Part I
Principles of Tumor Targeting

Drug Delivery in Oncology: From Basic Research to Cancer Therapy, First Edition.
Edited by Felix Kratz, Peter Senter, and Henning Steinhagen.

1
Limits of Conventional Cancer Chemotherapy

Klaus Mross and Felix Kratz

1.1
Introduction: The Era of Cancer Chemotherapy

The first effective anticancer drug that was developed was not worked out theoretically in a research laboratory, but took its beginning due to a tragic incidence during World War II. A German air raid in Bari, Italy led to the exposure and deaths of more than 1000 people to mustard gas (Figure 1.1).

The autopsies of the victims that Dr. Stewart Francis Alexander, an expert in chemical warfare, was subsequently deployed to investigate suggested that profound lymphoid and myeloid suppression had occurred after exposure. Dr. Alexander intuitively realized that since mustard gas primarily stopped the division of those types of somatic cells whose nature it was to divide fast, mustard gas should also potentially suppress the division of certain types of cancerous cells, which he noted in his report [1].

With this information in hand, two pharmacologists, Dr. Louis S. Goodman and Dr. Alfred Gilman, reasoned that mustard gas derivatives could be used to treat lymphoma, since lymphoma is a tumor of lymphoid cells. After setting up an animal model for lymphomas in mice, they were able to demonstrate that they could treat the tumor-bearing mice effectively with mustard agents. In a one-patient trial, they injected a related agent, the prototype nitrogen mustard anticancer chemotherapeutic, mustine, into a patient with non-Hodgkin's lymphoma and observed a dramatic reduction in the patient's tumor masses. Although this effect lasted only a few weeks, it was probably the first well-documented experimental trial that a cancer patient could be treated by a cytotoxic pharmacological agent, which has been in use under the brand name Mustargen® [2].

Shortly after World War II, Sidney Farber's work at the Harvard Medical School paved the way for the first rational design of an anticancer drug that earned him the name as the father of modern cancer chemotherapy (Figure 1.2). Farber had appreciated the work by Lucy Wills who had shown that folic acid seemed to stimulate the proliferation of acute lymphoblastic leukemia (ALL) cells when administered to children with this cancer. In collaboration with chemists at Lederle Laboratories, Farber probed folate analogs as antiproliferative agents

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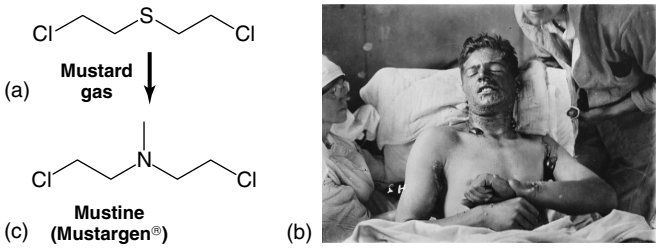


Figure 1.1 (a) Structure of mustard gas (1,5-dichloro-3-thiapentane), a highly toxic alkylating agent used as a vesicant warfare agent during World War I and II that produces severe burns and damage to the bone marrow and lymphoid system; (b) unidentified Canadian soldier with burns caused by mustard gas, *ca*. 1916–1918; (c) the structure of mustine (Mustargen), the first prototype of an alkylating agent, which has been used for treating Hodgkin's and non-Hodgkin's disease.

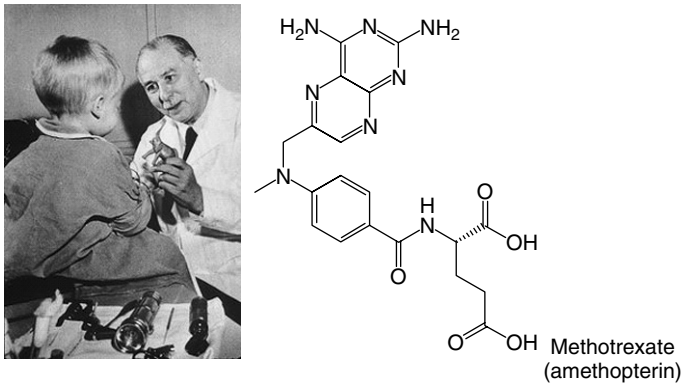


Figure 1.2 Sidney Farber discovers the first rationally designed anticancer agent, methotrexate, an inhibitor of the enzyme dihydrofolate reductase, which was successfully used to treat ALL and subsequently to successfully cure chorioncarcinomas (fast-growing solid tumors).

and discovered that certain analogs – first aminopterin and then amethopterin (now methotrexate) – were antagonists of folic acid and blocked the function of folate-requiring enzymes. In 1948, these agents became the first drugs to induce remissions in children with ALL. Although remissions were not long-lasting, the principle was clear – antifolates could suppress proliferation of malignant cells. It is somewhat surprising and in some ways bizarre that although Paul Ehrlich had set firm grounds with Salvarsan®, an arsenic-containing complex to treat syphilis successfully nearly 40 years earlier despite heavy protests from influential members of the scientific community, Farber met resistance once again to conducting his studies with a chemotherapeutic principle at a time when the commonly held medical belief was that leukemia was incurable and that the children should be allowed to die in peace. Even after Farber's 1948 report in the *New England Journal of Medicine* it was met with sarcasm and conspicuous astonishment [3].

As a deserved compensation, a decade later at the National Cancer Institute (NCI), Roy Hertz and Min Chiu Li discovered that the same methotrexate treatment alone could cure chorioncarcinoma (1958) – the first solid tumor to be cured by chemotherapy [4].

From the mid-1950s onwards, further progress in cancer chemotherapy was characterized primarily by four features: (i) further synthetic development of alkylating agents, antimetabolites, and platinum complexes, (ii) a federal initiative by the NCI that developed the methodologies and screening tools (e.g., cell line panels and animal models) for fostering a drug discovery program with a strong focus on identifying active natural products, (iii) establishment of standardized combination regimens that would prove to be more efficacious than single-agent therapy in several tumor indications, and (iv) clinical proof that adjuvant chemotherapy (i.e., treatment with anticancer agents after complete surgical resection of the tumor burden) significantly extended survival in several tumors indications, including those in a more advanced stage.

As a result, approximately 60–70% of anticancer chemotherapeutic agents are natural products or derived from them (Figure 1.3), as analyzed in depth by Newman and Cragg [5]. In their review article of 2007, they assessed the influence of natural products and their mimics as leads to anticancer drugs. By using data from the US Food and Drug Administration listings of antitumor drugs, coupled with previous data sources and with help from Japanese colleagues, they could show that over the whole category of anticancer drugs that entered clinical trials, these could be categorized as follows: biological "B" (18; 10%), natural product "N" (25; 14%), derived from a natural product "ND" (48; 28%), totally synthetic drug "S" (42; 24%), S/NM (14; 8%), made by total synthesis, but the pharmacophore is/was from a natural product "S*" (20; 11%), S*/NM (6; 4%), and vaccine "V"

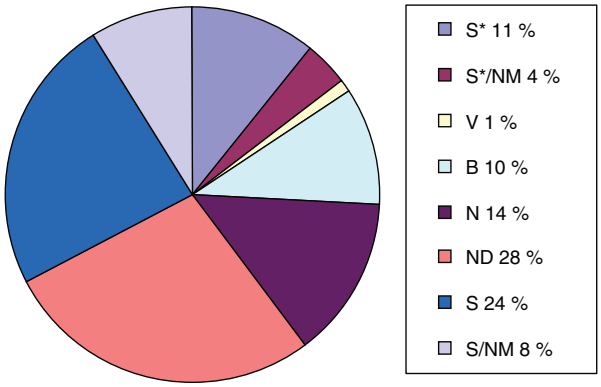


Figure 1.3 All available anticancer drugs, 1940s to June 2006, by source (N = 175). The major categories: B, biological; usually a large (more than 45 residues) peptide or protein either isolated from an organism/cell line or produced by biotechnological means; N, natural product; ND, derived from a natural product and is usually a semisynthetic modification; NM, natural product mimic; S, totally synthetic drug, often found by random screening/modification of an existing agent; S*, made by total synthesis, but the pharmacophore is/was from a natural product; V, vaccine. (Modified from [5].)

(2; 1%). If one removes the biologicals and vaccines, reducing the overall number to 155 (100%), the number of naturally inspired agents (i.e., N, ND, S/NM, S*, S*/NM) is 113 (72.9%).

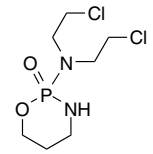
Although chemotherapeutic anticancer agents are classified into alkylating agents, antimetabolites, anthracyclines, plant alkaloids, microtubule inhibitors or modulators, topoisomerase inhibitors, and other antitumor agents, the modes of action can be diverse and manifold, resulting finally in a cytotoxic and/or cytostatic effect by affecting cell division, DNA synthesis and function, or apoptosis (programmed cell death). Some typical representatives of the different classes of antineoplastic agents are depicted in Figure 1.4.

