Cancer and AIDS

Research and Treatments

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Chapter 1

Experimental Cancer Treatment

Experimental cancer treatments are medical therapies intended or claimed to treat cancer by improving on, supplementing or replacing conventional methods (surgery, chemotherapy, radiation, and immunotherapy).

The entries listed below vary between theoretical therapies to unproven controversial therapies. Many of these treatments are alleged to only help against specific forms of cancer. It is not a list of treatments widely available at hospitals.

Bacterial treatments

Chemotherapeutic drugs have a hard time penetrating tumors to kill them at their core because these cells may lack a good blood supply. Researchers have been using anaerobic bacteria, such as *Clostridium novyi*, to consume the interior of oxygen-poor tumours. These should then die when they come in contact with the tumour's oxygenated sides, meaning they would be harmless to the rest of the body. A major problem has been that bacteria don't consume all parts of the malignant tissue. However combining the therapy with chemotheraputic treatments can help to solve this problem.

Another strategy is to use anaerobic bacteria that have been transformed with an enzyme that can convert a non-toxic prodrug into a toxic drug. With the proliferation of the bacteria in the necrotic and hypoxic areas of the tumour the enzyme is expressed solely in the tumour. Thus a systemically applied prodrug is metabolised to the toxic drug only in the tumour. This has been demonstrated to be effective with the non pathogenic anaerobe *Clostridium sporogenes*.

Drug therapies

HAMLET (human alpha-lactalbumin made lethal to tumor cells)

HAMLET (human alpha-lactalbumin made lethal to tumor cells) is a molecular complex derived from human breast milk that kills tumor cells by a process resembling programmed cell death (apoptosis). It has been tested in humans with skin papillomas and bladder cancer.

Dichloroacetate

Dichloroacetate (DCA) has been found to shrink tumors *in vitro* in rats, and has a plausible scientific mechanism: DCA appears to reactivate suppressed mitochondria in some types of oxygen-starved tumor cells, and thus promotes apoptosis. Because it was tested for other conditions, DCA is known to be relatively safe, available, and inexpensive, and it can be taken by mouth as a pill, which is convenient. Early animal studies received attention in the media, and some doctors began controversially using the chemical off-label. As of 2008, no patients had been treated with DCA in a clinical trial, and so its actual effectiveness is unknown.

Quercetin

In vitro, quercetin shows some antitumor activity. Cultured skin and prostate cancer cells showed significant mortality (compared to nonmalignant cells) when treated with a combination of quercetin and ultrasound Note that ultrasound also promotes topical absorption by up to 1,000 times, making the use of topical quercetin and ultrasound wands an interesting proposition.

High dietary intake of fruits and vegetables is associated with reduction in cancer, and some scientists suspect quercetin may be partly responsible. Research shows that quercetin influences cellular mechanisms in vitro and in animal studies, and there is limited evidence from human population studies that quercetin may reduce the risk of lung cancer.

Insulin potentiation therapy

In insulin potentiation therapy (IPT), insulin is a controversial supplement to low-dose chemotherapy. Its proponents claim insulin therapy increases the uptake of chemotherapeutic drugs by malignant cells, permitting the use of lower total drug doses and reducing side effects.

The first clinical trial, involving 30 women with breast cancer, combined insulin with low-dose methotrexate (a common chemotherapy drug) resulted in greatly increased stable disease, and much reduced progressive disease, compared with insulin or low-dose methotrexate alone.

Drugs that restore p53 activity

Several drug therapies are being developed based on p53, the tumour suppressor gene that protects the cell in response to damage and stress. It is like you deciding what to do with a damaged car: p53 brings everything to a halt, and then decides whether to fix the cell or whether the cell is beyond repair and should be destroyed. This protective function of p53 is disabled in most cancer cells, allowing them to multiply without check. Restoration of p53 activity in tumours (where possible) has been shown to inhibit tumour growth and can even shrink the tumour.

As p53 protein levels are usually kept low, one could block its degradation and allow large amounts of p53 to accumulate, thus stimulating p53 activity and its antitumour effects. Drugs that utilize this mechanism include nutlin and MI-219, which are both in phase I clinical trials. There are also other drugs that are still in the preclinical stage of testing, such as RITA and MITA.

Gene therapy

Introduction of tumor suppressor genes into rapidly dividing cells has been thought to slow down or arrest tumor growth. Adenoviruses are a commonly utilized vector for this purpose. Much research has focused on the use of adenoviruses which cannot reproduce, or reproduce only to a limited extent, within the patient to ensure safety via the avoidance of cytolytic destruction of noncancerous cells infected with the vector. However, new studies focus on adenoviruses which can be permitted to reproduce, and destroy cancerous cells in the process, since the adenoviruses' ability to infect normal cells is substantially impaired, potentially resulting in a far more effective treatment. Another use of gene therapy is the introduction of enzymes into these cells that make them susceptible to particular chemotherapy agents; studies with introducing thymidine kinase in gliomas, making them susceptible to aciclovir, are in their experimental stage.

Telomerase therapy

Because most malignant cells rely on the activity of the protein telomerase for their immortality, it has been proposed that a drug which inactivates telomerase might be effective against a broad spectrum of malignancies. At the same time, most healthy tissues in the body express little if any telomerase, and would function normally in its absence. Currently, Inositol hexaphosphate, which is available over-the-counter, is undergoing testing in cancer research due to its telomerase-inhibiting abilities.

A number of research groups have experimented with the use of telomerase inhibitors in animal models, and as of 2005 and 2006 phase I and II human clinical trials are underway. Geron Corporation, is currently conducting two clinical trials involving telomerase inhibitors. One uses a vaccine (GRNVAC1) and the other uses a lipidated drug (GRN163L).

Radiation therapies

Photodynamic therapy

Photodynamic therapy (PDT) is generally a non-invasive treatment using a combination of light and a photosensitive drug, such as 5-ALA, Foscan, Metvix, Tookad, WST09, WST11, Photofrin or Visudyne. The drug is triggered by light of a specific wavelength.

Hyperthermia therapy

Localized and whole-body application of heat has been proposed as a technique for the treatment of malignant tumours. Intense heating will cause denaturation and coagulation of cellular proteins, rapidly killing cells within a tumour.

More prolonged moderate heating to temperatures just a few degrees above normal can cause more subtle changes. A mild heat treatment combined with other stresses can cause cell death by apoptosis. There are many biochemical consequences to the heat shock response within in cell, including slowed cell division and increased sensitivity to ionizing radiation therapy.

There are many techniques by which heat may be delivered. Some of the most common involve the use of focused ultrasound (FUS or HIFU), microwave heating, induction heating, magnetic hyperthermia or direct application of heat through the use of heated saline pumped through catheters. Experiments have been done with carbon nanotubes that selectively bind to cancer cells. Lasers are then used that pass harmlessly through the body, but heat the nanotubes, causing the death of the cancer cells. Similar results have also been achieved with other types of nanoparticles including gold-coated nanoshells and nanorods which exhibit certain degrees of 'tunability' of the absorption properties of the nanoparticles to the wavelength of light for irradiation. The success of this approach to cancer treatment rests on the existence of an 'optical window' in which biological tissue (i.e. healthy cells) are completely transparent at the wavelength of the laser light while nanoparticles are highly absorbing at the same wavelength. Such a 'window' exists in the so-called near infrared region of the electromagnetic spectrum. In this way, the laser light can pass through the system without harming healthy tissue and only diseased cells, where the nanoparticles reside, get hot and are killed.

Magnetic hyperthermia makes use of magnetic nanoparticles, which can be injected into tumours and then generate heat when subjected to an alternating magnetic field.

One of the challenges in thermal therapy is delivering the appropriate amount of heat to the correct part of the patient's body. A great deal of current research focuses on precisely positioning heat delivery devices (catheters, microwave and ultrasound applicators, etc.) using ultrasound or magnetic resonance imaging, as well as of developing new types of nanoparticles that make them particularly efficient absorbers while offering little or no concerns about toxicity to the circulation system. Clinicians also hope to use advanced imaging techniques to monitor heat treatments in real time—heat-induced changes in tissue are sometimes perceptible using these imaging instruments.

Non-invasive RF cancer treatment

This preclinical treatment involves using radio waves to heat up tiny metals which are implanted in cancerous tissue. Gold nanoparticles or carbon nanotubes are the most likely candidate. Promising preclinical trials have been conducted, although clinical trials may not be held for another few years.

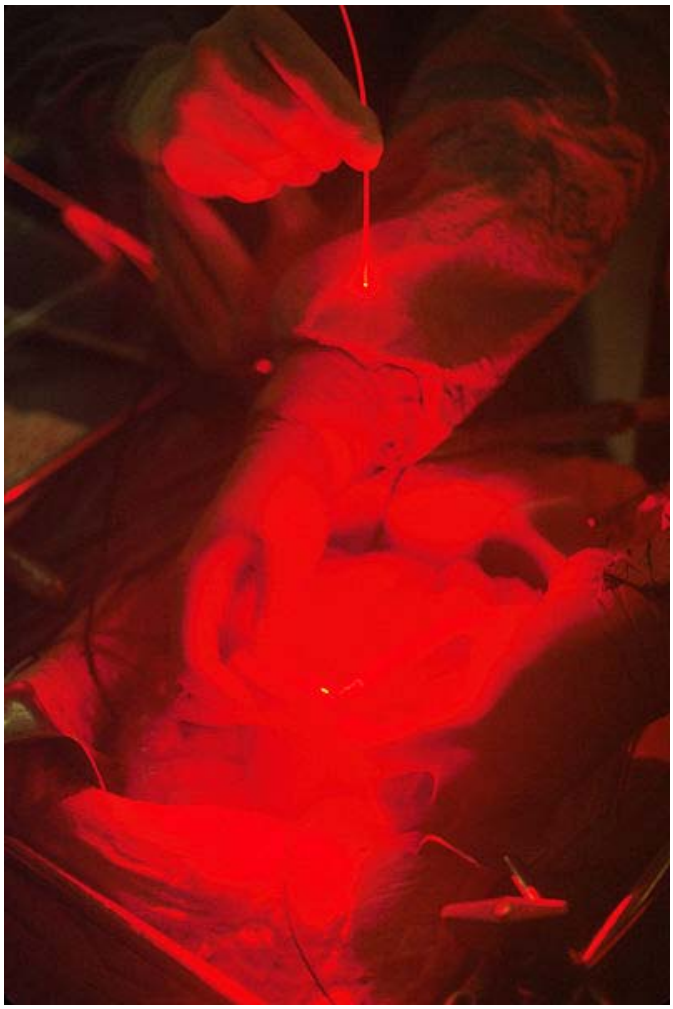
Complementary and alternative treatments

Complementary and alternative medicine (CAM) treatments are the diverse group of medical and health care systems, practices, and products that are not part of conventional medicine and have not been shown to be effective. *Complementary medicine* usually refers to methods and substances used along with conventional medicine, while *alternative medicine* refers to compounds used instead of conventional medicine. CAM use is common among people with cancer.

Most complementary and alternative medicines for cancer have not been rigorously studied or tested. Some alternative treatments that have been proven ineffective continue to be marketed and promoted.

Chapter 2

Photodynamic Therapy



Close up of surgeons' hands in an operating room with a "beam of light" traveling along fiber optics for photodynamic therapy. Its source is a laser beam which is split at two different stages to create the proper "therapeutic wavelength". A patient would be given a photo sensitive drug (photofrin) containing cancer killing substances which are absorbed by cancer cells. During the surgery, the light beam is positioned at the tumor site, which then activates the drug that kills the cancer cells, thus photodynamic therapy (PDT).

Photodynamic therapy (PDT), matured as a feasible medical technology in the 1980s at several institutions throughout the world, is used to eradicate premalignant and early-stage cancer and reduce the tumour size in end-stage cancers involving three key components: a photosensitizer, light, and tissue oxygen.

It is an approved treatment for wet macular degeneration, and is also being investigated for treatment of psoriasis.

Treatment of internal organs may be achieved through the use of endoscopes and fiber optic catheters to deliver light, and intravenously-administered photosensitizers.

A great deal of research and clinical study is now underway to determine optimal combinations of photosensitizers, light sources, and treatment parameters for a wide variety of different cancers.

It is currently being tested as a treatment for severe acne.

History

The German physician Friedrich Meyer–Betz performed the first study with what was first called **photoradiation therapy** (PRT) with porphyrins in humans in 1913. Meyer–Betz tested the effects of haematoporphyrin-PRT on his own skin.

Thomas Dougherty of Roswell Park Cancer Center, among others worldwide, became a highly visible advocate and educator. Early patients were treated at Roswell, Los Angeles Children's Hospital, Los Angeles County Hospital, and other clinics and Hospitals in the USA and overseas.

It was John Toth, as product manager for Cooper Medical Devices Corp/Cooper Lasersonics, who acknowledged the "photodynamic chemical effect" of the therapy with early clinical argon dye lasers and wrote the first "white paper" renaming the therapy as "Photodynamic Therapy" (PDT). This was done to support efforts in setting up 10 clinical sites in Japan where the term "radiation" had negative connotations. PDT received even greater interest as result of Thomas Dougherty helping expand clinical trials and forming the International Photodynamic Association, in 1986.

Mechanism of action

A photosensitizer is a chemical compound that can be excited by light of a specific wavelength. This excitation uses visible or near-infrared light. In photodynamic therapy, either a photosensitizer or the metabolic precursor of one is administered to the patient. The tissue to be treated is exposed to light suitable for exciting the photosensitizer. Usually, the photosensitizer is excited from a ground singlet state to an excited singlet state. It then undergoes intersystem crossing to a longer-lived excited triplet state. One of the few chemical species present in tissue with a ground triplet state is molecular oxygen. When the photosensitizer and an oxygen molecule are in proximity, an energy transfer

can take place that allows the photosensitizer to relax to its ground singlet state, and create an excited singlet state oxygen molecule. Singlet oxygen is a very aggressive chemical species and will very rapidly react with any nearby biomolecules. (The specific targets depend heavily on the photosensitizer chosen.) Ultimately, these destructive reactions will kill cells through apoptosis or necrosis.

This mechanism is identical to the mechanism of the disease erythropoietic protoporphyria, which causes blistering in response to sun exposure due to a genetic defect in the same metabolic pathway.

Example treatment of skin cancer

As an example, consider PDT as a treatment for basal cell carcinoma (BCC). BCC is the most common form of skin cancer in humans. Conventional treatment of BCC involves surgical excision, cryogenic treatment with liquid nitrogen, or localized chemotherapy with 5-fluorouracil or other agents.

A PDT treatment would involve the following steps.

- A photosensitizer precursor (aminolevulinic acid (ALA) or methyl aminolevulinate (MAL) or levulinic acid (LA)) is applied.
- A waiting period of a few hours is allowed to elapse, during which time
    - ALA will be taken up by cells, and
    - ALA will be converted by the cells to protoporphyrin IX, a photosensitizer.
- The physician shines a bright red light (from an array of light-emitting diodes or a diode laser) on the area to be treated. The light exposure lasts a few minutes to a few tens of minutes.
    - Protoporphyrin IX absorbs light, exciting it to an excited singlet state;
    - Intersystem crossing occurs, resulting in excited triplet protoporphyrin IX;
    - Energy is transferred from triplet protoporphyrin IX to triplet oxygen, resulting in singlet (ground state) protoporphyrin IX and excited singlet oxygen;
    - Singlet oxygen reacts with biomolecules, fatally damaging some cells in the treatment area.
- Within a few days, the exposed skin and carcinoma will scab over and flake away.
- In a few weeks, the treated area has healed, leaving healthy skin behind. For extensive malignancies, repeat treatments may be required. It is also common to experience pain from the area treated.
- After the treatment the patient will need to avoid excessive exposure to sunlight for a period of time.

Advantages and limitations

Unlike chemotherapy for cancer the effect of PDT can be localised. Specificity of treatment is achieved in three ways.

- First, light is delivered only to tissues that a physician wishes to treat. In the absence of light, there is no activation of the photosensitizer and no cell killing.
- Second, photosensitizers may be administered in ways that restrict their mobility.
- Finally, photosensitizers may be chosen which are selectively absorbed at a greater rate by targeted cells. ALA is taken up much more rapidly by metabolically active cells. Since malignant cells tend to be growing and dividing much more quickly than healthy cells, the ALA targets the unhealthy cells.

PDT can be much cheaper than the alternative radiotherapy or surgical operation and after care. Post operative recovery is typically hours or days rather than weeks.

A major limitation of PDT is that the light needed to activate most photosensitizers can not penetrate through more than one third of an inch (1 cm) of tissue using standard laser technology and low powered LED technology. Laser application of PDT is generally limited to the treatment of tumours on or under the skin, or on the lining of some internal organs. Moreover it is less effective in treatment of large tumours and metastasis for the same reason. However, new high-powered LED technology has been lab-tested to provide a depth of 2 inches from surface in a simulated breast tissue. Also, hollow needles have been used by some units to get the light into deeper tissues.

Photosensitizers

A wide array of photosensitizers for PDT exist. They can be divided into porphyrins, chlorophylls and dyes. Some examples include aminolevulinic acid (ALA), Silicon Phthalocyanine Pc 4, m-tetrahydroxyphenylchlorin (mTHPC), and mono-L-aspartyl chlorin e6 (NPe6).

Several photosensitizers are commercially available for clinical use, such as Photofrin, Visudyne, Levulan, Foscan, Metvix, Hexvix®, Cysview™, and Laserphyrin, with others in development, e.g. Antrin, Photochlor, Photosens, Photrex, Lumacan, Cevira, Visonac, BF-200 ALA. Amphinex.

Although these photosensitizers can be used for wildly different treatments, they all aim to achieve certain characteristics:

- High absorption at long wavelengths
    - Tissue is much more transparent at longer wavelengths (~700-850 nm). Absorbing at longer wavelengths would allow the light to penetrate deeper, and allow the treatment of larger tumors.
- High singlet oxygen quantum yield
- Low photobleaching
- Natural fluorescence
    - Many optical dosimetry techniques, such as fluorescence spectroscopy, depend on the drug being naturally fluorescent
- High chemical stability
- Low dark toxicity

    - The photosensitizer should not be harmful to the target tissue until the treatment beam is applied.
- Preferential uptake in target tissue

The major difference between different types of photosensitizers is in the parts of the cell that they target. Unlike in radiation therapy, where damage is done by targeting cell DNA, most photosensitizers target other cell structures. For example, mTHPC has been shown to localize in the nuclear envelope and do its damage there. In contrast, ALA has been found to localize in the mitochondria and Methylene Blue in the lysosomes.

Targeted PDT

Some photosensitisers naturally accumulate in the endothelial cells of vascular tissue allowing 'vascular targeted' PDT, but there is also research to target the photosensitiser to the tumour (usually by linking it to antibodies or antibody fragments). It is currently only in pre-clinical studies.

Other research

To allow treatment of deeper tumours some researchers are using internal chemiluminescence to activate the photosensitiser.

PDT is currently in clinical trials to be used as a treatment for severe acne. Initial results show have shown for it to be effective as a treatment only for severe acne, though some question whether it is better than existing acne treatments. The treatment causes severe redness and moderate to severe pain and burning sensation.

Chapter 3

Hyperthermia Therapy

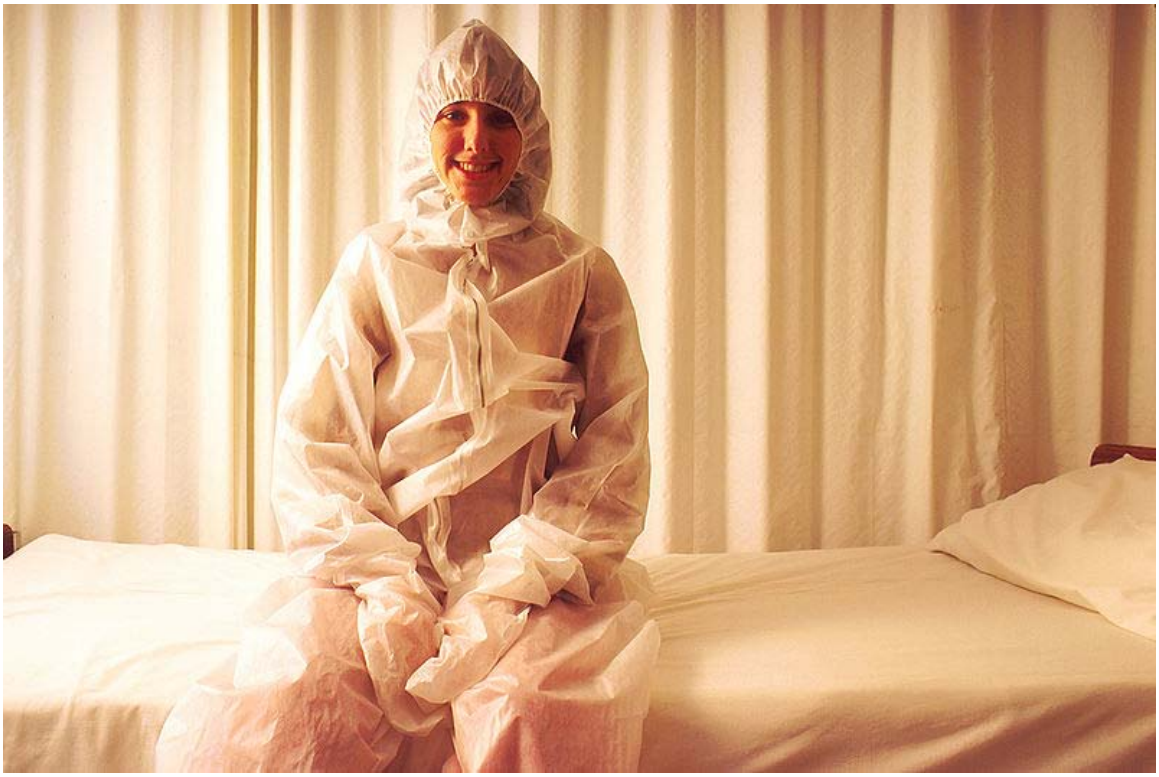
Hyperthermia therapy is a type of medical treatment in which body tissue is exposed to high temperatures to damage and kill cancer cells or to make cancer cells more sensitive to the effects of radiation and certain anti-cancer drugs. When combined with radiation therapy, it is called **thermoradiography**.

Local hyperthermia for certain small tumors is generally accepted, similar to surgically removing a tumor. Whole-body hyperthermia is generally considered to be a promising but experimental cancer treatment.

Hyperthermia is only useful for certain kinds of cancer, and is not in widespread use. Hyperthermia is most effective when used alongside conventional therapies, so it is normally used as an adjuvant therapy. The most effective uses are currently being studied.

Mechanism

Hyperthermia may kill or weaken tumor cells, and is controlled to limit effects on healthy cells. Tumor cells, with a disorganized and compact vascular structure, have difficulty dissipating heat. Hyperthermia may therefore cause cancerous cells to undergo apoptosis in direct response to applied heat, while healthy tissues can more easily maintain a normal temperature.



Whole-body suit used in hyperthermia therapy

Even if the cancerous cells do not die outright, they may become more susceptible to ionizing radiation therapy or to certain chemotherapy drugs, which may allow such therapy to be given in smaller doses.

Intense heating will cause denaturation and coagulation of cellular proteins, rapidly killing cells within a tumour. More prolonged moderate heating to temperatures just a few degrees above normal can cause more subtle changes. A mild heat treatment combined with other stresses can cause cell death by apoptosis. There are many biochemical consequences to the heat shock response within in cell, including slowed cell division and increased sensitivity to ionizing radiation therapy.

Hyperthermia can kill cells directly, but its more important use is in combination with other treatments for cancer. Hyperthermia increases blood flow to the warmed area, perhaps doubling perfusion in tumors, while increasing perfusion in normal tissue by ten times or even more. This enhances the delivery of medications. Hyperthermia also increases oxygen delivery to the area, which may make radiation more likely to damage and kill cells, as well as preventing cells from repairing the damage induced during the radiation session.

Cancerous cells are not inherently more susceptible to the effects of heat. When compared in *in vitro* studies, normal cells and cancer cells show the same responses to heat. However, the vascular disorganization of a solid tumor results in an unfavorable microenvironment inside tumors. Consequently, the tumor cells are already stressed by

low oxygen, higher than normal acid concentrations, and insufficient nutrients, and are thus significantly less able to tolerate the added stress of heat than a healthy cell in normal tissue.

Mild hyperthermia, which provides temperatures equal to that of a naturally high fever, may stimulate natural immunological attacks against the tumor, as part of a natural physiological response called thermotolerance.

Moderate hyperthermia, which heats cells in the range of 40 to 42 °C, damages cells directly, in addition to making the cells radiosensitive and increasing the pore size to improve delivery of large-molecule chemotherapeutic and immunotherapeutic agents (molecular weight greater than 1,000 Daltons), such as monoclonal antibodies and liposome-encapsulated drugs. Cellular uptake of certain small molecule drugs is also increased. Most local and regional cancer treatments are in this temperature range.

Very high temperatures, above 50 °C (122 °F), are used for ablation (direct destruction) of some tumors. This generally involves inserting a metal tube directly into the tumor, and heating the tip until the tissue next to the tube has been killed.

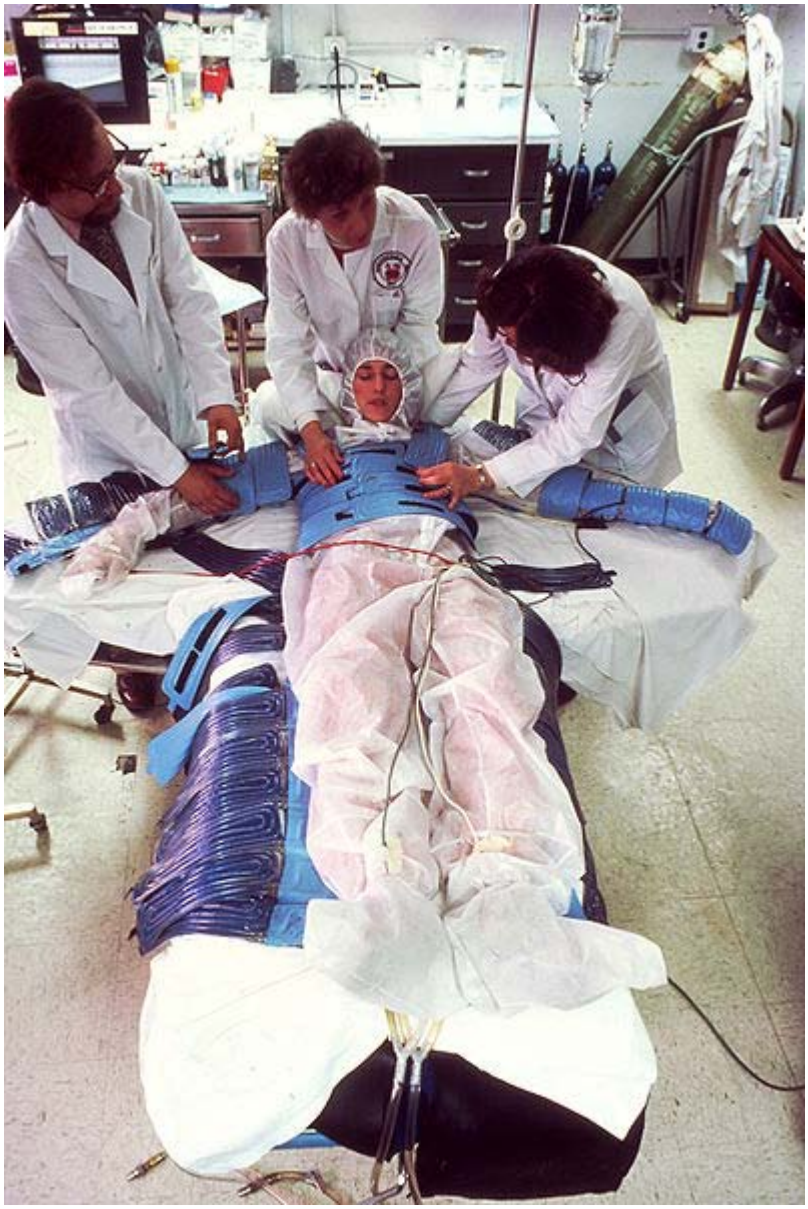
Heat sources

There are many techniques by which heat may be delivered. Some of the most common involve the use of focused ultrasound (FUS or HIFU), infrared sauna, microwave heating, induction heating, magnetic hyperthermia, infusion of warmed liquids, or direct application of heat such as through sitting in a hot room or wrapping a patient in hot blankets.

Types

- *Local hyperthermia* heats a very small area, usually the tumor itself. In some instances, the goal is to kill the tumor by "cooking" it, without damaging anything else. The heat may be created with microwave, radiofrequency, ultrasound energy or using magnetic hyperthermia. Depending on the location of the tumor, the heat may be applied to the surface of the body, inside normal body cavities, or deep in tissue through the use of needles or probes. One relatively common type is radiofrequency ablation of small tumors. This is easiest to achieve when the tumor is on a superficial part of the body, which is called *superficial hyperthermia*, or when needles or probes are inserted directly into the tumor, which is called *interstitial hyperthermia*.
- *Regional hyperthermia* heats a larger part of the body, such as an entire organ or limb. Usually, the goal is to weaken cancer cells so that they are more likely to be killed by radiation and chemotherapeutic medications. This may use the same techniques as local hyperthermia treatment, or it may rely on blood perfusion. In blood perfusion, the patient's blood is removed from the body, heated up, and returned to blood vessels that lead directly through the desired body part. Normally, chemotherapy drugs are infused at the same time. One specialized type

of this approach is continuous hyperthermic peritoneal perfusion (CHPP), which is used to treat difficult cancers within the peritoneal cavity (the abdomen), including primary peritoneal mesothelioma and stomach cancer. Hot chemotherapy drugs are pumped directly into the peritoneal cavity to kill the cancer cells.



Patient undergoing whole-body hyperthermia with doctors looking on

- *Whole-body hyperthermia* heats the entire body to temperatures of about 39 to 41 °C. It is typically used to treat metastatic cancer (cancer that spread to many parts of the body). Techniques include infrared hyperthermia domes which include the whole body apart from the head, putting the patient in a very hot room, or wrapping the patient in hot, wet blankets.

Treatment

Moderate hyperthermia treatments usually maintain the temperature for about an hour or so.

The schedule for treatments depends on the effect desired. After being heated, cells develop resistance to heat, which persists for about three days and reduces the likelihood that they will die from direct cytotoxic effects of the heat. This suggests a maximum treatment schedule of about twice a week. However, if the desired goal is increased radiosensitivity in a poorly oxygenated tumor, rather than directly killing the cells, then application of heat with every radiation treatment is acceptable.

Controlling temperatures

One of the challenges in thermal therapy is delivering the appropriate amount of heat to the correct part of the patient's body. For this technique to be effective, the temperatures must be high enough, and the temperatures must by sustained long enough, to damage or kill the cancer cells. However, if the temperatures are too high, or if they are kept elevated for too long, then serious side effects, including death, can result. The smaller the place that is heated, and the shorter the treatment time, the lower the side effects.

To minimize damage to healthy tissue and other adverse effects, physicians carefully monitor the temperature of the affected area. The goal is to keep local temperatures under 44 °C (111 °F) to avoid damage to surrounding tissues, and the whole body temperatures under 42 °C (108 °F), which is the upper limit compatible with life. These temperatures compare to the normal human body temperature, taken internally, of about 37.6 °C (99.6 °F).

A great deal of current research focuses on precisely positioning heat-delivery devices (catheters, microwave and ultrasound applicators, etc.) using ultrasound or magnetic resonance imaging, as well as developing new types of nanoparticles that make them particularly efficient absorbers while offering little or no concerns about toxicity to other tissues. Clinicians also hope to use advanced imaging techniques to monitor heat treatments in real time; heat-induced changes in tissue are sometimes perceptible using these imaging instruments.

The thermoacoustic (TA) effect refers to the generation of acoustic waves by electromagnetic (EM) irradiation, such as optical or microwave/radio frequency waves. In the past ten years, thermoacoustic tomography (TAT) using pulsed EM excitation has undergone tremendous growth. Energy deposition inside biological tissue through the absorption of incident EM pulses will create a transient temperature rise on the order of 10 mK. In the thermoelastic mechanism of acoustic generation, a sound or stress wave is produced as a consequence of the expansion induced by the temperature variation. Thermoacoustic signals are temperature dependent, which is an ideal characteristic for use in monitoring biological tissue temperature. The thermoacoustic pressure has the following expression

$$P=\mu_a H ß c^2/c_p,$$

where μ_a is the microwave absorption coefficient, H is the heating function can be written as the product of a spatial absorption function and a temporal illumination function, ß is the isobaric volume expansion coefficient, c_0 is the speed of sound, c_p is the heat capacity. The thermal expansion coefficient defines the fractional changes in the volume of a material with temperature-normally its value increases almost linearly with temperature except for the lowest temperatures. Thus, the thermoacoustic pressure can be written in the following forms:

$$P=(A+BT)*P_0$$

where A and B is a constant,whichi can be gotten by the linearship between temperature and thermal expansion coefficient. T is the temperature, P_0 is the thermoacoustic pressure at baseline temperature. The equation demonstrates that the thermoacoustic pressure is directly proportional to temperature where its variation is the reaction of sample thermodynamic parameter changes with heat.

This character of thermoacoustic signals give us a new method to monitor thermotherapy temperature, has the potential to be developed into a viable alternative to current clinical temperature monitoring device for microwave thermotherapy.

Adverse effects

External application of heat may cause blisters, which generally heal quickly, and burns, which do not. All techniques may result in pain or fatigue. Perfusion and moderate or high levels of hyperthermia can cause swelling, blood clots, and bleeding. Whole-body hyperthermia, which is the riskiest treatment, usually results in diarrhea, nausea, vomiting, fatigue, and other symptoms of sunstroke; it may also cause cardiovascular problems.

Effectiveness

By itself, hyperthermia is generally ineffective, with only small numbers of patients receiving lasting benefit. However, it may significantly increase the effectiveness of other treatments.

When combined with radiation, hyperthermia is particularly effective at increasing the damage to acidic, poorly oxygenated parts of a tumor, and cells that are preparing to divide. Hyperthermia treatment is most effective when provided at the same time, or within an hour, of the radiation. Irradiation alone produces a complete response in about 30% of patients. Combining irradiation and hyperthermia increases the complete response rate to about 70% of patients. In the past decade hyperthermia treatments in conjunction with radiation have been used with curative intent in patients with early stage cancers of the breast, head and neck, and prostate. In his observations, James Bicher recorded complete response rates were 82% for breast patients, 88% for head and neck,

and 93% for prostate patients. Projected 5 year survival rates were 80% for breast patients, 88% for head and neck, 87% for prostate patients.

