

General Design Considerations and Specifications for a Curative Set of Drugs

Pattern Recognition Tumor Targeting

The consistent and specific cure or control of cancer will require multiple drugs, administered in combination. The drugs must be targeted to abnormal patterns of normal cellular machinery that effect or reflect malignant behavior. Enough patterns must be targeted to address all malignant cell types that could realistically evolve in the patient. Drugs and technologies are needed that enable the detection and destruction of cells, if and only if, the cells express the target pattern. [\[i\]](#)

- Drug specificity must be based on abnormal patterns of normal biomolecules that effect or reflect proliferation and/or invasiveness in an abnormal context.
- Drug specificity must be for the pattern, not the individual biomolecules that comprise the pattern.
- The drugs must not have significant toxicity for cells that lack the target patterns. The drugs should ideally be at least 100 times more toxic to cells that express the target pattern.
- The drugs must kill or irreversibly inactivate cells that express the target patterns.

Target patterns must be comprised of elements characteristic of:

- The combination of proliferation and invasiveness; or
- The combination of invasiveness and the potential for cell proliferation; or
- Invasiveness alone [\[ii\]](#)

- The role of specificity is to enable comprehensiveness without patient toxicity.
- The mechanisms of tumor cell killing must be multiple, redundant and independent.
- The duration of therapy must be sufficiently long to address the episodic nature of the expression of malignant behavior by cancer cells.
- The drugs must kill or inactivate malignant cells at a rate significantly faster than the rate of cancer cell growth.

Absolute specificity is not necessary and not desirable as a design goal. Nonspecific bystander killing can greatly simplify the achievement of comprehensiveness and should be incorporated as a design feature.

- The drugs must be compatible and not interfere with each other.
- The drugs must either be given concurrently in combination or sequentially with close enough timing to preclude the emergence of drug resistance.
- The drugs should be suitable for long term, continuous, IV infusion.
- The drugs should be chemically stable.
- The drugs should have low allergenicity and be nonpyrogenic (not cause fever)
- The drugs should preferably be non-carcinogenic.
- The drug should have a large margin of safety.
- The set of drugs in combination must be safe.
- The set of drugs should be effective against all forms of solid cancer.
- The drugs should be modular in design.

All of these points need elaboration, but we will only focus on the most important issues. The nature of invasiveness imposes critical constraints on the design of a curative set of anticancer drugs. The machinery that executes invasiveness can reside on tumor cells and/or in the tumor cell environment. Therefore, comprehensiveness requires a set of drugs that kill tumor cells regardless of whether the targeting elements of invasiveness are present on tumor cells or in the environment.

This implies a requirement for drug targeting approaches that generate a zone of anticancer activity in the local volume that surrounds the targeting elements of invasiveness.

The component functions of malignant behavior, proliferation and invasiveness, are generally not synchronous. They occur at different times, and only intermittently. This, combined with the low percentage of cells that are actually replicating at a given time in most tumors, imposes an important practical constraint on the design of anticancer drugs. The drugs must be effective regardless of whether the cancer cells are actively proliferating or not, at the time of drug exposure. This implies that the molecular targets of the drugs must be present essentially throughout the cell cycle. It should be noted that this conclusion is diametrically opposite to conventional wisdom. Most anticancer drugs have been targeted to only actively replicating cells. Such drugs are inefficient. Only a small fraction of cancer cells engage in proliferation at a given point in time.

There are two good solutions to deal with the separation in time between the expression of invasiveness and proliferation:

- If target pattern specificity is for invasiveness alone [iv], then deliver effector agents that are toxic for cells in general. [v] In this case, the cancer cells will be killed regardless of whether they are actively proliferating or not.
- Target invasiveness and the potential for proliferation. Deliver effector agents to the target pattern that specifically and irreversibly arrests both the potential for cell proliferation and cell proliferation. Most normal cells in the body lack the potential for cell proliferation. Most normal cells don't have the required machinery and are programmed not to be able to acquire the machinery needed to divide. [vi] By contrast, all malignant cells have the potential to proliferate.