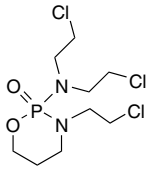
With a few exceptions, these antitumor agents are delivered intravenously (melphalan, busulfan, and capecitabine can be administered orally). In some cases, isolated limb perfusion (used in melanoma and soft-tissue sarcoma), or isolated infusion of chemotherapy into the liver or the lung have been used. The main purpose of these approaches is to deliver a very high dose of chemotherapy to tumor sites without causing overwhelming systemic damage. Depending on the patient, cancer, stage of cancer, type of chemotherapy, and dosage, intravenous chemotherapy may be given on either an inpatient or an outpatient basis. For continuous, frequent, or prolonged intravenous chemotherapy administration, various systems may be surgically inserted into the vasculature to maintain access. Commonly used systems are the Hickman line, the Port-a-Cath®, or the PICC (peripherally inserted central catheter) line. These have a lower infection risk, are much less prone to phlebitis or extravasation, and abolish the need for repeated insertion of peripheral cannulae.

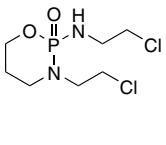
Tumors that responded best to cytostatic or cytotoxic agents were those with a fast doubling time of the order of a few days that include chorioncarcinoma, lymphoma, leukemia, rhabdosarcoma, and testicular cancers.

The response rates of the most common solid tumors – breast, lung, prostate, ovarian, liver, colorectal, gastric, and colorectal cancer – were far less encouraging. Over the past two decades, chemotherapy of these tumors has gradually but consistently been improved by the use of new developed drugs and optimized combinations of chemotherapeutic agents. An illustrative example is colon cancer. When only 5-Fluorouracil (5-FU) was available in the 1980s and early 1990s, the mean survival time was 12 months, which was even better than best supportive care (that had a survival time of 6 months). With the development of oxaliplatin and irinotecan, and optimizing the schedule of the combinations with 5-FU and folinic acid, the mean survival time has increased to more than 18 months. With the development of the two monoclonal antibodies (monoclonal antibody mAbs) cetuximab (Erbitux®) and bevacizumab (Avastin®) the mean survival time has reached 24 months or more for some subtypes. The rationale for using a combination of drugs is manifold. (i) By combining the drugs below their respective maximum tolerated dose as single agents, the overall systemic toxicity for the patient during chemotherapy cycles can be reduced. (ii) The individual tumor cells are in different stages of the cell cycle (G_2, S, M, G_1, G_0), such that some are proliferating, differentiating, or resting (quiescent) (Figure 1.5 and Table 1.1). As anticancer agents

ALKYLATING AGENTS


Cyclophosphamide


Trofosfamide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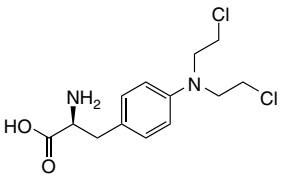
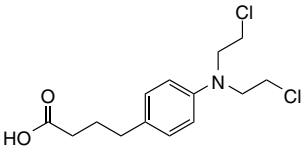
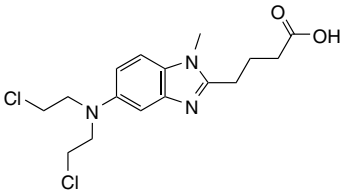
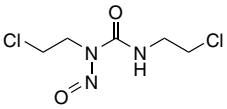
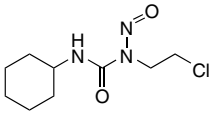
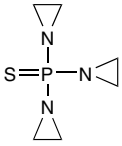

Melphalan

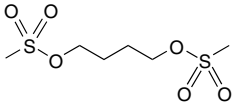

Chlorambucil

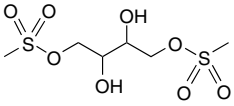

Bendamustine


Carmustine

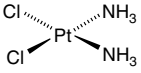

Lomustine

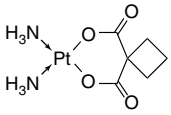

Thiotepa

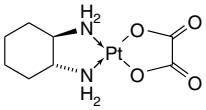

Busulfan


Treosulfan

PLATINUM COMPLEXES

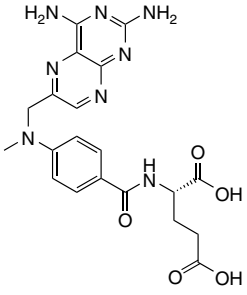

Cisplatin


Carboplatin


Oxaliplatin

ANTIMETABOLITES

Folic acid antagonists


Methotrexate

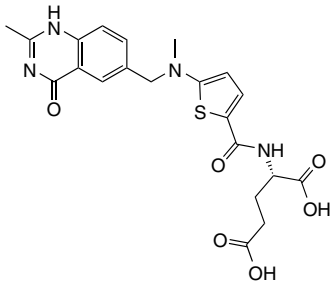
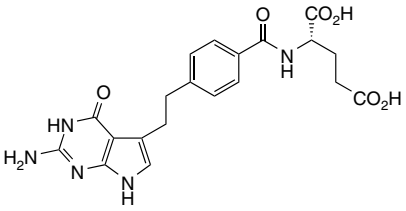
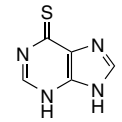

Raltitrexed

Figure 1.4 Chemical structures of the major representative classes of conventional anticancer agents used routinely in cancer chemotherapy.

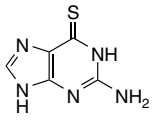


Permetrexed

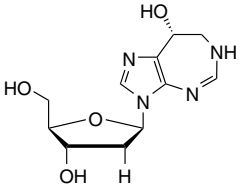
Purine analogs



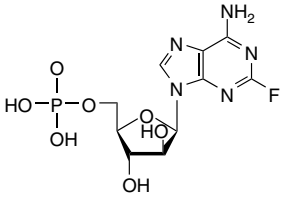
Mercaptopurine



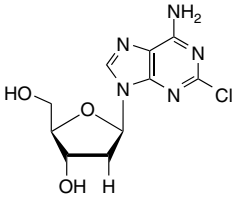
Thioguanine



Pentostatin

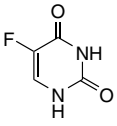


Fludarinbinphosphate

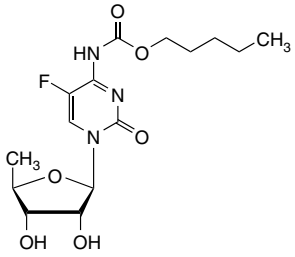


Cladribin

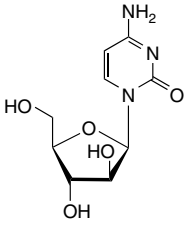
Pyrimidine analogs



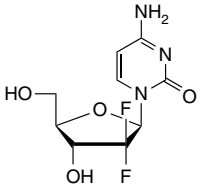
5-Fluorouracil



Capecitabine



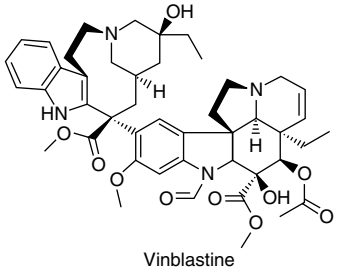
Cytarabine



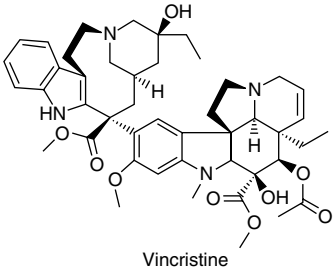
Gemcitabine

MICROTUBULE INHIBITORS

Vinca alkaloids

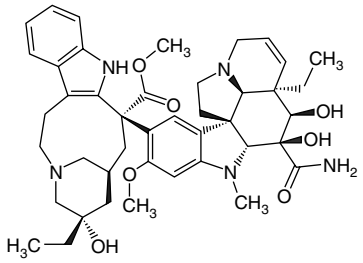


Vinblastine



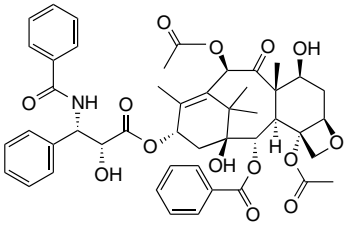
Vincristine

Figure 1.4 (*continued*)

