Chapter 10

Three Smart Chemotherapies

Oncology 101

I once attended a seminar at Sloan-Kettering Cancer Center on diet and life span. The speaker showed the curves comparing mice and human. Except for the time, the curves under various conditions such as the incidence of cancer etc. are extremely similar. He showed that under starvation diet for mice, which cannot be performed for human, the mice would live twice as long, or equivalent to a human of over 160 years. Under laboratory control and kept relatively healthy, the mice eventually all die of cancer. I wonder if any human being would want to live very long under starvation diet and then die of cancer. A recent drug, Resveratol, currently under clinical trials by Glaxo Smith Klein, is chemically similar to what contained in the skin of red grapes, may offer to enhance the expression of certain survival genes that may be triggered by the starvation, may deliver some health benefits without the forced starvation.

Since the human genome project, we have learned a great deal about our 32,000 genes. From that genome database, perhaps around 200 are onco (tumor) genes, and many of them are really onto (fetal) genes. Dr. Thomas often liked to give the following example; when a fertilized mouse egg starts to replicate, from one to two to four to eight, we can break this eight-cell cluster into eight individual cells and insert them into the uterus of eight mice under IVF procedure and they will yield eight identical mice. This growing of eight cells from one involves a tote potential replication, which is typically a form of tumor growth. That is, without such a form of replication, life as we know it could not get started. I like to give a more shocking example. As each of our cells contains a complete set of DNA except for certain immune genes, which we ourselves assemble mostly during our childhood, why not grow a placenta on the tip of our nose? You would say that's ridiculous. Why not? Well, to figure out why not would lead us to realize that most of our genes are not expressed, and those expressed genes are not expressing all the time or in all tissue types. A good example is the fetal genes, which are not allowed to express beyond certain fetal stage. Control of genes, mostly to prevent them from expressing, therefore, is

really the name of the game. As we get older, the control mechanisms become weaker and imperfect, so that a great variety of forbidden expression becomes active and the cancer incidence increases. The starved, long living mice finally all succumbed to cancer, all. This is Oncology 101.

My Initiation in Cancer

Indeed cancer is a most dreadful disease. I got involved in the topic because my former teacher Professor Tang, a foremost giant in philosophy and oriental studies, got lung cancer. At that time, my wife was a member of the clinical review committee at Sloan-Kettering Cancer Center, and she could offer no special treatment or suggestion. I started to learn tumor biology in a hurry, and starting from the elements of the periodic table, came up with radioactive bromine to replace a methyl (CH_3) of a DNA base. This bromine would do an atomic inner shell ionization by "k-capture", initiating an "Auger cascade", and deliver ~10^8 Rad or megaGray in a radius under 10 atomic diameter. Such a high dose with an exceedingly small damaging sphere would do absolutely no harm everywhere in the cell except in its DNA (later I learned that it will also disrupt the lysosome if it occurs there). Most important, uptake of the compound can also be made tumor specific for certain cancers. I presented this idea to Dr. Frederick Seitz, then the president of the Rockefeller University and chairman of the Sloan-Kettering Research Institute.

"What do you think of this approach?" I asked.

"I like it." he answered.

"Would you please take it?"

"No."

"Anything wrong with my idea?"

"I don't see any thing wrong."

"I don't want money, fame, or anything, I just want it developed."

"I know."

"You have so many professionals, why don't you want to do it?"

"You must do it." he answered.

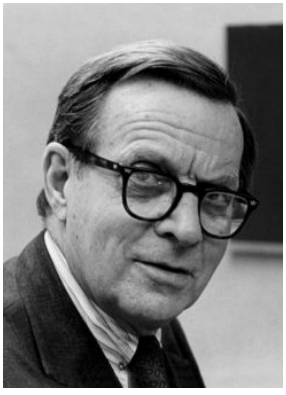
"But I have never taken a class in biology, nor do I know much about chemistry; I taught astronomy and did work in solid state physics, I know nothing in the biomedical field."

The thought of my leading a field of which I know absolutely nothing sounded ridiculous. Now looking back, I rarely work on anything based on knowledge existing in the field. Perhaps that explains why I failed so often but did obtain a few breaks here and there amongst the failures.

"You are young, you can change, and I will support you", said Dr. Seitz. And this started a deep friendship and association of over 30 years.

Dr. Seitz sent me to see Lewis Thomas, then the CEO at Sloan Kettering. Dr. Thomas made me his consultant and issued me a badge to allow me run around the Institute to find possible collaborations. He called my approach "Nuclear Chemotherapy". It could deliver a heavy dose exclusively to the tumor gene, not to kill the cell, only to disrupt the gene sufficiently to keep it from replication.

Nuclear Chemotherapy was the first of my three attempts, but the work, the Smart Chemo (1), was interrupted for reasons other than science and technology. Its story will be told later in this Chapter.



Dr. Lewis Thomas

As a consultant to Dr. Thomas, then the CEO of Sloan-Kettering, I mainly

worked there with two groups, one was the medical physics department and the other was with Dr. Lawrence Helson, an attending pediatrician whose lab happened to be on the same floor I was working and we have since become partners and have jointly done several patent disclosures.