Drugs that can specifically and irreversibly arrest both cell replication and the potential for cell replication are known. Examples include certain minor groove binding DNA cross-linking agents. Cross-linking agents chemically connect adjacent strands of DNA together. This blocks cell replication. DNA strands must separate for DNA replication to occur. If such cells try to replicate cell death occurs. Cells have evolved elaborate mechanisms to repair DNA damage. However, certain types of drugs can cause lesions that cannot be repaired.

MCM proteins

An evolutionarily conserved set of proteins, called MCM proteins, is an excellent marker for cells that have the potential for cell proliferation. These proteins surround parts of the cellular DNA like a ring around a finger. MCM proteins are involved in DNA licensing and are absolutely required for cell proliferation. Most cells in the body do not have MCM proteins and lack the capacity to divide. All malignant cells must express MCM proteins in order to be malignant.

A drug that irreversibly binds to MCM proteins and inhibits MCM function would be very valuable as an effector agent. MCM proteins are perhaps the ideal marker for cells that have the potential to proliferate. Targeting all MCM positive cells would result in fatal toxicity. But that's not what we're talking about. We're talking about killing only MCM positive cells that are involved in invasive processes. It's an engineering problem that is well within the scope of existing science and technology to develop an MCM targeted agent. We do not think that an MCM-targeted drug is absolutely needed to cure cancer. Delay in development should not compromise the bigger program. But an MCM-targeted agent would likely be of tremendous clinical value and could be ideal for the cure of brain cancers and could simplify the cure of cancer in general.

Drug penetration into tumors

A major area that must be addressed is the poor penetration of drugs into tumors. If the cancer drugs can't reach the tumor cells they cannot kill them. There are significant physical barriers to the penetration of drugs into tumors, including chaotic and inadequate blood perfusion, high fluid pressure within tumors, protein binding, and impairment of diffusion by the extracellular matrix. [vii] The extracellular matrix is a protein and carbohydrate scaffolding that surrounds and supports cells. Bulk tumor and localized lesions can be surgically resected or treated with local therapy. However, even small lesions, (0.1 to 0.2 mm thick) can present substantial barriers to drug penetration. [viii] Many anticancer drugs have failed simply because they could not reach the tumor cells at high enough concentrations.

There are no really good solutions to the problem of limited and uneven drug penetration into tumors. The physical properties (i.e. molecular weight) of drugs are constrained by chemical requirements that must be satisfied to achieve specificity and antitumor activity. Using high drug concentrations will increase the rate of drug penetration into tumors, but will also increase drug exposure to normal tissues and increase toxicity. The problem is not at the level of drug escape from blood vessel into tumors. The leakiness of tumor blood vessels is well documented. [ix] The problem is due to the limited and uneven blood flow to tumors and the slow rates of drug diffusion. Neither is amenable to major manipulation. We cannot change the laws of physics. Just as the Wright Brothers had to deal with gravity we must accept and address the problem of poor drug penetration into tumors.

Time is the key

The best and probably only solution is to eliminate altogether the rate of drug penetration into tumors as a factor that defines clinical activity. Time is the key. Given sufficient time the rate of drug diffusion becomes unimportant. [x] It is therefore necessary to devise drugs and treatment strategies that enable prolonged drug treatment, without significant patient toxicity. A prolonged treatment time is also required for the comprehensive detection of malignant cells, as malignant behavior can be episodic. What were talking about is the being able to give drugs continuously for perhaps as long as six months. Pumps suitable to the task are routinely used by diabetics. But the drugs must be sufficiently safe for long-term intravenous infusion. (The task of making the drugs suitable for administration by mouth so complicates drug design that it is not worth doing. Efforts in that direction would delay by years the development of a cure for cancer.)