Whole body hyperthermia cannot safely reach the temperatures necessary to improve the effectiveness of radiation, and thus is not used with radiation, but it may be useful for chemotherapy and immunotherapy.

History

The application of heat to treat certain conditions, including possible tumors, has a long history. Ancient Greeks, Romans, and Egyptians used heat to treat breast masses; this is still a recommended self-care treatment for breast engorgement. Medical practitioners in ancient India used regional and whole-body hyperthermia as treatments.

During the 19th century, tumor shrinkage after a high fever due to infection had been reported in a small number of cases. Typically, the reports documented the rare regression of a soft tissue sarcoma after erysipelas (an acute streptococcus bacterial infection of the skin; a different presentation of an infection by "flesh-eating bacteria") was noted. Efforts to deliberately recreate this effect led to the development of Coley's toxin. A sustained high fever after induction of illness was considered critical to treatment success. This treatment is generally considered both less effective than modern treatments and, when it includes live bacteria, inappropriately dangerous.

Around the same period Westermark used localized hyperthermia to produce tumor regression in patients. Encouraging results were also reported by Warren when he treated patients with advanced cancer of various types with a combination of heat, induced with pyrogenic substance, and x-ray therapy. Out of 32 patients, 29 improved for 1 to 6 months.

Properly controlled clinical trials on deliberately induced hyperthermia began in the 1970s.

Future directions

Hyperthermia may be combined with gene therapy, particularly using the heat shock protein 70 promoter.

Two major technological challenges make hyperthermia therapy complicated: the ability to achieve a uniform temperature in a tumor, and the ability to precisely monitor the temperatures of both the tumor and the surrounding tissue. Advances in devices to deliver uniform levels of the precise amount of heat desired, and devices to measure the total dose of heat received, are hoped for.

In locally advanced adenocarcinoma of middle and lower rectum, regional hyperthermia added to chemoradiotherapy achieved good results in terms of rate of sphincter sparing surgery.

Chapter 4

Alternative Cancer Treatments

Alternative cancer treatments describes alternative and complementary treatments for cancer that have not been approved by the government agencies responsible for the regulation of therapeutic goods. They include diet and exercise, chemicals, herbs, devices, and manual procedures. The treatments may be untested or unsupported by evidence, either because no proper testing has been conducted, or because testing did not demonstrate statistically significant efficacy. Concerns have been raised about the safety of some of them.

Alternative cancer treatments are typically contrasted with experimental cancer treatments, which are treatments for which experimental testing is currently underway. All currently approved chemotherapeutic cancer treatments were considered experimental cancer treatments before their safety and efficacy testing was completed.

Such therapies can be categorized broadly into three groups: alternative treatments offered as a substitute to standard medical treatment; alternative treatments as an addition to standard treatment; and treatments proposed in the past that have been found in clinical trials to be useless and/or unsafe. Some of these obsolete or disproven treatments continue to be promoted, sold, and used.

Background

Since the 1940s, medical science has developed chemotherapy, radiation therapy, adjuvant therapy and the newer targeted therapies, as well as refining surgical techniques for removing cancer. Before the development of these modern, evidence-based treatments, 90% of cancer patients died within five years. With mainstream treatments, only 34% of cancer patients die within five years. However, while generally prolonging life or permanently curing cancer, most effective, mainstream forms of cancer treatment have side effects ranging from unpleasant to fatal, and permanent cures are not guaranteed. These side effects create appeal for alternative treatments for cancer, which purport to cause fewer side effects or to increase survival rates.

Alternative cancer treatments have typically not undergone properly conducted, well-designed clinical trials, or the results have not been published due to publication bias (a refusal to publish results showing a treatment does not work). Among those that have been published, the methodology is often poor. A 2006 systematic review of 214 articles

covering 198 clinical trials of alternative cancer treatments concluded that almost none conducted dose-ranging studies, which are necessary to ensure that the patients are being given a useful amount of the treatment. These kinds of treatments appear and vanish frequently, and have throughout history.

Complementary versus alternative treatments

Complementary and alternative cancer treatments are often grouped together, but this grouping is controversial. Definitions vary, but generally speaking they are the same methods that are called "complementary" when given alongside mainstream treatments and "alternative" when they are not. Complementary therapies receive more support within the mainstream medical community than alternative treatments.

Complementary treatments are used in conjunction with proven mainstream treatments. They tend to be pleasant for the patient, not involve substances with any pharmacological effects, inexpensive, and intended to treat side effects rather than to kill cancer cells. Medical massage and self-hypnosis to treat pain are examples of complementary treatments. A 2006 systematic review of the effectiveness of complementary techniques in reducing pain concluded that although several seemed promising, conclusive evidence was lacking.

Alternative treatments, by contrast, are used in place of mainstream treatments. The most popular alternative cancer therapies are various, generally strict diets, including the macrobiotic diet. Other therapies include mind-body interventions, bioelectromagnetics, nutritional supplements, and herbs. The popularity and prevalence of different treatments varies widely by region.

People who choose alternative treatments

People who choose alternative treatments tend to believe that evidence-based medicine is ineffective, while still believing that their own health could be improved. They are impressed by physiological and other scientific-sounding information, prefer a healthcare model that treats the patient as an integrated, whole person, and are loyal to their alternative healthcare providers.

Cancer patients who choose complementary or alternative treatments in addition to conventional treatments believe themselves less likely to die than patients who choose only conventional treatments. They feel a greater sense of control over their destinies, and report less anxiety and depression.

However, patients who use alternative treatments have a poorer survival time, even after controlling for type and stage of disease. This may be because patients who accurately perceive that they are likely to survive do not attempt unproven remedies, and patients who accurately perceive that they are unlikely to survive are attracted to unproven remedies. Among patients who believe their condition to be untreatable by evidence-based medicine, "desperation drives them into the hands of anyone with a promise and a

smile." Con artists have long exploited fear, ignorance, and desperation to strip dying people of their money, comfort, and dignity.

About half the practitioners who dispense complementary or alternative treatments are physicians, although they tend to be generalists rather than oncologists. As many as 60% of physicians have referred their patients to a complementary or alternative practitioner for some purpose.

Examples of alternative treatment

None of the cancer treatments on this list have substantial evidence for their effectiveness in treating cancer. Some have shown some benefits as complementary therapy, to reduce pain. Vitamin C, perhaps the most well-known and controversial, is undergoing a clinical trial based on *in vitro* findings and theoretical speculation as to its *in vivo* effectiveness. Very few suppliers of alternative medicines have undertaken scientifically controlled clinical trials for their products, although occasional preliminary testing, or testing as adjuvant therapy, has been performed. For this reason, alternative therapies generally rely on testimonial or anecdotal evidence. In the United States, FDA regulations forbid the makers of unproven products from claiming efficacy against cancer.

Under consideration

- Quercetin
- Vitamin C megadosage by intravenous infusion Oral vitamin C, regardless of dose, is disproven. This is a type of redox therapy.
- Medicinal mushrooms
- Selenium (Selenomethionine and Se-methylselenocysteine)
- Nicholas Gonzalez's metabolic program

Mixed results

- Coley's Toxins

Unknown

- Essiac tea
- Budwig diet, a diet emphasizing flaxseed oil, milk, fruits, vegetables, and fiber. More likely to be useful for preventing cancer rather than treating it.
- Sodium Bicarbonate

Disproven or scientifically implausible

Chemical substances

- Homeopathy (disproven), tiny amounts of substances, ritually diluted according to 18th century standards

- Laetrile (disproven), also known as B-17, is a cyanide-containing extract of crushed apricot pits
- Di Bella Multitherapy (disproven), a mixture of vitamins, melatonin, and other chemicals.
- Emanuel Revici's catabolic/anabolic approach (disproven)
- Hoxsey method (disproven), a caustic, escharotic paste of herbs and arsenic that is banned in the US as "worthless", "discredited" and "quackery".
- 714X, a water-based solution purported to kill "somatids", which the inventor claims cause disease
- Escharotics such as Cansema or "black salve", usually a paste that kills any skin or tissue it is applied to
- Protocel and Cancell (disproven)
- Krebiozen (disproven), diluted blood from horses
- Hydrazine sulfate, a toxic, synthetic drug
- Proteolytic enzyme therapy
- William Koch's "glyoxide antitoxin" (fraud), also called "recrystallized synthetic toxin", which proved to be distilled water
- Radio-Sulfo Brew, a poultice made of Limburger cheese (fraud)
- Livingston-Wheeler, or Virginia Livingston's *Progenitor cryptocides* treatments, made from the patient's urine, to kill a non-existent bacteria claimed to be the cause of cancer
- Lawrence Burton's Immuno-Augmentative Therapy, claimed to energize the immune system
- Antineoplastons (disproven)
- Chaparral
- Shark cartilage

Diets

- Gerson therapy (dangerous and disproven), a combination of diet and enemas
- Johanna Brandt's "Grape Cure" (scientifically implausible), a diet of water and grapes
- Beverly Hills diet (scientifically implausible), a largely fruitarian diet
- Macrobiotic diet (scientifically implausible), a primarily vegetarian diet with no refined or processed foods (may be useful for cancer prevention, but not treatment)
- Edgar Cayce's diet (scientifically implausible), which prefers alkaline foods to acidic ones
- Juice fasting (scientifically implausible)

Electrical or physical treatments

- Orgone accumulators (fraud), a metal and cardboard box that the client sat in
- Magnet therapy (disproven), applying magnets to the body
- The Rife Machine (scientifically implausible), a radio frequency energy 'beam ray' tube machine

Energy and psychological treatments

- Anti-cancer psychotherapy (disproven), claiming that a "cancer personality" caused cancer, which could be cured through talk therapy, e.g., that of the Simonton Cancer Center, Bernie Siegel's "Exceptional Cancer Patients" (ECaP) or Deepak Chopra
- Therapeutic touch (scientifically implausible), a type of energy therapy
- Imagining successful outcomes (scientifically implausible), such as visualizing cancer cells dying, in cancer guided imagery

Examples of complementary therapy

- Acupuncture Useful to control certain symptoms, but does not kill cancer cells.
- Psychotherapy may reduce anxiety and improve quality of life
- Massage therapy may reduce pain

Alternative theories of cancer

Some alternative cancer treatments are based on unproven or disproven theories of how cancer begins or is sustained in the body. Some common concepts are:

- **Mind-body connection**: This idea says that cancer forms because of, or can be controlled through, the person's mental and emotional state. Treatments based on this idea are mind–body interventions. Proponents say that cancer forms because the person is unhappy or stressed, or that a positive attitude can cure cancer after it has formed. A typical claim is that stress, anger, fear, or sadness depresses the immune system, whereas that love, forgiveness, confidence, and happiness cause the immune system to improve, and that this improved immune system will destroy the cancer. This belief is not supported by any scientific research: people with weak immune systems have about the same rate of cancer as people with healthy immune systems. In fact, many cancers require the support of an active immune system to establish the tumor microenvironment necessary for a tumor to grow.
- **Toxin theory of cancer**: In this idea, the body's metabolic processes are overwhelmed by normal, everyday byproducts. These byproducts, called "toxins", are said to build up in the cells and cause cancer and other diseases through a process sometimes called *autointoxication* or *autotoxemia*. Treatments following this approach are usually aimed at detoxification or body cleansing, such as enemas.
- **Low activity by the immune system**: This claim asserts that if only the immune system were strong enough, it would kill the "invading" or "foreign" cancer. Unfortunately, most cancer cells retain normal cell characteristics, making them appear to the immune system to be a normal part of the body. These treatments often focus on substance said to increase the immune system's activity.

- **Supposed situations within the body**: In this idea, the body is incapable of coping with transient or local differences. For example, proponents will say that a lack of oxygen causes cancer.
- **Hypothetical microorganisms**: While infections are a significant cause of certain kinds of cancer (e.g., Hepatitis B can cause liver cancer, and some human papillomaviruses cause cervical cancer), these stories usually assert that the harmless bacteria and fungi normally present in or on the body cause cancer, or that organisms only detectable by the proponent cause cancer.

Regulatory action

Government agencies around the world routinely investigate purported alternative cancer treatments in an effort to protect their citizens from fraud and abuse.

In 2008, the United States Federal Trade Commission recently acted against companies that made unsupported claims that their products, some of which included highly toxic chemicals, could cure cancer. Targets included Omega Supply, Native Essence Herb Company, Daniel Chapter One, Gemtronics, Inc., Herbs for Cancer, Nu-Gen Nutrition, Inc., Westberry Enterprises, Inc., Jim Clark's All Natural Cancer Therapy, Bioque Technologies, Inc., Cleansing Time Pro, and Premium-essiac-tea-4less.

Chapter 5

Insulin Potentiation Therapy

Insulin potentiation therapy (IPT) is an alternative medicine pharmacologic strategy for the chemotherapy of cancer using insulin and low-dose chemotherapy.

The therapeutic approach is said to take advantage of the endogenous molecular biology of cancer cells, specifically insulin and insulin like growth factor secretion, and the interaction of these biochemicals with their specific receptors. By using insulin in conjunction with chemotherapy drugs, significantly less drug (about 10-15 % of a standard dose) can be targeted more specifically and more effectively to cancer cell populations, thus virtually eliminating dose-related side effects while claiming enhancing antineoplastic effects.

Controversy regarding effectiveness

Some physicians have labeled insulin potentiation therapy a form of quackery and have warned against its use.

Claimed explanatory molecular biology

The proponents of IPT give the following explanation of the biology of cancer and its cells in order to understand the mechanisms of IPT, which relies upon insulin, the most integral component of IPT, having three significant actions upon cancer cells described below, as well as also dropping blood sugar levels and thus the energy source for cancer. Low blood glucose (below 60 mg/dl) also stimulates secretion of growth hormone, and growth hormone probably helps to strengthen the immune system.

Differentiation between cancer and normal cells

Insulin biologically differentiates cancer cells from normal cells based on insulin receptor concentration.

Insulin can serve to distinguish and differentiate cancer cells from healthy cells in several way. Produced in the pancreas, one of its many functions is the regulation of blood glucose levels. Chiefly, insulin activates a glucose transport protein within all cells –

whether they be cancerous or healthy - which allows glucose, the energy source, to enter, thus lowering the blood glucose level.

Like anything else, cancer needs energy to grow. The growth of cancer is abnormally rapid, its sole purpose being to spread, therefore it has a voracious appetite compared to normal cells. Cancer cells have developed the ability to produce insulin and insulin-like growth factor (IGF) themselves; this way they can autonomously increase their glucose uptake.

Being able to produce its own insulin makes cancer different from normal cells, but there is a second abnormality that insulin highlights. Every cell in the body has insulin receptors on the outer surface of its membrane - from 100-100,000 receptors per cell. But cancer cells have a much higher concentration of receptors. Breast cancer cells - for example - have six times more insulin receptors and ten times more IGF receptors per cell than normal cells. As an added boost, insulin is able to react with its own receptors and is also able to cross-react with and activate the IGF receptors on cancer cells. This means that insulin will affect cancer cells sixteen times as strongly as it effects normal tissues. Something else to take into consideration is that ligand effect is a function of receptor concentration. In a particular tissue, the more receptors there are for a certain ligand – such as insulin – the greater the effect of that ligand on that tissue.

By activating the insulin and IGF receptors on cancer cells through the administration of insulin during an IPT treatment, the biological differences of cancer cells can be highlighted – a vital consideration for the safety of cancer chemotherapy.

Modification of cancer cell metabolism

Not only does insulin provide cancer cells with the means to grow, it has also been proven that IGFs are the most potent mitogen - promoter of cell division - for cancer growth.

Now why would growth be a favorable effect in a treatment, which is trying to kill cancer? The answer lies in the killing mechanism of chemotherapy medications. The standard pharmacologic treatment for cancer involves drugs, which are designed to attack cells that are dividing, cell division being the means by which tissue "grows." Cancer cells are rapidly dividing cells, and are constantly going through cell division. There are several phases to cell division, the one called the S-Phase being when cells replicate DNA. There are some chemotherapy agents that are "S-Phase dependent:" they attack cells that are in the S-phase of cell division, not cells in the resting phase.

Unfortunately hair cells, red and white blood cells and cells found in the digestive tract also fall into this category of rapidly dividing cells - the reason why the side effects related to standard chemotherapy are associated with these areas. In order to get a tumoral response in conventional chemotherapy, a high dose of drugs have to be used and unfortunately healthy cells are affected as well. The chemotherapy drugs by themselves cannot differentiate between rapidly dividing cancer cells and rapidly dividing healthy

cells. By implementing insulin in conjunction with chemotherapy drugs, the cancer cells are highlighted as being different based on receptor concentration and are promoted to grow, which makes it likely that more of them will be in the S-phase cycle. These effects allow for the powerful chemo agents to target the cancer cells more specifically, sparing healthy cells and therefore chemo-related side-effects.

Increase in cell membrane permeability

The third effect that insulin has on cancer cells is to activate enzyme activity in the cell membrane making them more permeable.

Cell membranes are largely made up of triglycerides, which are built of fatty acids. The more saturated that a fatty acid is, the higher the melting point (example: butter [a saturated fat with a higher melting point] is solid at room temperature, whereas olive oil [an unsaturated fat with a lower melting point] is a liquid). The enzyme that insulin activates is called delta-9 desaturase and the action of this enzyme is to de-saturate - to make a saturated fat into an unsaturated fat. Delta-9 desaturase - once it has been activated by insulin - de-saturates the fatty acids that make up the cell membrane of cancer cells. This fatty acid – saturated stearic acid– has a melting point of 65 °C. Stearic acid once it has been de-saturated, becomes mono-unsaturated oleic acid, which has a melting point of 5 °C. At physiologic temperatures (the temperature of the body, about 37.5 °C) tristearin – triglyceride with three stearic acids attached that composes the cancer cell membrane - is going to be more "waxy" than "oily" because of its higher melting point. This makes for a less permeable cell membrane. On the other hand, once the insulin has activated the enzyme delta-9 desaturase, the cell membrane of cancer cells is composed of triolein – the triglyceride with three oleic acids attached – with a melting point of 5 °C. This cell membrane will be more permeable at physiologic temperatures. The chemotherapy drugs are thus able to enter the cancer cells more easily because of the increased cell membrane permeability, providing the required intracellular dose intensity to kill the cancer.

Insulin is used in IPT to enhance anticancer drug cytoxicity and safety, via 1) an effect of biological differentiation based on insulin receptor concentration, 2) an effect of metabolic modification to increase the S-phase fraction in cancer cells, enhancing their susceptibility to cell-cycle phase-specific agents, and 3) a membrane permeability effect to increase the intracellular dose intensity of the drugs. Significantly less drug can thus be targeted more specifically and more effectively to cancer cells, all this occurring with a virtual elimination of the dose-related side effects.

Supportive research

In-vitro studies have shown how IPT works supporting the informal clinical work that has been conducted on hundreds of patients worldwide.

A clinical trial of IPT for treating breast cancer was done in Uruguay and concluded that "The group treated with insulin + methotrexate responded most frequently with stable disease" compared to being treated with methotrexate alone or insulin alone.

In 2000, the National Cancer Institute's Cancer Advisory Panel on Complementary and Alternative Medicine (CAPCAM) invited Drs. Perez Garcia and Ayre to present IPT to them as part of the National Cancer Institute's (NCI's) Best Case Series program.. However CAPCAM have not in the time since undertaken any further research into IPT.

Chapter 6

Management of Cancer

Cancer can be treated by surgery, chemotherapy, radiation therapy, immunotherapy, monoclonal antibody therapy or other methods. The choice of therapy depends upon the location and grade of the tumor and the stage of the disease, as well as the general state of the patient (performance status). A number of experimental cancer treatments are also under development.

Complete removal of the cancer without damage to the rest of the body is the goal of treatment. Sometimes this can be accomplished by surgery, but the propensity of cancers to invade adjacent tissue or to spread to distant sites by microscopic metastasis often limits its effectiveness. The effectiveness of chemotherapy is often limited by toxicity to other tissues in the body. Radiation can also cause damage to normal tissue.

Because "cancer" refers to a class of diseases, it is unlikely that there will ever be a single "cure for cancer" any more than there will be a single treatment for all infectious diseases. Angiogenesis inhibitors were once thought to have potential as a "silver bullet" treatment applicable to many types of cancer, but this has not been the case in practice.

Types of treatments

Surgery

In theory, non-hematological cancers can be cured if entirely removed by surgery, but this is not always possible. When the cancer has metastasized to other sites in the body prior to surgery, complete surgical excision is usually impossible. In the Halstedian model of cancer progression, tumors grow locally, then spread to the lymph nodes, then to the rest of the body. This has given rise to the popularity of local-only treatments such as surgery for small cancers. Even small localized tumors are increasingly recognized as possessing metastatic potential.

Examples of surgical procedures for cancer include mastectomy for breast cancer, prostatectomy for prostate cancer, and lung cancer surgery for non-small cell lung cancer. The goal of the surgery can be either the removal of only the tumor, or the entire organ. A single cancer cell is invisible to the naked eye but can regrow into a new tumor, a process called recurrence. For this reason, the pathologist will examine the surgical specimen to

determine if a margin of healthy tissue is present, thus decreasing the chance that microscopic cancer cells are left in the patient.

In addition to removal of the primary tumor, surgery is often necessary for staging, e.g. determining the extent of the disease and whether it has metastasized to regional lymph nodes. Staging is a major determinant of prognosis and of the need for adjuvant therapy.

Occasionally, surgery is necessary to control symptoms, such as spinal cord compression or bowel obstruction. This is referred to as palliative treatment.

If surgery is possible and appropriate, it is commonly performed before other forms of treatment, although the order does not affect the outcome. In some instances, surgery must be delayed until other treatments are able to shrink the tumor.

Radiation therapy

Radiation therapy (also called radiotherapy, X-ray therapy, or irradiation) is the use of ionizing radiation to kill cancer cells and shrink tumors. Radiation therapy can be administered externally via external beam radiotherapy (EBRT) or internally via brachytherapy. The effects of radiation therapy are localised and confined to the region being treated. Radiation therapy injures or destroys cells in the area being treated (the "target tissue") by damaging their genetic material, making it impossible for these cells to continue to grow and divide. Although radiation damages both cancer cells and normal cells, most normal cells can recover from the effects of radiation and function properly. The goal of radiation therapy is to damage as many cancer cells as possible, while limiting harm to nearby healthy tissue. Hence, it is given in many fractions, allowing healthy tissue to recover between fractions.

Radiation therapy may be used to treat almost every type of solid tumor, including cancers of the brain, breast, cervix, larynx, lung, pancreas, prostate, skin, stomach, uterus, or soft tissue sarcomas. Radiation is also used to treat leukemia and lymphoma. Radiation dose to each site depends on a number of factors, including the radiosensitivity of each cancer type and whether there are tissues and organs nearby that may be damaged by radiation. Thus, as with every form of treatment, radiation therapy is not without its side effects.

Chemotherapy

Chemotherapy is the treatment of cancer with drugs ("anticancer drugs") that can destroy cancer cells. In current usage, the term "chemotherapy" usually refers to *cytotoxic* drugs which affect rapidly dividing cells in general, in contrast with *targeted therapy*. Chemotherapy drugs interfere with cell division in various possible ways, e.g. with the duplication of DNA or the separation of newly formed chromosomes. Most forms of chemotherapy target all rapidly dividing cells and are not specific to cancer cells, although some degree of specificity may come from the inability of many cancer cells to repair DNA damage, while normal cells generally can. Hence, chemotherapy has the

potential to harm healthy tissue, especially those tissues that have a high replacement rate (e.g. intestinal lining). These cells usually repair themselves after chemotherapy.

Because some drugs work better together than alone, two or more drugs are often given at the same time. This is called "combination chemotherapy"; most chemotherapy regimens are given in a combination.

The treatment of some leukaemias and lymphomas requires the use of high-dose chemotherapy, and total body irradiation (TBI). This treatment ablates the bone marrow, and hence the body's ability to recover and repopulate the blood. For this reason, bone marrow, or peripheral blood stem cell harvesting is carried out before the ablative part of the therapy, to enable "rescue" after the treatment has been given. This is known as autologous stem cell transplantation. Alternatively, hematopoietic stem cells may be transplanted from a matched unrelated donor (MUD).

Targeted therapies

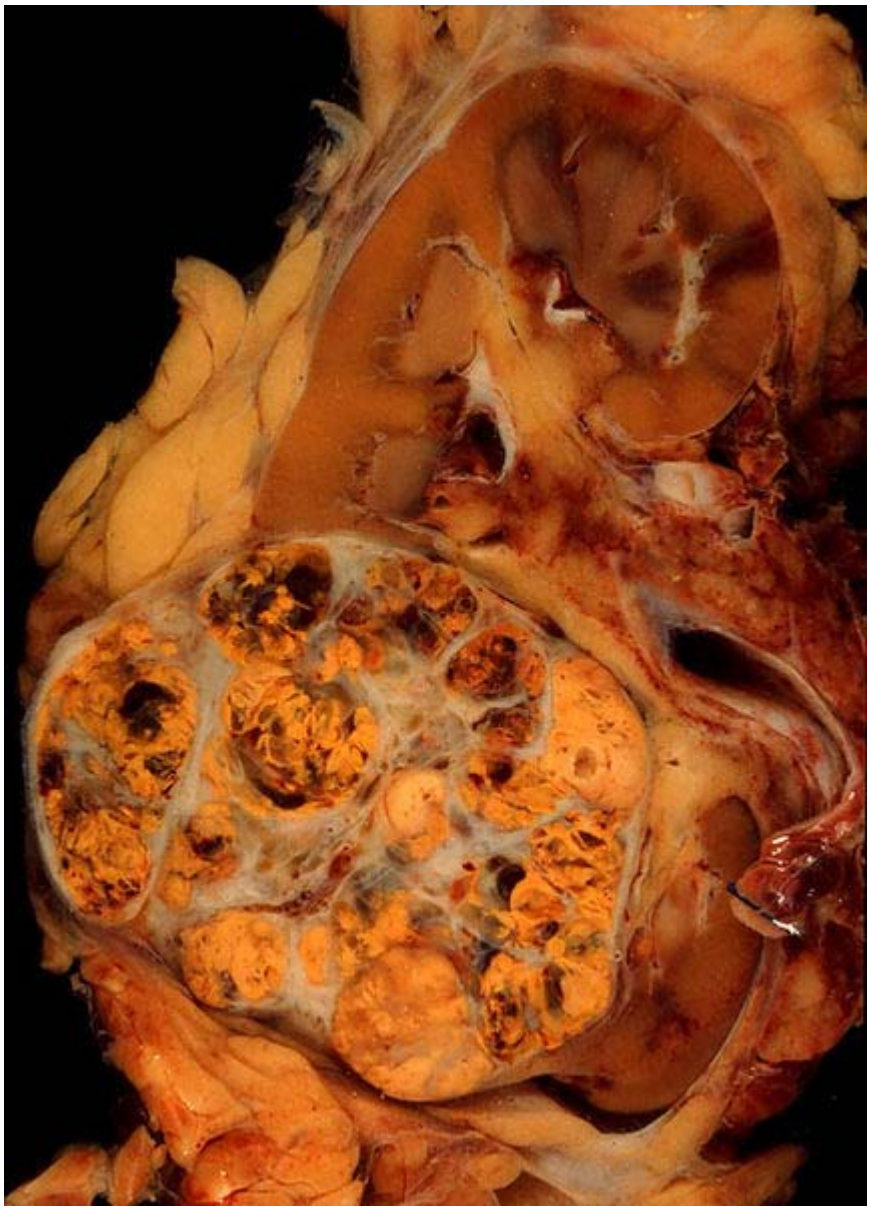
Targeted therapy, which first became available in the late 1990s, has had a significant impact in the treatment of some types of cancer, and is currently a very active research area. This constitutes the use of agents specific for the deregulated proteins of cancer cells. Small molecule targeted therapy drugs are generally inhibitors of enzymatic domains on mutated, overexpressed, or otherwise critical proteins within the cancer cell. Prominent examples are the tyrosine kinase inhibitors imatinib (Gleevec/Glivec) and gefitinib (Iressa).

Monoclonal antibody therapy is another strategy in which the therapeutic agent is an antibody which specifically binds to a protein on the surface of the cancer cells. Examples include the anti-HER2/neu antibody trastuzumab (Herceptin) used in breast cancer, and the anti-CD20 antibody rituximab, used in a variety of B-cell malignancies.

Targeted therapy can also involve small peptides as "homing devices" which can bind to cell surface receptors or affected extracellular matrix surrounding the tumor. Radionuclides which are attached to these peptides (e.g. RGDs) eventually kill the cancer cell if the nuclide decays in the vicinity of the cell. Especially oligo- or multimers of these binding motifs are of great interest, since this can lead to enhanced tumor specificity and avidity.

Photodynamic therapy (PDT) is a ternary treatment for cancer involving a photosensitizer, tissue oxygen, and light (often using lasers). PDT can be used as treatment for basal cell carcinoma (BCC) or lung cancer; PDT can also be useful in removing traces of malignant tissue after surgical removal of large tumors.

Immunotherapy



A renal cell carcinoma (lower left) in a kidney specimen

Cancer immunotherapy refers to a diverse set of therapeutic strategies designed to induce the patient's own immune system to fight the tumor. Contemporary methods for generating an immune response against tumours include intravesical BCG immunotherapy for superficial bladder cancer, and use of interferons and other cytokines to induce an immune response in renal cell carcinoma and melanoma patients. Vaccines to generate specific immune responses are the subject of intensive research for a number of tumours, notably malignant melanoma and renal cell carcinoma. Sipuleucel-T is a vaccine-like strategy in late clinical trials for prostate cancer in which dendritic cells from the patient are loaded with prostatic acid phosphatase peptides to induce a specific immune response against prostate-derived cells.

Allogeneic hematopoietic stem cell transplantation ("bone marrow transplantation" from a genetically non-identical donor) can be considered a form of immunotherapy, since the donor's immune cells will often attack the tumor in a phenomenon known as graft-versus-tumor effect. For this reason, allogeneic HSCT leads to a higher cure rate than autologous transplantation for several cancer types, although the side effects are also more severe.

The cell based immunotherapy in which the patients own Natural Killer cells(NK) and Cytotoxic T-Lymphocytes(CTL) are used has been in practice in Japan since 1990. NK cells and CTLs primarily kill the cancer cells when they are developed. This treatment is given together with the other modes of treatment such as Surgery, radiotherapy or Chemotherapy and called as Autologous Immune Enhancement Therapy (AIET)

Hormonal therapy

The growth of some cancers can be inhibited by providing or blocking certain hormones. Common examples of hormone-sensitive tumors include certain types of breast and prostate cancers. Removing or blocking estrogen or testosterone is often an important additional treatment. In certain cancers, administration of hormone agonists, such as progestogens may be therapeutically beneficial.

Angiogenesis inhibitors

Angiogenesis inhibitors prevent the extensive growth of blood vessels (angiogenesis) that tumors require to survive. Some, such as bevacizumab, have been approved and are in clinical use. One of the main problems with anti-angiogenesis drugs is that many factors stimulate blood vessel growth in cells normal or cancerous. Anti-angiogenesis drugs only target one factor, so the other factors continue to stimulate blood vessel growth. Other problems include route of administration, maintenance of stability and activity and targeting at the tumor vasculature.

Symptom control

Although the control of the symptoms of cancer is not typically thought of as a treatment directed at the cancer, it is an important determinant of the quality of life of cancer patients, and plays an important role in the decision whether the patient is able to undergo other treatments. Although doctors generally have the therapeutic skills to reduce pain, nausea, vomiting, diarrhea, hemorrhage and other common problems in cancer patients, the multidisciplinary specialty of palliative care has arisen specifically in response to the symptom control needs of this group of patients. This is an especially important aspect of care for those patients whose disease is not a good candidate for other forms of treatment. As most treatments for cancer involve significantly unpleasant side effects, a patient with little realistic hope of a cure may choose to seek palliative care only, eschewing more radical therapies in exchange for a prolonged period of normal living.

Pain medication, such as morphine and oxycodone, and antiemetics, drugs to suppress nausea and vomiting, are very commonly used in patients with cancer-related symptoms.

Improved antiemetics such as ondansetron and analogues, as well as aprepitant have made aggressive treatments much more feasible in cancer patients.

Chronic pain due to cancer is almost always associated with continuing tissue damage due to the disease process or the treatment (i.e. surgery, radiation, chemotherapy). Although there is always a role for environmental factors and affective disturbances in the genesis of pain behaviors, these are not usually the predominant etiologic factors in patients with cancer pain. Furthermore, many patients with severe pain associated with cancer are nearing the end of their lives and palliative therapies are required. Issues such as social stigma of using opioids, work and functional status, and health care consumption are not likely to be important in the overall case management. Hence, the typical strategy for cancer pain management is to get the patient as comfortable as possible using opioids and other medications, surgery, and physical measures. Doctors have been reluctant to prescribe narcotics for pain in terminal cancer patients, for fear of contributing to addiction or suppressing respiratory function. The palliative care movement, a more recent offshoot of the hospice movement, has engendered more widespread support for preemptive pain treatment for cancer patients.

Cancer-related fatigue is a very common problem for cancer patients, and has only recently become important enough for oncologists to suggest treatment, even though it plays a significant role in many patients' quality of life.

Research

Clinical trials, also called research studies, test new treatments in people with cancer. The goal of this research is to find better ways to treat cancer and help cancer patients. Clinical trials test many types of treatment such as new drugs, new approaches to surgery or radiation therapy, new combinations of treatments, or new methods such as gene therapy.

A clinical trial is one of the final stages of a long and careful cancer research process. The search for new treatments begins in the laboratory, where scientists first develop and test new ideas. If an approach seems promising, the next step may be testing a treatment in animals to see how it affects cancer in a living being and whether it has harmful effects. Of course, treatments that work well in the lab or in animals do not always work well in people. Studies are done with cancer patients to find out whether promising treatments are safe and effective.

Patients who take part may be helped personally by the treatment they receive. They get up-to-date care from cancer experts, and they receive either a new treatment being tested or the best available standard treatment for their cancer. At the same time, new treatments also may have unknown risks, but if a new treatment proves effective or more effective than standard treatment, study patients who receive it may be among the first to benefit. There is no guarantee that a new treatment being tested or a standard treatment will produce good results. In children with cancer, a survey of trials found that those enrolled in trials were on average not more likely to do better or worse than those on standard

treatment; this confirms that success or failure of an experimental treatment cannot be predicted.