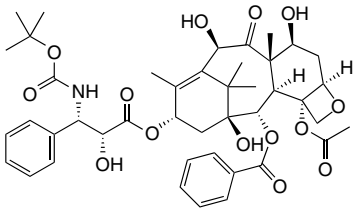


Vindesine

Taxanes

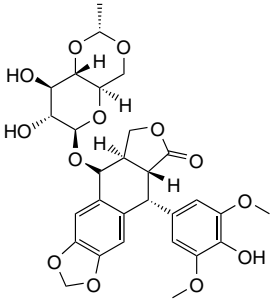


Paclitaxel

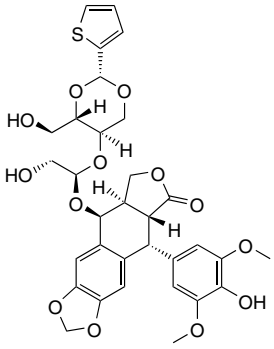


Docetaxel

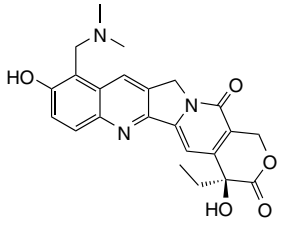
TOPOISOMERASE I AND II INHIBITORS



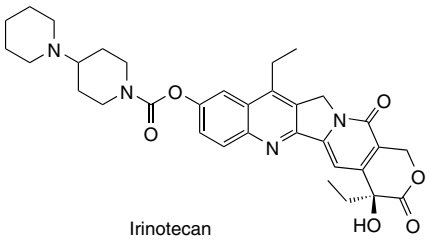
Etoposide



Teniposide



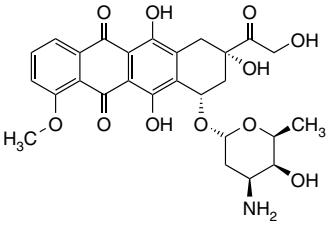
Topotecan



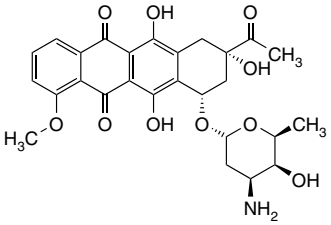
Irinotecan

Figure 1.4 (*continued*)

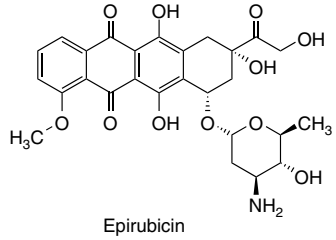
CYTOSTATIC ANTIBIOTICS



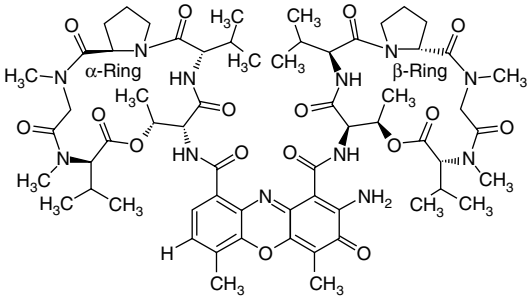
Doxorubicin



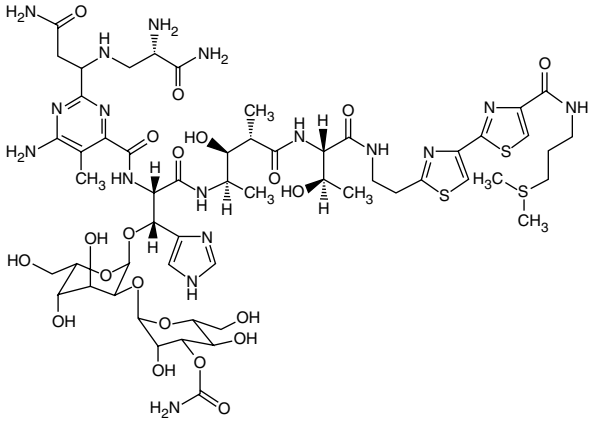
Daunorubicin



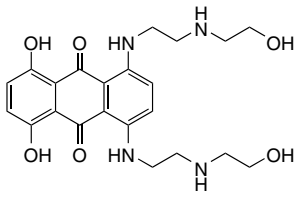
Epirubicin



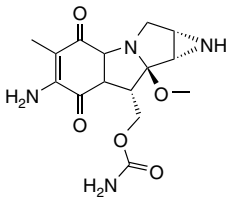
Dactinomycin



Bleomycin



Mitoxantrone



Mitomycin C

Figure 1.4 (*continued*)

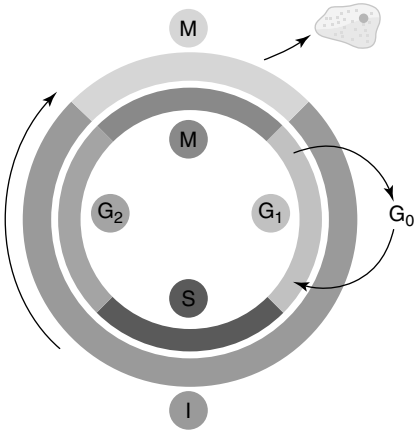


Figure 1.5 Schematic of the cell cycle. Outer ring: I = interphase, M = mitosis; inner ring: M = mitosis, G_1 = gap 1, G_2 = gap 2, S = synthesis; not in ring: G_0 = gap 0/resting. The duration of mitosis in relation to the other phases has been exaggerated in this diagram. Also see Table 1.1. (Adapted from *en.wikipedia.org/wiki/Cell_cycle*.)

Table 1.1 Phases of the cell cycle (also see Figure 1.5).

Phase	Abbreviation	Description
Gap 0	G_0	a resting phase where the cell has left the cycle and has stopped dividing.
Gap 1	G_1	cells increase in size in gap 1 and G_1 checkpoint control mechanisms make preparations for DNA synthesis
Synthesis	S	DNA replication occurs during this phase
Gap 2	G_2	during the gap between DNA synthesis and mitosis, the cell will continue to grow; the G_2 checkpoint control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.
Mitosis	M	cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cells; a checkpoint in the middle of mitosis (metaphase checkpoint) ensures that the cell is ready to complete cell division

Adapted from *en.wikipedia.org/wiki/Cell_cycle*.

inhibit tumor growth at different stages of the cell cycle, it is logical to assume that a combination of selected anticancer drugs acting at different stages of the cell cycle will result in an overall improved cell kill of the heterogeneous tumor cell population that on the whole is asynchronous with respect to tumor proliferation. (iii) Tumor cells become resistant to a single agent, thus by using different drugs

(phase-specific, phase-unspecific, as well as cycle-specific) concurrently it would be easier to target and kill individual tumor cells at their respective checkpoints, and the likelihood of the tumor developing resistance to the combination would be suppressed by applying several anticancer drugs simultaneously.

The major breakthrough for this approach was achieved around 1965 when James Holland, Emil Freireich, and Emil Frei rationalized that cancer chemotherapy should follow the strategy of antibiotic therapy of using combinations of drugs, each with a different mechanism of action, to inhibit the tumor cell population in the different stages of their cell cycle and also to prevent the emergence of resistance. Holland, Freireich, and Frei simultaneously administered methotrexate (an antifolate), vincristine (a vinca alkaloid), 6-mercaptopurine (6-MP), and prednisone – together referred to as the POMP regimen – and induced long-term remissions in children with ALL. With subsequent incremental refinements of these original protocols in the United Kingdom and Germany, ALL in children has become a largely curable disease and was extended to lymphomas where a combination of a nitrogen mustard, vincristine, procarbazine, and prednisone – known as the MOPP regimen – can cure patients with Hodgkin's and non-Hodgkin's lymphoma.

Currently, nearly all successful cancer chemotherapy regimens use this paradigm of multiple drugs given simultaneously, mainly in curative chemotherapy protocols (R-CHOP, BEACOPP, ABVD, COPP-ABVD, FAC, BEP, etc.), but also first-line and second-line chemotherapy regimens in palliative settings (solid tumors with metastases) generally include combinations with two or three drugs for maximizing the therapeutic effect (e.g., FOLFOX, FOLFIRI, ECF, DCF, FLOT, CDDP/GEM, CDDP/VP-16, CBDCA/PAC, IFO/DOX, etc.) (Table 1.2).

Despite the relatively slow progress in treating the most common solid tumors, especially once metastasized, another important strategy for the use of chemotherapy emerged – adjuvant therapy. If the tumor could be removed or the tumor burden reduced by surgery, then anticancer agents should be able to destroy any remaining malignant cells or micrometastases post-therapy, thus reducing the probability of tumor remission and/or the formation of metastases. This notion was nourished by the observation in animal models that anticancer drugs were most effective in eliminating tumors of smaller volume. It was again Emil Frei who first demonstrated this effect – high doses of methotrexate prevented the recurrence of osteosarcoma following surgical removal of the primary tumor [6]. Similarly, 5-FU was later shown to improve survival in colon cancer stage II and III (above all in stage III with lymph node metastasis) when used as an adjuvant to surgery in treating patients with colon cancer, and Gianni Bonadonna at the Istituto Nazionale Tumori di Milano, Italy, demonstrated that adjuvant chemotherapy after complete surgical resection of breast tumors significantly extended survival with CMF (cyclophosphamide/methotrexate/5-FU) even in more advanced cancer [7]. In subsequent years, adjuvant chemotherapy for treating breast cancer relied on an anthracycline-based regimen (FAC (5-FU/doxorubicin (adriamycin)/cyclophosphamide) or AC (doxorubicin (adriamycin)/cyclophosphamide)) followed by taxanes (docetaxel or paclitaxel) with or without trastuzumab (Herceptin®) in HER2/*neu*-positive tumors [8].

Table 1.2 Examples of commonly used cancer chemotherapy regimens in first-line, second-line, and palliative treatment.