Intensity of therapy

Clinically significant malignant behavior, (proliferation and invasiveness) occurs at the interface between tumor and non-tumor tissues, at the invasive fronts, and in the proximity of blood vessels and lymphatic channels. Lymphatic channels are like highways that tumor cells can take to reach the lymph nodes. Tumor cells that remain deep within the bulk of a tumor and distant to a blood supply are destined to death. The metabolic rate of the tumor cells determines the thickness of the cuff of viable tumor cells surrounding blood vessels, but according to Dr. Folkman's work it rarely exceeds 0.2 mm. [xi] Contrary to conventional

wisdom, to cure cancer there is no need for drugs to penetrate rapidly and deeply into tumors. The important pathology that sustains malignancy occurs within a limited zone around blood vessels (or other blood conduits as in the case of vasculogenic mimicry. [xii])

As a practical matter, cancer therapy must be sufficiently intense so that the rate of tumor cell death exceeds the rate of tumor cell growth. In most tumors the rate of cell replication is just slightly greater than the rate of cell death. As a result even though most tumor cells are replaced about every five days, it generally takes several months for tumors to double in size. Computer simulations predict that very small changes in the probability of tumor cell survival (PCS) can have an enormous impact on the rate of tumor growth. [xiii] If $PCS < 0.5$ there will be tumor shrinkage, a $PCS = 0.51$ will give slow tumor growth (doubling times of months), a $PCS = 0.57$, typical of Burkitt's Lymphoma will give a doubling time of approximately three days. The message is quite clear. A very minor but sustained decrease in the probability of tumor cell survival per cell division can have a profound impact.

Peeling the onion

To address the issue of poor drug penetration into tumors requires the development of approaches in which drugs are given continuously for prolonged periods of time. Cancer cells that engage in malignant behavior, and that are exposed to the drugs will be killed. This in turn will expose new layers of malignant cells to the drugs. The process can be likened to peeling away layers of an onion. You cannot use this type of approach today because current cancer drugs are too toxic for prolonged use. Some other approaches that can be used to increase the zone of cell death caused by anticancer drugs are described later. However, prolonged treatment times will still be required.

Inhibition of angiogenesis

It should be noted that by targeting a comprehensive set of patterns characteristic of proliferation and invasiveness (or invasiveness alone), the drugs will arrest angiogenesis, vasculogenic mimicry and the co-option of existing blood vessels. [xiv] These are the three mechanisms by which tumors can acquire a blood supply. Drug penetration will not be an issue. These processes occur close to existing blood flow. This will deprive tumors of new sources of blood supply, constrain tumor size, and provide time for the prolonged course of drug therapy to completely eradicate the malignant cells. There will be a dual effect: cancer cell death from lack of oxygen and cancer cell death from the toxic drugs delivered to the malignant cells.

There is a requirement for PRTT in order to consistently and specifically starve tumors of their blood supply. The machinery that cancer cells can use to secure a blood supply is identical to the machinery that cancer cells can use to engage in malignant behavior. The requirements to completely shut of the ability of tumors to acquire a blood supply are exactly the same as that of our hypothetical cancer-curing machine. Identical. Shutting of angiogenesis alone is not sufficient. Melanoma cells grow very well in the brains of mice treated with angiogenesis inhibitors. Unable to form new blood vessels the melanoma cells just grow along existing vessels. [xv]

There may be a single factor or set of individual factors like VEGF that are required for and specific to angiogenesis, but no such factor exists for vascular co-option.

Vascular co-option is fundamentally an invasive process of malignant cells. Patterns must be targeted to arrest vascular co-option.

The need for improved drug delivery technologies

To be able to give multiple drugs continuously for prolonged periods of time, without significant patient toxicity, requires markedly improved drug delivery technologies. Today you cannot give 5 to 10 anticancer drugs, each at effective doses, for months on end. It would kill the patient. This problem needs to be addressed in the drug design.

Regardless of the technologies employed to achieve pattern recognition tumor targeting, the drugs must localize to the tumor and tumor cells. The efficiency of drug delivery and drug retention are among the most important factors that define antitumor activity. It is therefore instructive to examine theoretically perfect, ideal drug targeting, so that the underlying principles can be incorporated into drug design.

Ideal drug targeting

Perfect drug targeting would involve the specific and irreversible retention of all drug molecules that contact the tumor without drug accumulation at non-tumor sites. In the theoretically perfect case, the tumor would act like a black hole for the drug. Although, in practice this cannot be achieved, we can develop technologies that approach the limits of ideal targeting. The room for improvement and potential clinical benefits are huge. With improved drug delivery technologies, the total effective dose of anticancer drugs required to treat patients could be reduced thousands to millions of times. [xvi] Clinical toxicity and unpleasant side effects would be expected to decrease proportionately.