Ultrasound energy

Ultrasound energy is being studied as a form of therapy.

Exosome research

Exosomes are lipid-covered microvesicles shed by solid tumors into bodily fluids, such as blood and urine. Current research is being done attempting to use exosomes as a detection and monitoring method for a variety of cancers., The hope is to be able to detect cancer with a high sensitivity and specificity via detection of specific exosomes in the blood or urine. The same process can be used to more accurately monitor a patients treatment progress as well. Enzyme linked lectin specific assay or ELLSA has been proven to directly detect melanoma derived exosomes from fluid samples. Previously, exosomes had been measured by total protein content in purified samples and by indirect immunomodulatory effects. ELLSA directly measures exosome particles in complex solutions, and has already been found capable of detecting exosomes from other sources, including ovarian cancer and tuberculosis-infected macrophages.

Exosomes secreted by tumors are also believed to be responsible for triggering programmed cell death (apoptosis) of immune cells; interrupting T-cell signaling required to mount an immune response; inhibiting the production of anti-cancer cytokines, and has implications in the spread of metastasis and allowing for angiogenesis. Studies are currently being done with Lectin Affinity Plasmapheresis (LAP), LAP is a blood filtration method which selectively targets the tumor based exosomes and removes them from the bloodstream. It is believed that decreasing the tumor secreted exosomes in a patients bloodstream will slow down progression of the cancer while at the same time increase the patients own immune response.

Complementary and alternative

Complementary and alternative medicine (CAM) treatments are the diverse group of medical and health care systems, practices, and products that are not part of conventional medicine and have not been shown to be effective. "Complementary medicine" refers to methods and substances used along with conventional medicine, while "alternative medicine" refers to compounds used instead of conventional medicine. CAM use is common among people with cancer; a 2000 study found that 69% of cancer patients had used at least one CAM therapy as part of their cancer treatment. Most complementary and alternative medicines for cancer have not been rigorously studied or tested. Some alternative treatments which have been investigated and shown to be ineffective continue to be marketed and promoted.

Special circumstances

In pregnancy

The incidence of concurrent cancer during pregnancy has risen due to the increasing age of pregnant mothers and due to the incidental discovery of maternal tumors during prenatal ultrasound examinations.

Cancer treatment needs to be selected to do least harm to both the woman and her embryo/fetus. In some cases a therapeutic abortion may be recommended.

Radiation therapy is generally out of the question, and chemotherapy always poses the risk of miscarriage and congenital malformations. Little is known about the effects of medications on the child.

Even if a drug has been tested as not crossing the placenta to reach the child, some cancer forms can harm the placenta and make the drug pass over it anyway. Some forms of skin cancer may even metastasize to the child's body.

Diagnosis is also made more difficult, since computed tomography is infeasible because of its high radiation dose. Still, magnetic resonance imaging works normally. However, contrast media cannot be used, since they cross the placenta.

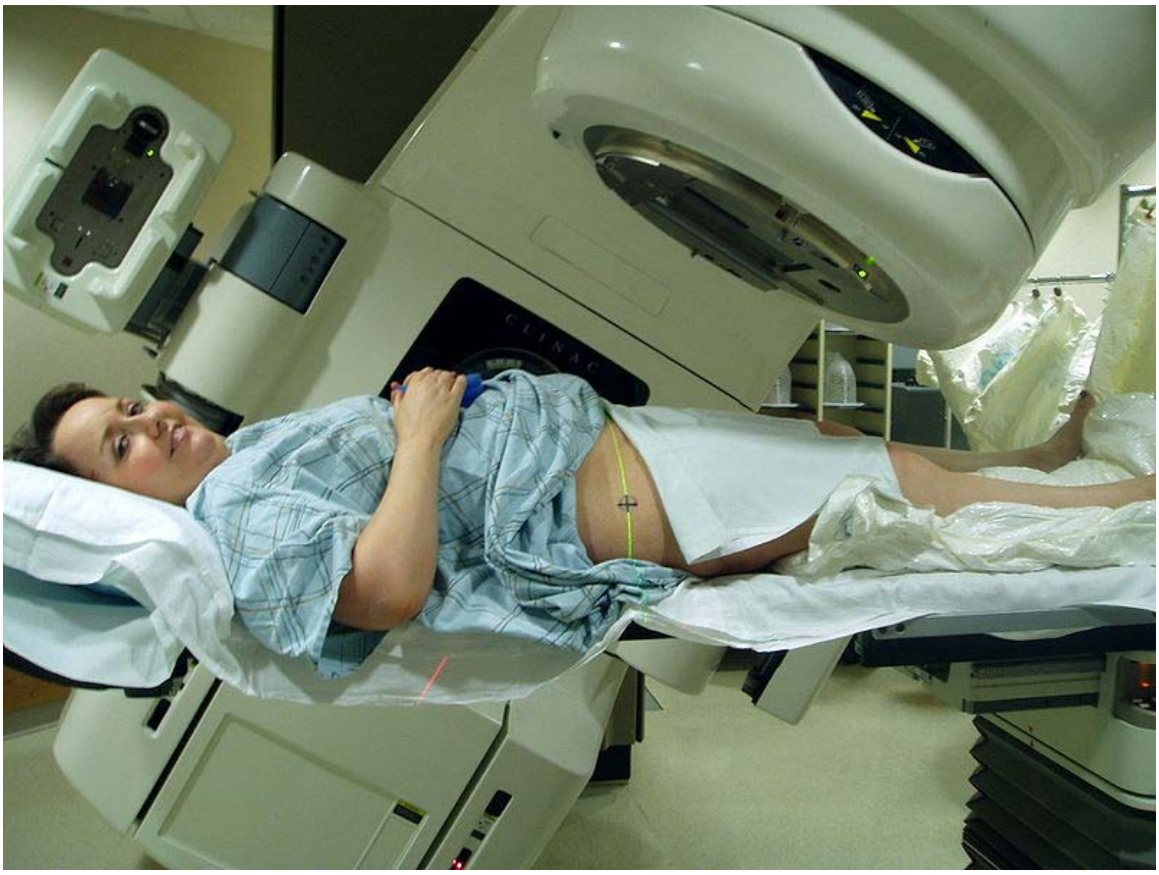
As a consequence of the difficulties to properly diagnose and treat cancer during pregnancy, the alternative methods are either to perform a Cesarean section when the child is viable in order to begin a more aggressive cancer treatment, or, if the cancer is malignant enough that the mother is unlikely to be able to wait that long, to perform an abortion in order to treat the cancer.

In utero

Fetal tumors are sometimes diagnosed while still in utero. Teratoma is the most common type of fetal tumor, and usually is benign.

Chapter 7

Radiation Therapy



Radiation therapy of the pelvis. Lasers and a mould under the legs are used to determine exact position.

Axesse Radiotherapy

Radiation therapy (in the USA), **radiation oncology**, or **radiotherapy** (in the UK, Canada and Australia), sometimes abbreviated to XRT, is the medical use of ionizing radiation as part of cancer treatment to control malignant cells (not to be confused with radiology, the use of radiation in medical imaging and diagnosis). Radiotherapy may be used for curative or adjuvant treatment. It is used as palliative treatment (where cure is not possible and the aim is for local disease control or symptomatic relief) or as therapeutic treatment (where the therapy has survival benefit and it can be curative). Total body irradiation (TBI) is a radiotherapy technique used to prepare the body to receive a bone marrow transplant. Radiotherapy has several applications in non-malignant conditions, such as the treatment of trigeminal neuralgia, severe thyroid eye disease, pterygium, pigmented villonodular synovitis, prevention of keloid scar growth, and prevention of heterotopic ossification. The use of radiotherapy in non-malignant conditions is limited partly by worries about the risk of radiation-induced cancers.

Radiotherapy is used for the treatment of malignant cancer, and may be used as a primary or adjuvant modality. It is also common to combine radiotherapy with surgery, chemotherapy, hormone therapy, Immunotherapy or some mixture of the four. Most common cancer types can be treated with radiotherapy in some way. The precise treatment intent (curative, adjuvant, neoadjuvant, therapeutic, or palliative) will depend on the tumor type, location, and stage, as well as the general health of the patient.

Radiation therapy is commonly applied to the cancerous tumor. The radiation fields may also include the draining lymph nodes if they are clinically or radiologically involved with tumor, or if there is thought to be a risk of subclinical malignant spread. It is necessary to include a margin of normal tissue around the tumor to allow for uncertainties in daily set-up and internal tumor motion. These uncertainties can be caused by internal movement (for example, respiration and bladder filling) and movement of external skin marks relative to the tumor position.

To spare normal tissues (such as skin or organs which radiation must pass through in order to treat the tumor), shaped radiation beams are aimed from several angles of exposure to intersect at the tumor, providing a much larger absorbed dose there than in the surrounding, healthy tissue.

Brachytherapy, in which a radiation source is placed inside or next to the area requiring treatment, is another form of radiation therapy that minimizes exposure to healthy tissue during procedures to treat cancers of the breast, prostate and other organs.

Mechanism of action

Radiation therapy works by damaging the DNA of cancerous cells. This DNA damage is caused by one of two types of energy, photon or charged particle. This damage is either direct or indirect ionizing the atoms which make up the DNA chain. Indirect ionization happens as a result of the ionization of water, forming free radicals, notably hydroxyl radicals, which then damage the DNA. In the older, most common form of radiation therapy, Intensity-modulated radiotherapy (IMRT) (photons), most of the radiation effect is through free radicals. Because cells have mechanisms for repairing single-strand DNA damage, double-stranded DNA breaks prove to be the most significant technique to cause cell death. Cancer cells generally are undifferentiated and stem cell-like, they reproduce more, and have a diminished ability to repair sub-lethal damage compared to most healthy differentiated cells. This single-strand DNA damage is then passed on through cell division, accumulating damage to the cancer cell's DNA, causing them to die or reproduce more slowly.

One of the major limitations of photon radiotherapy is that the cells of solid tumors become deficient in oxygen. Solid tumors can outgrow their blood supply, causing a low-oxygen state known as hypoxia. Oxygen is a potent radiosensitizer, increasing the effectiveness of a given dose of radiation by forming DNA-damaging free radicals. Tumor cells in a hypoxic environment may be as much as 2 to 3 times more resistant to radiation damage than those in a normal oxygen environment. Much research has been

devoted to overcoming hypoxia including the use of high pressure oxygen tanks, blood substitutes that carry increased oxygen, hypoxic cell radiosensitizer drug's such as misonidazole and metronidazole, and hypoxic cytotoxins (tissue poisons), such as tirapazamine.

Direct damage to cancer cell DNA occurs through high-LET (linear energy transfer) charged particles such as proton, boron, carbon or neon ions which have an antitumor effect which is independent of tumor oxygen supply because these particles act mostly via direct energy transfer usually causing double-stranded DNA breaks. Due to their relatively large mass, protons and other charged particles have little lateral side scatter in the tissue; the beam does not broaden much, stays focused on the tumor shape and delivers small dose side-effects to surrounding tissue. They also more precisely target the tumor using the Bragg peak effect. The cyclotron's, dielectric wall accelerator (DWA), or Still River Systems's super conducting high field magnet (two new compact protron replacements) provide the energy source for charged particle therapy. These particles can be charged to different amounts to provide the desired tissue penetration. This procedure avoids healthy tissue because it releases its energy at the last few milimeters calibrated to be at the target tumor and stops. Because IMRT has little mass it cannot be controlled to as fine a degree as charged particles and is still damaging healthy cells when it exits the body. This is critically important in almost all cases where the close proximity of other organs makes any stray ionization very damaging example: (head and neck cancers). This damage causes secondary inducted cancers . This x-ray exposure is especially bad for children, due to their growing bodies. They have a 30% chance of a second malignancy after 5 years post initial RT.

Dose

The amount of radiation used in photon radiation therapy is measured in gray (Gy), and varies depending on the type and stage of cancer being treated. For curative cases, the typical dose for a solid epithelial tumor ranges from 60 to 80 Gy, while lymphomas are treated with 20 to 40 Gy.

Preventative (adjuvant) doses are typically around 45 - 60 Gy in 1.8 - 2 Gy fractions (for Breast, Head, and Neck cancers.) Many other factors are considered by radiation oncologists when selecting a dose, including whether the patient is receiving chemotherapy, patient comorbidities, whether radiation therapy is being administered before or after surgery, and the degree of success of surgery.

Delivery parameters of a prescribed dose are determined during treatment planning (part of dosimetry). Treatment planning is generally performed on dedicated computers using specialized treatment planning software. Depending on the radiation delivery method, several angles or sources may be used to sum to the total necessary dose. The planner will try to design a plan that delivers a uniform prescription dose to the tumor and minimizes dose to surrounding healthy tissues.

Fractionation

The total dose is fractionated (spread out over time) for several important reasons. Fractionation allows normal cells time to recover, while tumor cells are generally less efficient in repair between fractions. Fractionation also allows tumor cells that were in a relatively radio-resistant phase of the cell cycle during one treatment to cycle into a sensitive phase of the cycle before the next fraction is given. Similarly, tumor cells that were chronically or acutely hypoxic (and therefore more radioresistant) may reoxygenate between fractions, improving the tumor cell kill. Fractionation regimes are individualised between different radiotherapy centres and even between individual doctors. In North America, Australia, and Europe, the typical fractionation schedule for adults is 1.8 to 2 Gy per day, five days a week. In some cancer types, prolongation of the fraction schedule over too long can allow for the tumor to begin repopulating, and for these tumor types, including head-and-neck and cervical squamous cell cancers, radiation treatment is preferably completed within a certain amount of time. For children, a typical fraction size may be 1.5 to 1.8 Gy per day, as smaller fraction sizes are associated with reduced incidence and severity of late-onset side effects in normal tissues.

In some cases, two fractions per day are used near the end of a course of treatment. This schedule, known as a concomitant boost regimen or hyperfractionation, is used on tumors that regenerate more quickly when they are smaller. In particular, tumors in the head-and-neck demonstrate this behavior.

One of the best-known alternative fractionation schedules is Continuous Hyperfractionated Accelerated Radiotherapy (CHART). CHART, used to treat lung cancer, consists of three smaller fractions per day. Although reasonably successful, CHART can be a strain on radiation therapy departments.

Another increasingly well-known alternative fractionation schedule, used to treat breast cancer, is called Accelerated Partial Breast Irradiation (APBI). APBI can be performed with either brachytherapy or with external beam radiation. APBI normally involves two high-dose fractions per day for five days, compared to whole breast irradiation, in which a single, smaller fraction is given five times a week over a six-to-seven-week period.

Implants can be fractionated over minutes or hours, or they can be permanent seeds which slowly deliver radiation until they become inactive.

Effect on different types of cancer

Different cancers respond differently to radiation therapy.

The response of a cancer to radiation is described by its radiosensitivity. Highly radiosensitive cancer cells are rapidly killed by modest doses of radiation. These include leukemias, most lymphomas and germ cell tumors. The majority of epithelial cancers are only moderately radiosensitive, and require a significantly higher dose of radiation (60-70Gy) to achieve a radical cure. Some types of cancer are notably radioresistant, that is,

much higher doses are required to produce a radical cure than may be safe in clinical practice. Renal cell cancer and melanoma are generally considered to be radioresistant.

It is important to distinguish the radiosensitivity of a particular tumor, which to some extent is a laboratory measure, from the radiation "curability" of a cancer in actual clinical practice. For example, leukemias are not generally curable with radiotherapy, because they are disseminated through the body. Lymphoma may be radically curable if it is localised to one area of the body. Similarly, many of the common, moderately radioresponsive tumors are routinely treated with curative doses of radiotherapy if they are at an early stage. For example: non-melanoma skin cancer, head and neck cancer, breast cancer, non-small cell lung cancer, cervical cancer, anal cancer, prostate cancer. Metastatic cancers are generally incurable with radiotherapy because it is not possible to treat the whole body.

Before treatment, a CT scan is often performed to identify the tumor and surrounding normal structures. The patient is then sent for a simulation so that molds can be created to be used during treatment. The patient receives small skin marks to guide the placement of treatment fields.

The response of a tumor to radiotherapy is also related to its size. For complex reasons, very large tumors respond less well to radiation than smaller tumors or microscopic disease. Various strategies are used to overcome this effect. The most common technique is surgical resection prior to radiotherapy. This is most commonly seen in the treatment of breast cancer with wide local excision or mastectomy followed by adjuvant radiotherapy such as brachytherapy. Another method is to shrink the tumor with neoadjuvant chemotherapy prior to radical radiotherapy. A third technique is to enhance the radiosensitivity of the cancer by giving certain drugs during a course of radiotherapy. Examples of radiosensiting drugs include: Cisplatin, Nimorazole, and Cetuximab.

History of radiation therapy

Radiation therapy has been in use as a cancer treatment for more than 100 years, with its earliest roots traced from the discovery of x-rays in 1895 by Wilhelm Röntgen.

The field of radiation therapy began to grow in the early 1900s largely due to the groundbreaking work of Nobel Prize-winning scientist Marie Curie, who discovered the radioactive elements polonium and radium. This began a new era in medical treatment and research. Radium was used in various forms until the mid-1900s when cobalt and caesium units came into use. Medical linear accelerators have been used too as sources of radiation since the late 1940s.

With Godfrey Hounsfield’s invention of computed tomography (CT) in 1971, three-dimensional planning became a possibility and created a shift from 2-D to 3-D radiation delivery; CT-based planning allows physicians to more accurately determine the dose distribution using axial tomographic images of the patient's anatomy. Orthovoltage and

cobalt units have largely been replaced by megavoltage linear accelerators , useful for their penetrating energies and lack of physical radiation source.

The advent of new imaging technologies, including magnetic resonance imaging (MRI) in the 1970s and positron emission tomography (PET) in the 1980s, has moved radiation therapy from 3-D conformal to intensity-modulated radiation therapy (IMRT) and image-guided radiation therapy (IGRT) Tomotherapy. These advances allowed radiation oncologists to better see and target tumors, which have resulted in better treatment outcomes, more organ preservation and fewer side effects.

Types of radiation therapy

Historically, the three main divisions of radiotherapy are external beam radiotherapy (EBRT or XRT) or teletherapy, brachytherapy or sealed source radiotherapy, and systemic radioisotope therapy or unsealed source radiotherapy. The differences relate to the position of the radiation source; external is outside the body, brachytherapy uses sealed radioactive sources placed precisely in the area under treatment, and systemic radioisotopes are given by infusion or oral ingestion. Brachytherapy can use temporary or permanent placement of radioactive sources. The temporary sources are usually placed by a technique called afterloading. In afterloading a hollow tube or applicator is placed surgically in the organ to be treated, and the sources are loaded into the applicator after the applicator is implanted. This minimizes radiation exposure to health care personnel. Particle therapy is a special case of external beam radiotherapy where the particles are protons or heavier ions. Intraoperative radiotherapy or IORT is a special type of radiotherapy that is delivered immediately after surgical removal of the cancer. This method has been employed in breast cancer (TARGeted Introperative radioTherapy or TARGIT), brain tumors and rectal cancers.

External beam radiotherapy

The following three sections refer to treatment using x-rays.

Conventional external beam radiotherapy

Conventional external beam radiotherapy (2DXRT) is delivered via two-dimensional beams using linear accelerator machines. 2DXRT mainly consists of a single beam of radiation delivered to the patient from several directions: often front or back, and both sides. *Conventional* refers to the way the treatment is *planned* or *simulated* on a specially calibrated diagnostic x-ray machine known as a simulator because it recreates the linear accelerator actions (or sometimes by eye), and to the usually well-established arrangements of the radiation beams to achieve a desired *plan*. The aim of simulation is to accurately target or localize the volume which is to be treated. This technique is well established and is generally quick and reliable. The worry is that some high-dose treatments may be limited by the radiation toxicity capacity of healthy tissues which lay close to the target tumor volume. An example of this problem is seen in radiation of the prostate gland, where the sensitivity of the adjacent rectum limited the dose which could

be safely prescribed using 2DXRT planning to such an extent that tumor control may not be easily achievable. Prior to the invention of the CT, physicians and physicists had limited knowledge about the true radiation dosage delivered to both cancerous and healthy tissue. For this reason, 3-dimensional conformal radiotherapy is becoming the standard treatment for a number of tumor sites.

Stereotactic Radiation

Stereotactic radiation is a specialized type of external beam radiation therapy. It uses focused radiation beams targeting a well-defined tumor using extremely detailed imaging scans. Radiation oncologists perform stereotactic treatments, often with the help of a neurosurgeon for tumors in the brain or spine.

There are two types of stereotactic radiation. **Stereotactic radiosurgery** (SRS) is when doctors use a single or several stereotactic radiation treatments of the brain or spine. **Stereotactic body radiation therapy** (SBRT) refers to one or several stereotactic radiation treatments with the body, such as the lungs.

Some doctors say an advantage to stereotactic treatments are they deliver the right amount of radiation to the cancer in a shorter amount of time than traditional treatments, which can often take six to 11 weeks. Plus treatments are given with extreme accuracy, which should limit the effect of the radiation on healthy tissues. One problem with stereotactic treatments is that they are only suitable for certain small tumors.

Stereotactic treatments can be confusing because many hospitals call the treatments by the name of the manufacturer rather than calling it SRS or SBRT. Brand names for these treatments include Axesse, Cyberknife, Gamma Knife, Novalis, Primatom, Synergy, X-Knife, TomoTherapy and Trilogy. This list changes as equipment manufacturers continue to develop new, specialized technologies to treat cancers.

Virtual simulation, 3-dimensional conformal radiotherapy, and intensity-modulated radiotherapy

The planning of radiotherapy treatment has been revolutionized by the ability to delineate tumors and adjacent normal structures in three dimensions using specialized CT and/or MRI scanners and planning software.

Virtual simulation, the most basic form of planning, allows more accurate placement of radiation beams than is possible using conventional X-rays, where soft-tissue structures are often difficult to assess and normal tissues difficult to protect.

An enhancement of virtual simulation is **3-Dimensional Conformal Radiotherapy (3DCRT)**, in which the profile of each radiation beam is shaped to fit the profile of the target from a beam's eye view (BEV) using a multileaf collimator (MLC) and a variable number of beams. When the treatment volume conforms to the shape of the tumor, the relative toxicity of radiation to the surrounding normal tissues is reduced, allowing a

higher dose of radiation to be delivered to the tumor than conventional techniques would allow.

Intensity-Modulated Radiation Therapy (IMRT) is an advanced type of high-precision radiation that is the next generation of 3DCRT. IMRT also improves the ability to conform the treatment volume to concave tumor shapes, for example when the tumor is wrapped around a vulnerable structure such as the spinal cord or a major organ or blood vessel. Computer-controlled x-ray accelerators distribute precise radiation doses to malignant tumors or specific areas within the tumor. The pattern of radiation delivery is determined using highly tailored computing applications to perform optimization and treatment simulation (Treatment Planning). The radiation dose is consistent with the 3-D shape of the tumor by controlling, or modulating, the radiation beam's intensity. The radiation dose intensity is elevated near the gross tumor volume while radiation among the neighboring normal tissue is decreased or avoided completely. The customized radiation dose is intended to maximize tumor dose while simultaneously protecting the surrounding normal tissue. This may result in better tumor targeting, lessened side effects, and improved treatment outcomes than even 3DCRT.

3DCRT is still used extensively for many body sites but the use of IMRT is growing in more complicated body sites such as CNS, head and neck, prostate, breast and lung. Unfortunately, IMRT is limited by its need for additional time from experienced medical personnel. This is because physicians must manually delineate the tumors one CT image at a time through the entire disease site which can take much longer than 3DCRT preparation. Then, medical physicists and dosimetrists must be engaged to create a viable treatment plan. Also, the IMRT technology has only been used commercially since the late 1990s even at the most advanced cancer centers, so radiation oncologists who did not learn it as part of their residency program must find additional sources of education before implementing IMRT.

Proof of improved survival benefit from either of these two techniques over conventional radiotherapy (2DXRT) is growing for many tumor sites, but the ability to reduce toxicity is generally accepted. Both techniques enable dose escalation, potentially increasing usefulness. There has been some concern, particularly with 3DCRT, about increased exposure of normal tissue to radiation and the consequent potential for secondary malignancy. Overconfidence in the accuracy of imaging may increase the chance of missing lesions that are invisible on the planning scans (and therefore not included in the treatment plan) or that move between or during a treatment (for example, due to respiration or inadequate patient immobilization). New techniques are being developed to better control this uncertainty—for example, real-time imaging combined with real-time adjustment of the therapeutic beams. This new technology is called image-guided radiation therapy (IGRT) or four-dimensional radiotherapy.

Particle Therapy

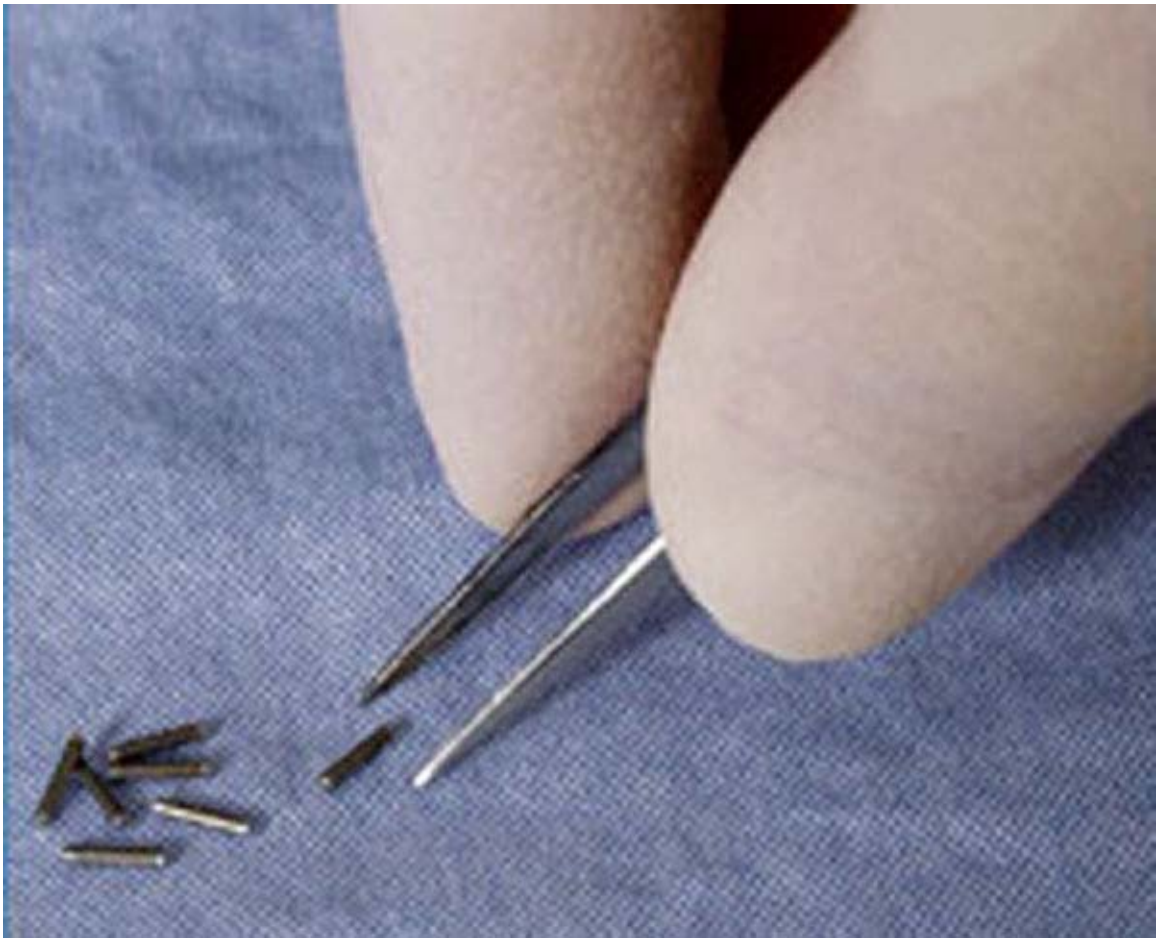
In particle therapy (Proton therapy), energetic ionizing particles (protons or carbon ions) are directed at the target tumor. The dose increases while the particle penetrates the

tissue, up to a maximum (the Bragg peak) that occurs near the end of the particle's range, and it then drops to (almost) zero. The advantage of this energy deposition profile is that less energy is deposited into the healthy tissue surrounding the target tissue.

Brachytherapy

Researcher Cate Yashar, M.D. With SAVI brachytherapy device.

Brachytherapy (internal radiotherapy) is delivered by placing radiation source(s) inside or next to the area requiring treatment. Brachytherapy is commonly used as an effective treatment for cervical, prostate, breast, and skin cancer and can also be used to treat tumours in many other body sites. As with stereotactic radiation, brachytherapy treatments are often known by their brand names. For example, brand names for breast cancer brachytherapy treatments include SAVI, MammoSite, and Contura. Brand names for prostate cancer include Proxcelan, TheraSeed, and I-Seed.



Brachytherapy seeds used to treat prostate cancer

In brachytherapy, radiation sources are precisely placed directly at the site of the cancerous tumour. This means that the irradiation only affects a very localized area – exposure to radiation of healthy tissues further away from the sources is reduced. These characteristics of brachytherapy provide advantages over external beam radiotherapy - the tumour can be treated with very high doses of localized radiation, whilst reducing the probability of unnecessary damage to surrounding healthy tissues. A course of brachytherapy can often be completed in less time than other radiotherapy techniques. This can help reduce the chance of surviving cancer cells dividing and growing in the intervals between each radiotherapy dose.

As one example of the localized nature of breast brachytherapy, the SAVI device delivers the radiation dose through multiple catheters, each of which can be individually controlled. This approach decreases the exposure of healthy tissue and resulting side effects, compared both to external beam radiotherapy and older methods of breast brachytherapy.

Radioisotope Therapy (RIT)

Systemic radioisotope therapy is a form of targeted therapy. Targeting can be due to the chemical properties of the isotope such as radioiodine which is specifically absorbed by the thyroid gland a thousandfold better than other bodily organs. Targeting can also be achieved by attaching the radioisotope to another molecule or antibody to guide it to the target tissue. The radioisotopes are delivered through infusion (into the bloodstream) or ingestion. Examples are the infusion of metaiodobenzylguanidine (MIBG) to treat neuroblastoma, of oral iodine-131 to treat thyroid cancer or thyrotoxicosis, and of hormone-bound lutetium-177 and yttrium-90 to treat neuroendocrine tumors (peptide receptor radionuclide therapy). Another example is the injection of radioactive glass or resin microspheres into the hepatic artery to radioembolize liver tumors or liver metastases.

A major use of systemic radioisotope therapy is in the treatment of bone metastasis from cancer. The radioisotopes travel selectively to areas of damaged bone, and spare normal undamaged bone. Isotopes commonly used in the treatment of bone metastasis are strontium-89 and samarium (^{153}Sm) lexidronam.

In 2002, the United States Food and Drug Administration (FDA) approved ibritumomab tiuxetan (Zevalin), which is an anti-CD20 monoclonal antibody conjugated to yttrium-90. In 2003, the FDA approved the tositumomab/iodine (^{131}I) tositumomab regimen (Bexxar), which is a combination of an iodine-131 labelled and an unlabelled anti-CD20 monoclonal antibody. These medications were the first agents of what is known as radioimmunotherapy, and they were approved for the treatment of refractory non-Hodgkins lymphoma.

Side effects

Radiation therapy is in itself painless. Many low-dose palliative treatments (for example, radiotherapy to bony metastases) cause minimal or no side effects, although short-term pain flare up can be experienced in the days following treatment due to oedema compressing nerves in the treated area. Treatment to higher doses causes varying side effects during treatment (acute side effects), in the months or years following treatment (long-term side effects), or after re-treatment (cumulative side effects). The nature, severity, and longevity of side effects depends on the organs that receive the radiation, the treatment itself (type of radiation, dose, fractionation, concurrent chemotherapy), and the patient.

Most side effects are predictable and expected. Side effects from radiation are usually limited to the area of the patient's body that is under treatment. One of the aims of modern radiotherapy is to reduce side effects to a minimum, and to help the patient to understand and to deal with those side effects which are unavoidable.

The main side effects reported are fatigue and skin irritation, like a mild to moderate sun burn. The fatigue often sets in during the middle of a course of treatment and can last for

weeks after treatment ends. The skin irritation will also go away, but it may not be as elastic as it was before.

Acute side effects

Damage to the epithelial surfaces. Epithelial surfaces may sustain damage from radiation therapy. Depending on the area being treated, this may include the skin, oral mucosa, pharyngeal, bowel mucosa and ureter. The rates of onset of damage and recovery from it depend upon the turnover rate of epithelial cells. Typically the skin starts to become pink and sore several weeks into treatment. The reaction may become more severe during the treatment and for up to about one week following the end of radiotherapy, and the skin may break down. Although this moist desquamation is uncomfortable, recovery is usually quick. Skin reactions tend to be worse in areas where there are natural folds in the skin, such as underneath the female breast, behind the ear, and in the groin.

If the head and neck area is treated, temporary soreness and ulceration commonly occur in the mouth and throat. If severe, this can affect swallowing, and the patient may need painkillers and nutritional support/food supplements. The esophagus can also become sore if it is treated directly, or if, as commonly occurs, it receives a dose of collateral radiation during treatment of lung cancer.

The lower bowel may be treated directly with radiation (treatment of rectal or anal cancer) or be exposed by radiotherapy to other pelvic structures (prostate, bladder, female genital tract). Typical symptoms are soreness, diarrhoea, and nausea.

Swelling (edema or oedema). As part of the general inflammation that occurs, swelling of soft tissues may cause problems during radiotherapy. This is a concern during treatment of brain tumors and brain metastases, especially where there is pre-existing raised intracranial pressure or where the tumor is causing near-total obstruction of a lumen (e.g., trachea or main bronchus). Surgical intervention may be considered prior to treatment with radiation. If surgery is deemed unnecessary or inappropriate, the patient may receive steroids during radiotherapy to reduce swelling.

Infertility. The gonads (ovaries and testicles) are very sensitive to radiation. They may be unable to produce gametes following **direct** exposure to most normal treatment doses of radiation. Treatment planning for all body sites is designed to minimize, if not completely exclude dose to the gonads if they are not the primary area of treatment.