Abbreviation for the combination protocol	Drugs used in the regimen	Tumor indication
FOLFOX	folinic acid 5-FU oxaliplatin	colon cancer
FOLFIRI	folinic acid 5-FU irinotecan	colon cancer
ECF	epirubicin cisplatin 5-FU	gastric cancer
DCF	docetaxel cisplatin 5-FU	gastric cancer
FLOT	5-FU leucovorin (folinic acid) oxaliplatin taxotere (docetaxel)	gastric cancer
R-CHOP	rituximab cyclophosphamide hydroxy-daunomycin (doxorubicin) oncovin (vincristine) prednisone (cortisone)	non-Hodgkin's lymphoma
FAC	5-FU adriamycin (doxorubicin) cyclophosphamide	breast cancer
BEP	bleomycin etoposide cisplatin	testicular cancer
BEACOPP	bleomycin etoposide adriamycin cyclophosphamide oncovine (vincristine) procarbacin prednisolone (cortisone)	Hodgkin's lymphoma
ABVD	adriamycin (doxorubicin) bleomycin vinblastine decarbacin	Hodgkin's lymphoma

1.2 Dilemma and Challenge of Treating Malignant Diseases

One of the main dilemmas of treating solid tumors is that they are not detected early enough and once diagnosed have often formed metastases. If they cannot be treated by surgery in combination with radiotherapy or neoadjuvant chemotherapy, the prognosis for curing the patient, mostly expressed in the literature as at least a 5-year tumor-free interval, remains highly unsatisfactory. Current chemotherapy regimens applied alone or in combination with novel agents such as mAbs and signal transduction inhibitors are to date the best option of inhibiting or reducing the size of the primary tumor and/or metastases. Chemotherapy regimens are generally applied in cycles (ranging from a 1- to 4-week interval), with the frequency and duration of treatments limited by toxicity to the patient. Most commonly, chemotherapy acts by killing cells that divide actively – one of the main properties of most cancer cells. This means that they also harm cells that divide rapidly under normal circumstances, such as cells in the bone marrow, digestive tract, and hair follicles (see Section 1.3).

It is instructive to understand the rationale for repeated doses that must be administered to continue to inhibit tumor growth or reduce the size of the tumor. As only a fraction of the cells in a tumor die with each treatment, it is obligatory that repeated and optimized chemotherapy cycles must be administered to obtain the best therapeutic outcome.

This principle is known as ''log cell kill,'' often also referred to as ''fractional cell kill,'' and is a generally accepted hypothesis for hematological cancers that states that during every cycle of chemotherapy or radiotherapy the same fraction of tumor cells is killed, but not the same number.

Howard E. Skipper laid the foundation for the log cell kill hypothesis in 1964 when carrying out experiments with mice suffering from leukemia [9]. Leukemia cells that grow exponentially result in a straight line when plotted on a semilogarithmic scale over time, reflecting the doubling of tumor cells. When the mice were treated with constant doses of anticancer agents, it was observed that the number of leukemia cells diminished logarithmically; if, for example, 99% of leukemia cells were killed after the first administration, this is equivalent to a decrease from 10^9 to 10^7 cells, which corresponds to 2 orders of magnitude (log steps). A second administration will also result in a 99% cell kill, but the number of tumor cells is only reduced from 10^7 to 10^5, which is only 10 million cells compared to the 1 billion cells in the first cycle. In other words, in this idealized model, the fraction of cells that are killed remains constant, but the number of cells killed over time constantly decreases.

Transferring the log cell kill hypothesis to solid tumors is not as straightforward as it appears at first glance (Figure 1.6).

With modern diagnostics, a tumor is detectable when it reaches a size of 1 cm^3 after 30 doubling cycles, which corresponds to 1 g and 10^9 cancer cells. Only 10 further doubling steps are necessary for the tumor to reach a size of 1 kg (10^{12} cancer cells). In this time interval tumor symptoms start emerging.

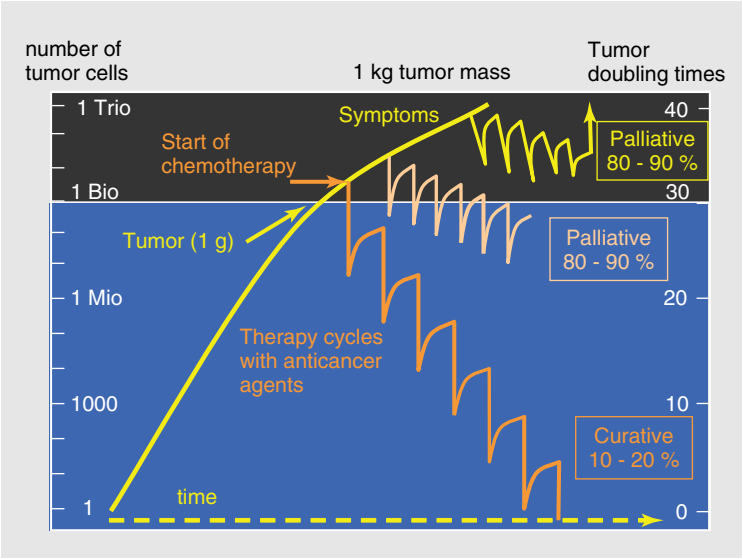


Figure 1.6 Tumor growth curve of a solid tumor. Once the tumor comprises approximately 1 billion tumor cells, its size is around 1 cm^3 (1 g) and it becomes detectable. The initial tumor cell has to perform 30 doubling steps to reach this size (which can take months to years considering that the tumor doubling times for human tumors lies in the range of 5–200 days), and a mere 10 further doublings are needed to reach a mass of 1 kg assuming tumor growth occurs exponentially. This generally does not take place because of an insufficient growing vasculature in large tumors, leading to a lack of supply of nutrients and tumor necrosis. Of note is that according to the log cell kill hypothesis many cycles of chemotherapy are necessary to eliminate all of the tumor cells and only in 10–20% of cases are cures achieved. Palliative treatment is particularly disappointing with large tumors where only a relatively small fraction of tumor cells respond to anticancer agents.

These insights are the reason why during curative, adjuvant, or palliative chemotherapy the doses and cycles of anticancer agents should not be reduced or discontinued even if the tumor or tumor lesions are no longer detectable (assuming that the schedule is tolerated by the patient). The log cell kill hypothesis can additionally be viewed as a theoretical basis for further treating patients for longer periods although diagnostically a complete remission has been achieved.

However, the log cell kill hypothesis is not strictly valid for solid tumors, if at all, only for those that are fast growing, but in most cases the effect of cytostatic or cytotoxic agents on tumor growth can be described by the so-called Gompertz growth curve. This implies that tumor growth diminishes with increasing size of the tumor, which is noted in the semilogarithmic plot by a decreasing slope of the tumor growth curve. With increasing tumor size many tumor cells remain in the G_0 phase (quiescent phase) of the cell cycle because of an insufficient growing vasculature leading to a lack of supply of nutrients and tumor necrosis. In this phase, the response to treatment with anticancer agents is significantly reduced

and the initial cycle of chemotherapy only manages to kill a fraction of the tumor cells, mostly those proliferating in the periphery of the tumor. As a consequence, the tumor mass is reduced, and quiescent cells are reactivated to enter the cell cycle and multiply. This is the reason why the response in the second or third cycle of palliative treatment is often better than in the first one because a higher percentage of tumor cells are killed.

Unfortunately, in this advanced stage of the disease further reduction of tumor size is seldom achieved because a population of tumor cells has developed chemoresistance. Intrinsic or acquired chemoresistance is a major problem in cancer therapy. In the majority of cases the cancer cells develop resistance against a spectrum of anticancer agents, a phenomenon called multidrug resistance (MDR). A number of biochemical mechanisms have been described that are responsible for the MDR phenotype, including changes in the cellular target of the respective drug, alterations in enzymatic activation and detoxification mechanisms, defective apoptotic pathways, membrane changes as well as elimination of the drug from the tumor cell through the action of drug efflux pumps, such as P-glycoprotein, multiple resistance protein (MRP), and breast cancer resistance protein (BCRP), which belong to the ATP-binding cassette transporter family [10].

In addition, tissue penetration into necrotic areas of the tumor is hampered. Solid tumors are heterogeneous and form a complex society of cells in different microenvironments. This includes variable vascular density, different intratumoral blood pressure, and regions of hypoxic, acidic, and necrotic areas. These factors have an influence on the tissue penetration of drug as shown for the anticancer drug in Figure 1.7, which is shown for three different preclinical tumors in mice with regard to the intratumoral distribution of doxorubicin [11]. The immunofluorescence images after administration show the blood vessels in red, hypoxic areas in green, and doxorubicin in blue. The penetration length for doxorubicin from the nearest blood vessels varies considerably within a 100 µm range and doxorubicin is unable to accumulate in hypoxic areas.

1.3 Adverse Effects

The side-effects associated with cancer chemotherapy can be classified as acute toxicities (patient-felt toxicities, which appear directly after administration or delayed after a few hours or days, e.g., nausea, vomiting, gastrointestinal symptoms, dyspnea, fever, skin reactions), cycle-dependent toxicities (e.g., bone marrow toxicities, stomatitis, mucositis, alopecia), or long-term or cumulative toxicities (e.g., cardiotoxicity, nephrotoxicity, neurotoxicity).