The anticancer drugs will generally localize to tumors by binding to one or more receptors. Drug binding to a receptor can be viewed like a key fitting into a lock. At a given time, only a small fraction of tumor associated target receptors may be drug accessible due to limitations of drug penetration into tumors. Some areas will be near the blood supply and bathed in drug. Other tumor areas will be poorly perfused by blood and receive little or no drug.

Currently, chemotherapy is given to patients at high concentrations to try to force drug into poorly perfused areas of the tumor. The entire tumor is regarded as the target. This approach may be best for conventional chemotherapy, but is not suited to our goal of approaching ideal drug delivery.

To approach ideal drug targeting, focus should be on maximizing drug delivery to the drug accessible target receptors and minimizing drug deposition elsewhere in the body. With long enough treatment times, all tumor associated target receptors will eventually become drug

accessible. This is a new way of thinking about the problem. On a given day our target is not the entire tumor. Our target is just that part of the tumor that drug can easily and freely reach after escaping from the tumor blood vessels. We want to give just enough drug to bind the drug accessible target receptors and kill the drug accessible tumor. Over a long enough period of time drug will eventually get to all the malignant cells. As tumor cells are killed new layers of cancer cells will become drug accessible

An upper limit to the rate of tumor drug delivery is provided by the average concentration of the drug in the blood times the rate of blood flow into the tumor. The rate of tumor blood flow varies but is within the approximate range of 0.15 to 0.6 ml/min/ gram (or ~ ml) of tumor. [xvii] This implies that 1 ml of tumor tissue is perfused with approximately 200 to 800 ml of blood per day. This means that given perfect targeting, (complete drug retention) and a 24 hour time period, the drug concentration in the tumor can become ~ 200 to 800 times greater than the average blood concentration. Typical potential doubling times for tumors are on the order of magnitude of 5 days. [xviii] Cell production is almost balanced by the rate of cell death so that actual tumor doubling times are typically several months. This implies that with ideal targeting and prolonged infusion times, about 1000 to 4000 fold increase in drug concentration within the (drug accessible) bulk tumor, over the average blood concentration, is the maximum theoretically possible.

Viewed from another perspective, with perfect drug targeting, as little as 7 ml of (drug accessible) tumor could clear all drug from the entire volume of blood in the body within 24 hours.

In principle, perfect drug targeting is unattainable. However, many examples of almost theoretically perfect targeting exist in nature. Nerve gas provides an ugly, but compelling man made example of nearly perfect, theoretically ideal targeting. Infinitesimally small amounts of nerve gas can be rapidly fatal because the toxic chemical binds rapidly, specifically and irreversibly to receptors in the brain and nervous system. The receptors act like a black hole for the nerve gas molecules. The underlying chemical principles are known and can be adapted to targeting anticancer drugs.

Approaches towards the goal of perfect drug targeting involve:

- Employing drugs that bind specifically, and very tightly (or irreversibly) to their target receptors. We want drugs that after binding to their target receptor stay bound for a long time.
- Administering these drugs, in the smallest quantities sufficient to saturate drug accessible target receptors
- Decreasing nonspecific drug binding and nonspecific cellular uptake
- Increasing the quantity of drug receptors that specifically trap drugs within the tumor (exponential pattern recognition targeting)
- Prolonged treatment times

The chemical principles that can enable drugs to bind tightly and remain bound to receptors are well understood. Approaches include: high energy, single-site binding; multivalent binding, tight-slow-binding, and covalent binding. Applications of these approaches will be discussed in more detail in later sections.

Nonspecific drug binding

Many drugs engage in extensive, nonspecific, protein binding. Nonspecific binding can dramatically decrease the concentration of free drug in solution, alter drug distribution and change drug uptake patterns. To the extent possible, this issue should be addressed by the optimization of drug design. An additional approach is to administer at high concentrations, nontoxic displacer agents that competitively inhibit nonspecific drug binding. In most cases existing drugs with low toxicity and established safety profiles can probably be found that will be suitable displacer agents. If not, the design of such agents poses no special challenge. The issue of non-specific binding is all too frequently neglected in cancer drug development.