Late side effects

Late side effects occur months to years after treatment and are generally limited to the area that has been treated. They are often due to damage of blood vessels and connective tissue cells. Many late effects are reduced by fractionating treatment into smaller parts.

Fibrosis
: Tissues which have been irradiated tend to become less elastic over time due to a diffuse scarring process.

Epilation (Hair Loss)
: Epilation may occur on any hair bearing skin with doses above 1 Gy. It only occurs within the radiation field/s. Hair loss may be permanent with a single dose of 10 Gy, but if the dose is fractionated permanent hair loss may not occur until dose exceeds 45 Gy.

Dryness
: The salivary glands and tear glands have a radiation tolerance of about 30 Gy in 2 Gy fractions, a dose which is exceeded by most radical head and neck cancer treatments. Dry mouth (xerostomia) and dry eyes (xerophthalmia) can become irritating long-term problems and severely reduce the patient's quality of life. Similarly, sweat glands in treated skin (such as the armpit) tend to stop working, and the naturally moist vaginal mucosa is often dry following pelvic irradiation.

Lymphedema
: Lymphedema, a condition of localized fluid retention and tissue swelling, can result from damage to the lymphatic system sustained during radiotherapy. It is the most commonly reported complication in breast radiotherapy patients.

Cancer
: Radiation is a potential cause of cancer, and secondary malignancies are seen in a very small minority of patients - usually less than 1/1000. It usually occurs 20 - 30 years following treatment, although some haematological malignancies may develop within 5 - 10 years. In the vast majority of cases, this risk is greatly outweighed by the reduction in risk conferred by treating the primary cancer. The cancer occurs within the treated area of the patient.

Heart disease
: Radiation has potentially excess risk of death from heart disease seen after some past breast cancer RT regimens.

Cognitive decline
: In cases of radiation applied to the head radiation therapy may cause cognitive decline.

Radiation Proctitis
: This can involve long-term effects on the rectum including bleeding, diarrhoea and urgency and is associated with radiotherapy to pelvic organs. Pelvic radiotherapy can also cause radiation cystitis when the bladder is affected

Cumulative side effects

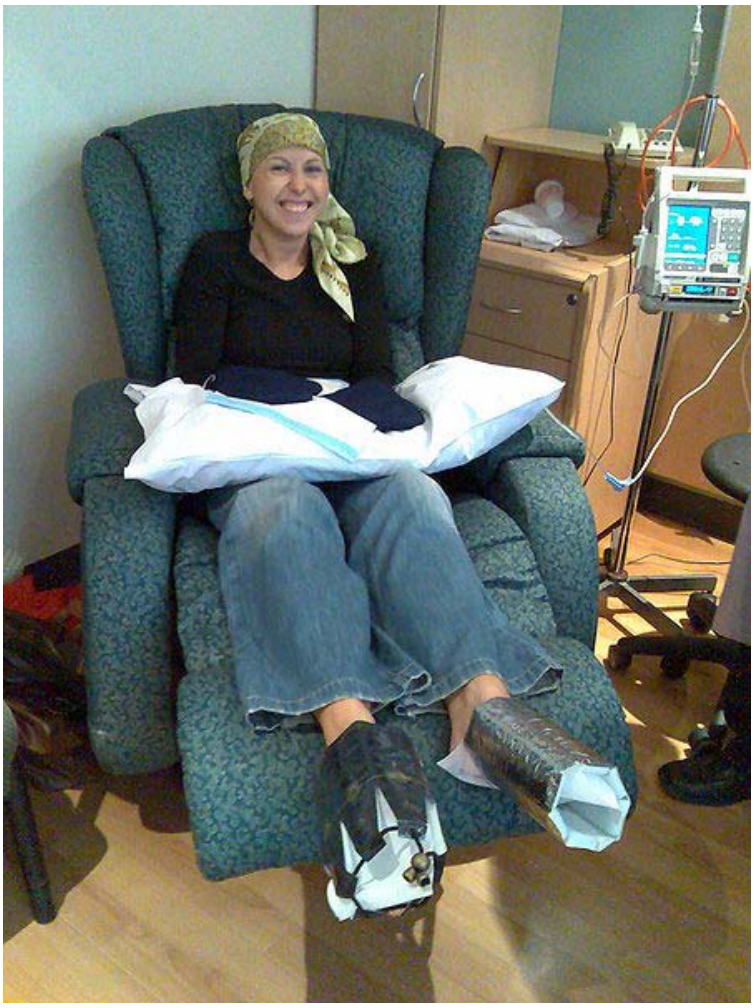
Cumulative effects from this process should not be confused with long-term effects—when short-term effects have disappeared and long-term effects are subclinical, reirradiation can still be problematic.

Radiation therapy accidents

There are rigorous procedures in place to minimise the risk of accidental overexposure of radiotherapy to patients. However, mistakes do occasionally occur; for example, the radiation therapy machine Therac-25 was responsible for at least six accidents between 1985 and 1987, where patients were given up to one hundred times the intended dose; two people were killed directly by the radiation overdoses. From 2005 to 2010, a hospital in Missouri overexposed 76 patients (most with brain cancer) during a five-year period because new radiation equipment had been set up incorrectly. Although medical errors are exceptionally rare, radiation oncologists, medical physicists and other members of the radiation therapy treatment team are working diligently to eliminate them. ASTRO has launched a safety initiative called Target Safely that, among other things, aims to record errors nation wide so that doctors can learn from each and every mistake and prevent them from happening. ASTRO also publishes a list of questions for patients to ask their doctors about radiation safety to ensure every treatment is as safe as possible.

Chapter 8

Chemotherapy



A woman being treated with docetaxel chemotherapy for breast cancer. Cold mittens and wine coolers are placed on her hands and feet to reduce harm to her nails.

Chemotherapy, in the most simple sense, is the treatment of an ailment by chemicals especially by killing micro-organisms or cancerous cells. In popular usage, it refers to antineoplastic drugs used to treat cancer or the combination of these drugs into a cytotoxic standardized treatment regimen. In its non-oncological use, the term may also

refer to antibiotics (*antibacterial chemotherapy*). In that sense, the first modern chemotherapeutic agent was arsphenamine, an arsenic compound discovered in 1909 and used to treat syphilis. This was later followed by sulfonamides (sulfa drugs) and penicillin.

Most commonly, chemotherapy acts by killing cells that divide rapidly, one of the main properties of most cancer cells. This means that it also harms cells that divide rapidly under normal circumstances: 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, hence also immunosuppression), mucositis (inflammation of the lining of the digestive tract), and alopecia (hair loss).

Other uses of cytostatic chemotherapy agents (including the ones mentioned below) are the treatment of autoimmune diseases such as multiple sclerosis, dermatomyositis, polymyositis, lupus, rheumatoid arthritis and the suppression of transplant rejections.

Newer anticancer drugs act directly against abnormal proteins in cancer cells; this is termed targeted therapy.

History

The use of minerals and plant-based medicines are believed to date back to prehistoric medicine.

The first use of drugs to treat cancer, however, was in the early 20th century, although it was not originally intended for that purpose. Mustard gas was used as a chemical warfare agent during World War I and was studied further during World War II. During a military operation in World War II, a group of people were accidentally exposed to mustard gas and were later found to have very low white blood cell counts. It was reasoned that an agent that damaged the rapidly growing white blood cells might have a similar effect on cancer. Therefore, in the 1940s, several patients with advanced lymphomas (cancers of certain white blood cells) were given the drug by vein, rather than by breathing the irritating gas. Their improvement, although temporary, was remarkable. That experience led researchers to look for other substances that might have similar effects against cancer. As a result, many other drugs have been developed to treat cancer, and drug development since then has exploded into a multibillion-dollar industry, although the principles and limitations of chemotherapy discovered by the early researchers still apply.

Principles

Cancer is the uncontrolled growth of cells coupled with malignant behavior: invasion and metastasis. Cancer is thought to be caused by the interaction between genetic susceptibility and environmental toxins.

In the broad sense, most *chemotherapeutic* drugs work by impairing mitosis (cell division), effectively targeting fast-dividing cells. As these drugs cause damage to cells

they are termed *cytotoxic*. Some drugs cause cells to undergo apoptosis (so-called "self programmed cell death").

Scientists have yet to identify specific features of malignant and immune cells that would make them uniquely targetable (barring some recent examples, such as the Philadelphia chromosome as targeted by imatinib). This means that other fast-dividing cells, such as those responsible for hair growth and for replacement of the intestinal epithelium (lining), are also often affected. However, some drugs have a better side effect profile than others, enabling doctors to adjust treatment regimens to the advantage of patients in certain situations.

As chemotherapy affects cell division, tumors with high *growth fractions* (such as acute myelogenous leukemia and the aggressive lymphomas, including Hodgkin's disease) are more sensitive to chemotherapy, as a larger proportion of the targeted cells are undergoing cell division at any time. Malignancies with slower growth rates, such as indolent lymphomas, tend to respond to chemotherapy much more modestly.

Drugs affect "younger" tumors (i.e., more differentiated) more effectively, because mechanisms regulating cell growth are usually still preserved. With succeeding generations of tumor cells, differentiation is typically lost, growth becomes less regulated, and tumors become less responsive to most chemotherapeutic agents. Near the center of some solid tumors, cell division has effectively ceased, making them insensitive to chemotherapy. Another problem with solid tumors is the fact that the chemotherapeutic agent often does not reach the core of the tumor. Solutions to this problem include radiation therapy (both brachytherapy and teletherapy) and surgery.

Over time, cancer cells become more resistant to chemotherapy treatments. Recently, scientists have identified small pumps on the surface of cancer cells that actively move chemotherapy from inside the cell to the outside. Research on p-glycoprotein and other such chemotherapy efflux pumps, is currently ongoing. Medications to inhibit the function of p-glycoprotein are undergoing testing as of June, 2007 to enhance the efficacy of chemotherapy.

Treatment schemes

There are a number of strategies in the administration of chemotherapeutic drugs used today. Chemotherapy may be given with a curative intent or it may aim to prolong life or to palliate symptoms.

Combined modality chemotherapy is the use of drugs with other cancer treatments, such as radiation therapy or surgery. Most cancers are now treated in this way. *Combination chemotherapy* is a similar practice that involves treating a patient with a number of different drugs simultaneously. The drugs differ in their mechanism and side effects. The biggest advantage is minimising the chances of resistance developing to any one agent.

In *neoadjuvant chemotherapy* (*preoperative* treatment) initial chemotherapy is designed to shrink the primary tumour, thereby rendering local therapy (surgery or radiotherapy) less destructive or more effective.

Adjuvant chemotherapy (*post*operative treatment) can be used when there is little evidence of cancer present, but there is risk of recurrence. This can help reduce chances of developing resistance if the tumour does develop. It is also useful in killing any cancerous cells which have spread to other parts of the body. This is often effective as the newly growing tumours are fast-dividing, and therefore very susceptible.

Palliative chemotherapy is given without curative intent, but simply to decrease tumor load and increase life expectancy. For these regimens, a better toxicity profile is generally expected.

All chemotherapy regimens require that the patient be capable of undergoing the treatment. Performance status is often used as a measure to determine whether a patient can receive chemotherapy, or whether dose reduction is required. Because only a fraction of the cells in a tumor die with each treatment (fractional kill), repeated doses must be administered to continue to reduce the size of the tumor. Current chemotherapy regimens apply drug treatment in cycles, with the frequency and duration of treatments limited by toxicity to the patient.

Types

The majority of chemotherapeutic drugs can be divided in to alkylating agents, antimetabolites, anthracyclines, plant alkaloids, topoisomerase inhibitors, and other antitumour agents. All of these drugs affect cell division or DNA synthesis and function in some way.

Some newer agents do not directly interfere with DNA. These include monoclonal antibodies and the new tyrosine kinase inhibitors e.g. *imatinib mesylate* (*Gleevec* or *Glivec*), which directly targets a molecular abnormality in certain types of cancer (chronic myelogenous leukemia, gastrointestinal stromal tumors). These are examples of targeted therapies.

In addition, some drugs that modulate tumor cell behaviour without directly attacking those cells may be used. Hormone treatments fall into this category.

Where available, Anatomical Therapeutic Chemical Classification System codes are provided for the major categories.

Alkylating agents (L01A)

Alkylating agents are so named because of their ability to alkylate many nucleophilic functional groups under conditions present in cells. Cisplatin and carboplatin, as well as oxaliplatin, are alkylating agents. They impair cell function by forming covalent bonds

with the amino, carboxyl, sulfhydryl, and phosphate groups in biologically important molecules.

Other agents are mechlorethamine, cyclophosphamide, chlorambucil, ifosfamide. They work by chemically modifying a cell's DNA.

Anti-metabolites (L01B)

Anti-metabolites masquerade as purines ((azathioprine, mercaptopurine)) or pyrimidines—which become the building blocks of DNA. They prevent these substances from becoming incorporated in to DNA during the "S" phase (of the cell cycle), stopping normal development and division. They also affect RNA synthesis. Due to their efficiency, these drugs are the most widely used cytostatics.

Plant alkaloids and terpenoids (L01C)

These alkaloids are derived from plants and block cell division by preventing microtubule function. Microtubules are vital for cell division, and, without them, cell division cannot occur. The main examples are vinca alkaloids and taxanes.

Vinca alkaloids (L01CA)

Vinca alkaloids bind to specific sites on tubulin, inhibiting the assembly of tubulin into microtubules (M phase of the cell cycle). They are derived from the Madagascar periwinkle, *Catharanthus roseus* (formerly known as *Vinca rosea*). The vinca alkaloids include:

- Vincristine
- Vinblastine
- Vinorelbine
- Vindesine

Podophyllotoxin (L01CB)

Podophyllotoxin is a plant-derived compound which is said to help with digestion as well as used to produce two other cytostatic drugs, etoposide and teniposide. They prevent the cell from entering the G1 phase (the start of DNA replication) and the replication of DNA (the S phase). The exact mechanism of its action is not yet known.

The substance has been primarily obtained from the American Mayapple (*Podophyllum peltatum*). Recently it has been discovered that a rare Himalayan Mayapple (*Podophyllum hexandrum*) contains it in a much greater quantity, but, as the plant is endangered, its supply is limited. Studies have been conducted to isolate the genes involved in the substance's production, so that it could be obtained recombinantly.

Taxanes (L01CD)

The prototype taxane is the natural product paclitaxel, originally known as Taxol and first derived from the bark of the Pacific Yew tree. Docetaxel is a semi-synthetic analogue of paclitaxel. Taxanes enhance stability of microtubules, preventing the separation of chromosomes during anaphase.

Topoisomerase inhibitors (L01CB and L01XX)

Topoisomerases are essential enzymes that maintain the topology of DNA. Inhibition of type I or type II topoisomerases interferes with both transcription and replication of DNA by upsetting proper DNA supercoiling.

- Some type I topoisomerase inhibitors include *camptothecins*: irinotecan and topotecan.

- Examples of type II inhibitors include amsacrine, etoposide, etoposide phosphate, and teniposide. These are semisynthetic derivatives of epipodophyllotoxins, alkaloids naturally occurring in the root of American Mayapple (*Podophyllum peltatum*).

Antineoplastics (L01D)

These include the immunosuppressant dactinomycin (which is used in kidney transplantations), doxorubicin, epirubicin, bleomycin and others.

Newer and experimental approaches

Isolated infusion approaches

Isolated limb perfusion (often used in melanoma), or isolated infusion of chemotherapy into the liver or the lung have been used to treat some tumours. The main purpose of these approaches is to deliver a very high dose of chemotherapy to tumor sites without causing overwhelming *systemic* damage. These approaches can help control solitary or limited metastases, but they are by definition *not* systemic, and, therefore, do not treat distributed metastases or micrometastases.

Targeted delivery mechanisms

Specially targeted delivery vehicles aim to increase effective levels of chemotherapy for tumor cells while reducing effective levels for other cells. This should result in an increased tumor kill and/or reduced toxicity.

Specially targeted delivery vehicles have a differentially higher affinity for tumor cells by interacting with tumor-specific or tumour-associated antigens.

In addition to their targeting component, they also carry a payload - whether this is a traditional chemotherapeutic agent, or a radioisotope or an immune stimulating factor. Specially targeted delivery vehicles vary in their stability, selectivity, and choice of target, but, in essence, they all aim to increase the maximum effective dose that can be delivered to the tumor cells. Reduced systemic toxicity means that they can also be used in sicker patients, and that they can carry new chemotherapeutic agents that would have been far too toxic to deliver via traditional systemic approaches.

Nanoparticles

Nanoparticles have emerged as a useful vehicle for poorly soluble agents such as paclitaxel. Protein-bound paclitaxel (e.g., Abraxane) or nab-paclitaxel was approved by the U.S. Food and Drug Administration (FDA) in January 2005 for the treatment of refractory breast cancer. This formulation of paclitaxel uses human albumin as a vehicle and not the Cremophor vehicle used in Taxol. Nanoparticles made of magnetic material can also be used to concentrate agents at tumour sites using an externally applied magnetic field.

Dosage

Dosage of chemotherapy can be difficult: If the dose is too low, it will be ineffective against the tumor, whereas, at excessive doses, the toxicity (side effects, neutropenia) will be intolerable to the patient. This has led to the formation of detailed "dosing schemes" in most hospitals, which give guidance on the correct dose and adjustment in case of toxicity. In immunotherapy, they are in principle used in smaller dosages than in the treatment of malignant diseases.

In most cases, the dose is adjusted for the patient's body surface area, a measure that correlates with blood volume. The BSA is usually calculated with a mathematical formula or a nomogram, using a patient's weight and height, rather than by direct measurement.

Delivery

Most chemotherapy is delivered intravenously, although a number of agents can be administered orally (e.g., melphalan, busulfan, capecitabine). In some cases, isolated limb perfusion (often used in melanoma), 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 tumour sites without causing overwhelming systemic damage.

Depending on the patient, the cancer, the stage of cancer, the type of chemotherapy, and the 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 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.

Harmful and lethal toxicity from chemotherapy limits the dosage of chemotherapy that can be given. Some tumors can be destroyed by sufficiently high doses of chemotherapeutic agents. However, these high doses cannot be given because they would be fatal to the patient.

Adverse effects

Chemotherapeutic techniques have a range of side effects that depend on the type of medications used. The most common medications mainly affect the fast-dividing cells of the body, such as blood cells and the cells lining the mouth, stomach, and intestines. Common side effects include:

- Depression of the immune system, which can result in potentially fatal infections. Although patients are encouraged to wash their hands, avoid sick people, 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, such as sepsis, or as localized outbreaks, such as shingles. Sometimes, chemotherapy treatments are postponed because the immune system is suppressed to a critically low level.
- Fatigue. The treatment can be physically exhausting for the patient, who might already be very tired from cancer-related fatigue. It may produce mild to severe anemia. Treatments to mitigate anemia include hormones to boost blood production (erythropoietin), iron supplements, and blood transfusions.
- 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.
- 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 doesn't eat or drink enough, 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.
- Hair loss. Some medications that kill rapidly dividing cells cause dramatic hair loss; other medications may cause hair to thin. These are temporary effects: hair usually starts growing back a few weeks after the last treatment, sometimes with a tendency to curl that may be called a "chemo perm".

Damage to specific organs may occur, with resultant symptoms:

- Cardiotoxicity (heart damage)
- Hepatotoxicity (liver damage)
- Nephrotoxicity (kidney damage)
- Ototoxicity (damage to the inner ear), producing vertigo
- Encephalopathy (brain dysfunction)

Immunosuppression and myelosuppression

Virtually all chemotherapeutic regimens can cause depression of the immune system, often by paralysing the bone marrow and leading to a decrease of white blood cells, red blood cells, and platelets. The latter two, when they occur, are improved with blood transfusion. Neutropenia (a decrease of the neutrophil granulocyte count below 0.5 x 10^9/litre) can be improved with synthetic G-CSF (granulocyte-colony stimulating factor, e.g., filgrastim, lenograstim).

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, meaning *allogenic* or *autologous* bone marrow cell transplants are necessary. (In autologous BMTs, cells are removed from the patient before the treatment, multiplied and then re-injected afterwards; in *allogenic* BMTs the source is a donor.) However, some patients still develop diseases because of this interference with bone marrow.

In Japan the government has approved the use of some medicinal mushrooms like *Trametes versicolor*, to counteract depression of the immune system in patients undergoing chemotherapy.

Nausea and vomiting

Chemotherapy-induced nausea and vomiting (CINV) is common with many treatments and some forms of cancer. However, some chemotherapy regimens do not have this side effect, and very effective drugs to stop or noticeably reduce this adverse effect are available.

A class of 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 hours 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 (Zofran). The newest 5-HT_3 inhibitor, palonosetron, also prevents delayed nausea and vomiting, which occurs during the 2–5 days after treatment. Since some patients have trouble swallowing pills, these drugs are often available by injection, as orally disintegrating tablets, or as transdermal patchs.

The substance P inhibitor aprepitant, which became available in 2005, is also effective in controlling the nausea of cancer chemotherapy.

Some studies and patient groups say that the use of cannabinoids derived from marijuana during chemotherapy greatly reduces the associated nausea and vomiting, and enables the patient to eat. Some synthetic derivatives of the active substance in marijuana (Tetrahydrocannabinol or THC) such as Marinol may be practical for this application. Natural marijuana, known as medical cannabis is also used and recommended by some oncologists, though its use is regulated and not legal everywhere.

Secondary neoplasm

Development of secondary neoplasia after successful chemotherapy and/or radiotherapy treatment can occur. The most common secondary neoplasm is secondary acute myeloid leukemia, which develops primarily after treatment with alkylating agents or topoisomerase inhibitors. Survivors of childhood cancer are more than 13 times as likely to get a secondary neoplasm during the 30 years after treatment than the general population. Not all of this increase can be attributed to chemotherapy.

Infertility

Some types of chemotherapy are gonadotoxic and may cause infertility. Chemotherapies with high risk include procarbazine and other alkylating drugs such as cyclophosphamide, ifosfamide, busulfan, melphalan, chlorambucil and chlormethine. Drugs with medium risk include doxorubicin and platinum analogs such as cisplatin and carboplatin. On the other hand, therapies with low risk of gonadotoxicity include plant derivatives such as vincristine and vinblastine, antibiotics such as bleomycinand dactinomycin and antimetabolites such as methotrexate, mercaptopurine and 5-fluoruracil.

Patients may choose between several methods of fertility preservation prior to chemotherapy, including cryopreservation of semen, ovarian tissue, oocytes or embryos. As more than half of cancer patients are elderly, this adverse effect is only relevant for a minority of patients.

Other side effects

In particularly large tumors, such as large lymphomas, some patients develop 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, damaged fingernails, a dry mouth (xerostomia), water retention, and sexual impotence. Some medications can trigger allergic or pseudoallergic reactions.

Some patients report fatigue or non-specific neurocognitive problems, such as an inability to concentrate; this is sometimes called post-chemotherapy cognitive impairment, referred to as "chemo brain" by patients' groups.

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).

Efficacy

Chemotherapy is highly effective in some cancers, useless in others, and unnecessary in still others.

Taking all forms of cancer together, people who receive chemotherapy increase their odds of living five years after diagnosis by about two percentage points (e.g., from about 61% being alive after five years to about 63% of them being alive after five years). However, this overall rate obscures the wide variation. Cytotoxic chemotherapy produces much larger gains for some forms of cancer, including testicular cancer (about 40% of the men who live five years after diagnosis are alive because of chemotherapy), lymphomas (about 13%), and cervical cancer (12%). By contrast, chemotherapy is essentially useless in other cancers, including prostate cancer, melanoma of the skin, multiple myeloma, bladder cancer, kidney cancer, and pancreatic cancer: people who receive chemotherapy for these conditions are just as likely to die within five years as people who do not. Chemotherapy only slightly improves survival for some of the most common forms of cancer, including breast cancers (1.5%) and lung cancers (1.5%).

In other animals

Chemotherapy is used in veterinary medicine similar to in human medicine.

Chapter 9

Targeted Therapy

Targeted therapy is a type of medication that blocks the growth of cancer cells by interfering with specific targeted molecules needed for carcinogenesis and tumor growth, rather than by simply interfering with rapidly dividing cells (e.g. with traditional chemotherapy). Targeted cancer therapies may be more effective than current treatments and less harmful to normal cells.

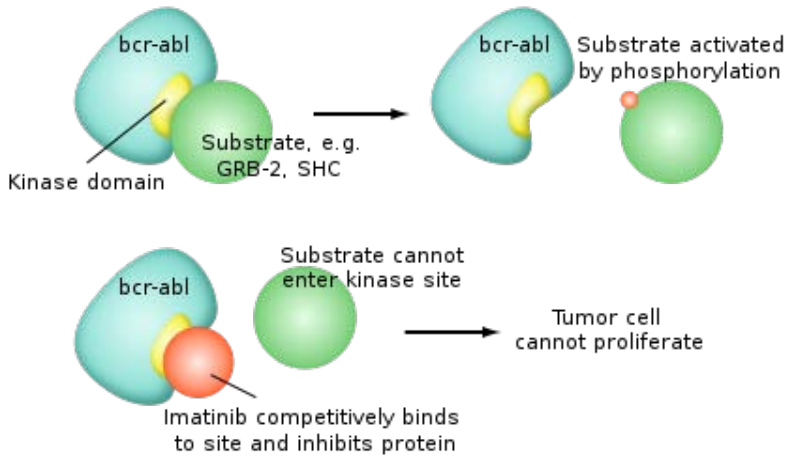
The definitive experiments that showed that targeted therapy would reverse the malignant phenotype of tumor cells involved treating Her2/neu transformed cells with monoclonal antibodies in vitro and in vivo by Mark Greene's laboratory.

Some have challenged use of the term, stating that drugs usually associated with the term are insufficiently selective. The phrase occasionally appears in scare quotes.

Types

The main categories of targeted therapy are *small molecules* and *monoclonal antibodies.*

Small molecules



Mechanism of imatinib

- Imatinib mesylate (Gleevec, also known as STI–571) is approved for chronic myelogenous leukemia, gastrointestinal stromal tumor and some other types of cancer. Early clinical trials indicate that imatinib may be effective in treatment of dermatofibrosarcoma protuberans.
- Gefitinib (Iressa, also known as ZD1839), targets the epidermal growth factor receptor (EGFR) tyrosine kinase and is approved in the U.S. for non small cell lung cancer. EGFR is also overexpressed in the cells of other solid tumors, such as lung and breast cancers. This leads to inappropriate activation of the apoptotic Ras signal transduction cascade, eventually leading to uncontrolled cell proliferation.Gefitinib inhibits EGFR tyrosine kinase by binding to the adenosine triphosphate (ATP)-binding site of the enzyme. Thus the function of the EGFR tyrosine kinase in activating the Ras signal transduction cascade is inhibited; and malignant cells are inhibited.
- Erlotinib (marketed as Tarceva). Erlotinib inhibits epidermal growth factor receptor, and works through a similar mechanism as gefitinib. Erlotinib has been shown to increase survival in metastatic non small cell lung cancer when used as second line therapy. Because of this finding, erlotinib has replaced gefitinib in this setting.
- Bortezomib (Velcade) is an apoptosis-inducing drug that causes cancer cells to undergo cell death by interfering with proteins. It is approved in the U.S. to treat multiple myeloma that has not responded to other treatments.
- The selective estrogen receptor modulator tamoxifen has been described as the foundation of targeted therapy.
- Newer BCL-2 antagonists, such as Obatoclax, ABT-263, and Gossypol.
- PARP inhibitors (e.g. Iniparib, Olaparib in clinical trials)
- Janus kinase inhibitors
- Apatinib is a selective VEGF Receptor 2 inhibitor which has shown encouraging anti-tumor activity in a broad range of malignancies in clinical trials. Apatinib is currently in clinical development for metastatic gastric carcinoma, metastatic breast cancer and advanced hepatocellular carcinoma.
- salinomycin has demonstrated potency in killing cancer stem cells in both laboratory-created and naturally occurring breast tumors in mice.

Monoclonal antibodies

Several are in development and a few have been licenced by the FDA. Examples of licenced monoclonal antibodies include:

- Rituximab (marketed as MabThera or Rituxan) targets CD20 found on B cells. It is used in non Hodgkin lymphoma
- Trastuzumab (Herceptin) targets the Her2/neu (also known as ErbB2) receptor expressed in some types of breast cancer
- Cetuximab (marketed as Erbitux) targets the epidermal growth factor receptor. It is used in the treatment of colon cancer and non-small cell lung cancer.
- Bevacizumab (marketed as Avastin) targets circulating VEGF ligand. It is approved for use in the treatment of colon cancer, breast cancer, non-small cell

lung cancer, and is investigational in the treatment of sarcoma. Its use for the treatment of brain tumors has been recommended.

Progress and future

Many oncologists believe that targeted therapies are the chemotherapy of the future. As solid tumor cancer continues to be viewed as a chronic condition, methods for long-term treatment, with less side-effects, continue to be investigated.

In the U.S., the National Cancer Institute's *Molecular Targets Development Program* (MTDP) to identify and evaluate molecular targets that may be candidates for drug development.

The next stage of targeted therapies will focus on finding which patients will respond to which targeted therapies. This is called the identification of "sub-populations", stratified medicine or even personalized medicine. The route to identify these sub-populations is through biomarkers and surrogate endpoints.

Chapter 10

Cancer Immunotherapy

Cancer immunotherapy is the use of the immune system to reject cancer. The main premise is stimulating the patient's immune system to attack the malignant tumor cells that are responsible for the disease. This can be either through immunization of the patient (eg. by administering a cancer vaccine, such as Dendreon's Provenge), in which case the patient's own immune system is trained to recognize tumor cells as targets to be destroyed, or through the administration of therapeutic antibodies as drugs, in which case the patient's immune system is recruited to destroy tumor cells by the therapeutic antibodies.

Since the immune system responds to the environmental factors it encounters on the basis of discrimination between self and non-self, many kinds of tumor cells that arise as a result of the onset of cancer are more or less tolerated by the patient's own immune system since the tumor cells are essentially the patient's own cells that are growing, dividing and spreading without proper regulatory control.

In spite of this fact, however, many kinds of tumor cells display unusual antigens that are either inappropriate for the cell type and/or its environment, or are only normally present during the organisms' development (e.g. fetal antigens). Examples of such antigens include the glycosphingolipid GD2, a disialoganglioside that is normally only expressed at a significant level on the outer surface membranes of neuronal cells, where its exposure to the immune system is limited by the blood-brain barrier. GD2 is expressed on the surfaces of a wide range of tumor cells including neuroblastoma, medulloblastomas, astrocytomas, melanomas, small-cell lung cancer, osteosarcomas and other soft tissue sarcomas. GD2 is thus a convenient tumor-specific target for immunotherapies.

Other kinds of tumor cells display cell surface receptors that are rare or absent on the surfaces of healthy cells, and which are responsible for activating cellular signal transduction pathways that cause the unregulated growth and division of the tumor cell. Examples include ErbB2, a constitutively active cell surface receptor that is produced at abnormally high levels on the surface of breast cancer tumor cells.

Monoclonal antibody therapy

Antibodies are a key component of the adaptive immune response, playing a central role in both in the recognition of foreign antigens and the stimulation of an immune response to them. It is not surprising therefore, that many immunotherapeutic approaches involve the use of antibodies. The advent of monoclonal antibody technology has made it possible to raise antibodies against specific antigens such as the unusual antigens that are presented on the surfaces of tumors.

A number of therapeutic monoclonal antibodies have been approved for use in humans; approvals mentioned here are by the U.S. Food and Drug Administration (FDA).

Cancer immunotherapy:Monoclonal antibodies

Antibody	Brand name	Approval date	Type	Target	Approved treatment(s)
Alemtuzumab	Campath	2001	humanized	CD52	Chronic lymphocytic leukemia
Bevacizumab	Avastin	2004	humanized	vascular endothelial growth factor	colorectal cancer
Cetuximab	Erbitux	2004	chimeric	epidermal growth factor receptor	colorectal cancer
Gemtuzumab ozogamicin	Mylotarg	2000	humanized	CD33	acute myelogenous leukemia (with calicheamicin)

Ibritumomab tiuxetan	Zevalin	2002	murine	CD20	non-Hodgkin lymphoma (with yttrium-90 or indium-111)
Panitumumab	Vectibix	2006	human	epidermal growth factor receptor	colorectal cancer
Rituximab	Rituxan, Mabthera	1997	chimeric	CD20	non-Hodgkin lymphoma
Trastuzumab	Herceptin	1998	humanized	ErbB2	breast cancer

Alemtuzumab

Alemtuzumab is an anti-CD52 humanized IgG1 monoclonal antibody indicated for the treatment of Chronic lymphocytic leukemia(CLL), the most frequent form of leukaemia in Western countries. The function of CD52 is unknown, but it is found on >95% of peripheral blood lymphocytes and monocytes. Upon binding to CD52, alemtuzumab initiates its cytotoxic effect by complement fixation and antibody-dependent cell-mediated cytotoxicity mechanisms. Alemtuzumab therapy is also indicated for T-prolymphocytic leukaemia (TPPL), for which no standard treatment exists. This is a highly aggressive tumour, with a median survival of 7.5 months.

Bevacizumab

Bevacizumab is a humanized IgG1 monoclonal antibody which binds to and sterically interferes with the vascular endothelial growth factor-A (VEGF-A), preventing receptor activation. A marked increase in VEGF expression is thought to play a role in tumor angiogenesis. Bevacizumab is indicated for colon cancer; but has been applied to numerous other cancers in small scale studies, especially renal cell carcinoma. Results obtained showed that bevacizumab increased the duration of survival, progression-free survival, the rate of response and the duration of response in a statistically relevant manner.

Cetuximab

Cetuximab is a chimeric IgG1 monoclonal antibody which targets the extracellular domain of the epidermal growth factor receptor (EGFR). It functions by competitively

inhibiting ligand binding, thereby preventing EGFR activation, and is indicated for the treatment of colorectal cancer. Studies have also been carried out on numerous other malignancies, especially non-small cell lung cancer and head and neck cancer. As a single agent, cetuximab showed a response rate of 10.8% in patients with EGFR overexpressed metastatic colon cancer. Other anti-EGFR monoclonal antibodies in development include: ABX-EGF, hR3, and EMD 72000. Although they hold significant promise for the future, as of yet none of the agents are currently beyond phase I clinical trials.