All the above anticancer chemotherapeutics are essentially cytotoxic regardless of whether they are of synthetic or of natural origin. Patients receiving these agents experience severe side-effects that limit the doses that can be administered and hence limit the beneficial effects. The therapeutic window is in general narrow due to the fact that the cytotoxic agents used are low-molecular-weight compounds

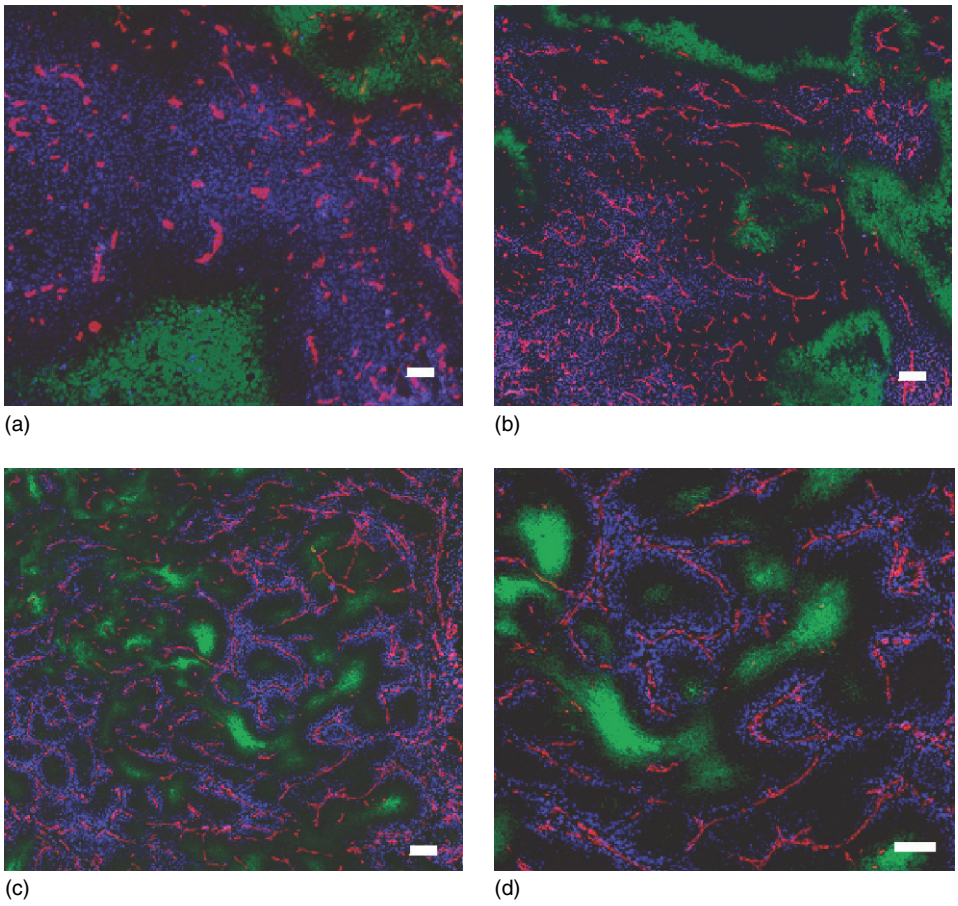


Figure 1.7 Representative three-color composite images showing the perivascular distribution of doxorubicin (blue) in relation to blood vessels (red) and hypoxic regions (green) in three different tumors growing in the right flank of mice: (a) human prostate PC-3 carcinoma, (b) mouse mammary sarcoma EMT6, and (c) and (d) 16/C mammary carcinoma. Bar: 100 µm. (Reproduced with permission from [11].)

(typically smaller than 1000 Da) that diffuse rapidly into healthy tissues with relatively small amounts of the drug reaching the target site. They are characterized by a rapid clearance, basically being eliminated from the circulation within minutes or hours, metabolized in the liver, and excreted via the bile duct or the kidneys.

Most commonly, chemotherapy acts by killing cells that are dividing – one of the main properties of most cancer cells, but not of all. The growth fraction can vary between 5 and 100%. The grading of a tumor depends on the behavior of the growth fraction, which can be roughly estimated by the Ki-67-positive fraction – an antigen that can be used as a proliferation marker for cancer cells. This means that cytostatic/cytotoxic agents also harm cells that divide rapidly under normal circumstances, in particular cells in the bone marrow, digestive tract, and

hair follicles; this results in the most common side-effects of chemotherapy – myelosuppression (decreased production of blood cells), mucositis (inflammation of the lining of the digestive tract), and alopecia (hair loss).

Clinical investigators realized that the ability to manage these toxicities was crucial to the success of cancer chemotherapy. Most chemotherapeutic agents cause profound suppression of the bone marrow. This is reversible, but takes time to recover. Support with platelet and red cell transfusions as well as broad-spectrum antibiotics in the case of infection during this period is crucial to allow the patient to recover. The success of chemotherapy depends heavily on additional supportive care. This is independent from normal-dose chemotherapy or high-dose chemotherapies, including hematopoietic stem cell support (either autologous or allogeneic). Supportive care is a necessary part of a chemotherapy plan.

1.3.1 Common Side-Effects

Cancer chemotherapy has a broad range of side-effects that depend on the type of medications used. The most common side-effects are described below:

1.3.1.1 Depression of the Immune System

Virtually all chemotherapeutic regimens can cause depression of the immune system, often by affecting the bone marrow – a compartment with a very strong proliferation – subsequently leading to a decrease of white blood cells, red blood cells, and platelets. The latter two, when they occur, are improved with blood or platelet transfusion. Neutropenia (a decrease of the neutrophil granulocyte count below $0.5 \times 10^9/l$) can be improved with synthetic granulocyte colony-stimulating factor (G-CSF; e.g., filgrastim, lenograstim). The prophylactic use of G-CSF depends on the risk estimation for fever and infection. In cases where the risk estimation is greater than 10% (variables: age, chemotherapeutic drug, dosage, pretreatment, etc.), a prophylactic treatment with G-CSF is justified. More important in cases of fever of unknown origin is the early use of antibiotics. The therapeutic use of G-CSF in cases of neutropenia or neutropenic fever is less validated.

Depression of the immune system can result in potentially fatal infections. Although patients are encouraged to wash their hands, avoid people with infections, and to take other infection-reducing steps, about 85% of infections are due to naturally occurring microorganisms in the patient's own gut and skin. This may manifest as systemic infections (e.g., sepsis) or as localized outbreaks (e.g., shingles). Sometimes, chemotherapy treatments are postponed because the immune system is suppressed to a critically low level.

In very severe myelosuppression, which occurs in some regimens, almost all the bone marrow stem cells (cells that produce white and red blood cells) are destroyed and allogenic or autologous bone marrow cell transplants are necessary. In autologous bone marrow cell transplants the cells are removed from the patient before the treatment, multiplied, and then reinjected afterwards; in allogenic bone marrow cell transplants the source is a donor. The initial tremendously high

mortality in the early days of an allogeneic hematopoietic cell transplantation has been successfully decreased during recent years.

In Japan, the government has approved the use of some medicinal mushrooms (e.g., *Trametes versicolor*) to counteract depression of the immune system in patients undergoing chemotherapy (*http://www.cancer.org/docroot/ETO/content/ETO_5_3X_Coriolous_Versicolor.asp*).

The United States' top-ranked hospital, the University of Texas MD Anderson Cancer Center, has reported that polysaccharide-K (PSK; an extract from *T. versicolor*) is a ''promising candidate for chemoprevention due to the multiple effects on the malignant process, limited side effects, and safety of daily oral doses for extended periods of time'' (*http://cancer.ucsd.edu/Outreach/PublicEducation/CAMs/coriolusversicolor.asp*). PSK is already used in pharmaceuticals designed to complement chemotherapy. The MD Anderson has also reported that there are 40 human studies, 55 animal studies, 37 *in vitro* studies, and 11 reviews published concerning *T. versicolor* or its extract PSK (*http://www.mdanderson.org/education-and-research/resources-for-professionals/clinical-tools-and-resources/cimer/therapies/herbal-plant-biologic-therapies/coriolus-versicolor-scientific.html*).

1.3.1.2 **Fatigue**

The treatment can be physically exhausting for the patient who might already be very tired from cancer-related fatigue. Chemotherapy can cause or potentiate fatigue and may produce mild to severe anemia. Therapeutic options to mitigate anemia include hormones to boost blood production (erythropoietin), iron supplements, and blood transfusions.

1.3.1.3 **Tendency to Bleed Easily**

Medications that kill rapidly dividing cells or blood cells are likely to reduce the number of platelets in the blood, which can result in bruises and bleeding. Extremely low platelet counts may be temporarily boosted through platelet transfusions. Sometimes, chemotherapy treatments are postponed to allow platelet counts to recover. Extremely low platelet counts can be expected in all dose-intense chemotherapy protocols, mainly for those protocols with hematopoietic stem cell rescue and all induction chemotherapy protocols for malignant hematological disorders, when an aplasia is part of the treatment strategy. Because chemotherapy is not administered routinely in extreme dose intensities in solid tumors, this adverse effect can be well managed.