In summary

It is a solvable engineering problem within the scope of existing science and technology to develop drugs and drug delivery approaches that approach theoretically perfect drug targeting. The maximum theoretically possible tumor drug concentration is about one thousand times greater than the time-average blood concentration. [xix] It should be noted that the biological result can be enormously magnified if the antitumor activity is a multiplicative or nonlinear function of the concentrations of the drugs. For example, systems can be designed where a ten times higher drug concentration results in a million times more cell killing power.

Basic processes in drug action

Medicinal chemistry or the chemistry of drugs is a very complicated subject. There are an unlimited number of different chemical structures and potential drug structures. Having said that, there are only a few types of things that a drug molecule can do:

- It can dissolve in a solvent.
- It can bind to a receptor.
- It can be chemically modified or changed.
- It can act as a catalyst and trigger a chemical reaction.
- It can do combinations of the above.

Components of a drug molecule can engage in similar processes. The art and science of drug design is about using these basic processes to make a molecular machine that can carry out a desired goal. For PRTT the goal is to detect a pattern of proteins and then cause cell death.

Pattern recognition tumor targeting requires drugs that are able to detect multiple inputs corresponding to the individual components of the pattern, and kill cells if and only if the

complete pattern is detected. The methods of PRTT involve multi-functional drugs in which different components endow distinct properties and functionality. The different components or modules are like Lego™ pieces.

Key modular components of pattern recognition targeting technologies include:

- **Targeting ligands:** Chemical groups that specifically bind to a target receptor
- **Triggers:** Chemical groups that can undergo specific biotransformation and change the chemistry and functionality of the drug. A trigger is like a switch that can turn on or off.
- **Linkers:** Relatively inert components that connect different groups in a molecule. Linkers are like steel beams of a building, they provide support and structure to the drug.
- **Effector agents:** Groups that exert the desired pharmacological effect such as cell killing
- **Male ligands and female adaptors:** Chemical groups that can bind specifically and tightly to each other in a lock and key like manner.

Additional functionality can be added in a modular fashion to address issues such as drug solubility or drug uptake into cells. These modular components exist or can be developed using well-established technology. I am not talking about things that involve the need for major new scientific discoveries or technological advances. The pieces largely exist. What is novel is putting the pieces together to target patterns of proteins.

Modularity of drug design and standardized chemistries for connecting the modules can greatly increase the efficiency and economics of drug development. Functional components can be individually developed and optimized. The functional properties of modules within the whole drug will undoubtedly differ from that of sub-assemblies of modular components, so additional rounds of optimization will be required. Modifications can be made to individual modular components to optimize each final drug. Modularity in drug design also allows for separation between function and specificity. Targeting specificity can be varied in a modular fashion. In addition, the key modular components can be used in a number of different PRTT technologies.

A standardized Lego™ like modular approach to drug design and synthesis should markedly decrease the time and cost required to develop a set of drugs for the cure of cancer. Currently, each new drug is individually created from scratch. The modular approach can allow the same template to be used on multiple different drugs merely by varying key modular components.

Chemistry is a reasonably mature science

The fundamental principles are well understood. It all comes down to thermodynamics or laws about energy change. Using deductive logic, it is usually possible to make highly reliable quantitative predictions about the properties and reactions of particular chemical structures. The techniques to test these predictions also generally exist. The interactions of

drugs with biological systems can be difficult to accurately predict, but are easy enough to study experimentally. Today it is generally not a problem to synthesize chemical compounds. It may take longer than one would like. It may cost more money than one would like, but synthetic chemistry is almost always up to the task. Anyway in designing drugs, one can usually utilize chemical structures that are known and that will not pose major synthetic problems.