Gemtuzumab ozogamicin

Gemtuzumab ozogamicin is an "immuno-conjugate" of an anti-CD33 antibody chemically linked to calicheamicin, a cytotoxic agent. It is indicated for the treatment of acute myeloid leukaemia (AML). The patient group most likely to benefit from gemtuzumab is young adults, and trials have reported high complete responses (85%), when combined with intensive chemotherapy. There are minimal side-effects associated with Gemtuzumab therapy.

Rituximab

Rituximab is a chimeric monoclonal antibody specific for CD20. CD20 is widely expressed on B-cells. Although the function of CD20 is relatively unknown it has been suggested that CD20 could play a role in calcium influx across plasma membrane, maintaining intracellular calcium concentration and allowing for the activation of B cells. The exact mode of action of rituximab is also unclear, but it has been found to have a general regulatory effect on the cell cycle and on immune-receptor expression. Experiments involving primates showed that treatment with anti-CD20 reduced peripheral B-cells by 98%, and peripheral lymph node and bone marrow B-cells by up to 95%.

Trastuzumab

Trastuzumab is a monoclonal IgG1 humanized antibody specific for the epidermal growth factor receptor 2 protein (HER2). It received FDA-approval in 1998, and is clinically used for the treatment of breast cancer. The use of Trastuzumab is restricted to patients whose tumours over-express HER-2, as assessed by immunohistochemistry (IHC) and either chromogenic or Fluorescent in situ hybridisation (FISH), as well as numerous PCR-based methodologies.

HER-2 is a member of the epidermal growth factor receptor (EGFR) family of transmembrane tyrosine kinases, and is normally involved in regulation of cell proliferation and differentiation. Amplification or overexpression of HER-2 is present in 25-30% of breast carcinomas and has been associated with aggressive tumour phenotype, poor prognosis, non-responsiveness to hormonal therapy and reduced sensitivity to conventional chemotherapeutic agents.

Radioimmunotherapy

Radioimmunotherapy involves the use of radioactively conjugated murine antibodies against cellular antigens. Most research currently involved their application to lymphomas, as these are highly radio-sensitive malignancies. To limit radiation exposure, murine antibodies were especially chosen, as their high immunogenicity promotes rapid clearance from the body.

Ibritumomab tiuxetan

Ibritumomab tiuxetan is a murine antibody chemically linked to a chelating agent which binds yttrium-90. ^{90}Y is a beta radiator, has a half-life of 64 h and a tissue penetration of 1-5 millimetres. Its use has been investigated, primarily in the treatment of follicular lymphoma.

Tositumomab/iodine (^{131}I) tositumomab regimen

Tositumomab is a murine IgG2a anti-CD20 antibody. Iodine (^{131}I) tositumomab is covalently bound to Iodine 131. ^{131}I emits both beta and gamma radiation, and is broken down rapidly in the body. Clinical trials have established the efficacy of a sequential application of tositumomab and iodine (^{131}I) tositumomab in patients with relapsed follicular lymphoma.

Advances in immunotherapy

The development and testing of second generation immunotherapies are already under way. While antibodies targeted to disease-causing antigens can be effective under certain circumstances, in many cases, their efficacy may be limited by other factors. In the case of cancer tumors, the microenvironment is immunosuppressive, allowing even those tumors that present unusual antigens to survive and flourish in spite of the immune response generated by the cancer patient, against his or her own tumor tissue. Certain members of a group of molecules known as cytokines, such as Interleukin-2 also play a key role in modulating the immune response, and have been tried in conjunction with antibodies in order to generate an even more devastating immune response against the tumor. While the therapeutic administration of such cytokines may cause systemic inflammation, resulting in serious side effects and toxicity, a new generation of chimeric molecules consisting of an immune-stimulatory cytokine attached to an antibody that targets the cytokine's activity to a specific environment such as a tumor, are able to generate a very effective yet localized immune response against the tumor tissue, destroying the cancer-causing cells without the unwanted side-effects. A different type of chimeric molecule is an artificial T cell receptor.

The targeted delivery of cytokines by anti-tumor antibodies is one example of using antibodies to delivery payloads rather than simply relying on the antibody to trigger an immune response against the target cell. Another strategy is to deliver a lethal radioactive dose directly to the target cell, which has been utilized in the case of the Zevalin

therapeutic. A third strategy is to deliver a lethal chemical dose to the target, as used in the Mylotarg therapeutic. Engineering the antibody-payload pair in such a way that they separate after entry into a cell by endocytosis can potentially increase the efficacy of the payload. One strategy to accomplish this is the use of a disulfide linkage which could be severed by the reducing conditions in the cellular interior. However, recent evidence suggests that the actual intracellular trafficking of the antibody-payload after endocytosis is such to make this strategy not generally applicable. Other potentially useful linkage types include hydrazone and peptide linkages.

Latest research

In 2001, two U.S. based non-profit organizations, the Cancer Research Institute and the Ludwig Institute for Cancer Research, formed the Cancer Vaccine Collaborative, a unique global network of clinical trial sites with special expertise in immunology, built to centrally design and coordinate early-stage clinical trials to be run in parallel in order to identify more quickly the optimal combination of reagents, or vaccine components, necessary for a successful therapeutic cancer vaccine. The Cancer Vaccine Collaborative has to-date (June 2009) completed or is currently running more than 40 clinical trials of different therapeutic cancer vaccines, including 37 phase I, 6 phase II, and 1 fully-randomized phase II clinical trials, and has published more than 130 scientific papers in peer-reviewed journals. Nearly all of these trials featured vaccines targeting various forms of the cancer-testes antigen, NY-ESO-1, a highly-immunogenic, prototypical protein marker limited in expression to a wide variety of cancer types but not in normal tissue, with the exception of the immune-privileged testes. Vaccines tested in Cancer Vaccine Collaborative trials have induced integrated immune responses composed of target-specific antibodies and CD4+ and CD8+ T lymphocytes, all of which are held to be essential for effective long-term control of cancer. Insights from these trials have generated a strong framework for the selection of components that will likely comprise an ideal therapeutic cancer vaccine, including: multiple cancer-antigens in various forms delivered with potent adjuvants and all administered in a prime-boost setting in conjunction with a modulator of cancer immunosuppression.

In June 2008, it was announced that US doctors from the Clinical Research Division led by Cassian Yee at Fred Hutchinson Cancer Research Center in Seattle have for the first time successfully treated a skin cancer patient by using immune cells cloned from his own immune system which were then re-injected into him. The patient, who was suffering from advanced skin cancer, was free from tumours within eight weeks of being injected with billions of his own immune cells in the first case of its kind. Experts say that this case could be a landmark in the treatment of cancer in general. Larger trials are now under way.

More new research is being conducted by Drs. Richard O'Reilly and Michel Sadelain. Drs. O'Reilly and Sadelain have done extensive research at Memorial Sloan-Kettering Cancer Center hospital and are among leaders of the cancer adoptive Immunotherapy field

Topical immunotherapy

Dermatologists use new creams and injections in the management of benign and malignant skin tumors. Topical immunotherapy utilizes an immune enhancement cream (imiquimod) which is an interferon producer causing the patient's own killer T cells to destroy warts, actinic keratoses, basal cell carcinoma, squamous cell carcinoma, cutaneous T cell lymphoma, and Superficial spreading melanoma. Injection immunotherapy uses mumps, candida or trichophytin antigen injections to treat warts (HPV induced tumors).

Natural products

Some types of natural products have shown promise to stimulate the immune system. Research suggests that mushrooms like Reishi and *Agaricus blazei* may be able to stimulate the immune system. Research has shown that *Agaricus blazei* may be a potent stimulator of natural killer cells. *Agaricus blazei* is rich in proteoglucans and beta-glucans, which are potent stimulators of macrophages.

Research ss the compounds in medicinal mushrooms most responsible for up-regulating the immune system and providing an anti-cancer effect, are a diverse collection of polysaccharide compounds, particularly beta-glucans. Beta-glucans are known as "biological response modifiers", and their ability to activate the immune system is well documented. Specifically, beta-glucans stimulate the innate branch of the immune system. Research has shown beta-glucans have the ability to stimulate macrophage, NK cells, T cells, and immune system cytokines. The mechanisms in which beta-glucans stimulate the immune system is only partially understood. One mechanism in which beta-glucans are able to activate the immune system, is by interacting with the Macrophage-1 antigen (CD18) receptor on immune cells.

Highly purified compounds isolated from medicinal mushrooms such as lentinan (isolated from Shiitake), and Polysaccharide-K, (isolated from *Trametes versicolor*), have become incorporated into the health care system of a few countries, such as Japan. Japan's Ministry of Health, Labour and Welfare approved the use of Polysaccharide-K in the 1980s, to stimulate the immune systems of patients undergoing chemotherapy. In Australia, a pharmaceutical based on a mixture of several mycological extracts including lentinan and Polysaccharide-K is sold commercially as MC-S.

Chapter 11

Discovery and Development of CCR5-Receptor Antagonists

The life cycle of the Human Immunodeficiency Virus (HIV) presents potential targets for drug therapy, one of them being the viral entry pathway. The C-C motif chemokine receptors CCR5 and CXCR4 are the main chemokine receptors involved in the HIV entry process. These receptors belong to the seven transmembrane G-protein-coupled receptor (GPCR) family and are predominantly expressed on human T-cells, dendritic cells and macrophages. They play an important role as co-receptors that HIV type 1 (HIV-1) uses to attach to cells before viral fusion and entry into host cells. HIV isolates can be divided into R5 and X4 strains. R5 strain is when the virus uses the co-receptor CCR5 and X4 strain is when it uses CXCR4. The location of CCR5 receptors at the cell surface, both large and small molecules have the potential to interfere with the CCR5-viral interaction and inhibit viral entry into human cells. The following summarizes the discovery and developing of small molecule antagonists of CCR5 receptor.

History

Since the discovery of HIV in the 1980s, remarkable progress has been made in the development of novel antiviral drugs. The trigger for the discovery of the CCR5 antagonists was the observation that a small percentage of high-risk populations showed either resistance or delayed development of the disease. This population was found to have a mutation (CCR5-Δ32) in the gene that codes for the CCR5 receptor which results in almost complete resistance against HIV-1 infection and scientists then discovered the key role of the cell surface receptors CCR5 and CXCR4 in successful viral fusion and infection. In 1996, it was demonstrated that CCR5 serves as a co-receptor for the most commonly transmitted HIV-1 strains, R5. This type of virus is predominant during the early stages of infection and remains the dominant form in over 50% of late stage HIV-1 infected patients, however R5 strains can eventually evolve into X4 as the disease progresses. This information led to the development of a new class of HIV drugs called CCR5 antagonists.

Mechanism of action

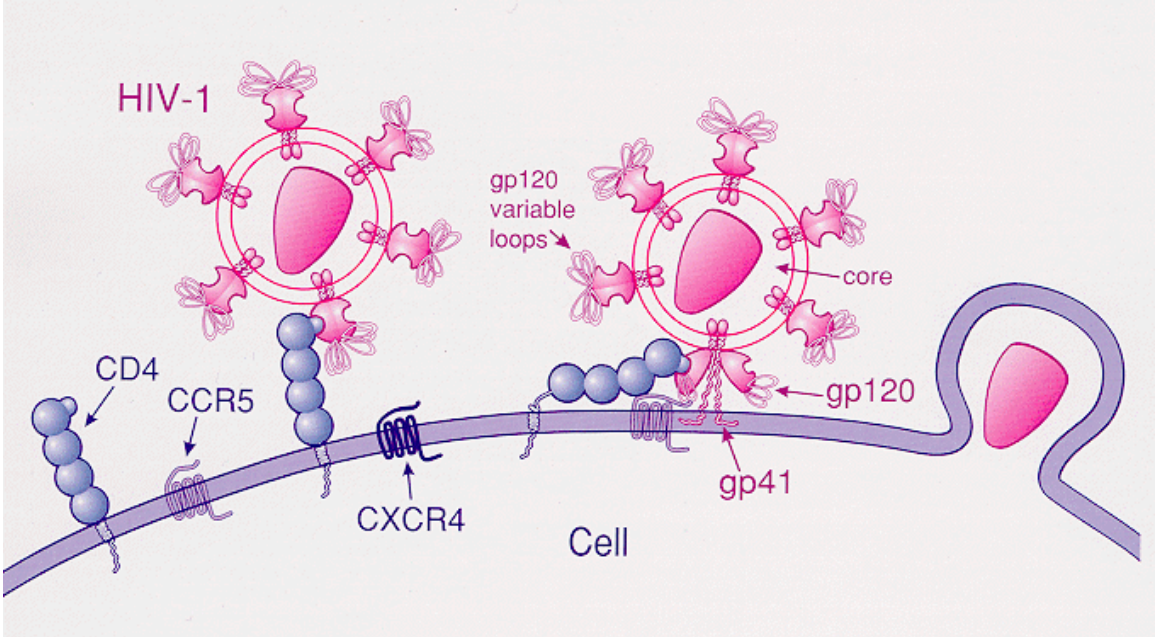


Figure 1 HIV entry into CD4+ cell via CCR5 co-receptor

HIV enters host cells in the blood by attaching itself to receptors on the surface of the CD4+ cell. Viral entry to the CD4+ cell begins with attachment of the R5 HIV-1 glycoprotein 120 (gp120) to the CD4+ T-cell receptor, which produces a conformational change in gp120 and allows it to bind to CCR5, thereby triggering glycoprotein 41 (gp41) mediated fusion of the viral envelope with the cell membrane and the nucleocapsid enters the host cell (Figure 1). CCR5 co-receptor antagonists prevent HIV-1 from entering and infecting immune cells by blocking CCR5 cell-surface receptor. Small molecule antagonists of CCR5 bind to a hydrophobic pocket formed by the transmembrane helices of the CCR5 receptor. They are thought to interact with the receptor in an allosteric manner locking the receptor in a conformation that prohibits its co-receptor function.

Drug development

As mentioned, the CCR5 receptor is a G-protein coupled receptor (GPCR) and before the discovery of CCR5's role in HIV infection many pharmaceutical companies had already built a substantial collection of compounds that target GPCRs. Some of these compounds provided an excellent starting point for CCR5 antagonists but then needed further optimization to gain better potency, pharmacokinetic properties and better CCR5 selectivity. One of the biggest problems was the affinity for the hERG ion channel, as inhibition of hERG can lead to QT interval prolongation. Abnormality in the interval can increase the risk of developing ventricular arrhythmias. Many CCR5 antagonists have been introduced by pharmaceutical companies but few of them have actually reached human efficacy studies, for example AstraZeneca, Novartis, Merck and Takeda have used these GPRC compound collections to develop a potent CCR5 antagonist but none of them have reached clinical trials. Three pharmaceutical companies were racing for the

first approved small molecule CCR5 antagonist; GlaxoSmithKline(GSK), with their compound aplaviroc, Schering-Plough with vicriviroc and Pfizer with maraviroc. All the compounds reached clinical trials in humans but only maraviroc has been approved by the U.S. Food and Drug Administration (FDA). In the following section the development of these three compounds will be discussed.

Aplaviroc

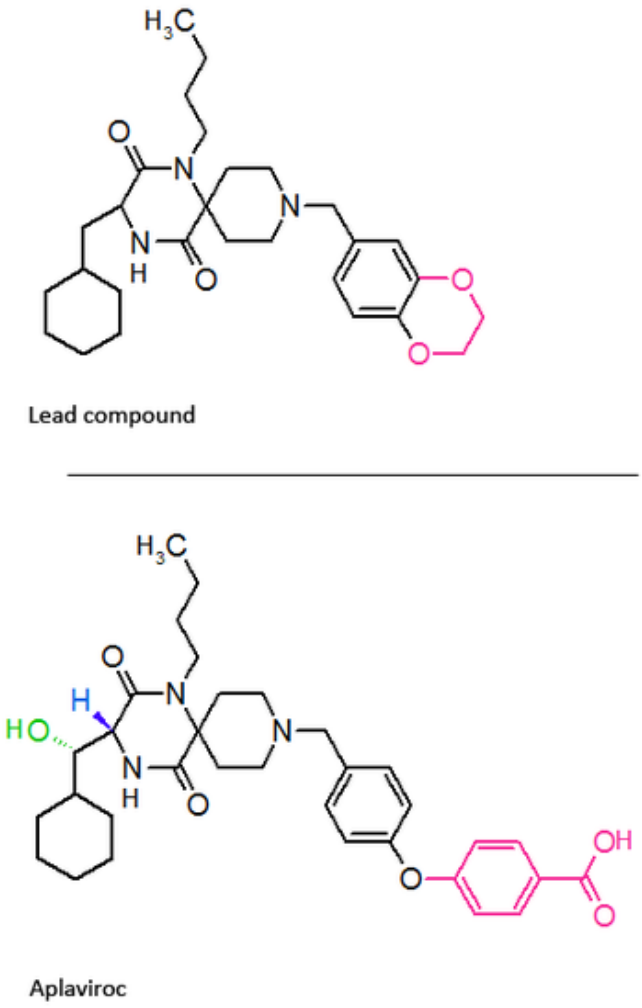


Figure 2 Molecular structure of aplaviroc and its lead compound

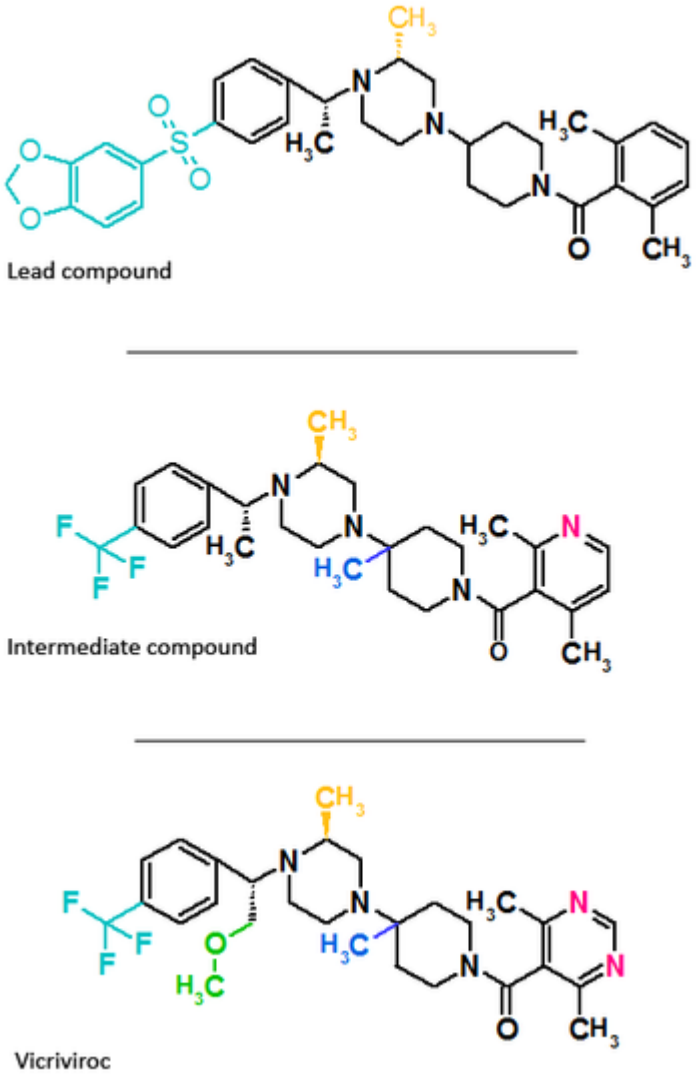


Figure 3 Molecular structure of vicriviroc and its lead compounds

Aplaviroc is originated from a class of spirodiketopiperazine derivatives. Figure 2 shows the molecular structure of the lead compound and the final compound aplaviroc. The lead compound showed good potency in blocking CCR5 in a number of R5 HIV strains and against multi-drug resistant strains. The problem with this compound was not its CCR5 selectivity but the oral bioavailability. This led to further development of the molecule and the result was a compound named aplaviroc. Unfortunately despite the promising preclinical and early clinical results some severe liver toxicity was observed in the treatment of naive and treatment-experienced patients that led to the discontinuation in further development of aplaviroc.

Vicriviroc

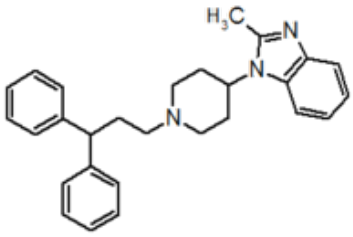
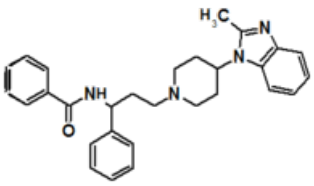
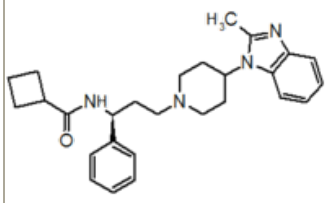
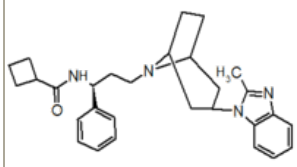
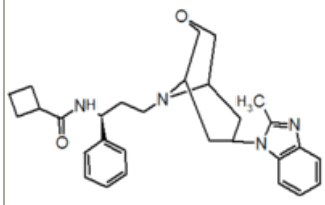
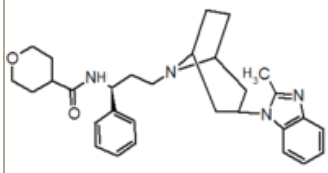
Schering-Plough identified an active compound during screening. Figure 3 shows the molecular structure of the lead compound, intermediate compound and the final compound vicriviroc. The lead compound contained a piperazine scaffold and was a potent muscarinic acetylcholine receptor (M2) antagonist with modest CCR5 activity. The changes that were made on the left hand side of the lead compound and the addition of a methyl group on the piperazine group ((S)-methylpiperazine) resulted in the intermediate compound that had good affinity for CCR5 receptors but very little affinity for muscarinic activity, however, the compound did show affinity for the hERG ion channel. Further reconstruction led to the development of the final compound vicriviroc, when Schering discovered that the pyridyl N-oxide on the intermediate could be replaced by 4,6-dimethylpyrimidine carboxamide. Vicriviroc had an excellent selectivity for CCR5 receptors over muscarinic and hERG affinity was greatly reduced. Phase I clinical trial of vicriviroc gave promising results, so a phase II study in the treatment of naive patients was initiated. The phase II study was discontinued since there was a viral breakthrough in the vicriviroc group compared to the control group. These results suggested that vicriviroc was not effective in the treatment of treatment-naive patients compared to current treatments. Another phase II clinical study was performed in treatment-experienced patients. The results were that vicriviroc did have strong antiviral activity but five instances of cancer among the participants were reported, however, the study was continued since there was lack of causal association of the malignancies and vicriviroc. In late 2009 vicriviroc was assigned in phase III studies in treatment-experienced patients, and in phase II studies in treatment for naive patients.

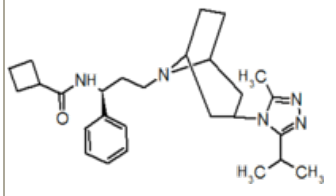
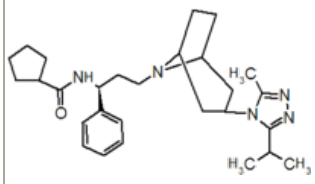
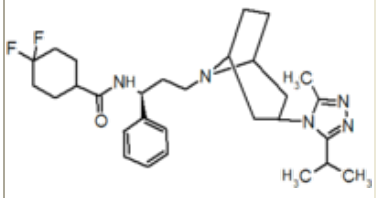
Maraviroc

Pfizer turned to high-throughput screening in their search for a good starting point for a small molecule CCR5 antagonist. Their screening resulted in a compound that presented weak affinity and no antiviral activity but represented a good starting point for further optimization. Compounds 1-9 in table 1 show the development of maraviroc in few steps. The chemical structure of the starting molecule is presented as compound 1. Their first focus was to minimize CYP2D6 activity of the molecule and to reduce its lipophilicity. They replaced the imidazopyridine with benzimidazole and the benzhydril group was swapped out for a benzamide. The outcome was compound 2. That compound showed good binding potency and the start of an antiviral activity. Further SAR (structure-activity relationship) optimization of the amide region and identifying the enantiomeric preference led to the cyclobutyl amide structure in compound 3. However, the problem with the CYP2D6 activity of the compound was still unacceptable so they had to perform further SAR optimization that determined that the [3.2.1]-azabicycloamine (topane) could replace the aminopiperidine moiety. This change in the chemical structure led to compound 4. Compound 4 had no CYP2D6 activity while preserving excellent binding affinity and antiviral activity. Although compound 4 showed promising results it demonstrated 99% inhibition on the hERG ion channel. That inhibition was unacceptable since it can lead to QTc interval prolongation. The research team then did a few modifications to see which part of the molecule played a role in the hERG affinity.

Compound 5 shows an analogue that they synthesized which contained an oxygen bridgehead in the tropane ring; however, that reconstruction did not have an effect on the hERG affinity. They then focused on the polar surface area in the molecule to dial out the hERG affinity. These efforts resulted in compound 6. That compound preserved desired antiviral activity and was selective against the hERG inhibition but the problem was its bioavailability. Reduction in the lipophilicity, by replacing the benzimidazol group with a substituted triazole group gave compound 7. Compound 7 had shown a significant reduction in lipophilicity and maintained the antiviral activity but again, with the introduction of a cyclobutyl group the compound showed hERG inhibition. Changing the ring size in compound 7 from a cyclobutyl unit to a cyclopentyl unit in compound 8 led to a significant increase in antiviral activity and loss of hERG affinity. Further development led to discovery of a 4,4'-difluorocyclohexylamide also known as maraviroc. Maraviroc preserved excellent antiviral activity, whilst demonstrating no significant hERG binding affinity. The lack of hERG binding affinity was predicted to be because of the large size of the cyclohexyl group and the high polarity of the fluoro substituents. In August 2007 the FDA approved the first CCR5 antagonist, maraviroc discovered and developed by Pfizer.

Table 1 Represents the molecular structures in the development of maraviroc

		
Compound 1	Compound 2	Compound 3
		
Compound 4	Compound 5	Compound 6

		
Compound 7	Compound 8	Compound 9 (Maraviroc)

Pharmacophore

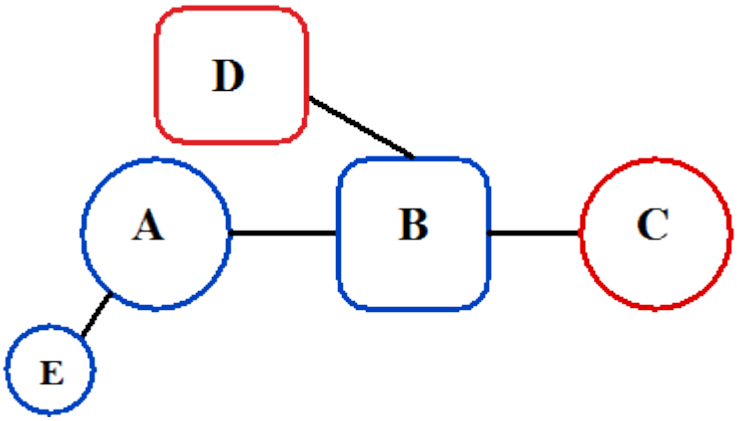


Figure 4 Predictive pharmacophore model for piperidine- and piperazine-based CCR5 antagonists

The predictive pharmacophore model was developed for a large series of piperidine- and piperazine-based CCR5 antagonists by Schering-Plough Research Institute. Their hypothesis consisted of mostly five features, two hydrogen bond acceptors, marked C and D in figure 4 and three hydrophobic groups, A, B and E in figure 4. Part B usually has a basic nitrogen group. The model was validated using diverse set of six CCR5 antagonists from five different pharmaceutical companies. The best model correctly predicted these compounds as being highly active. It is possible to use the model as a tool in virtual screening for new small molecular CCR5 antagonists and also to predict biological activities of compounds prior to undertaking their costly synthesis.

Binding

CCR5 is a member of G-protein-coupled, seven transmembrane segment receptors. The structure of the receptor comprises seven-helix bundle in the transmembrane region, these regions are labeled I-VII in figure 5 and 6. The CCR5 antagonists are predicted to bind to a putative binding pocket which is buried inside the transmembrane domain, enclosed by the seven transmembrane helices. The binding pocket is very hydrophobic with multiple aromatic residues lining the pocket. The key residues are tryptophan 86 and 248 (Trp86, Trp248), tyrosine 108 and 251 (Tyr108, Tyr251), phenylalanine 109 (phe109), threonine

195 (Thr195), isoleucine 198 (Ile198), glutamic acid 283 (Glu283). CCR5 antagonists are very different in shape and electrostatic potential although they all share the same binding pocket. The interesting thing about the binding of these molecules is that they exhibit significantly different binding modes, although they all establish an extensive interaction network with CCR5.

Binding of aplaviroc

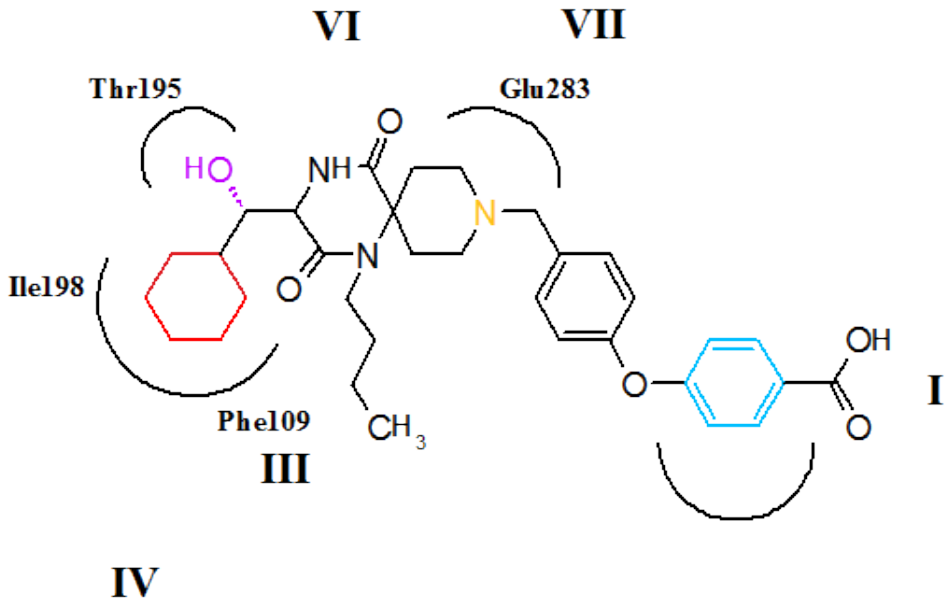


Figure 5 Putative binding of aplaviroc to the CCR5 receptor

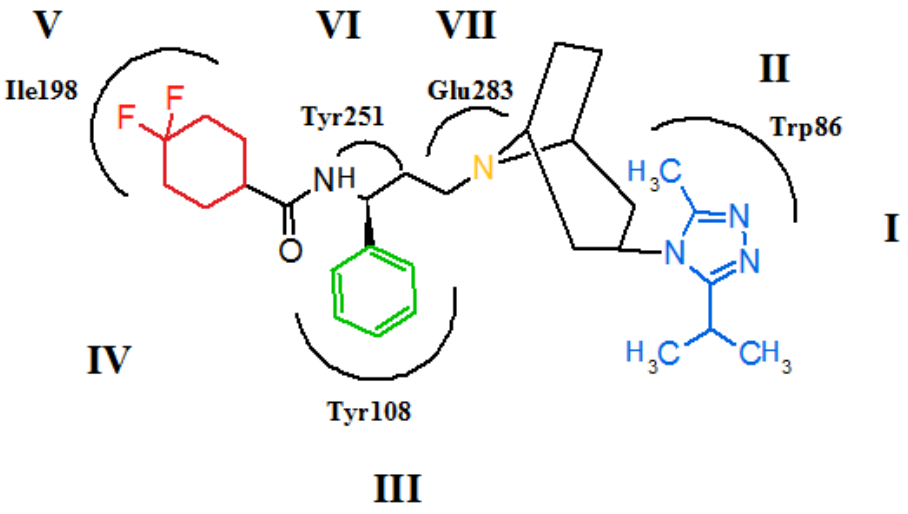


Figure 6 Putative binding of maraviroc to the CCR5 receptor

The putative binding mode for aplaviroc is shown in figure 5. The key saltbridge interaction between aplaviroc and Glu283 is predicted to be quite weak compared to other CCR5 antagonists. The hydroxyl group on aplaviroc forms a strong hydrogen bond to the polar residue Thr195. This H-bond interaction is the strongest with aplaviroc compared to other CCR5 antagonists. The cyclohexyl group in the aplaviroc structure is predicted to interact with the receptor in a hydrophobic pocket formed by Ile198, Thr15 and Phe109 and is thought to show quite strong hydrophobic interactions. The researchers predict that the t-butyl group of aplaviroc is buried within the helical bundle through strong hydrophobic interaction with multiple aromatic residues of the CCR5 receptor.

Binding of maraviroc

The putative binding mode for maraviroc is shown in figure 6. The strongest interaction is estimated to be between maraviroc and glutamic acid (Glu283) through a strong salt-bridge interaction. The interaction between tryptophan (Trp86) and maraviroc involves T-shaped π-π stacking while the interaction with phenylalanine (Phe109) is predicted to be hydrophopic. Tyrosine (Tyr108) is thought to interact with the phenyl group on maraviroc through a parallel displaced interaction. The interaction between maraviroc and isoleucine (Ile198) is predicted to be mostly hydrophobic in nature and the interaction between maraviroc and tyrosine (Tyr251) is very limited.

Unique binding of aplaviroc

Aplaviroc has a unique feature of preserving two of the natural chemokine protein ligands binding to CCR5 and subsequent activation, whereas maraviroc and the other antagonists almost fully block chemokine-CCR5 interactions. This kind of interference is so far considered to be safe, and individuals that naturally lack CCR5 do not show any obvious health problems. However, to limit the toxicity and side effects of CCR5 antagonists it would be ideal to be able to preserve the chemokine receptor function. Consequently, it should be of interest to design inhibitors that specifically disrupt CCR5-gp120 binding but do not affect the CCR5 chemokine activation.