1.3.1.4 **Gastrointestinal Distress**

Nausea and vomiting are common side-effects of chemotherapeutic medications that kill fast-dividing cells. This can also produce diarrhea or constipation. Malnutrition and dehydration can result when the patient is unable to eat or drink sufficient amounts, or when the patient vomits frequently because of gastrointestinal damage. This can result in rapid weight loss or occasionally in weight gain, if the patient eats too much in an effort to allay nausea or heartburn. Weight gain can also be caused by some steroid medications. These side-effects can frequently

be reduced or eliminated with antiemetic drugs. Self-care measures, such as eating frequent small meals and drinking clear liquids, or ginger tea, are often recommended. This is a temporary effect and frequently resolves within a week of finishing treatment. Chemotherapy-induced nausea and vomiting is common with many treatments and some forms of cancer. Drugs called 5-HT_3 antagonists are the most effective antiemetics, and constitute the single greatest advance in the management of nausea and vomiting in patients with cancer. These drugs block one or more of the nerve signals that cause nausea and vomiting. During the first 24 h after chemotherapy, the most effective approach appears to be blocking the 5-HT_3 nerve signal. Approved 5-HT_3 inhibitors include dolasetron, granisetron, and ondansetron. The newest 5-HT_3 inhibitor, palonosetron, also prevents delayed nausea and vomiting, which occurs during 2–5 days after treatment [12]. Since some patients have trouble swallowing pills, these drugs are often available by injection, as orally disintegrating tablets, or as patches. The substance P inhibitor aprepitant (Emend®), which became available in 2005, is also effective in controlling the nausea of cancer chemotherapy [13]. A few studies indicate that the use of cannabinoids derived from marijuana during chemotherapy greatly reduces nausea and vomiting, and enables the patient to eat [13]. Some synthetic derivatives of the active substance in marijuana (tetrahydrocannabinol) such as Marinol® may be practical for this application. Natural marijuana, known as medical cannabis is also used and recommended by some oncologists, although its use is regulated and not legal everywhere [14].

1.3.1.5 Hair Loss

Some medications that kill rapidly dividing cells cause dramatic hair loss; other medications may cause hair to thin out. These are temporary effects – hair growth usually returns a few weeks after the last treatment, sometimes with a tendency to curl (a ''chemo perm''). Hair loss seems more a psychological problem because it is immediately visible that a person is under anticancer treatment. It is a kind of stigma that can be very distressing for the patient. All treatments to avoid hair loss are only partly successful. The best results have been seen with cool caps, which limit the circulation of the cytostatic agents, thus preventing damage to the hair follicle in the scalp. Nevertheless, the procedure itself is associated with some pain during the cooling procedure. For all longer infusion protocols with cytostatic agents, this procedure is not effective in avoiding hair loss.

1.3.2 Damage to Specific Organs

Damage to specific organs may occur, with resultant symptoms.

1.3.2.1 Cardiotoxicity

The myocardium consists of cells that have limited regenerative capability, which may render the heart susceptible to permanent adverse effects from chemotherapeutic agents. The effects of antineoplastic agents on the heart can be predictable or

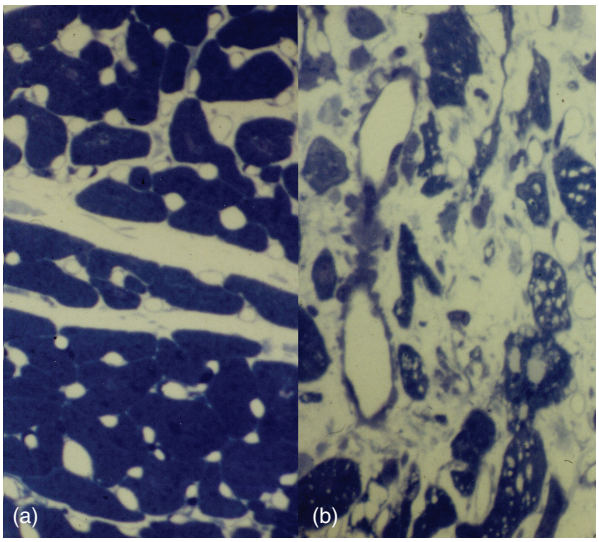


Figure 1.8 Normal (a) and damaged (b) cardiac tissue in mice: destruction of the myocytes and vacuolization. (Reproduced with permission from [17], © Zuckschwerdt Verlag.)

unpredictable, fixed or cumulative [15]. The anthracyclines are feared because acute toxicities include supraclavicular tachycardia, ventricle ectopy, myopericarditis, electrocardiogram changes, cardiomyopathy, and sudden death. Severe irreversible cardiotoxicity is rarely seen at grades 2–4. The most important cardiotoxicity is cumulative late cardiomyopathy, which generally occurs 5 or more years after anthracycline therapy [16]. This kind of toxicity is dose dependent, symptomatic with a progressive decrease in the left ventricular function often resulting in congestive heart failure. Figure 1.8 histologically depicts the damage to the myocytes of the hearts of mice under cumulative treatment with doxorubicin [17].

This is especially a problem in young children because the heart of these patients is much more vulnerable than in older patients. Anthracyclines are an important part of curative therapy for treating most malignant tumors of children [18]. The problem of injury to the heart has probably been underestimated in the past. In the case of anthraquinones such as mitoxantrone, similar but less cardiac damage has been observed. The use of vinca alkaloids has also been associated with heart toxicity related to the vasoconstrictive properties of this group of drugs. Under treatment with 5-FU, myocardial ischemia has been observed. 5-FU has been shown to induce a plethora of cardiac abnormalities. The putative mechanisms of ischemia and other cardiac toxic effects of 5-FU are not known, but there is evidence that coronary vasospasms may play a critical role [19].

1.3.2.2 **Hepatotoxicity**

Hepatotoxicity is mainly judged by liver function tests because of the ease of determining liver-associated enzyme activities. Apart from hepatic damage by

cytostatic agents, other potential causes of abnormal liver function must be considered, such as other medications, alcohol, chemicals, infections, and localized or diffuse infiltrating liver metastases. The spectrum of liver toxicities ranges from the usually incidental elevations of transaminases observed, for example, with nitrosurea to life-threatening massive hepatic necrosis observed with dacarbazine. At doses higher than 16 mg/kg busulfan, veno-occlusive disease develops in a significant number of patients undergoing hematopoietic stem cell transplantation. 6-MP-induced hepatotoxicity occurs in a variety of clinical settings when the dosage exceeds the usual daily dose of 2 mg/kg. The histological pattern includes features of both intrahepatic cholestasis and parenchymal cell necrosis. Bilirubin increases with a moderate increase of transaminases and alkaline phosphates. Since liver function tests do not adequately reflect the degree of hepatic injury, the presence or absence of liver damage is best assessed by serial fine needle biopsies of the liver. Several antineoplastic drugs are metabolized in the liver and excreted via the bile ducts, and are thus relatively nontoxic to the liver. Transient liver enzyme elevations with normalization is the most common reaction profile [20, 21].

1.3.2.3 **Nephrotoxicity**

The most prominent drugs that are toxic for the kidney are cisplatin, ifosfamide, methotrexate, and mitomycin. Chemotherapeutic agents can produce a variety of acute and chronic kidney toxicities. Awareness of the toxicity potential of each anticancer drug in use is important. Either the glomeruli, tubules, or renal vasculature might be at risk, with clinical manifestations ranging from asymptomatic serum creatinine to acute renal failure requiring dialysis. Several factors can potentiate renal dysfunction and contribute to the nephrotoxic potential of antineoplastic drugs: the use of several other drugs, other comorbidities, diabetes mellitus, and heart failure, which might contribute to a decrease of renal function. An acute, mainly proximal tubular impairment occurs with platinum complexes. Proximal tubular damage is marked by a considerable reduction in the reabsorbtive capacities for sodium and water, which is followed by disruption of glomerular filtration and impaired distal tubular function. Forcing diuresis is the method of choice. For the alkylating agents ifosfamide and cyclophosphamide, forced diuresis, and splitting the dose in the case of ifosfamide, is the method of choice for protecting the kidney. For mitomycin, renal failure and microangiopathic hemolytic anemia termed thrombocytopenic purpura/hemolytic uremic syndrome is well known. Ten percent of all patients treated with mitomycin C can develop this severe syndrome. Azacitidine, a pyrimidine analog, has been observed to induce a proximal tubular defect in up to 70% of patients. Methotrexate-induced nephrotoxicity has been managed with high-dose leucovorin, hemodialysis, and with the recombinant enzyme carboxypeptidase G_2, which cleaves methotrexate to produce inactive metabolites [22].