The basic chemistry of PRTT is simple. With just a few modular components one can design drugs that will detect patterns and kill cells if and only if the pattern is detected. There are a variety of different approaches available. By deductive logic from established principles of chemistry we can be confident in the basic designs. However, there is no substitute for building, testing, correcting errors and optimizing the designs based on empirical data. This is what engineering is all about. However, just as the designers of the Hoover Dam knew from basic principles the dam would hold back its lake Meade water load, so too we can know that PRTT drugs can be successfully developed. We do not mean to minimize the amount of work involved. We anticipate that it will take about five years and a coordinated multi-institutional, multi-disciplinary effort to develop 5 to 10 drugs targeted to patterns. This is a significant project. But this is what nature demands of us in order to develop therapy that can consistently and specifically cure or control cancer.

A variety of Pattern Recognition Tumor Targeting technologies are available including:

- Targeted delivery of a targeted toxin
- Independently targeted synergistic agents
- Targeted, Trigger-Dependent Agents
- Multivalent PRTT
- Exponential PRTT

Although presented as distinct technologies, a single drug can incorporate features from several of these PRTT technologies.

Target patterns will generally be comprised of two or three different proteins. There are only a couple of different ways that a drug can detect a protein. The drug can bind to the protein in a lock and key like manner. Alternatively, if the protein is an enzyme, then it can activate a trigger on the drug. [xx] In designing a drug to target a particular pattern of proteins, one must decide which mechanisms of detection will be employed, protein binding, trigger activation, or both.

Targeting ligands are the modular components of the drug that bind to the protein receptors. A different targeting ligand is needed for each type of protein receptor. It is like needing a different key for different locks. Targeting ligands exist for many of the important proteins involved in proliferation and invasiveness. Much of traditional drug development over the years involved the development of targeting ligands. Very powerful technologies have been developed to identify and optimize targeting ligands. As in the case of synthetic

chemistry, it may take more time than we like, it may cost more money than we like, but existing technology is almost always up to the task of providing us with suitable targeting ligands that can bind tightly and specifically to a protein receptor.

Triggers are the modular components of the drugs that are activated by enzymes or other agents. A different trigger is needed for each different enzyme. Although there can be modularity in trigger design. The same basic trigger structure can be used for many different enzymes. Varying a modular component of the trigger can change enzyme specificity. Much of biochemistry is about enzymes and the reactions they catalyze. There is a tremendous base of knowledge in the area. Many of the important enzymes involved in proliferation and invasiveness have been extensively studied. We know what the chemical requirements are for a trigger to be acted upon by the enzymes. From a practical point of view, the enzymatic reactions that are of interest in PRTT design all result in the cleavage of a chemical bond. Using well-established principles of chemistry and known chemical reactions the enzymatic cleavage of a chemical bond can be used to trigger all kinds of useful results. Again this is not new science. Triggers and the chemistry of triggers are well known in the field of medicinal chemistry. Techniques to develop highly specific and useful triggers exist today.

Linkers are modular components of the drug that connect the different parts together. Linkers are like a scaffolding or skeleton for the drug. Linker chemistry is absolutely routine. Methods for chemically connecting different modules to the linker system are routine. There are subtleties to linker design. As in all aspects of drug design there are specifications that the linkers must meet. But this is all just ordinary engineering.

Effector agents are the modular components that actually carry out the final desired drug result. Generally this will be killing of the cancer cells. There are thousands of known drugs that can be used as effector agents to kill the cancer cells. Killing cells is easy. The hard part is killing just cancer cells. That's what PRTT is designed to do. Effector agents do not need to be toxic drugs. In some situations the effector agent may serve another function that contributes to pattern targeting or that amplifies the response. For example, an effector agent can be delivered that makes the tumor look like a bacterial infection to the immune system. The net result will be a massive immune attack against the tumor cells. This is discussed in more detail later. My point now is that effector agents exist. It is a well define engineering task to select a suitable set to deliver to the tumor using PRTT.

Male ligand and female adaptors also exist and will be discussed in a later section.

Footnotes and References

[i] Glazier, A.; "Pattern Recognition Tumor Targeting"; [Transactions of the Integrated Bio-medical Informatics and Enabling Technologies Symposium \(TIBETS\) \(2004\) 1:61-78](#)

[ii] As a practical matter, invasiveness can serve as an excellent marker for malignant behavior provided that the drugs are not used during periods of physiological invasiveness such as wound healing.

