukaemia cell lines. The team also studied the kinetics of tumour growth and developed protocols for monotherapy and combination chemotherapy in seminal experiments during the 1960s, which were crucial to the preclinical testing of numerous agents. High dose chemotherapy was advocated by this group. The early screening *in vivo* models were L1210 and P388 murine leukaemia, used in 1955-1975 with some success for identifying agents for the treatment of leukaemia and lymphoma. Today the US National Cancer Institute holds a bank of over 60 human cancer cell lines.

"Skipper gained some experience in anti-cancer agents with nitrogen mustard"

Etoposide (EP)
There is evidence that extracts from *Podophyllum* plants have an ancient history as medicinal plants as recorded, by Dioscorides, a Greek physician and pharmacologist. Investigations began in the early 1950s to explore this potential using extracts of the rhizome of *Podophyllum peltatum* (American mandrake) at the Sandoz laboratories in Basel, Switzerland. Over two decades of research by a team led by Hartmann Stahelin and Albert von Wartberg was required to prepare the semi-synthetic derivative etoposide which showed cytotoxic potency and prolonged survival on the L1210 leukaemic mouse model in 1966, and clinical trials were conducted in 1971. However, it would be a further 12 years for FDA approval. There were intensive studies on the mechanism by which etoposide acts and studies by Minocha and Long in 1983 showed that etoposide inhibits TIM 2 by binding to DNA to cause strand breakage and inhibit DNA replication. Etoposide has been used in chemotherapy, often in combination for testicular cancer, ovarian cancer, lung cancer, leukaemias and neuroblastoma. Side effects include bone marrow suppression, allergic reactions and increased risk of infection.

Platinum compounds – cisplatin and carboplatin

First investigated by Barnett Rosenberg, a biophysics researcher, in 1965 at Michigan State University, who observed that cell division of *E. coli* ceased during electrolysis using platinum electrodes. Further work performed identified a soluble cytotoxic compound released from the platinum electrodes as cisplatin and found positive results with a sarcoma mouse model but nephrotoxicity at high doses. Clinical trials began in 1972 at Indiana University led by Lawrence Einhorn with highly successful results using combination chemotherapy with cisplatin, vinblastine and bleomycin in patients with metastatic testicular cancer. FDA approval was achieved in 1978 and also for patients with ovarian and bladder cancer. Analogues, notably carboplatin, were developed later by a research team at the Institute of Cancer Research in London, UK and reported in 1983 showing less toxic side effects. Carboplatin can be used in ovarian, lung and head and neck cancers but is less effective than cisplatin in certain testicular cancers. Cisplatin has also been used alone, or in combination, for cervical cancer and certain brain tumours, but side effects may be common and are often serious.

Concluding comments

This has been a fascinating period for chemotherapy with a range of approaches adopted to destroy cancer cells, and the use of natural plant sources to derive anti-cancer agents, synthesise analogues, and collaboration with pharmaceutical companies. The trials and tribulations of cancer drug development is well exemplified by Taxol and etoposide and likewise good fortune in the discovery of vinblastine and cisplatin.

It is the initiative, dedication and scientific quality of the research scientists and oncologists included in this short review which is so impressive. Innumerable people have made invaluable contributions in the war on cancer, which nevertheless continues. IBMS

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To see the references, view the article online at **thebiomedicalscientist.net**