1.3.2.4 **Pulmonary Side-Effects**

Early-onset chemotherapy-induced lung injury can be classified as inflammatory interstitial pneumonitis, pulmonary edema, bronchospasm, or pleural effusions [23]. In late-onset chemotherapy-induced lung injury, present more than 2 months

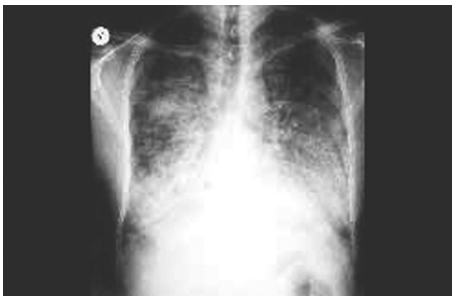


Figure 1.9 Respiratory distress syndrome induced in a patient receiving gemcitabine (strong infiltration in both lungs induced by gemcitabine, 1000 mg/m^2). (Reproduced with permission from [24].)

after the completion of therapy, the most common manifestation is pulmonary fibrosis. The agents with the highest incidence include bleomycin, busulfan, carmustine (bis-chloronitrosourea; BCNU®), and mitomycin. In rare cases even gemcitabine can cause pulmonary damage, as shown in Figure 1.9 [24].

Finally, chemotherapy-induced pulmonary toxicity remains an elusive entity to diagnose. Clinicians should be ever vigilant in watching the clinical signs and symptoms of lung injury, and include this in their differential diagnosis.

1.3.2.5 **Vascular Adverse Effects**

It has long been recognized that thrombosis and thromboembolic disease may complicate cancer-associated symptoms. There is increasing evidence that vascular toxicity is associated with the administration of chemotherapeutic agents. Such vascular toxicities encompass a heterogeneous group of disorders, including asymptomatic arterial lesions, pulmonary veno-occlusive disease, hepato veno-occlusive disease, Budd–Chiari syndrome, Raynaud's phenomenon, myocardial infarction and ischemia, thrombotic microangiopathy, thrombosis, thromboembolic events, hypotension, hypertension, acral erythema, leukocytoclastic vasculitis, and retinal toxicity. The origin of cytotoxic drug-induced vascular disorders is not clear. One possible mechanism is damage to the endothelial cell by antineoplastic agents or their metabolites. Bleomycin causes a direct toxic effect on endothelial cells in capillaries and small arterioles. Another possible mechanism is drug-induced perturbation of the clotting system or platelet activation. It is apparent that a variety of vascular disorders can be seen after chemotherapies. However, it is not always apparent whether their manifestations are related to the cytotoxic drugs or the malignancy itself and further research in this field is necessary [25].

1.3.2.6 **Tissue Damage (Extravasation)**

Some of the chemotherapeutic agents are strong tissue-damaging agents, which occurs when the drug is not administered intravenously [26]. The symptoms of extravasation reactions can range from pain, localized tissue inflammation, necrosis to the ulceration of skin and underlying structures (Figure 1.10). Most lesions heal

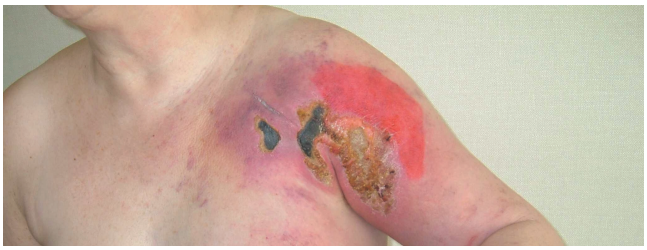


Figure 1.10 Severe tissue ulceration by anthracycline extravasation; epirubicin was administered in the tissue due to a misleading Port-a-Cath needle. (Reproduced with permission from [27].)

poorly and slowly. Vesicant (ulcerogenic) drugs have the capacity to induce the formation of blisters and cause tissue destruction.

Irritant drugs can cause pain with or without inflammation reaction. Nonvesicant drugs rarely produce acute reactions or tissue damage. Chemotherapeutic agents that bind to nucleic acids such as anthracyclines cause direct cell death with necrosis. The drugs that do not bind to nucleic acids may undergo clearance, which limits the degree of tissue injury. A high vesicant potential is known for actinomycin, daunorubicin, doxorubicin, epirubicin, idarubicin, mechlroethamine, vinblastine, vincristine, vindesine, and vinorelbine. The drug amount and the localization of the paravasate determines the amount of tissue damage/irritation. Since extravasation is one of the most serious complications when administering chemotherapeutic agents, oncologists need to take all precautions and care that accidents are avoided. Each location, either in hospitals or outpatients wards or doctor's offices, where intravenous administrations of chemotherapy is carried out should have written guidelines and standard operating procedures for minimizing tissue damage [28].

1.3.2.7 **Neurological Side-Effects**

Microtubule-stabilizing agents (MTSAs), including the taxanes and epothilones, stabilize microtubules, block mitosis, and induce cell death [29]. A major toxicity associated with this mechanism is peripheral neuropathy. The mechanism of MTSA-induced peripheral neuropathy is not fully understood. As the neurons require that proteins and other components be actively transported along long axons from the neuron's cell body to its distal synapses, MTSA treatment disturbs this active transport. The incidence of peripheral neuropathy depends on the dose of MTSA per treatment cycle. There is a significant difference in the incidence of peripheral neuropathy depending on treatment schedules. Weekly paclitaxel is more neurotoxic than 3-week schedules. The onset of peripheral neuropathy generally depends on the cumulative dose of MTSAs. Other risk factors may include diabetes mellitus, platinum compounds (especially cisplatin), and older patients who are more prone to MTSA-induced peripheral neuropathy. To date, the best strategy is to stop treatment with any potentially neurotoxic drug as soon as neurotoxicity becomes clinically apparent. Administering potentially neuroprotective

drugs to prolong treatment with MTSA has failed and unfortunately there are no effective drugs available that reduce this drug-induced toxicity. The reversibility of neurotoxicity is very limited [30].

1.3.2.8 Secondary Neoplasms

The development of secondary neoplasms after successful chemotherapy and or radiotherapy treatment can occur. The most common secondary neoplasm is secondary acute myeloid leukemia (myelodysplastic syndrome), which develops primarily after treatment with alkylating agents or topoisomerase inhibitors. Other studies have shown a 13.5-fold increase from the general population in the incidence of secondary neoplasm occurrence after 30 years from treatment.

1.3.2.9 Infertility

Distinct chemotherapy regimens are gonadotoxic and may cause infertility. Chemotherapies with high risk include procarbazine and alkylating drugs such as cyclophosphamide, ifosfamide, busulfan, melphalan, chlorambucil, and mechloroethamine. Drugs with medium risk include doxorubicin and platinum analogs such as cisplatin and carboplatin [31]. Therapies with a low risk of gonadotoxicity include plant derivatives such as vincristine and vinblastine, antibiotics such as bleomycin and dactinomycin, and antimetabolites such as methotrexate, 6-MP, and 5-FU.

Patients may choose between several methods of fertility preservation prior to chemotherapy, including cryopreservation of semen, ovarian tissue, oocytes, or embryos [32].

1.3.2.10 Other Side-Effects

Patients with particularly large tumors, such as large lymphomas, can develop a tumor lysis syndrome from the rapid breakdown of malignant cells. Although prophylaxis is available and is often initiated in patients with large tumors, this is a dangerous side-effect that can lead to death if left untreated.

Less-common side-effects include pain, red skin (erythema), dry skin, hand and foot syndrome damaged fingernails, a dry mouth (xerostomia), water retention, and sexual impotence. Some medications can trigger allergic or pseudoallergic reactions.

Some patients report fatigue or nonspecific neurocognitive problems, such as an inability to concentrate; this is sometimes called postchemotherapy cognitive impairment (referred to as ''chemo brain'' by patient groups) [33]. Specific chemotherapeutic agents are associated with organ-specific toxicities, including cardiovascular disease (e.g., doxorubicin), interstitial lung disease (e.g., bleomycin), and occasionally secondary neoplasm (e.g., MOPP therapy for Hodgkin's disease).

1.4 Supportive Care

The development of new drugs to prevent nausea (the prototype of which was ondansetron) was of great practical use as was the design of indwelling intravenous

catheters (e.g., Hickman lines and Port-a-Cath systems) that allowed safe administration of chemotherapy as well as supportive therapies via an intravenous line. Supportive care includes treatment of infections, treatment with growth factors, treatment with blood products, and treatment of pain, diarrhea, psychological derangement, depression, and anxiety by oncologists, psycho-oncologists, and specialized nursing staff.

1.5 New Approaches Complementing Current Cancer Chemotherapy

Despite the success of certain anticancer agents in curing certain malignant diseases, mostly hematological cancers or neoplasms with a low prevalence, and the gradual improvement of treating the most common solid tumors with combination therapy in the adjuvant or palliative setting, oncologists appeared to have hit a wall in terms of achieving major breakthroughs with conventional cytostatic agents.