Other CCR5 antagonists

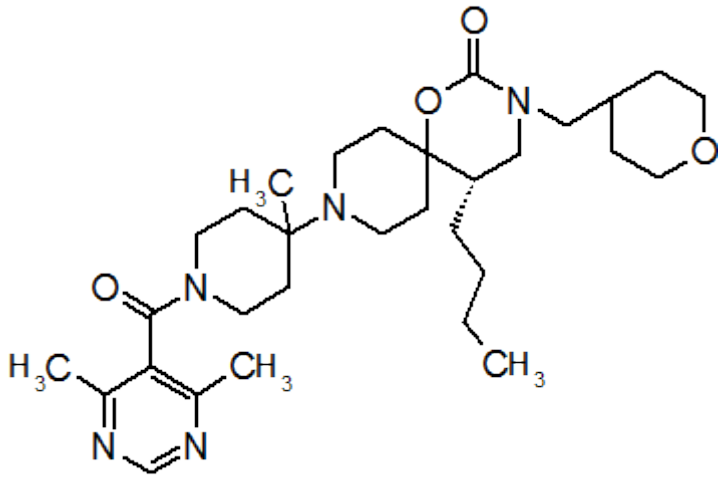


Figure 7 Molecular structure of compound A

Development of new CCR5 antagonists continues, both for their antiviral effects and also for potential utility in a variety of autoimmune indications. Researchers at Ono have discovered a novel series of potent CCR5 small molecule antagonists. Lead optimization was pursued by balancing opposing trends of metabolic stability and potency. Combination of the spiropiperidine template with pharmacophore elements from both aplaviroc, and Schering's CCR5 antagonist program, led to the initial lead compound in this series. Further development of that lead compound led to the discovery of compound A in figure 7 - A compound that possesses a good selectivity and pharmacokinetic properties.

The CCR5 antagonist INCB009471 has nanomolar activity against HIV-1 in vitro. This compound demonstrated potent and prolonged antiviral activity against R5-tropic HIV-1 when given 200 mg once daily dose for 14 days. These findings supported further clinical development of INCB009471 and they have since progressed to phase IIb clinical trials. As of 2009 the study of this compound is inactive and no further studies are planned at this time.

Chapter 12

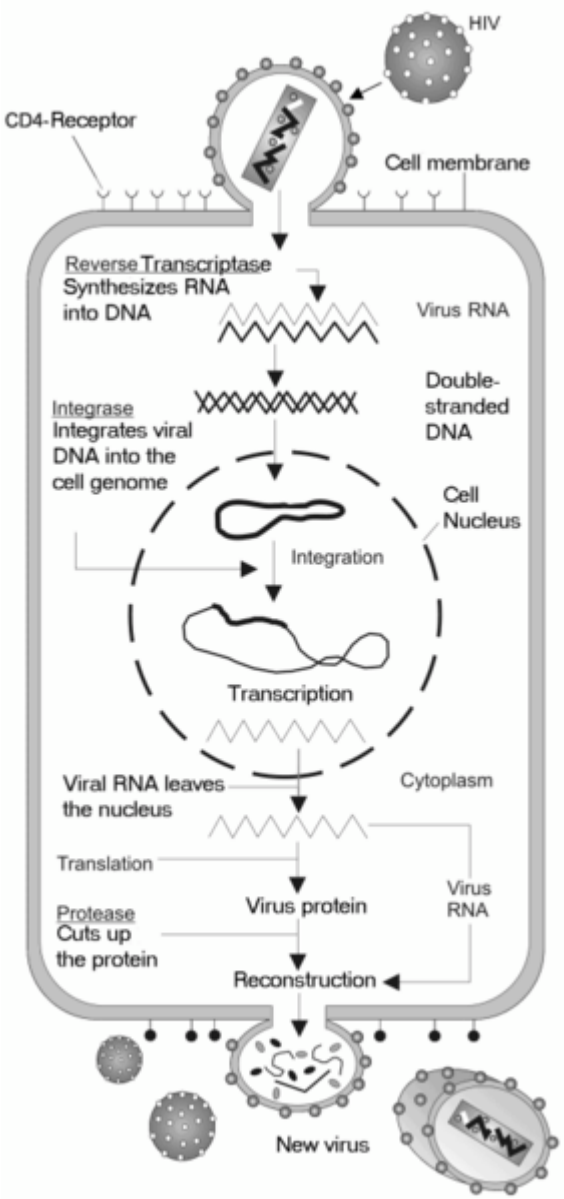
Discovery and Development of HIV-Protease Inhibitors

Many major physiological processes depend on regulation of proteolytic enzyme activity and there can be dramatic consequences when equilibrium between an enzyme and its substrates is disturbed. In this prospective, the discovery of small-molecule ligands, like protease inhibitors, that can modulate catalytic activities has an enormous therapeutic effect. Hence, inhibition of the HIV protease is one of the most important approaches for the therapeutic intervention in HIV infection and their development is regarded as major success of structure-based drug design. They are highly effective against HIV and have, since the 1990s, been a key component of anti-retroviral therapies for HIV/AIDS.

History

Human immunodeficiency virus (HIV) is a lentivirus that has two major species, HIV-1 which causes the majority of the epidemic, and HIV-2, a close relative whose distribution is concentrated in western Africa. HIV infection was first described in 1981 in San Francisco and New York City. In 1985, HIV was identified as the causative agent of acquired immune deficiency syndrome (AIDS) and its complete genome was immediately available. This knowledge paved the way for the development of selective inhibitors. HIV-2 carries a slightly lower risk of transmission than HIV-1 and infection tends to progress more slowly to AIDS. In common usage HIV usually implies HIV-1. HIV-1 protease is one of the best known aspartic proteases, and an attractive target for the treatment of AIDS. After the discovery of HIV protease it only took 10 years for its first inhibitor to reach the market. The first reports of highly selective antagonists against the HIV protease were revealed in 1987. Phase I trials of saquinavir began in 1989 and it was the first HIV protease inhibitor to be approved for prescription use in 1995. Four months later, two other protease inhibitors, ritonavir and indinavir, were approved. In 2009, ten protease inhibitors have reached the market for treatment against HIV but one protease inhibitor, amprenavir, was withdrawn from the market in 2004.

Life cycle of HIV



The HIV replication cycle

HIV belongs to the class of viruses called retroviruses, which carry genetic information in the form of RNA. HIV infects T cells that carry the CD4 antigen on their surface. When HIV infects its target cell it requires fusion of the viral and cellular membranes. The first step is the interaction between envelope proteins of the virus (gp120, gp41) and specific host-cell surface receptors (e.g. CD4 receptor) on the target cell. Then the virus binds to the chemokine coreceptors CXCR4 or CCR5, resulting in conformational changes in the envelope proteins. This fusion creates a pore through which the viral capsid enters the cell. Following entry into the cell the RNA of the virus is reverse-transcribed to DNA by the first virally encoded enzyme, the reverse transcriptase. The viral DNA enters the nucleus where it is integrated into the genetic material of the cell by the integrase, a

second virally encoded enzyme. Activation of the host cell leads to the transcription of the viral DNA into mRNA. The mRNA is then translated into viral proteins and the third virally encoded enzyme, namely HIV protease, is required to cleave a viral polyprotein precursor into individual mature proteins. The viral RNA and viral proteins assemble at the surface of the cell into new virions. The virions bud from the cell and are released to infect other cells. All infected cells are eventually killed because of this extensive cell damage, from the destruction of the host's genetic system to the budding and release of virions.

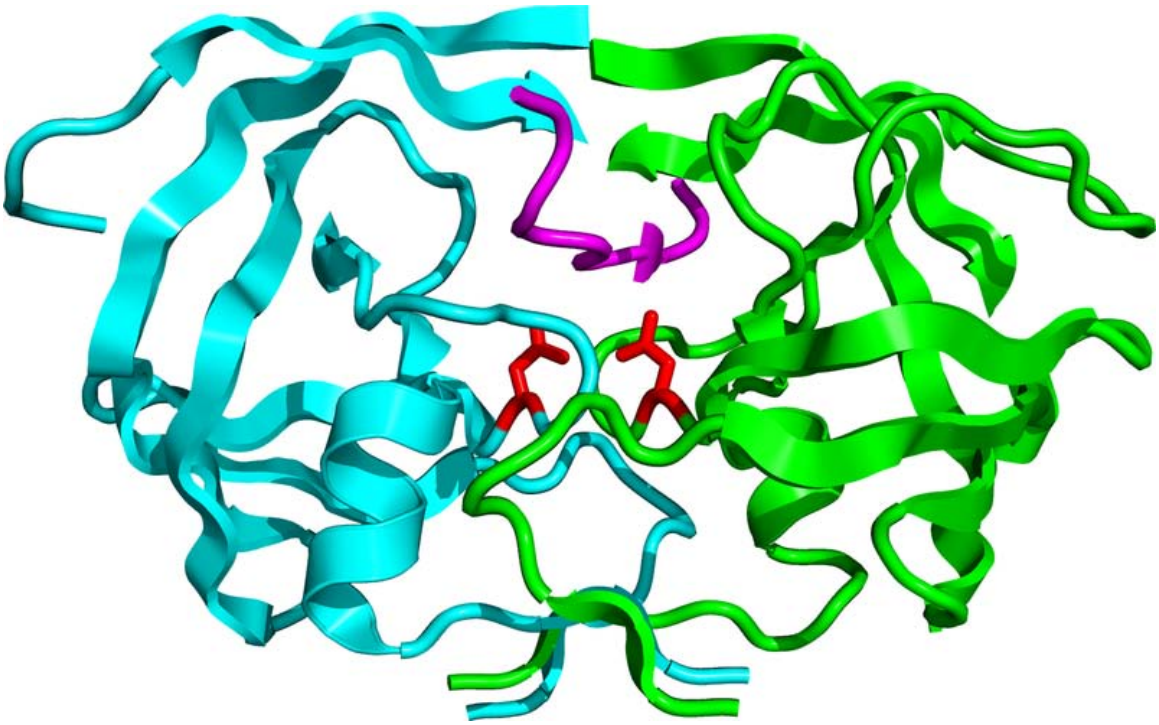
Mechanism of action

There are several steps in the HIV life cycle that may be interfered with, thus stopping the replication of the virus. A very critical step is the proteolytic cleavage of the polypeptide precursors into mature enzymes and structural proteins catalyzed by HIV protease. HIV protease inhibitors are peptide-like chemicals that competitively inhibit the action of the virus aspartyl protease. These drugs prevent proteolytic cleavage of HIV Gag and Pol polyproteins that include essential structural and enzymatic components of the virus. This prevents the conversion of HIV virus particles into their mature infectious form. Protease inhibitors can alter adipocyte metabolism causing lipodystrophy, a common side effect associated with the use of most HIV protease inhibitors. Many mechanisms have been proposed, for example inhibition of adipocyte differentiation, triglyceride accumulation and increased lipolysis. Theories considering the effect of protease inhibitors on insulin-stimulated glucose uptake have also been linked to the lipodystrophic syndrome. It is possible that protease inhibitors can cause a decrease in insulin-stimulated tyrosine phosphorylation of IRS-1, representing inhibition of early steps in insulin signaling. Decreased adiponectin secretion and induced expression of interleukin-6 associated with HIV protease inhibitors may also contribute to inhibition of insulin-stimulated glucose uptake.

Design

Protease inhibitors were designed to mimic the transition state of the protease's actual substrates. A peptide linkage consisting of –NH-CO- is replaced by an hydroxyethylen group (-CH_2-CH(OH)-) which the protease is unable to cleave. HIV protease inhibitors fit the active site of the HIV aspartic protease and were rationally designed utilizing knowledge of the aspartyl protease's mode of action. The most promising transition state mimic was hydroxyethylamine which lead to the discovery of the first protease inhibitor, saquinavir. Following that discovery, other HIV protease inhibitors were designed using the same principle.

Binding site



A schematic structure of a HIV-1 protease. The monomers are shown in green and cyan, the Asp-25 and Asp-25′ residues are shown in red, and Ile50 and Ile50′ residues linked to a water molecule are shown in purple.

The HIV protease is a C2-symmetric homodimeric enzyme consisting of two 99 amino acid monomers. Each monomer contributes an aspartic acid residue that is essential for catalysis, Asp-25 and Asp-25′. The HIV protease has the sequence Asp-Thr-Gly, which is conserved among other mammalian aspartic protease enzymes. An extended beta-sheet region on the monomers, known as the flap, constitutes in part the substrate binding site with the two aspartyl residues lying on the bottom of a hydrophobic cavity. Each flexible flap contains three characteristic regions: side chains that extend outward (Met46, Phe53), hydrophobic chains extending inward (Ile47, Ile54), and a glycine rich region (Gly48, 49, 51, 52). Ile50 remains at the tip of the turn and when the enzyme is unliganded a water molecule makes hydrogen bonds to the backbone of Ile50 on each monomer.

HIV proteases catalyze the hydrolysis of peptide bonds with high sequence selectivity and catalytic proficiency. The mechanism of the HIV protease shares many features with the rest of the aspartic protease family although the full detailed mechanism of this enzyme is not fully understood. The water molecule seems to play a role in the opening and closing of the flaps as well as increasing the affinity between enzyme and substrate. The aspartyl residues are involved in the hydrolysis of the peptide bonds. The preferred cleavage site for this enzyme is the N-terminal side of proline residues, especially between phenylalanine and proline or tyrosine and proline.

Development

The first HIV protease inhibitor, saquinavir, is a peptidomimetic hydroxyethylamine and was marketed in 1995. It is a transition state analogue of a native substrate of the protease. The observation that HIV-1 protease cleaves the sequences containing the dipeptides Tyr-Pro or Phe-Pro was the basic design criterion. Addition of the decahydroisoquinoline (DIQ) group was one of the most significant modifications that led to the discovery of saquinavir. This substituent improves aqueous solubility and potency by limiting the conformational freedom of the inhibitor. Saquinavir is effective against both HIV-1 and HIV-2 and is usually well tolerated but high serum concentration is not achieved.

Ritonavir, a peptidomimetic HIV protease inhibitor, was marketed in 1996. It was designed to fit the C2-symmetry in the binding site of the protease. The developers of ritonavir, Abbott Laboratories, started with compounds that were active against the virus but had poor bioavailability. Some improvements were made, for example the terminal phenyl residues were removed and pyridyl groups put instead to add water solubility. The final product of these improvements was ritonavir. Significant gastrointestinal side effects and a large pill burden are ritonavir's main drawbacks and is therefore not used as a single treatment. However, it is a strong inhibitor of the cytochrome P450 enzyme mediated metabolism and it is only used in a combination therapy with other protease inhibitors for pharmacokinetic boosting.

Indinavir, which is a peptidomimetic hydroxyethylene HIV protease inhibitor, reached the market in 1996. The design of indinavir was guided by molecular modeling and the X-ray crystal structure of the inhibited enzyme complex. The terminal phenyl constituents contribute hydrophobic binding to increase potency. It is an analogue of the phenylalanine-proline cleavage site of the HIV Gag-polyprotein.

Nelfinavir was the first protease inhibitor that was not peptidomimetic. In the design process of nelfinavir, an orally bioavailable and nonpeptidic inhibitor, iterative protein cocrystal structure analysis of peptidic inhibitors was used and parts of the inhibitors were replaced by nonpeptidic substituents. Nelfinavir contains a novel 2-methyl-3-hydroxybenzamide group, whereas its carboxyl terminal contains the same DIQ group as saquinavir. Nelfinavir was marketed in 1997 and was the first protease inhibitor to be indicated for pediatric AIDS.

Amprenavir reached the market in 1999. It is an N,N-disubstituded amino-sulfonamide nonpeptide HIV protease inhibitor and shares some common features with previous protease inhibitors. It has a core similar to that of saquinavir but with different functional groups on both ends. On one end it has a tetrahydrofuran carbamate group and on the other end is an isobutylphenyl sulfonamide with an added amide. This structure results in fewer chiral centers, that makes it easier to synthesize and gives it enhanced aqueus solubility. That in turn gives better oral bioavailability. However, amprenavir was withdrawn from the market in 2004 since fosamprenavir, its prodrug, proved superior in many aspects.

Lopinavir was marketed in 2000 and was originally designed to diminish the interactions of the inhibitor with Val82 of the HIV-1 protease, a residue that is often mutated in the drug resistant strains of the virus. It is a peptidomimetic HIV protease inhbitor and its core is identical to that of ritonavir. Instead of the 5-thiazolyl end group in ritonavir, lopinavir has a phenoxyacetyl group and the 2-isopropylthiazolyl group in ritonavir was replaced by a modified valine in which the amino terminal had a six-membered cyclic urea attached.

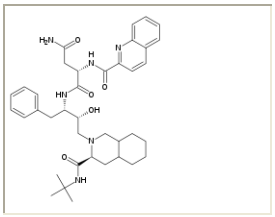
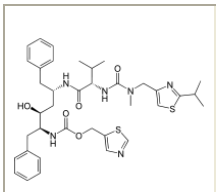
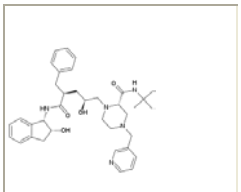
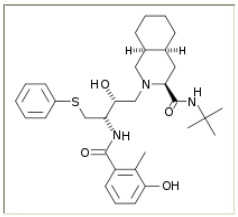
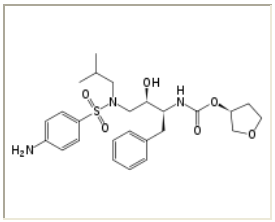
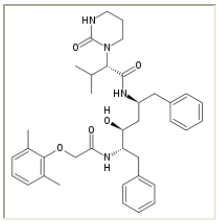
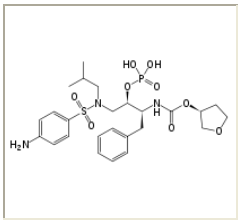
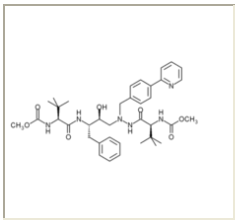
Fosamprenavir was marketed in 2003 and is a phosphoester prodrug that is rapidly and extensively metabolized to amprenavir. The solubility and bioavailability is better than of amprenavir which results in reduced daily pill burden.

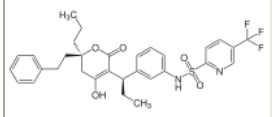
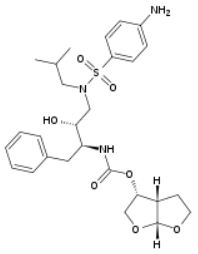
Atazanavir was marketed in 2003 and is an azapeptide protease inhibitor designed to fit the C2-symmetry of the enzyme binding site. Atazanavir showed better resistant profiles than previous HIV protease inhibitors. It is unique among the other protease inhibitors as it can only be absorbed in an acidic environment.

Tipranavir is a nonpeptidic HIV-1 protease inhibitor and reached the market in 2005. Unlike other HIV protease inhibitors on the market, tipranavir was developed from a nonpeptidic coumarin template and its antiprotease activity was discovered by high-throughput screening. This sulfonamide containing 5,6-dihydro-4-hydroxy-2-pyrone had emerged from screenings of 3-substituted coumarins and dihydropyrones. It possesses broad antiviral activity against multiple protease inhibitor resistant HIV-1.

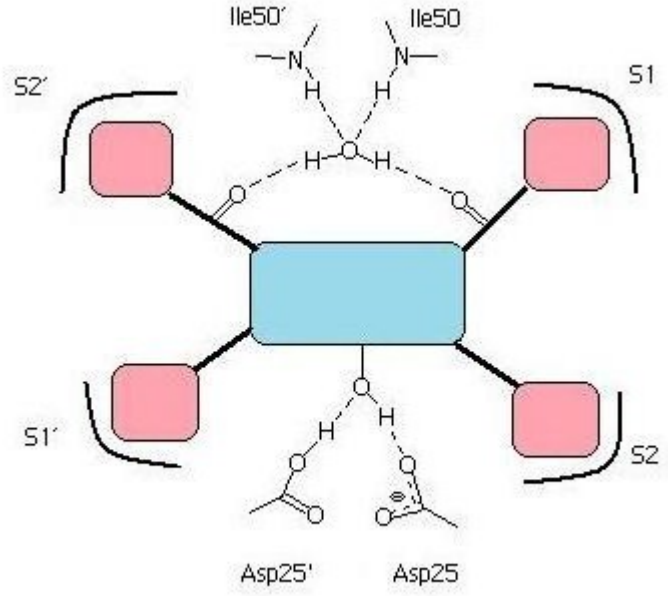
Darunavir reached the market in 2006 and is a nonpeptidic analogue of amprenavir, with a critical change at the terminal tetrahydrofuran (THF) group. Instead of a single THF group, darunavir contains two THF groups fused in the compound, to form a bis-THF moiety which makes it more effective than amprenavir. With this structural change, the stereochemistry around the bis-THF moiety confers orientational changes, that allows for continued binding with the protease which has developed a resistance for amprenavir. All the FDA approved protease inhibitors are listed below.

HIV protease inhibitors the FDA has approved

			
Saquinavir	Ritonavir	Indinavir	Nelfinavir
			

Amprenavir	Lopinavir	Fosamprenavir	Atazanavir
			
Tipranavir	Darunavir		

Structure-activity relationship



A simplified image of a protease inhibitor binding to the active site of the HIV-1 protease. The central core motif is shown in blue with the hydroxyl group forming hydrogen bonds with Asp-25 and Asp-25´. Hydrogen bonds also connect carbonyl groups on the inhibitor to the water molecule linked to Ile50 and Ile50´. Hydrophobic groups are shown in pink and their complementing pockets referred to as S1, S1´, S2 and S2´.

All the HIV protease inhibitors on the market contain a central core motif consisting of a hydroxyethylen scaffold, with the only exception being the central core of tipranavir, which is based on a coumarin scaffold. A very important group on the HIV protease inhibitors is a hydroxyl group on the core motif which forms a hydrogen bond with the carboxylic acid on the Asp-25 and Asp-25´ residues in the binding site. Hydrogen bonds between the water molecule, which is linked to Ile50 and Ile50', and carbonyl groups of the peptidomimetic inhibitors seem to connect them with the flap regions. On the other hand, on the nonpeptidic inhibitors, there is a proton acceptor which replaces the tetracoordinated water molecule and interacts directly with the two Ile50 residues on the flap of the enzyme. Specific pockets in the binding site of the HIV protease, often

referred to as S1,S1',S2 and S2', recognize hydrophobic amino acids on natural substrates. The potency of inhibitors bearing hydrophobic groups complementing these areas is therefore increased. Some residues in the enzyme binding site are capable of forming hydrogen bonds with hydrophilic groups on the inhibitor, for example with the THF moieties on amprenavir and darunavir. Since darunavir has a bis-THF moiety, instead of a single THF moiety like on amprenavir, it can form more hydrogen bonds and increase binding energy.

Resistance

Mutations that code for alterations of the conformational shape facilitate resistance of HIV to protease inhibitors. The locations of these mutations are primarily in the active site of the HIV protease enzyme as well as outside of the active site. Active site mutations have been shown to directly change the interactions of the inhibitors with the protease whilst non-active site mutations are considered to affect by other mechanisms, like influencing dimer stability and conformational flexibility. Over 100 single gene point mutations have been described, of which at least 26 are specific to protease inhibitors. Of these, there are about 15 primary or major mutations that are significant enough to change drug activity. Many mutated residues have been found in HIV-1 protease which cause drug resistance, for example Leu33 changes to Ile, Val, or Phe; Val82 to Ala, Phe, Leu, or Thr; Ile84 to Val; and Leu90 to Met. Different mutations affects different protease inhibitors. For instance, mutations at Leu90 evidently affect saquinavir and nelfinavir while indinavir activity is affected by mutations at Met46, Val82, and Ile84, and fosamprenavir is affected when Ile50 changes to Val and at Ile84. A combination of mutations can render high-level drug resistance but single mutations normally do not equate with drug resistance to protease inhibitors. The mutations can be divided into primary mutations and secondary mutations. Primary mutations often have only a small effect on resistance. The chemical structures of most protease inhibitors are quite similar, so it is not surprising that some primary mutations lead simultaneously to resistance to multiple protease inhibitors. Cross-resistance is one of the major problems of protease inhibitor treatment. Additional mutations emerging in the protease during continuous protease inhibitor therapy are commonly referred to as secondary mutations. This can lead to high-level protease inhibitor resistance.
There is no general strategy to tackle the problem of drug resistance. Researches directed towards development of new therapies to cure AIDS are focused on avoiding cross-resistance to drugs that are already on the market.

Current status

In November 2009 darunavir was still the most recent HIV protease inhibitor to reach the market. In 2006, GlaxoSmithKline discontinued the phase II clinical development of brecanavir, an investigational protease inhibitor for the treatment of HIV, due to insurmountable issues regarding formulation. In the summer of 2009, GlaxoSmithKline and Concert Pharmaceuticals announced their collaboration to develop and commercialise deuterium-containing medicines. One of them is CTP-518, a protease inhibitor for the treatment of HIV, expected to enter phase I clinical trials in the second

half of 2009. CTP-518 is a novel HIV protease inhibitor developed by replacing certain key hydrogen atoms of atazanavir with deuterium. Pre-clinical studies have demonstrated that this modification fully retains the antiviral potency but can evidently slow hepatic metabolism and thereby increase the half life and plasma trough levels. CTP-518, therefore, has the potential to be the first HIV protease inhibitor to eliminate the need to co-dose with a boosting agent, such as ritonavir.

Chapter 13

WHO Disease Staging System for HIV Infection and Disease in Adults and Adolescents

WHO Disease Staging System for HIV Infection and Disease in Adults and Adolescents was first produced in 1990 by the World Health Organization and updated in September 2005. It is an approach for use in resource limited settings and is widely used in Africa and Asia and has been a useful research tool in studies of progression to symptomatic HIV disease.

Following infection with HIV, the rate of clinical disease progression varies enormously between individuals. Many factors such as host susceptibility and immune function, health care and co-infections, as well as factors relating to the viral strain may affect the rate of clinical disease progression.

Revised World Health Organization (WHO) Clinical Staging of HIV/AIDS For Adults and Adolescents (2005)

(This is the interim African Region version for persons aged 15 years or more who have had a positive HIV antibody test or other laboratory evidence of HIV infection) (It must be noted that the UN defines adolescents as persons aged 10−19 years but for surveillance purposes, the category of adults and adolescents comprises people aged 15 years and over)

Primary HIV infection

- Asymptomatic
- Acute retroviral syndrome

Clinical stage 1

- Asymptomatic
- Persistent generalized lymphadenopathy

Clinical stage 2

- Moderate and unexplained weight loss (<10% of presumed or measured body weight)
- Recurrent respiratory tract infections (such as sinusitis, bronchitis, otitis media, pharyngitis)
- Herpes zoster
- Recurrent oral ulcerations
- Papular pruritic eruptions
- Angular cheilitis
- Seborrhoeic dermatitis
- Fungal finger nail infections

Clinical stage 3

Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations

- Unexplained chronic diarrhoea for longer than one month
- Unexplained persistent fever (intermittent or constant for longer than one month)
- Severe weight loss (>10% of presumed or measured body weight)
- Oral candidiasis
- Oral hairy leukoplakia
- Pulmonary tuberculosis (TB) diagnosed in last two years
- Severe presumed bacterial infections (e.g. pneumonia, empyema, meningitis, bacteraemia, pyomyositis, bone or joint infection)
- Acute necrotizing ulcerative stomatitis, gingivitis or periodontitis

Conditions where confirmatory diagnostic testing is necessary

- Unexplained anaemia (< 80 g/l), and or neutropenia (<500/µl) and or thrombocytopenia (<50 000/ µl) for more than one month

Clinical stage 4

Conditions where a presumptive diagnosis can be made on the basis of clinical signs or simple investigations

- HIV wasting syndrome
- Pneumocystis pneumonia
- Recurrent severe or radiological bacterial pneumonia
- Chronic herpes simplex infection (orolabial, genital or anorectal of more than one month's duration)
- Oesophageal candidiasis
- Extrapulmonary Tuberculosis
- Kaposi's sarcoma

- Central nervous system toxoplasmosis
- HIV encephalopathy

Conditions where confirmatory diagnostic testing is necessary

- Extrapulmonary cryptococcosis including meningitis
- Disseminated non-tuberculous mycobacteria infection
- Progressive multifocal leukoencephalopathy
- Candida of trachea, bronchi or lungs
- Cryptosporidiosis
- Isosporiasis
- Visceral herpes simplex infection
- Cytomegalovirus (CMV) infection (retinitis or of an organ other than liver, spleen or lymph nodes)
- Any disseminated mycosis (e.g. histoplasmosis, coccidiomycosis, penicilliosis)
- Recurrent non-typhoidal salmonella septicaemia
- Lymphoma (cerebral or B cell non-Hodgkin)
- Invasive cervical carcinoma
- Visceral leishmaniasis

Original proposal in 1990

Clinical Stage I

- Asymptomatic
- Generalised lymphadenopathy

Performance scale: 1: asymptomatic, normal activity.

Clinical Stage II

- Weight loss, < 10% of body weight
- Minor mucocutaneous manifestations (seborrheic dermatitis, prurigo, fungal nail infections, recurrent oral ulcerations, angular cheilitis)
- Herpes zoster within the last five years
- Recurrent upper respiratory tract infections (i.e. bacterial sinusitis)

And/or performance scale 2: symptomatic, normal activity.

Clinical Stage III

- Weight loss, > 10% of body weight
- Unexplained chronic diarrhoea > 1 month
- Unexplained prolonged fever (intermittent or constant), > 1 month
- Oral [candidiasis] ([thrush])
- Oral hairy leucoplakia

- Pulmonary tuberculosis
- Severe bacterial infections (i.e. pneumonia, pyomyositis)

And/or performance scale 3: bedridden < 50% of the day during last month.

Clinical Stage IV

The declaration of AIDS

- HIV wasting syndrome *
- Pneumocystis carinii pneumonia
- Toxoplasmosis of the brain
- Cryptosporidiosis with diarrhoea > 1 month
- Cryptococcosis, extrapulmonary
- Cytomegalovirus disease of an organ other than liver, spleen or lymph node (ex: retinitis)
- Herpes simplex virus infection, mucocutaneous (>1 month) or visceral
- Progressive multifocal leucoencephalopathy
- Any disseminated endemic mycosis
- Candidiasis of esophagus, trachea, bronchi
- Atypical mycobacteriosis, disseminated or lungs
- Non-typhoid Salmonella septicemia
- Extrapulmonary tuberculosis
- Lymphoma
- Kaposi's sarcoma
- HIV encephalopathy **

And/or performance scale 4: bedridden > 50% of the day during last month.

(*) HIV wasting syndrome: weight loss of > 10% of body weight, plus either unexplained chronic diarrhoea (> 1 month) or chronic weakness and unexplained prolonged fever (> 1 month).

(**) HIV encephalopathy: clinical findings of disabling cognitive and/or motor dysfunction interfering with activities of daily living, progressing over weeks to months, in the absence of a concurrent illness or condition other than HIV infection which could explain the findings.

Chapter 14

Circumcision and HIV

Over forty epidemiological studies have been conducted to investigate the **relationship between male circumcision and HIV infection**. Reviews of these studies have reached differing conclusions about whether circumcision could be used as a prevention method against HIV.

Experimental evidence was needed to establish a causal relationship between lack of circumcision and HIV, so three randomized controlled trials were commissioned as a means to reduce the effect of any confounding factors. Trials took place in South Africa, Kenya and Uganda. All three trials were stopped early by their monitoring boards on ethical grounds, because those in the circumcised group had a lower rate of HIV contraction than the control group. The results showed that circumcision reduced vaginal-to-penile transmission of HIV by 60%, 53%, and 51%, respectively. A meta-analysis of the African randomised controlled trials found that the risk in circumcised males was 0.44 times that in uncircumcised males, and reported that 72 circumcisions would need to be performed to prevent one HIV infection. The authors also stated that using circumcision as a means to reduce HIV infection would, on a national level, require consistently safe sexual practices to maintain the protective benefit.

As a result of these findings, the WHO and the Joint United Nations Programme on HIV/AIDS (UNAIDS) stated that male circumcision is an efficacious intervention for HIV prevention but should be carried out by well trained medical professionals and under conditions of informed consent (parents consent for their infant boys). Both the WHO and CDC indicate that circumcision may not reduce HIV transmission from men to women, and that data is lacking for the transmission rate of men who engage in anal sex with a female partner. The joint WHO/UNAIDS recommendation also notes that circumcision only provides partial protection from HIV and should never replace known methods of HIV prevention.

A meta-analysis of data from fifteen observational studies of men who have sex with men found "insufficient evidence that male circumcision protects against HIV infection or other STIs."

Some earlier reports had expressed the position that circumcision has little to no effect on HIV transmission among heterosexual couples. Furthermore, some have challenged the

validity of the African randomized controlled trials, prompting a number of researchers to question the effectiveness of circumcision as an HIV prevention strategy.

Earliest appearance in the literature

According to Alcena, it was he who first hypothesised that low rates of circumcision in Africa were partly responsible for the continent's high rate of HIV infection. He did this via a letter to the New York State Journal of Medicine in August 1986. He also alleges that the late Aaron J. Fink stole his idea when Fink published a letter to the New England Journal of Medicine entitled *A possible explanation for heterosexual male infection with AIDS*, in October 1986.

Observational studies

In 1989 Cameron found uncircumcised men 8.2 times more likely to have HIV. Since then over 40 epidemiological studies have been conducted to investigate the relationship between circumcision and HIV infection.

In 1994, de Vincenzi and Mertens surveyed previous studies that had links between circumcision status and HIV; they surveyed 23 in total. They criticised the Cameron study saying that it may have suffered from selection bias.

In 1995 Ntozi noted: "There are now two schools of thought about the link between lack of circumcision and HIV infection in Africa. One school is that of Bongaarts et al. (1989), Moses et al. (n.d.) and Caldwell and Caldwell (1994) who use geographical distribution evidence to argue that the association between lack of circumcision and a high level of HIV infection in Africa is so convincing that the likelihood of a link should be recognized and taken into account where possible in the battle against AIDS. Moses et al. (n.d.) have gone further to recommend circumcision interventions for Africa. In contrast, De Vincenzi and Mertens (1994) argue that the evidence for an association, at least from small-scale surveys, is doubtful and hence not conclusive enough to qualify circumcision as an intervention.