[iii] This is a highly conservative estimate as it ignores the fact that many tumor cells are not malignant and lack clonogenic potential.

[iv] As a practical matter, invasiveness can serve as an excellent marker for malignant behavior provided that the drugs are not used during periods of physiological invasiveness such as wound healing.

[v] An effector agent is the part of the drug that causes a desired pharmacological effect like cell death.

[vi] Normal cells that do have the potential to proliferate like bone marrow stem cells are vital to life.

[vii] Stohrer M, Boucher Y, Stangassinger M, Jain RK. "Oncotic pressure in solid tumors is elevated."; [Cancer Res. 2000 Aug 1;60\(15\):4251-5](#)

Jain RK.; "Delivery of molecular medicine to solid tumors: lessons from in vivo imaging of gene expression and function."; [J Control Release. 2001 Jul 6;74\(1-3\):7-25](#)

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[viii] Kyle AH, Huxham LA, Chiam AS, Sim DH, Minchinton AI.; "Direct assessment of drug penetration into tissue using a novel application of three-dimensional cell culture."; [Cancer Res. 2004 Sep 1;64\(17\):6304-9](#)

[ix] McDonald DM, Baluk P.; "Significance of blood vessel leakiness in cancer."; [Cancer Res. 2002 Sep 15;62\(18\):5381-5](#)

[x] The mechanism is not drug penetration into tumors, but rather the onion peeling effect described below.

[xi] Folkman , J.; "Angiogenesis and apoptosis."; [Semin Cancer Biol. 2003 13\(2\):159-67](#)

[xii] Folberg R, Maniotis AJ. "Vasculogenic mimicry."; [APMIS. 2004 Jul;112\(7-8\):508-25](#)

[xiii] Moore GW, Berman J.; "Cell growth simulations predicting polyclonal origins for 'monoclonal' tumors."; [Cancer Lett. 1991 Nov;60\(2\):113-9.](#)

[xiv] Folkman J; "Angiogenesis and apoptosis"; [Semin Cancer Biol. 2003 Apr;13\(2\):159-67](#)

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[xv] Leenders WP, Kusters B, Verrijp K, Maass C, Wesseling P, Heerschap A, Ruiters D, Ryan A, de Waal R.; "Antiangiogenic therapy of cerebral melanoma metastases results in sustained tumor progression via vessel co-option ."; *Clin Cancer Res.* 2004 Sep 15;10(18 Pt 1):6222-30.

[xvi] For example, five hundred molecules of bleomycin delivered into a cell can be lethal to the cell. A patient with advanced cancer may have 10^{12} tumor cells. With perfect targeting approximately 5×10^{14} would be required to treat the patient. In practice, patients are treated with bleomycin at doses hundreds of thousands of times higher.

Pron G, Mahrour N, Orlowski S, Tounekti O, Poddevin B, Belehradec J Jr, Mir LM.; "Internalisation of the bleomycin molecules responsible for bleomycin toxicity: a receptor-mediated endocytosis mechanism."; *Biochem Pharmacol.* 1999 Jan 1;57(1):45

[xvii] Brix G, Kiessling F, Lucht R, Darai S, Wasser K, Delorme S, Griebel J. "Microcirculation and microvasculature in breast tumors: pharmacokinetic analysis of dynamic MR image series."; *Magn Reson Med.* 2004 Aug;52(2):420-9

[xviii] Rew DA, Wilson GD.; "Cell production rates in human tissues and tumours and their significance. Part 1: an introduction to the techniques of measurement and their limitations."; *Eur J Surg Oncol.* 2000 ;26(3):227-38.

Rew DA, Wilson GD.; "Cell production rates in human tissues and tumours and their significance. Part II: clinical data."; *Eur J Surg Oncol.* 2000 ;26(4):405-17.

[xix] This is an estimate of the average value. Local concentrations within parts of the tumor may be higher.

[xx] An enzyme is a protein that catalyzes a specific chemical reaction. The enzyme makes the reaction proceed faster. In many cases the reaction may not occur at all in the absence of the enzyme.