An important asset at this point was the recognition that hormonal therapy was beneficial for several types of cancers derived from hormonally responsive tumors, including breast, prostate, endometrium, adrenal cortex, and endocrine tumors. Effective strategies for starving tumor cells of growth- and survival-promoting hormones was to use drugs that inhibit the production of those hormones (e.g., estrogens or testosterone for breast and prostate cancer, respectively) or to administer hormone receptor antagonists. Both classes are often used prior or after chemotherapy in the subpopulation of patients that have a positive hormone receptor status, and can inhibit tumor growth for many months and are also beneficial in preventing a tumor relapse after successful surgery or chemotherapy. For some tumors, such as endocrine tumors, an analog of the peptide hormone somatostatin, octreotide, is the best option of treating endocrine tumors of different origin and carcinoid tumors.

From the 1980s onwards, advances in molecular biology allowed a progressive elucidation of the mechanisms underlying cancer and a profound understanding of the genetic nature of cancer. The molecular and genetic approaches uncovered entirely new signaling networks of intra- and extracellular kinases, growth factor receptors, and antigens that regulate activities of tumor cells and tumor tissue, such as their proliferation and survival as well as angiogenesis – the formation of tumor blood vessels that are necessary for a solid tumor to grow once it has reached a size of approximately 1 cm^3.

As a result, the pharmaceutical and biotech industry invested heavily into a new drug generation and the expression ''targeted therapy'' was coined, referring to treating cancer by blocking the growth of cancer cells by interfering with specific cellular targets needed for carcinogenesis and tumor growth.

The two categories of targeted therapy are small molecules and monoclonal antibodies (mAbs). The most successful example of targeted development for small molecules is imatinib mesylate (Gleevec®) – a small molecule that inhibits the signaling molecule kinase Bcr–Abl kinase that causes chronic myelogenous leukemia

(CML). Gleevec® has dramatically improved the treatment of this malignancy. The next generation of drugs (dasatinib and nilotinib) has now been approved and is available, inducing a more effective molecular remission in CML than imatinib.

Subsequent developments for treating solid tumors have resulted in tyrosine kinase inhibitors such as gefitinib (Iressa®) [34], which targets the epidermal growth factor receptor (EGFR) tyrosine kinase and is approved for treating non-small-cell lung cancer (NSCLC), and erlotinib (Tarceva®), which acts by a similar mechanism as gefitinib. Both drugs work best for EGFR receptor kinase mutations in NSCLC [35]. This type of cancer is a distinct type of cancer where personalized medicine can be successfully used when the biomarker EGFR activating mutations is present. Another good example is the treatment of NSCLC with an anaplastic lymphoma kinase (ALK) inhibitor (crizotinib) in the case of (echinoderm microtubule-associated protein-like) EML4-ALK-positive tumors [36]. EML4-ALK is a fusion-type protein tyrosine kinase that is present in only about 5% of NSCLC patients. A crucial factor is the identification of this small subgroup through prospective tumor genotyping as a prerequisite for a successful treatment. Three other multikinase inhibitors (sunitinib, sorafenib, and pazopanib) are approved, but not for major tumor types, such as breast, lung, colon, and prostate cancer. The approvals comprise less-common tumors such as kidney cancer, gastrointestinal stromal tumor, and primary liver cancer, and to date it is unclear if these drugs can be successfully integrated in the treatment strategies of major tumors. Recent results showed that sunitinib failed in phase III studies in colon and breast cancer. Another interesting aspect is the fact that all new drugs failed in pancreatic cancer, and most in prostate cancer, indicating that these tumors cannot currently be treated effectively by targeted therapy.

Bortezomib (Velcade®) is an inhibitor of the proteasome – an intracellular protein complex that degrades unneeded or damaged proteins – and is approved to treat multiple myeloma that no longer responds to chemotherapy.

Another new class of targeted small molecules is represented by the histone deacetylase (HDAC) inhibitors that inhibit the proliferation of tumor cells by inducing cell cycle arrest, differentiation, and/or apoptosis. Histone acetylation and deacetylation play pivotal roles in the regulation of gene transcription. Vorinostat® is the first HDAC inhibitor to be approved for the treatment of cutaneous T cell lymphoma.

The alternative approach to conventional chemotherapy has been the development of mAbs that are directed toward tumor-associated antigens. The advent of chimeric or humanized, or human mAbs in which only the variable, the hypervariable, or none of the regions of the binding domain carry murine sequences, has resolved the initial drawback of provoking a immune reaction in cancer patients. As a consequence, six antibodies, trastuzumab (Herceptin®, used in the treatment of HER2/*neu* breast cancer), alemtuzumab (Campath®, targets the antigen CD52 expressed on in chronic lymphatic leukemia), rituximab (Rituxan®, used in the treatment of $CD20^+$ Hodgkin's lymphoma), bevacizumab (Avastin®, used in the treatment of colon cancer, breast cancer, and NSCLC inhibiting the vascular

endothelial growth factor receptor that is important for angiogenesis) [37], and cetuximab (Erbitux®) and panitumumab (Vectibix®), both of which target the EGFR and are used in the treatment of colon cancer, are approved and a large number of other antibodies are in clinical trials [38]. The next mAb that will very likely be approved is ipilimumab, which blocks cytotoxic T-lymphocyte-associated antigen-4 to potentiate an T-cell response for the treatment of metastatic melanoma [39].

Although the new generation of targeted therapies have undoubtedly improved the therapeutic options of treating cancer, four important issues need to be considered: (i) the use of approved targeted drugs have improved the overall survival of cancer patients by 3–12 months; (ii) treatment with these drugs causes side-effects that range from skin toxicity, cardiac toxicity, effusions, diarrhea, fatigue, and hypertension to other side-effects that can be severe, approaching grade 3 and 4 toxicity, which is encumbering for the patient and sometimes requires another treatment to ameliorate these side-effects; (iii) resistance against targeted therapy occurs just like with conventional anticancer agents; and (iv) targeted therapy is generally used in combination with conventional chemotherapy and the best therapeutic results are achieved in such regimens.

1.6 Conclusions and Perspectives

The era of cancer chemotherapy began in the 1940s with the first use of nitrogen mustards and folic acid antagonist drugs. Cancer drug development has developed since then into a multi-billion dollar industry. Conventional cytotoxic chemotherapy has shown the ability to cure some cancers, including testicular cancer, chorioncarcinoma, rhabdosarcoma, Hodgkin's disease, non-Hodgkin's lymphoma, and some leukemias. It has also proved effective in the adjuvant setting, in reducing the risk of recurrence after surgery for high-risk breast, colon, and lung cancers, among others. In the palliative setting, the continuing evaluation of combination therapies has improved the quality of live and over two decades has shown a slow but gradual increase in the overall survival rates of patients with solid tumors.

The overall impact of chemotherapy on cancer survival can be difficult to estimate, since improved cancer screening, prevention (e.g., antismoking campaigns), and detection all influence statistics on cancer incidence and mortality. To date, the World Health Organization predicts that deaths from cancer worldwide are projected to rise continuously, with an estimated 12 million deaths in 2030.

The addition of targeted therapies has significantly improved the treatment of a few malignancies such as CML, lung tumors with adeno cancer, kidney cancer, colon cancer, or multiple myeloma, and has in combination with classic anticancer agents improved the quality of life and the overall survival for patients with many solid tumor by approximately 3–12 months in the palliative setting. However, for some common solid tumors such as metastatic breast cancer the overall survival has remained more or less constant during nearly three decades, albeit several

subgroups (e.g., HER2/*neu* receptor-positive) have an significant advantage by additionally using a HER2/*neu* receptor-specific antibody (Herceptin®).

With the better understanding of carcinogenesis, angiogenesis, and signal transduction pathways there were high hopes that therapy with targeted therapies with or without conventional chemotherapy would revolutionize cancer therapy with malignant diseases being treated as a chronic disease with long-term improvement or stabilization of the disease. Unfortunately, we are still far from reaching this goal. With succeeding generations of tumor cells, differentiation is typically lost, growth becomes less regulated, and tumors become less responsive to most chemotherapeutic or targeted agents. Near the center of some solid tumors, cell division has effectively ceased, making them insensitive to chemotherapy. Further challenges for treating solid tumors are due to the fact that the chemotherapeutic agent often does not reach the core of the tumor. Finally, with increasing tumor mass and the formation of metastases, cancer cells become more resistant to chemotherapy treatments.

The three volume compendium gives an overview of the drug delivery systems that have been developed over the past decades with the aim of improving the therapeutic index of anticancer drugs. This is a central goal for treating malignant diseases with conventional anticancer drugs or targeted drugs, with the first drug delivery systems now approved and many others in clinical trials. Such drug delivery systems will be a valuable asset for the oncologist in his/her options of treating cancer patients as effectively as possible, very likely in combination with established clinical protocols. As we move toward a more personalized approach of treating over 100 different tumor indications, we should learn from the mistakes of putting all our eggs in one basket and disregarding long experience with conventional chemotherapy in the hope that new approaches, whether they are called targeted therapies, immunotherapy, gene therapy, or nanomedicine, will find a quick medical solution. It is more likely that all these fields will make advances and it will be a tailor-made combination of different therapeutic strategies that will achieve the best results when faced with such a complex disease as cancer.

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