Van Howe conducted a meta-analysis in 1999 and found circumcised men at a greater risk for HIV infection. He further speculated that circumcision may be responsible for the increased number of partners, and therefore, the increased risk. Van Howe's work was reviewed by O'Farrell and Egger (2000) who said Van Howe used an inappropriate method for combining studies, stating that re-analysis of the same data revealed that the presence of the foreskin was associated with increased risk of HIV infection (fixed effects OR 1.43, 95%CI 1.32 to 1.54; random effects OR 1.67, 1.25 to 2.24). Moses *et al.* (1999) also criticised Van Howe's paper, stating that his results were a case of "Simpson's paradox, which is a type of confounding that can occur in epidemiological analyses when data from different strata with widely divergent exposure levels are combined, resulting in a combined measure of association that is not consistent with the results for each of the individual strata." They concluded that, contrary to Van Howe's assertion, the evidence that lack of circumcision increases the risk of HIV "appears compelling".

Weiss, Quigley and Hayes carried out a meta-analysis on circumcision and HIV in 2000 and found as follows: "Male circumcision is associated with a significantly reduced risk of HIV infection among men in sub-Saharan Africa, particularly those at high risk of HIV. These results suggest that consideration should be given to the acceptability and feasibility of providing safe services for male circumcision as an additional HIV prevention strategy in areas of Africa where men are not traditionally circumcised."

The USAID document summarised research as of September 2002. It states:

A systematic review and meta-analysis of 28 published studies by the London School of Hygiene and Tropical Medicine, published in the journal AIDS in 2000, found that circumcised men are less than half as likely to be infected by HIV as uncircumcised men. A subanalysis of 10 African studies found a 71 percent reduction among higher-risk men. A September 2002 update considered the results of these 28 studies plus an additional 10 studies and, after controlling for various potentially confounding religious, cultural, behavioral, and other factors, had similarly robust findings. Recent laboratory studies in Chicago found HIV uptake in the inner foreskin tissue to be up to nine times more efficient than in a control sample of cervical tissue.

Siegried *et al.* (2003) surveyed 35 observational studies relating to HIV and circumcision: 16 conducted in the general population and 19 in high-risk populations. They state:

We found insufficient evidence to support an interventional effect of male circumcision on HIV acquisition in heterosexual men. The results from existing observational studies show a strong epidemiological association between male circumcision and prevention of HIV, especially among high-risk groups. However, observational studies are inherently limited by confounding which is unlikely to be fully adjusted for. In the light of forthcoming results from RCTs, the value of IPD analysis of the included studies is doubtful. The results of these trials will need to be carefully considered before circumcision is implemented as a public health intervention for prevention of sexually transmitted HIV.

In 2005, Siegfried *et al.* published a review including 37 observational studies. Most studies indicated an association between lack of circumcision and increased risk of HIV, but the quality of evidence was judged insufficient to warrant implementation of circumcision as a public health measure. The authors stated that the results of the three randomised controlled trials then underway would therefore provide essential evidence about the effects of circumcision as an HIV intervention.

Kiwanuka *et al.*'s (1996) study on the relationship between religion and HIV in rural Uganda was presented at the 1996 10th *International AIDS Conference* He said that: "Lower rates of HIV infection among Pentecostals appear to be associated with less alcohol consumption, sexual abstinence and fewer sexual partners, whereas the low HIV prevalence in Muslims appears to be associated with low reported alcohol consumption and male circumcision." Muslims, despite having the lowest rate of sexual abstinence and

the highest rate of having two or more sexual partners, had the lowest level of HIV infection compared with the other religious groups in the study (Catholics, Protestants, and Pentecostals). The factor in common between the Muslims (14.5% seropositive) and the Pentecostals (14.6% seropositive) was the lower alcohol consumption rate in these two groups than amongst Protestants (19.2%) and Catholics (19.9%).

Kelly *et al.* (1999) investigated the age of male circumcision and risk of prevalent HIV infection in rural Uganda and found that circumcision before the age of 12 resulted in a reduction to 0.39 of the odds of being infected. The degree of protection varied with the age at which circumcision was performed. Those circumcised at between 13 and 20 years had an odds ratio of 0.46, and those circumcised after the age of 20 at an odds ratio of 0.78. They concluded: "Prepubertal circumcision is associated with reduced HIV risk, whereas circumcision after age 20 years is not significantly protective against HIV-1 infection."

Buvé and colleagues (1999) investigated the reasons why the HIV prevalence rate among pregnant women in many large towns in Central, East and southern Africa was higher (>30%) than in the cities and towns of most of West Africa (<10%). Between June 1997 and March 1998 surveys were carried out and blood samples were taken in 4 sites. Kisumu (Kenya) and Ndola (Zambia), in Central/East Africa, were selected as the towns with high HIV prevalence, while the low-prevalence towns in West Africa were Cotonou (Benin) and Yaoundé (Cameroon). "In conclusion, differences in the rate of HIV spread between the East African and West African cities studied cannot be explained away by differences in sexual behaviour alone. In fact, behavioural differences seem to be outweighed by differences in HIV transmission probability."

Bailey et al. (1999) interviewed 188 circumcised and 177 uncircumcised consenting Ugandan men in one of four native languages during April and May, 1997. Non-Muslim circumcised men were found to have a higher risk profile than uncircumcised men. Muslims generally had a lower risk profile than other circumcised men except they were less likely to have ever used a condom or to have used a condom during the last sex encounter. Bailey et al. concluded that "these results suggest that differences between circumcised and uncircumcised men in their sex practices and hygienic behaviors do not account for the higher risk of HIV infection found among uncircumcised men. Further consideration should be given to male circumcision as a prevention strategy in areas of high prevalence of HIV and other sexually transmitted diseases. Studies of the feasibility and acceptability of male circumcision in traditionally non-circumcising societies are warranted."

Bonner (2001) reserved caution over using circumcision to prevent HIV: "Until we know why and how circumcision is protective, exactly what the relationship is between circumcision status and other STIs, and whether the effect seen in high-risk populations is generalisable to other groups, the wisest course is to recommend risk reduction strategies of proven efficacy, such as condom use."

At the 14th International AIDS conference in 2002, Changedia and Gilada reported that "Though circumcision offers protection in acquisition of HIV infection, our findings reveal that it does not reduce transmission of HIV in conjugal settings." Hunter *et al.* (1994), however, report that "Women whose husband or usual sex partner was uncircumcised had a threefold increase in risk of HIV, and this risk was present in almost all strata of potential confounding factors." Fonck *et al.* (2000) reported that "Partners of circumcised men had less-prevalent HIV infection."

The prevalence of circumcision varies across Africa. Studies have been conducted to assess the acceptability of promoting circumcision in place where they traditionally do not circumcise. In 2007, country consultations and planning to scale up male circumcision programmes took place in Botswana, Kenya, Lesotho, Malawi, Mozambique, Namibia, Rwanda, South Africa, Swaziland, Uganda, Tanzania, Zambia and Zimbabwe. Kebaabetswe *et al.* carried out interviews in nine geographically representative locations to determine the acceptability of male circumcision as well as the preferred age and setting for male circumcision in Botswana. Their conclusion was "Male circumcision appears to be highly acceptable in Botswana. The option for safe circumcision should be made available to parents in Botswana for their male children. Circumcision might also be an acceptable option for adults and adolescents, if its efficacy as an HIV prevention strategy among sexually active people is supported by clinical trials." Boyle criticised Kebaabetswe *et al.*'s proposal to introduce infant circumcision to Botswana saying that: "The proposal by Kebaabetswe and colleagues for the introduction of circumcision into Botswana is seriously flawed, and is irresponsible in failing to place the emphasis on safe sex practices. As described here, there are many medical, sexual, psychological, social, human rights, ethical, and legal aspects that must be considered. Reliance on circumcision to prevent HIV transmission is wishful fantasy, and can only result in a calamitous worsening of the HIV-AIDS epidemic."

Bailey *et al.* looked at the possible adverse effects of introducing male circumcision on a public health scale and the post operative satisfaction levels of 380 circumcisions on 18-24 year old consenting men. As to satisfaction; "At 30 days post-surgery, 99.3% of men reported being very satisfied and 0.7% somewhat satisfied with circumcision. None were dissatisfied." And with regard to adverse effects; "All were mild or moderate and resolved within hours or several days of detection." Their findings were presented at the *15th International AIDS Conference* held in Bangkok in 2004.

At the 15th International AIDS Conference in 2004, Connolly *et al.* presented their report detailing the effects of circumcision in South Africa. They reported that, among racial groups, "circumcised Blacks showed similar rates of HIV as uncircumcised Blacks, (OR: 0.8, $p = 0.4$) however other racial groups showed a strong protective effect, (OR: 0.3, $p = 0.01$)." They added "When the data are further stratified by age of circumcision, there is a slight protective effect between early circumcision and HIV among Blacks, OR: 0.7, $p = 0.4$." They conclude that "in general, circumcision offers slight protection." At the same conference, Thomas *et al.* (2004) reported that "male circumcision is not associated with HIV or STI prevention in a U.S. Navy population."

Reynolds *et al.* (2004) found that male circumcision was strongly protective against HIV-1 infection with circumcised men being almost seven times less at risk of HIV infection than uncircumcised men. They further state that: "The specificity of this relation suggests a biological rather than behavioural explanation for the protective effect of male circumcision against HIV-1."

Baeten *et al.* (2005) found that uncircumcised men were at a greater than twofold increased risk of acquiring HIV per sex act when compared with circumcised men. They conclude as follows:

"Moreover, our results strengthen the substantial body of evidence suggesting that variation in the prevalence of male circumcision may be a principal contributor to the spread of HIV-1 in Africa."

At the 2006 Conference on Retroviruses and Opportunistic Infections Quinn *et al.* presented their study, conducted in Rakai, Uganda, which observed a 30% reduction in male-to-female HIV transmission, suggesting some protective effect for the female partner.

Newell and Bärnighausen (2007) also stated there was "firm evidence that the risk of acquiring HIV is halved by male circumcision."

Mishra et al. (2006) used data collected from the Demographic and Health Surveys and found that HIV prevalence was "considerably higher in urban areas and for women, especially at younger ages. Adults in wealthier households, in polygamous unions, being widowed/divorced/separated, having multiple sex partners, and having reported STIs had higher HIV rates than other adults. No consistent relationship between male circumcision and HIV risk was observed in most countries."

Way et al. (2006) also used data from Demographic and Health Surveys in Burkina Faso, Cameroon, Ghana, Kenya, Lesotho, and Malawi and from AIDS Indicator Surveys in Tanzania and Uganda to conduct his study. They found that "With age, education, wealth status, and a number of sexual and other behavioral risk factors controlled statistically, in only one of the eight countries were circumcised men at a significant advantage. In the other seven countries, the association between circumcision and HIV status was not statistically significant for the male population as a whole."

Garenne (2006) has doubts about circumcision's value in reducing HIV, and Talbott (2007), in a controversial paper stated that cross country regression data pointed to prostitution as the key factor in the AIDS epidemic rather than circumcision. World Health Organization AIDS Prevention Team official Tim Farley disagreed with the findings of the paper, while Chris Surridge, PLoS One's managing editor, defended its publication. In 1999 the American Medical Association had stated, "behavioral factors are far more important in preventing these infections than the presence or absence of a foreskin."

If proper hygienic procedures are not adhered to, the circumcision operation itself can spread HIV. Brewer *et al.* (2007) report, "[circumcised] male and female virgins were substantially more likely to be HIV infected than uncircumcised virgins. Among adolescents, regardless of sexual experience, circumcision was just as strongly associated with prevalent HIV infection. However, uncircumcised adults were more likely to be HIV positive than circumcised adults." They concluded: "HIV transmission may occur through circumcision-related blood exposures in eastern and southern Africa."

Van Howe *et al.* criticise the drive to promote circumcision in Africa, asking "Why are circumcision proponents expending so much time and energy promoting mass circumcision to North Americans when their supposed aim is to prevent HIV in Africa? The circumcision rate is declining in the US, especially on the west coast; the two North American national paediatric organisations have elected not to endorse the practice, and the practice's legality has been questioned in both the medical and legal literature. 'Playing the HIV card' misdirects the fear understandably generated in North Americans by the HIV/AIDS pandemic into a concrete action: the perpetuation of the outdated practice of neonatal circumcision."

Connolly *et al.* (2008) found that "circumcision had no protective effect in the prevention of HIV transmission. This is a concern, and has implications for the possible adoption of the mass male circumcision strategy both as a public health policy and an HIV prevention strategy."

Sidler *et al.* (2008) say that using neonatal non-therapeutic circumcision to combat the HIV crisis in Africa is neither medically nor ethically justifiable. Furthermore, promoting circumcision might worsen the problem by creating a false sense of security and therefore undermining safe sex practices. Education, female economic independence, safe sex practices and consistent condom use are proven effective measures against HIV transmission.

Boiley *et al.* (2008) found that the protection of circumcision against STI contributes little to the overall effect of circumcision on HIV.

Men who have sex with men (MSM)

Millett *et al.* (2007) found no association in three major US cities between circumcision and HIV infection among Latino and black men who have sex with men (MSM) . They conclude as follows: "In these cross-sectional data, there was no evidence that being circumcised was protective against HIV infection among black MSM or Latino MSM."

Lagarde (2003) stated that "More than 70% of the non-circumcised men (NCM) stated that they would want to be circumcised if MC were proved to protect against sexually transmitted diseases (STD)." Lagarde cautioned that "Our results strongly suggest that interventions including MC should carefully address the false sense of security that it may provide."

A 2008 meta-analysis of 15 observational studies, including 53,567 gay and bisexual men from the United States, Britain, Canada, Australia, India, Taiwan, Peru and the Netherlands (52% circumcised), found that the rate of HIV infection was non-significantly lower among men who were circumcised compared with those who were uncircumcised. For men who engaged primarily in insertive anal sex, a protective effect was observed, but it too was not statistically significant. Observational studies included in the meta-analysis that were conducted prior to the introduction of highly active antiretroviral therapy in 1996 demonstrated a statistically significant protective effect for circumcised MSM against HIV infection. In response to the meta-analysis by Millett *et al.*, Vermund and Qian note that "circumcision would likely be insufficiently efficient to be universally effective in reducing HIV risk, and will have to be combined with other prevention modalities to have a substantial and sustained prevention effect."

Randomised controlled trials

Africa has a higher rate of adult HIV infection than anywhere else in the world. Three randomised controlled trials were commissioned to investigate whether circumcision could lower the rate of HIV contraction. All 3 were conducted in Africa.

The first study to be published was named ANRS-1265. It was funded by the French government's research agency, Agence Nationale de Recherches sur la SIDA (ANRS) and carried out in Orange Farm, Gauteng in South Africa. The purpose was to test the effect of adult male circumcision on HIV acquisition. The principal investigator was Dr. Bertran Auvert of Versailles University. The study enrolled 3,274 men aged 18–24. The participants were split into 2 equal groups. One group was circumcised straight away; the other group, serving as a control, was to be circumcised 21 months later. 146 of the original participants were found to have HIV at the start of the trial - to avoid stigmatization, they were not excluded. It was planned that all the men would visit the research clinic four times during this 21-month period, and that they would be tested for HIV each time. They were instructed not to have sex for six weeks after the operation, and asked at each clinic visit to provide detailed information about their sexual activity. The circumcision procedure used was the forceps-guided method , carried out by three local general practitioners in their surgical offices. After 17 months, 20 men had contracted HIV in the circumcised group and 49 in the control group. The trial was halted on ethical grounds. The results of the trial were published in November 2005.

The authors said, "Male circumcision provides a degree of protection against acquiring HIV infection, equivalent to what a vaccine of high efficacy would have achieved. Male circumcision may provide an important way of reducing the spread of HIV infection in sub-Saharan Africa."

Williams *et al.*(2006) looked at the potential impact of circumcision on HIV in Africa, based upon the South African RCT, saying that that male circumcision could substantially reduce the burden of HIV in Africa, particularly in southern Africa where the existing prevalence of male circumcision is low and the existing prevalence of HIV is high. More specifically it predicted that if full coverage with MC was achieved in sub-

Saharan Africa over the next ten years, MC could prevent approximately 2.0 (1.1 to 3.8) million new HIV infections over that ten year period and a further 3.7 million in the ten years after that.

The above conclusions drawn from the Orange Farm study have been criticised by Michel Garenne (2006) of the Institut Pasteur. In his critique, published on the PLoS Journal of Medicine, he concludes that: "'male circumcision should be regarded as an important public health intervention for preventing the spread of HIV' appears overstated. Even though large-scale male circumcision could avert a number of HIV infections, theoretical calculations and empirical evidence show that it is unlikely to have a major public health impact, apart from the fact that achieving universal male circumcision is likely to be more difficult than universal vaccination coverage or universal contraceptive use."

Mills and Siegfried (2006) point out that trials that are stopped early tend to over estimate treatment effects. They argued that a meta-analysis should be done before further feasibility studies are done.

The NIAID, part of the NIH, supported two further trials, conducted in Kenya and in Uganda. The primary objectives of these studies were to determine whether adult male circumcision can be administered safely, and whether it would reduce the risk of acquiring HIV infection through heterosexual contact. After an initial HIV screening and a medical exam, eligible men were randomly assigned either to receive circumcision immediately or to wait two years before circumcision. All participants were closely followed for two years to collect information about their health, sexual activity, and their and their partners' attitudes about circumcision; to counsel participants in HIV prevention and safe sex practices; and to check the HIV status of the volunteer. Participants in the Kenyan study were scheduled for six visits over the two-year follow-up, compared with four visits for the Ugandan trial participants. In addition to the study visits, men enrolled in the Kenyan trial were encouraged to receive all of their outpatient health care at the study clinics, which enabled researchers to collect information on the safety of the procedure and the number of other sexually transmitted diseases the men had during follow-up.

The Kenyan trial, also known as the UNIM trial (Universities of Nairobi, Illinois and Manitoba trial), began in February 2002, in Kisumu, Kenya. It was a collaborative effort between U.S., Canadian and Kenyan researchers, led by Dr. Robert Bailey, of the University of Illinois. Also involved were Stephen Moses, University of Manitoba, Jeckoniah Ndinya-Achola, University of Nairobi, and Kwango Agot, UNIM. The trial was funded by the NIAID and the Canadian Institutes of Health Research. This trial enrolled 2,784 men between 18 and 24 years old. The participants were assessed by HIV testing, medical examinations, and behavioural interviews during follow-ups at 1, 3, 6, 12, 18, and 24 months. The circumcision procedure used in the Ugandan trial is known as the sleeve method and takes about 30 minutes. The Ugandan trial used cauterization of the blood vessels to control bleeding and stitches to close the wound. 22 men in the

intervention group and 47 in the control group had tested positive for HIV when the study was stopped on ethical grounds.

The Ugandan trial began August, 2003 in Rakai, Uganda, with 4,996 men aged between 15 and 49 years old. It was led by Drs. Ronald Gray and Maria Wawer of Johns Hopkins Bloomberg School of Public Health and Drs. David Serwadda and Nelson Sewankambo of Makerere University in Kampala, Uganda. The circumcision procedure used in the Kenyan trial was the foreskin clamp method. The Kenyan trial procedure took about 25 minutes and used stitches to control bleeding and improve wound closure. Trained and certified physicians performed the circumcisions in well-equipped operating rooms. Post-operative follow-up visits were scheduled at 24–48 hours, 5–9 days, and 4–6 weeks. HIV testing, physical examination, and interviews were repeated at 4–6 weeks, 6-, 12-, and 24-month follow-up visits. After 24 months, 964 of the original 2387 men of the circumcised men had been retained of whom 22 had contracted HIV. 980 of the 2430 uncircumcised men had been retained of whom 45 had contracted HIV.

Both trials were stopped early on December 13, 2006 on ethical grounds after it found that those belonging to the control group had a greater number of men with HIV than the circumcised group.

On Wednesday, March 28, 2007, the World Health Organisation (WHO) and UNAIDS issued joint recommendations concerning male circumcision and HIV/AIDS. These recommendations are:

- Male circumcision should now be recognized as an efficacious intervention for HIV prevention.
- Promoting male circumcision should be recognized as an additional, important strategy for the prevention of heterosexually acquired HIV infection in men.

Kim Dickson, coordinator of the working group that authored the report, commented:

- Male circumcision "would have greatest impact" in countries where the HIV infection rate among heterosexual males is greater than 15 percent and fewer than 20 percent of males are circumcised.
- WHO further recommends that the procedure must be done by a trained physician.
- Protection is incomplete and men must continue to use condoms and have fewer partners.
- Newly circumcised men should abstain from sex for at least six weeks.

The World Health Organization (WHO) said: “Although these results demonstrate that male circumcision reduces the risk of men becoming infected with HIV, the UN agencies emphasize that it does not provide complete protection against HIV infection. Circumcised men can still become infected with the virus and, if HIV-positive, can infect their sexual partners. Male circumcision should never replace other known effective prevention methods and should always be considered as part of a comprehensive

prevention package, which includes correct and consistent use of male or female condoms, reduction in the number of sexual partners, delaying the onset of sexual relations, and HIV testing and counselling."

Others have also expressed concern that some may mistakenly believe they will be fully protected against HIV through circumcision and see circumcision as a safe alternative to other forms of protection, such as condoms.

Dowsett et al. urged caution over using cirumcision as a HIV prevention strategy saying that there were still questions that needed to be answered: "We need to investigate the effects of those other social and contextual factors that will be in play in real world settings – because the effectiveness of male circumcision will not be generated by the efficacy of the surgery alone." He contrasts the preventative effect of cirucmcision taken from the RCT's (55%) with the preventative effect of condoms (80-90%). He criticises the fact that the trials were not double-blinded - the participants knew their circumcision status and so this could have affected how the men responded behaviourally, psychologically and sexually. He criticised the randomisation measures used in the trial: sexual practices (number of partners, condom use) and sexual health measures (presence of STIs), saying that "Effective measures were not used, and differences related to sexual subjectivity, such as sexual network participation, pleasure preferences, body image, sexual history effects (e.g. abuse), partner preferences (younger, older, peers, groups) and so on were never assessed or analysed." He also asks how the extensive counselling and education might have influenced the participants' sexual activity. He adds that "all participants were subject to regular monitoring (e.g. behaviour surveys, clinical check-ups), which clearly might have enhanced compliance with suggested safety regimes and lowered risk-taking during the follow-up period. Such compliance cannot be guaranteed in real world settings." He also said the trials were subject to the Hawthorne effect.

An interim analysis from the Rakai Health Sciences Program in Uganda suggested that newly circumcised HIV positive men may be more likely to spread HIV to their female partners if they have sexual intercourse before the wound is fully healed. "Because the total number of men who resumed sex before certified wound healing is so small, the finding of increased transmission after surgery may have occurred by chance alone. However, we need to err on the side of caution to protect women in the context of any future male circumcision programme," said Dr Maria Wawer, the study's principal investigator.

Kalichman *et al.* (2007) argue that any protective effects cirucmcision could offer would be partially offset by increased HIV risk behavior, or "risk compensation" including reduction in condom use or increased numbers of sex partners. They note that circumcised men in the South African trial had 18% more sexual contacts than uncircumcised men at follow-up. They also said that because participants were given ongoing risk-reduction counseling and free condoms, it "reduced the utility of these trials for estimating the potential behavioral impact of male cirucmcision when implemented in a natural setting." They also criticised current models for failing to account for increased HIV risk behaviour. Increased HIV risk behaviour would mean more women would be

infected which would consequntly increase the risk of men. It would also mean that non-HIV STI's, which have been assoiated with increased HIV risk, would increase. Green *et al.* (2008) also disagree with using circumcision to prevent HIV, citing similar reasons.

Published meta-analyses, using data from the RCTs, have estimated the summary relative risk at 0.42 (95% CI 0.31-0.57), 0.44 (0.33-0.60) and 0.43 (0.32-0.59). (rate of HIV infection in circumcised divided by rate in uncircumcised men). Weiss *et al.* report that meta-analysis of "as-treated" figures from RCTs reveals a stronger protective effect (0.35; 95% CI 0.24-0.54) than if "intention-to-treat" figures are used. Byakika-Tusiime also estimated a summary relative risk of 0.39 (0.27-0.56) for observational studies, and 0.42 (0.33-0.53) overall (including both observational and RCT data). Weiss *et al.* report that the estimated relative risk using RCT data was "identical" to that found in observational studies (0.42). Byakika-Tusiime states that available evidence satisfies six of Hill's criteria, and concludes that the results of her analysis "provide unequivocal evidence that circumcision plays a causal role in reducing the risk of HIV infection among men." Mills *et al.* conclude that circumcision is an "effective strategy for reducing new male HIV infections", but caution that consistently safe sexual practices will be required to maintain the protective effect at the population level. Weiss *et al.* conclude that the evidence from the trials is conclusive, but that challenges to implementation remain, and will need to be faced.

Estimated impact of circumcision programs

Modelling of the population-level impact of circumcision has shown mixed results. Podder *et al.* found that although circumcision would not eliminate HIV, it would "significantly reduce" the HIV burden in a population, stating that reduction was more effective when circumcision was combined with anti-retroviral drugs than with condoms. Disease elimination was considered feasible when all interventions were combined. Hallet *et al.* predicted "dramatic" reductions in HIV if circumcision were scaled up alongside behaviour change programmes.

Studies of the cost-effectiveness of circumcision programmes have been similarly mixed. Kahn *et al.* studied sub-Saharan African settings with a high or moderate HIV prevalence, reporting that adult circumcision is "likely to be a cost-effective HIV prevention strategy" even when deployed among only a small fraction of the population. The authors concluded that circumcision "generates large net savings after adjustment for averted HIV medical costs". White *et al.* concluded that circumcision "is a cost-saving intervention in a wide range of scenarios of HIV and initial circumcision prevalence but the United Nations Joint Programme on HIV/AIDS/WHO recommended target age group should be widened to include older HIV-uninfected men and counselling should be targeted at both newly and already circumcised men to minimize risk compensation". The UNAIDS/WHO/SACEMA Expert Group on Modelling the Impact and Cost of Male Circumcision for HIV Prevention found "large benefits" of circumcision in settings with high HIV prevalence and low circumcision prevalence. The Group estimated "one HIV infection being averted for every five to 15 male circumcisions performed, and costs to avert one HIV infection ranging from US$150 to US$900 using a 10-y time horizon".

McAllister *et al.* estimated that consistent condom use is 95 times more cost effective than circumcision at reducing the rate of HIV in sub-Saharan Africa; the World Health Organisation states that circumcision is "highly cost-effective" in comparison to other HIV interventions when data from the South African trial are used, but less cost-effective when data from the Ugandan trial are used.

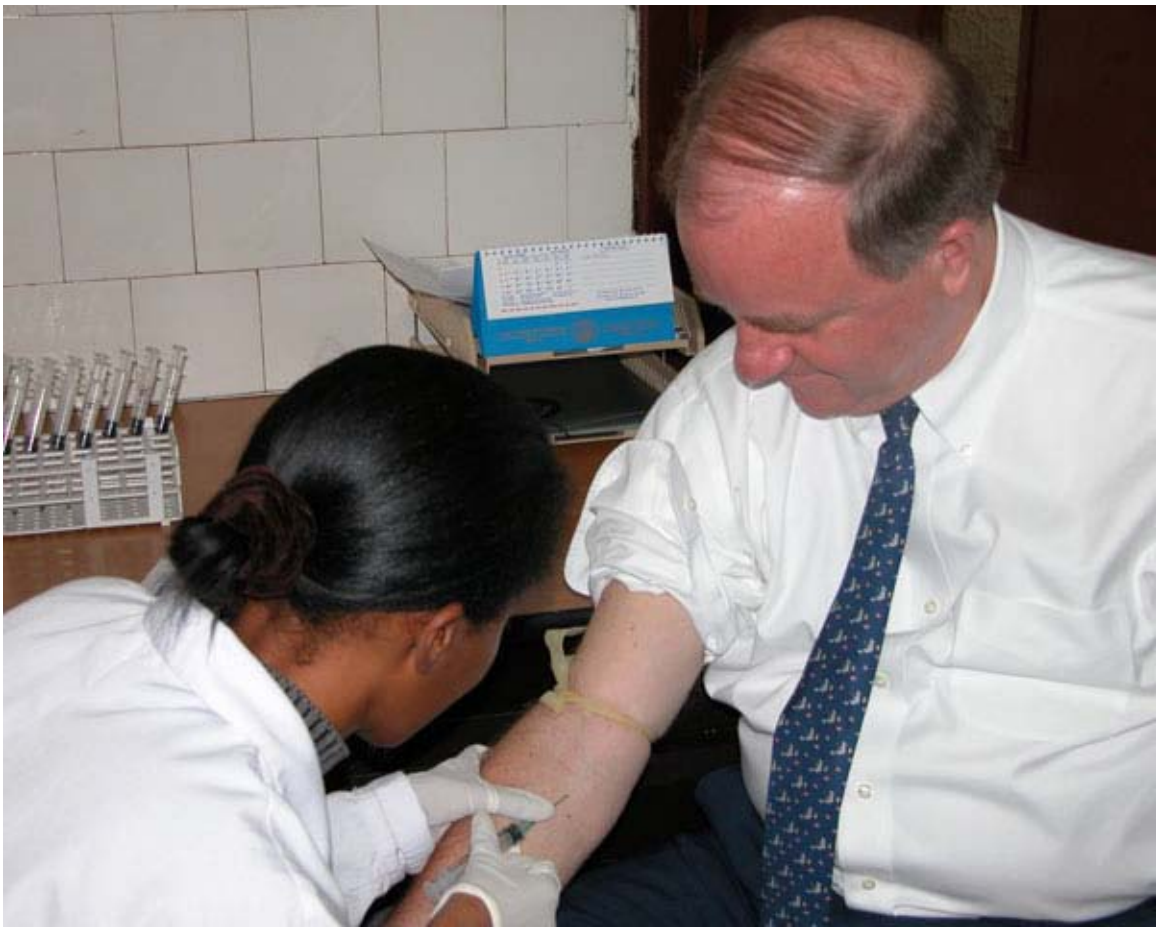
Langerhans cells and HIV transmission

Langerhans cells are part of the human immune system. Three studies identified high concentrations of Langerhans and other "HIV target" cells in the foreskin and Szabo and Short suggested that the Langerhans cells in the foreskin may provide an entry point for viral infection. McCoombe, Cameron, and Short also found that the keratin is thinnest on the foreskin and frenulum. Van Howe, Cold and Storms criticised Szabo and Short's suggestion as "pure speculation". Fleiss, Hodges and Van Howe had previously stated a belief that the prepuce has an immunological function. Waskett criticised their specific hypothesis on technical grounds. A study published in 2007 by de Witte and others said that langerin, produced by Langerhans cells, is a natural barrier to HIV-1 transmission by Langerhans cells.

Dowsett (2007) questioned why it was just males that were being encouraged to circumcise: "Langerhans cells occur in the clitoris, the labia and in other parts of both male and female genitals, and no one is talking of removing these in the name of HIV prevention."

Chapter 15

HIV Test



Randall L. Tobias, former U.S. Global AIDS Coordinator, being publicly tested for HIV in Ethiopia in an effort to reduce the stigma of being tested.

HIV tests are used to detect the presence of the human immunodeficiency virus in serum, saliva, or urine. Such tests may detect HIV antibodies, antigens, or RNA.

Terminology

The **window period** is the time from infection until a test can detect any change. The average window period with HIV-1 antibody tests is 25 days for subtype B. Antigen testing cuts the window period to approximately 16 days and NAT (Nucleic Acid Testing) further reduces this period to 12 days.

Performance of medical tests is often described in terms of:

- sensitivity: The percentage of the results that will be positive when HIV is present
- specificity: The percentage of the results that will be negative when HIV is not present.

All diagnostic tests have limitations, and sometimes their use may produce erroneous or questionable results.

- False positive: The test incorrectly indicates that HIV is present in a non-infected person.
- False negative: The test incorrectly indicates that HIV is absent in an infected person.

Nonspecific reactions, hypergammaglobulinemia, or the presence of antibodies directed to other infectious agents that may be antigenically similar to HIV can produce false positive results. Autoimmune diseases, such as systemic lupus erythematosus, have also rarely caused false positive results. Most false negative results are due to the window period; other factors, such as post-exposure prophylaxis, can rarely produce false negatives.

Principles

Screening donor blood and cellular products

Tests selected to screen donor blood and tissue must provide a high degree of confidence that HIV will be detected if present (that is, a high sensitivity is required). A combination of antibody, antigen and nucleic acid tests are used by blood banks in Western countries. The World Health Organization estimated that, as of 2000, inadequate blood screening had resulted in 1 million new HIV infections worldwide.

In the USA, since 1985, all blood donations are screened with an ELISA test for HIV-1 and HIV-2, as well as a nucleic acid test. These diagnostic tests are combined with careful donor selection. As of 2001, the risk of transfusion-acquired HIV in the U.S. was approximately one in 2.5 million for each transfusion.

Diagnosis of HIV infection

Tests used for the diagnosis of HIV infection in a particular person require a high degree of both sensitivity and specificity. In the United States, this is achieved using an algorithm combining two tests for HIV antibodies. If antibodies are detected by an initial test based on the ELISA method, then a second test using the Western blot procedure determines the size of the antigens in the test kit binding to the antibodies. The combination of these two methods is highly accurate (see below).

Human rights

The UNAIDS/WHO policy statement on HIV Testing states that conditions under which people undergo HIV testing must be anchored in a human rights approach that pays due respect to ethical principles. According to these principles, the conduct of HIV testing of individuals must be

- Confidential;
- Accompanied by counseling (for those who test positive);
- Conducted with the informed consent of the person being tested.

Confidentiality

Considerable controversy exists over the ethical obligations of health care providers to inform the sexual partners of individuals infected with HIV that they are at risk of contracting the virus. Some legal jurisdictions permit such disclosure, while others do not. More state funded testing sites are now using confidential forms of testing. This allows for monitoring of infected individuals easily, compared to anonymous testing that has a number attached to the positive test results. Controversy exists over privacy issues.

Anonymous Testing

Testing that has only a number attached to the specimen that will be delivered for testing. Items that are confirmed positive will not have the HIV infected individual's name attached to the specimen. Sites that offer this service advertise this testing option.

Routine testing recommendation

In the United States, one emerging standard of care is to screen all patients for HIV in all health care settings. In 2006, the Centers for Disease Control announced an initiative for voluntary, routine testing of all Americans aged 13–64 during health care encounters. An estimated 25% of infected individuals were unaware of their status; If successful the effort was expected to reduce new infections by 30% per year. The CDC recommends elimination of requirements for written consent or extensive pre-test counseling, as barriers to widespread routine testing.

Antibody tests

HIV **antibody tests** are specifically designed for routine diagnostic testing of adults; these tests are inexpensive and extremely accurate.

Window period

Antibody tests may give false negative (no antibodies were detected despite the presence of HIV) results during the *window period*, an interval of three weeks to six months between the time of HIV infection and the production of measurable antibodies to HIV seroconversion. Most people develop detectable antibodies approximately 30 days after infection, although some seroconvert later. The vast majority of people (97%) have detectable antibodies by three months after HIV infection; a six-month window is extremely rare with modern antibody testing. During the window period, an infected person can transmit HIV to others although their HIV infection may not be detectable with an antibody test. Antiretroviral therapy during the window period can delay the formation of antibodies and extend the window period beyond 12 months. This was not the case with patients that underwent treatment with post exposure prophylaxis (PEP). Those patients must take ELISA tests at various intervals after the usual 28 day course of treatment, sometimes extending outside of the conservative window period of 6 months. Antibody tests may also yield false negative results in patients with X-linked agammaglobulinemia; other diagnostic tests should be used in such patients.

Three instances of delayed HIV seroconversion occurring in health-care workers have been reported; in these instances, the health-care workers tested negative for HIV antibodies greater than 6 months postexposure but were seropositive within 12 months after the exposure. DNA sequencing confirmed the source of infection in one instance. Two of the delayed seroconversions were associated with simultaneous exposure to hepatitis C virus (HCV). In one case, co-infection was associated with a rapidly fatal HCV disease course; however, it is not known whether HCV directly influences the risk for or course of HIV infection or is a marker for other exposure-related factors.

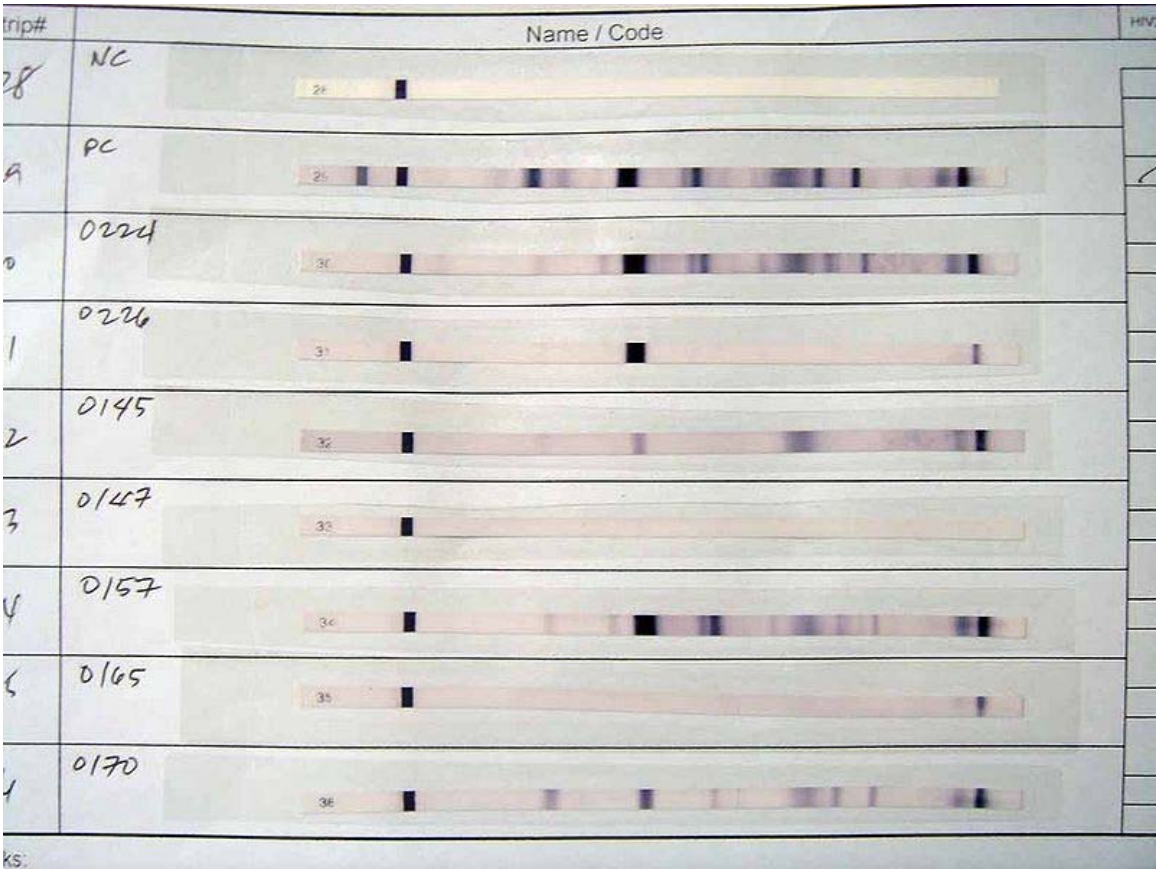
ELISA

The *enzyme-linked immunosorbent assay* (ELISA), or *enzyme immunoassay* (EIA), was the first screening test commonly employed for HIV. It has a high sensitivity.

In an ELISA test, a person's serum is diluted 400-fold and applied to a plate to which HIV antigens have been attached. If antibodies to HIV are present in the serum, they may bind to these HIV antigens. The plate is then washed to remove all other components of the serum. A specially prepared "secondary antibody" — an antibody that binds to human antibodies — is then applied to the plate, followed by another wash. This secondary antibody is chemically linked in advance to an enzyme. Thus the plate will contain enzyme in proportion to the amount of secondary antibody bound to the plate. A substrate for the enzyme is applied, and catalysis by the enzyme leads to a change in color or

fluorescence. ELISA results are reported as a number; the most controversial aspect of this test is determining the "cut-off" point between a positive and negative result.

Western blot



Western blot test results. The first two strips are a negative and a positive control, respectively. The others are actual tests.

Like the ELISA procedure, the western blot is an antibody detection test. However, unlike the ELISA method, the viral proteins are separated first and immobilized. In subsequent steps, the binding of serum antibodies to specific HIV proteins is visualized.

Specifically, cells that may be HIV-infected are opened and the proteins within are placed into a slab of gel, to which an electrical current is applied. Different proteins will move with different velocities in this field, depending on their size, while their electrical charge is leveled by a surfactant called sodium lauryl sulfate. Some commercially prepared Western blot test kits contain the HIV proteins already on a cellulose acetate strip. Once the proteins are well-separated, they are transferred to a membrane and the procedure continues similar to an ELISA: the person's diluted serum is applied to the membrane and antibodies in the serum may attach to some of the HIV proteins. Antibodies that do not attach are washed away, and enzyme-linked antibodies with the capability to attach to the person's antibodies determine to which HIV proteins the person has antibodies.

There are no universal criteria for interpreting the western blot test: The number of viral bands that must be present may vary. If no viral bands are detected, the result is negative. If at least one viral band for each of the GAG, POL, and ENV gene-product groups are present, the result is positive. The three-gene-product approach to western blot interpretation has not been adopted for public health or clinical practice. Tests in which less than the required number of viral bands are detected are reported as indeterminate: a person who has an indeterminate result should be retested, as later tests may be more conclusive. Almost all HIV-infected persons with indeterminate western blot results will develop a positive result when tested in one month; persistently indeterminate results over a period of six months suggests the results are not due to HIV infection. In a generally healthy low-risk population, indeterminate results on western blot occur on the order of 1 in 5,000 patients. However for those individuals that have had high-risk exposures to individuals where HIV-2 is most prevalent, Western Africa, an inconclusive western blot test may prove infection with HIV-2.

Rapid or point-of-care tests

A woman demonstrates the use of the OraQuick rapid HIV test

Rapid Antibody Tests are qualitative immunoassays intended for use as a point-of-care test to aid in the diagnosis of HIV infection. These tests should be used in conjunction with the clinical status, history, and risk factors of the person being tested. The specificity of Rapid Antibody Tests in low-risk populations has not been evaluated. These tests should be used in appropriate multi-test algorithms designed for statistical validation of rapid HIV test results.

If no antibodies to HIV are detected, this does not mean the person has not been infected with HIV. It may take several months after HIV infection for the antibody response to reach detectable levels, during which time rapid testing for antibodies to HIV will not be indicative of true infection status. For most people, HIV antibodies reach a detectable level after two to six weeks.

Although these tests have high specificity, false positives do occur. Any positive test result should be confirmed by a lab using the western blot.

Home Access Express HIV-1 Test is the only FDA-approved home test: the patient collects a few blood drops from a fingerstick, and mails the sample to a laboratory; results and counseling are obtained over the phone. All results are anonymous and confirmed before they are released.

OraQuick is an antibody test that provides results in 20 minutes. The blood, plasma or oral fluid is mixed in a vial with developing solution, and the results are read from a sticklike testing device. Usually detects HIV 1 and HIV 2.

Orasure is an HIV test that uses mucosal transudate from the tissues of cheeks and gums. It is an antibody test that first employs ELISA, then western blot.

Uni-Gold is a rapid HIV antibody test that provides results in 10–12 minutes. A drop of blood is placed on the device with developing solution. Uni-Gold is only FDA approved to test for HIV 1.

Clearview Complete HIV 1/2 and **Clearview HIV 1/2 Stat-Pak** are rapid tests for the detection of HIV 1 and HIV 2 antibodies in blood, serum, or plasma samples. Results are provided within 15 minutes.

There is also a **urine test**; it employs both the ELISA and the western blot techniques.

iDiagnostics Rapid HIV Test is, according only to their website, a non-FDA-approved home test. The company sells a blood test and a urine test produced by InTec PRODUCTS, INC. Similar to a home pregnancy test the patient collects a drop of blood/urine and drops the sample onto a cassette. Results are read visually in 15 minutes. The accuracy of this test has not been confirmed by the FDA, and it is not authorized for sale in the United States.

The INSTI HIV-1/HIV-2* Rapid Antibody Test is a rapid in vitro qualitative test for the detection of antibodies to Human Immunodeficiency Virus Type 1 in human whole blood, serum or plasma. The test is intended for use by trained personnel in medical facilities, clinical laboratories, emergency care situations, and physicians' offices as a screening assay capable of providing test results in less than 60 seconds. The assay is packaged as a kit containing INSTI Membrane Units, Sample Diluent, Color Developer and Clarifying Solution, and is available in point-of-care use packaging, or packaging suitable for laboratory use.

Reveal HIV is a rapid *in vitro* qualitative test for the detection of antibodies to HIV in whole blood, serum or plasma. Reveal is among the fastest rapid HIV test available and it detects signs of early infection better than some other rapid tests. Reveal HIV is approved in Canada, the United States, Europe, Africa, Asia, and South America.

Interpreting antibody tests

ELISA testing alone cannot be used to diagnose HIV, even if the test suggests a high probability that antibody to HIV-1 is present. In the United States, such ELISA results are not reported as "positive" unless confirmed by a Western Blot.

The ELISA antibody tests were developed to provide a high level of confidence that donated blood was *NOT* infected with HIV. It is therefore not possible to conclude that blood rejected for transfusion because of a *positive* ELISA antibody test is in fact infected with HIV. Sometimes, retesting the donor in several months will produce a *negative* ELISA antibody test. This is why a confirmatory Western Blot is always used before reporting a "positive" HIV test result.

Rare false positive results due to factors unrelated to HIV exposure are found more often with the ELISA test than with the Western Blot. False positives may be associated with medical conditions such as recent acute illnesses and allergies. A rash of false positive tests in the fall of 1991 was initially blamed on the influenza vaccines used during that flu season, but further investigation traced the cross-reactivity to several relatively non-specific test kits. A false positive result does not indicate a condition of significant risk to health. When the ELISA test is combined with Western Blot, the rate of false positives is extremely low, and diagnostic accuracy is very high (see below).

HIV antibody tests are highly sensitive, meaning they react preferentially with HIV antibodies, but not all positive or inconclusive HIV ELISA tests mean the person is infected by HIV. Risk history, and clinical judgement should be included in the assessment, and a confirmation test (Western blot) should be administered. An individual with an inconclusive test should be re-tested at a later date.

Accuracy of HIV testing

Modern HIV testing is highly accurate. The evidence regarding the risks and benefits of HIV screening was reviewed in July 2005 by the U.S. Preventive Services Task Force. The authors concluded that:

...the use of repeatedly reactive enzyme immunoassay followed by confirmatory Western blot or immunofluorescent assay remains the standard method for diagnosing HIV-1 infection. A large study of HIV testing in 752 U.S. laboratories reported a sensitivity of 99.7% and specificity of 98.5% for enzyme immunoassay, and studies in U.S. blood donors reported specificities of 99.8% and greater than 99.99%. With confirmatory Western blot, the chance of a false-positive identification in a low-prevalence setting is about 1 in 250 000 (95% CI, 1 in 173 000 to 1 in 379 000).

The specificity rate given here for the inexpensive enzyme immunoassay screening tests indicates that, in 1,000 positive HIV test results, about 15 of these results will be a false positive. Confirming the test result (i.e., by repeating the test, if this option is available) could reduce the ultimate likelihood of a false positive to about 1 result in 250,000 tests given. The sensitivity rating, likewise, indicates that, in 1,000 negative HIV test results, 3 will actually be a false negative result. However, based upon the HIV prevalence rates at most testing centers within the United States, the negative predictive value of these tests is extremely high, meaning that a negative test result will be correct more than 9,997 times in 10,000 (99.97% of the time). The very high negative predictive value of these tests is why the CDC recommends that a negative test result be considered conclusive evidence that an individual does not have HIV.

Of course, the actual numbers vary depending on the testing population. This is because interpreting of the results of any medical test (assuming no test is 100% accurate) depends upon the initial degree of belief, or the prior probability that an individual has, or does not have a disease. Generally the prior probability is estimated using the prevalence of a disease within a population or at a given testing location. The positive predictive value and negative predictive value of all tests, including HIV tests, take into account the prior probability of having a disease along with the accuracy of the testing method to determine a new degree of belief that an individual has or does not have a disease (also known as the posterior probability). The chance that a positive test accurately indicates an HIV infection increases as the prevalence or rate of HIV infection increases in the population. Conversely, the negative predictive value will decrease as the HIV prevalence rises. Thus a positive test in a high-risk population, such as people who frequently engage in unprotected anal intercourse with unknown partners, is more likely to correctly represent HIV infection than a positive test in a very low-risk population, such as unpaid blood donors.

It is important to remember that many studies have confirmed the accuracy of current methods of HIV testing in the United States, reporting false-positive rates of 0.0004 to 0.0007 and false-negative rates of 0.003 in the general population.

Antigen tests

The **p24 antigen test** detects the presence of the p24 protein of HIV (also known as CA), the capsid protein of the virus. Monoclonal antibodies specific to the p24 protein are mixed with the person's blood. Any p24 protein in the person's blood will stick to the monoclonal antibody and an enzyme-linked antibody to the monoclonal antibodies to p24 causes a color change if p24 was present in the sample.

This test is no longer used routinely in the US or the EU to screen blood donations since the objective was to reduce the risk of false negatives in the window period. Nucleic acid testing (NAT) is more effective for this purpose, and p24 antigen testing is no longer indicated if a NAT test is performed. The p24 antigen test is not useful for general diagnostics, as it has very low sensitivity and only works during a certain time period after infection before the body produces antibodies to the p24 protein.

Nucleic acid-based tests (NAT)

Nucleic-acid-based tests amplify and detect one or more of several target sequences located in specific HIV genes, such as HIV-I GAG, HIV-II GAG, HIV-env, or the HIV-pol. Since 2001, donated blood in the United States has been screened with nucleic-acid-based tests, shortening the window period between infection and detectability of disease to about 12 days. Since these tests are relatively expensive, the blood is screened by first pooling some 8-24 samples and testing these together; if the pool tests positive, each sample is retested individually. A different version of this test is intended for use in conjunction with clinical presentation and other laboratory markers of disease progress for the management of HIV-1-infected patients.

In the **RT-PCR test**, viral RNA is extracted from the patient's plasma and is treated with reverse transcriptase (RT) to convert the viral RNA into cDNA. The polymerase chain reaction (PCR) process is then applied, using two primers unique to the virus's genome. After PCR amplification is complete, the resulting DNA products are hybridized to specific oligonucleotides bound to the vessel wall, and are then made visible with a probe bound to an enzyme. The amount of virus in the sample can be quantified with sufficient accuracy to detect threefold changes.

In the **Quantiplex bDNA** or **branched DNA test**, plasma is centrifugated to concentrate the virus, which is then opened to release its RNA. Special oligonucleotides that bind to viral RNA and to certain oligonucleotides bound to the wall of the vessel are added. In this way, viral RNA is fastened to the wall. Then new oligonucleotides that bind at several locations to this RNA are added, and other oligonucelotides that bind at several locations to those oligonucleotides. This is done to amplify the signal. Finally, oligonucleotides that bind to the last set of oligonucleotides and that are bound to an enzyme are added; the enzyme action causes a color reaction, which allows quantification of the viral RNA in the original sample. Monitoring the effects of antiretroviral therapy by serial measurements of plasma HIV-1 RNA with this test has been validated for patients with viral loads greater than 25,000 copies per milliliter.

Other tests used in HIV treatment

The **CD4 T-cell count** is not an HIV test, but rather a procedure where the number of CD4 T-cells in the blood is determined.

A CD4 count does not check for the presence of HIV. It is used to monitor immune system function in HIV-positive people. Declining CD4 T-cell counts are considered to be a marker of progression of HIV infection. In HIV-positive people, AIDS is officially diagnosed when the count drops below 200 cells/μL or when certain opportunistic infections occur. This use of a CD4 count as an AIDS criterion was introduced in 1992; the value of 200 was chosen because it corresponded with a greatly increased likelihood of opportunistic infection. Lower CD4 counts in people with AIDS are indicators that prophylaxis against certain types of opportunistic infections should be instituted.

Low CD4 T-cell counts are associated with a variety of conditions, including many viral infections, bacterial infections, parasitic infections, sepsis, tuberculosis, coccidioidomycosis, burns, trauma, intravenous injections of foreign proteins, malnutrition, over-exercising, pregnancy, normal daily variation, psychological stress, and social isolation.

This test is also used occasionally to estimate immune system function for people whose CD4 T cells are impaired for reasons other than HIV infection, which include several blood diseases, several genetic disorders, and the side effects of many chemotherapy drugs.

In general, the lower the number of T cells the lower the immune system's function will be. Normal CD4 counts are between 500 and 1500 CD4+ T cells/microliter, and the counts may fluctuate in healthy people, depending on recent infection status, nutrition, exercise, and other factors. Women tend to have somewhat lower counts than men.

Criticisms of HIV tests

As a result of an increase in false positive rates with rapid oral HIV testing in 2005, New York City's Department of Health and Mental Hygiene added the option of testing finger-stick whole blood after any reactive result, before using a Western Blot test to confirm the positive result. Following a further increase of false positives in NYC DOHMH STD Clinics during the end of 2007 and beginning of 2008, their clinics opted to forgo further oral screenings, and instead reinsituted testing using finger-stick whole blood. Despite the increase in false positives in NYC DOHMH, the CDC still continues to support the use of noninvasive oral fluid specimens due to their popularity in health clinics and convenience of use. The director of the HIV control program for public health at Seattle King county, reported OraQuick failed to spot at least 8 percent of 133 people found to be infected with a comparable diagnostic test. Strategies implemented to determine quality control and false positive rates were implemented. It is to be understood that any reactive OraQuick test result is a preliminary positive result and will always require a confirmatory test, regardless of the mean of testing (venipuncture whole blood,

fingerstick whole blood or oral mucosal transudate fluid) Several other testing sites who did not experience a spike in false positive rates continue to use OraSure's OraQuick HIV Anti-body Testing.

HIV tests have been criticized by AIDS denialists (a fringe group that believes that HIV either does not exist or is harmless). The accuracy of serologic testing has been verified by isolation and culture of HIV and by detection of HIV RNA by PCR, which are widely accepted "gold standards" in microbiology. While AIDS denialists focus on individual components of HIV testing, the combination of ELISA and Western Blot used for the diagnosis of HIV is remarkably accurate, with very low false-positive and -negative rates as described above. The views of AIDS denialists are based on highly selective analysis of mostly outdated scientific papers; there is broad scientific consensus that HIV is the cause of AIDS.

Fraudulent testing

There have been a number of cases of fraudulent tests being sold via mail order or the Internet to the general public. In 1997, a California man was indicted on mail fraud and wire charges for selling supposed home test kits. In 2004, the US Federal Trade Commission asked Federal Express and US Customs to confiscate shipments of the Discreet home HIV test kits, produced by Gregory Stephen Wong of Vancouver, BC. In February 2005, the US FDA issued a warning against using the rapid HIV test kits and other home use kits marketed by Globus Media of Montreal, Canada.

Chapter 16

HIV Vaccine

An **HIV vaccine** is the theoretical vaccine which would be given to persons without HIV in order to vaccinate them against getting HIV, the virus which causes AIDS. No effective vaccine against HIV exists. As there is not a known cure for AIDS, the search for a vaccine has become part of medical approaches against the disease.

It has been known for many years that HIV is an extremely difficult virus to render harmless, and no cure presently exists. Research into a vaccine is one of several strategies to reduce the worldwide harm from AIDS, with other approaches based upon antiviral treatments such as highly active antiretroviral therapy (HAART), and social approaches such as safe sex.

There is evidence that a vaccine may be possible. Work with monoclonal antibodies (MAb) has proven that the human body can defend itself against HIV, and certain individuals remain asymptomatic for decades after HIV infection. More recently in 2009, a number of potential candidates for antibodies and early stage results from clinical trials have been announced by various teams. However these are early results, and have either not been developed to the point of human testing, or not fully peer reviewed and replicated by other teams, at this time.

Overview

The urgency of the search for a vaccine against HIV stems from the AIDS-related death toll of over 25 million people since 1981. Indeed, in 2002, AIDS became the primary cause of mortality due to an infectious agent in Africa.

Alternative medical treatments to a vaccine do exist. Highly active antiretroviral therapy (HAART) has been highly beneficial to many HIV-infected individuals since its introduction in 1996 when the protease inhibitor-based HAART initially became available. HAART allows the stabilization of the patient's symptoms and viremia, but they do not cure the patient of HIV, nor of the symptoms of AIDS. And, importantly, HAART does nothing to prevent the spread of HIV through people with undiagnosed HIV infections. Safer sex measures have also proven insufficient to halt the spread of AIDS in the worst affected countries, despite some success in reducing infection rates.

Therefore, an HIV vaccine is generally considered as the most likely, and perhaps the only way by which the AIDS pandemic can be halted. However, after over 20 years of research, HIV-1 remains a difficult target for a vaccine.

Difficulties in developing an HIV vaccine

In 1984, after the confirmation of the etiological agent of AIDS by scientists at the U.S. National Institutes of Health and the Pasteur Institute, the United States Health and Human Services Secretary Margaret Heckler declared that a vaccine would be available within two years.

However, the classical vaccination approaches that have been successful in the control of various viral diseases by priming the adaptive immunity to recognize the viral envelope proteins have failed in the case of HIV-1. Some have stated that an HIV vaccine may not be possible without significant theoretical advances.

There are a number of factors that cause development of an HIV vaccine to differ from the development of other classic vaccines:

- Classic vaccines mimic natural immunity against reinfection generally seen in individuals recovered from infection; there are almost no recovered AIDS patients.
- Most vaccines protect against disease, not against infection; HIV infection may remain latent for long periods before causing AIDS.
- Most effective vaccines are whole-killed or live-attenuated organisms; killed HIV-1 does not retain antigenicity and the use of a live retrovirus vaccine raises safety issues.
- Most vaccines protect against infections that are infrequently encountered; HIV may be encountered daily by individuals at high risk.
- Most vaccines protect against infections through mucosal surfaces of the respiratory or gastrointestinal tract; the great majority of HIV infection is through the genital tract.

HIV structure

The epitopes of the viral envelope are more variable than those of many other viruses. Furthermore, the functionally important epitopes of the gp120 protein are masked by glycosylation, trimerisation and receptor-induced conformational changes making it difficult to block with neutralising antibodies.

The ineffectiveness of previously developed vaccines primarily stems from two related factors.

- First, HIV is highly mutable. Because of the virus' ability to rapidly respond to selective pressures imposed by the immune system, the population of virus in an infected individual typically evolves so that it can evade the two major arms of

the adaptive immune system; humoral (antibody-mediated) and cellular (mediated by T cells) immunity.
- Second, HIV isolates are themselves highly variable. HIV can be categorized into multiple clades and subtypes with a high degree of genetic divergence. Therefore, the immune responses raised by any vaccine need to be broad enough to account for this variability. Any vaccine that lacks this breadth is unlikely to be effective.

The difficulties in stimulating a reliable antibody response has led to the attempts to develop a vaccine that stimulates a response by cytotoxic T-lymphocytes.

Another response to the challenge has been to create a single peptide that contains the least variable components of all the known HIV strains.

Animal model

The typical animal model for vaccine research is the monkey, often the macaque. Monkeys can be infected with SIV or the chimeric SHIV for research purposes. However, the well-proven route of trying to induce neutralizing antibodies by vaccination has stalled because of the great difficulty in stimulating antibodies that neutralise heterologous primary HIV isolates. Some vaccines based on the virus envelope have protected chimpanzees or macaques from homologous virus challenge, but in clinical trials, individuals who were immunised with similar constructs became infected after later exposure to HIV-1.

There are some differences between SIV and HIV that may introduce challenges in the use of an animal model.

As published on 27 November 2009 in Journal of Biology, there is new animal model strongly resembling that of HIV in humans. Generalized immune activation as a direct result of activated CD4+ T cell killing - performed in mice allows new ways of testing HIV behaviour.

Clinical trials to date

Several vaccine candidates are in varying phases of clinical trials.

Phase I

Most initial approaches have focused on the HIV envelope protein. At least thirteen different gp120 and gp160 envelope candidates have been evaluated, in the US predominantly through the AIDS Vaccine Evaluation Group. Most research focused on gp120 rather than gp41/gp160, as the latter are generally more difficult to produce and did not initially offer any clear advantage over gp120 forms. Overall, they have been safe and immunogenic in diverse populations, have induced neutralizing antibody in nearly 100% recipients, but rarely induced CD8+ cytotoxic T lymphocytes (CTL). Mammalian derived envelope preparations have been better inducers of neutralizing antibody than

candidates produced in yeast and bacteria. Although the vaccination process involved many repeated "booster" injections, it was very difficult to induce and maintain the high anti-gp120 antibody titers necessary to have any hope of neutralizing an HIV exposure.

The availability of several recombinant canarypox vectors has provided interesting results that may prove to be generalizable to other viral vectors. Increasing the complexity of the canarypox vectors by inclusion of more genes/epitopes has increased the percent of volunteers that have detectable CTL to a greater extent than did increasing the dose of the viral vector. Importantly, CTLs from volunteers were able to kill peripheral blood mononuclear cells infected with primary isolates of HIV, suggesting that induced CTLs could have biological significance. In addition, cells from at least some volunteers were able to kill cells infected with HIV from other clades, though the pattern of recognition was not uniform among volunteers. Canarypox is the first candidate HIV vaccine that has induced cross-clade functional CTL responses. The first phase I trial of the candidate vaccine in Africa was launched early in 1999 with Ugandan volunteers. The study determined the extent to which Ugandan volunteers have CTL that are active against the subtypes of HIV prevalent in Uganda, A and D.

Other strategies that have progressed to phase I trials in uninfected persons include peptides, lipopeptides, DNA, an attenuated Salmonella vector, lipopeptides, p24, etc. Specifically, candidate vaccines that induce one or more of the following are being sought:

- neutralizing antibodies active against a broad range of HIV primary isolates;
- cytotoxic T cell responses in a vast majority of recipients;
- strong mucosal immune responses.

Phase II

On December 13, 2004, the HIV Vaccine Trials Network (HVTN) began recruiting for the STEP study, a 3,000-participant phase II clinical trial of a novel HIV vaccine, at sites in North America, South America, the Caribbean and Australia. The trial was co-funded by the National Institute of Allergy and Infectious Diseases (NIAID), which is a division of the National Institutes of Health (NIH), and the pharmaceutical company Merck & Co. Merck developed the experimental vaccine called V520 to stimulate HIV-specific cellular immunity, which prompts the body to produce T cells that kill HIV-infected cells. In previous smaller trials, this vaccine was found to be safe, because of the lack of adverse effects on the patients. The vaccine showed induced cellular immune responses against HIV in more than half of volunteers.

V520 contains a weakened adenovirus that serves as a carrier for three subtype B HIV genes (*gag* / *pol* / *nef*). Subtype B is the most prevalent HIV subtype in the regions of the study sites. Adenoviruses are among the main causes of upper respiratory tract ailments such as the common cold. Because the vaccine contains only three HIV genes housed in a weakened adenovirus, study participants cannot become infected with HIV or get a respiratory infection from the vaccine. It was announced in September 2007 that the trial

for V520 would be discontinued after it determined that the vaccination was ineffective. The foremost issue facing the rAd5 adenovirus that was used is the high prevalence of the adenovirus-specific antibodies as a result of prior exposure to the virus. Adenovirus vectors and many other viral vectors currently used in HIV vaccines, will induce a rapid memory immune response against the vector. This results in an impediment to the development of a T cell response against the inserted antigen (HIV antigens) Additionally, it appears that V520 may have made some recipients more receptive to infection by HIV-1.

The HVTN expected to finish the study in 2009, but ceased further treatment administration and declared the vaccine ineffective at preventing HIV-infection in September 2007. The results of the trial have caused some to call for a reexamination of vaccine development strategies.

Phase III

In February 2003, VaxGen announced that their AIDSVAX vaccine was a failure in North America as there was not a statistically significant reduction of HIV infection within the study population. This same vaccine was retested in Thailand within a vaccine regimen called RV 144 beginning in 2003, with positive results. In both cases the vaccines targeted gp120 and were specific for the geographical regions. The Thai trial was the largest AIDS vaccine trial to date when it started.

In October 2009, the results of the RV 144 trial were published. Initial results, released in September 2009 prior to publication of complete results, were encouraging for scientists in search of a vaccine. The study involved 16,395 participants who did not have HIV infection, 8197 of whom were given treatment consisting of two experimental vaccines targeting HIV types B and E that are prevalent in Thailand, while 8198 were given a placebo. The participants were tested for HIV every six months for three years. After three years, the vaccine group saw HIV infection rates reduced by more than 30% compared with those in the placebo group. However, after taking into account the seven people who had HIV infections at the time of their vaccination (two in the placebo group, five in the vaccine group) the percentage dropped to 26%.

Planned clinical trials

Novel approaches, including modified vaccinia Ankara (MVA), adeno-associated virus, Venezuelan equine encephalitis (VEE) replicons, and codon-optimized DNA have proven to be strong inducers of CTL in macaque models, and have provided at least partial protection in some models. Most of these approaches are in, or will soon enter, clinical studies.

Economics of vaccine development

A June 2005 study estimates that $682 million is spent on AIDS vaccine research annually.

Economic issues with developing an AIDS vaccine include the need for advance purchase commitment (or advance market commitments) because after an AIDS vaccine has been developed, governments and NGOs may be able to bid the price down to marginal cost.

Classification of all theoretically possible HIV vaccines

Any theoretically possible HIV vaccines must inhibit or stop the HIV virion replication cycle. So, the targets of the vaccine are the following phases of the HIV virion cycle:

- Phase I. Free state
- Phase II. Attachment
- Phase III. Penetration
- Phase IV. Uncoating
- Phase V. Replication
- Phase VI. Assembling
- Phase VII. Releasing

So, the possible approaches for the HIV vaccine are the following (in the bracket specified the *Phases* were it is possible to do).

Filtering of the virions from blood (Phase I)

- Biological approach for removing the HIV virions from the blood.
- Chemical approach for removing the HIV virions from the blood.
- Physical approach for removing the HIV virions from the blood.

Different approaches to catch the virion (Phase I-III, VI, VII)

- Phagocytosis of the HIV virions.
- Chemical or organic based capture (creation of any skin or additional membrane around the virion) of HIV virions
- Chemical or organic attachments to the virion

Different approaches to destroy or damage the virion or its parts (Phase I-VII)

Here, "damage" means inhibiting or stopping the ability of virion to process any of the *Phase II-VII*. Here are the different classification of methods:

- By nature of method:
  - Physical methods (*Phase I-VII*)
  - Chemical and biological methods (*Phase I-VII*)
- By damaging target of the HIV virion structure:
  - Damaging the Docking Glycoprotein gp120 (*Phase I-III, VI, VII*)
  - Damaging the Transmembrane Glycoprotein gp41 (*Phase I-III, VI, VII*)

- Damaging the virion matrix (*Phase I-III, VI, VII*)
- Damaging the virion Capsid (*Phase I-III, VI, VII*)
- Damaging the Reverse Transcriptase (*Phase I-VII*)
- Damaging the RNA (*Phase I-VII*)

Blocking the replication (Phase I)

- Insertion into blood chemical or organic compounds which binds to the gp120. Hypothetically, it can be pieces of the CD4 cell membranes with receptors. Any chemical and organic alternative (with ability to bind the gp120) of this receptors also can be used.
- Insertion into blood chemical or organic compounds which binds to the receptors of the CD4 cells.

Inhibiting process of phases (drugs already used for this approach)

- Biological, chemical or physical approach to inhibit the *Attachment*
- Biological, chemical or physical approach to inhibit the *Penetration*
- Biological, chemical or physical approach to inhibit the *Uncoating* including introducing the mutation into the HIV
- Biological, chemical or physical approach to inhibit the *Replication* including introducing the mutation into the HIV
- Biological, chemical or physical approach to inhibit the *Assembling* including introducing the mutation into the HIV
- Biological, chemical or physical approach to inhibit (capping) the *Releasing*

Methods of the inhibiting of the functionality of the infected cell (*Phase VI- VII*)

Inhibiting the life functions of the infected cell:

- Inhibiting the metabolism of the infected cell
- Inhibiting the energy exchange of the infected cell

Future work

According to Gary J. Nabel of the Vaccine Research Center in Bethesda, Maryland, several hurdles must be overcome before scientific research will culminate in a definitive AIDS vaccine. First, greater translation between animal models and human trials must be established. Second, new, more effective, and more easily produced vectors must be identified. Finally, and most importantly, there must arise a robust understanding of the immune response to potential vaccine candidates. Emerging technologies that enable the identification of T-cell-receptor specificities and cytokine profiles will prove invaluable in hastening this process.