Gus Kinzel tried to be helpful for my work at Sloan and asked me to see if there were any issues or items desired by the research staffs. I asked around and found that medical physics wanted to have a high field MRI and the department head wanted to be selected to join the National Academy of Engineering, of which Gus was the founding chairman. I made an appointment for Gus to visit the department. As Gus went to see the department head, the first sentence he uttered was "May I have the honor to invite you to join my Academy?". Shortly after, every few days the department secretary would send me materials to update the resume.

During the few years I worked at Sloan, I befriended a few professors across the street at the Rockefeller University. One was Dr. Stanford Moore, who was trained as an aeronautical engineer, but shared a Nobel Prize with Stein in protein chemistry. He wanted to hear stories in astronomy and could not have enough of my isotope enrichment schemes. The other was Bruce Merrifield, who had basal carcinoma on the face because he had too much X-ray exposures as a teenager to treat his acmes of the face. Basal carcinoma was the first case I tried to treat, or suggested to treat, as a real disease. I suggested the use of 5FU, but it was absolutely useless for Merrifield, then I suggested ^{77}BrdC and ^{77}BrdU mixed in skin cream with 10^6 Gray from Auger electrons, but he worried that it might induce more malignant mutation. In the process, Bruce and I became close friends. Then for a period of a couple of years I avoided talking to him because he won a Nobel Prize in Chemistry. When asked why, I told him that as an old friend, I should not "crowd in". But two years had passed and the crowd had quieted down, and I wanted him to join our board. He said that he absolutely hated such activities, and had refused many such invitations. But precisely because of this reputation, I wanted him to make an exception and he agreed. Upon joining the board, he did, however, give me the most trouble; no matter what I did technically, it was not good enough to meet his satisfaction. Then our chairman Dr. Seitz made a comment that during the Second World War, a well known statement was that "Enemy of good is the best". Good implies the product or effort is useful, but the best implies to do something not attainable, and therefore becomes the enemy. This statement made Merrifield realizing the difference between academic endeavor and industrial work, and modified his standard toward our development.

Conventional Chemotherapy

Therapy with chemical agents, or chemo, may or may not terminate most of the transformed tumor populations. There are more than 500 known kineases that can be activated in a cell, and each chemoagent could only target a group of kinases or pathways and often the cell could find an alternative kinase/pathway to survive the chemo. This is particularly true for more primitive cells where more genes can be expressing and the out-of-controlled, transformed activities are far more "aggressive" with respect to metastarsis in establishing new colonies. Differentiated tissues typically have a well detifined set of vital kinases/pathways according to their functions, as a result, they can be more sensitive to a particular group of chemoagents. But in general, there is no chemoagent that can deliver a chemo-surgery to a tissue or organ and as a result could eliminate all transformed cells originating from that tissue or organ. By combining several chemoagents to function in synergy to target a particular set of transformed cells, carries with it the greatly increased toxicity to all the normal cell population that must survive, at least with some large fraction from such an enhanced treatment. This is the "side effects" which must not overwhelm the patient. Note that as the side effects would greatly weaken the patient, if the first wave of conventional treatment is not sufficiently successful, most patients would have difficulty to undergo a second wave of similar treatment when the body is much weakened.

An obvious indication of killing fast growing cells under chemotherapy is the lost of hair because hair follicles have a relatively short cell cycle. Two decades ago a nurse applied an ice pack to the blood uptake position of the head to greatly reduce the temperature and thus the metabolic rate of the hair follicles and could reduce much of the patient hair loss. It's not practiced today because of the reduction of chemoagent uptake can include the targeted transformed follicle cells. In reality, the shortest cell cycles are the immune cells so that patients under chemotherapy to hit the class of fast growing cells are usually compromised in immunity. Also cells in the digestive system such as the gastro-intestinal lining tissues which have a relative short cell cycle and the common side effects of chemotherapy include nauseating and vomiting because of the harm done to the GI epidermoid tissues under chemotherapy and need to be repaired and re-grown before returning to their normal functions. These side effects are particular pronounced for chemotherapies for leukaemia, lymphoma such as the Hodgkin's disease etc. where most of the targets for chemoagents are the fastest growing cells. By forcing the relatively slow growing tissues to enter the G_0 state, the side effects of the chemotherapy can be further reduced.

Chemo with Booster

The Helson Couple

My oncology associate Dr. Larry Helson is well known to use relatively heavy dose on the chemotherapeutic agents at the beginning of the treatment. Typically the patients prior to the treatment are in fairly good physical condition and can tolerate the harsher poisons much better than those whose body had already undergone various treatments. In fact, most "five-year survival" patients who survive through the initial batch of treatment and can be considered as cured. Patients often do not survive the "patch-up" treatments that follow the poor initial reponse because by then, the weakened body can sustain only a much reduced harshness with a rather poor prognosis statistically. Dr. Helson has been highly enthusiastic on a chemo-booster compound "curcumin" in conventional chemotherapy. Under most chemoagents, the drug tries to reach the tumor population hanging on the G_2/S stage and can usually be reached with toxic DNA analogs as if they were normal DNA material. But empirically the tumor cells have several survival pathways such as AKT, STAT3, P53, BCL2, BCLXL, NFKB, etc to survive. These are the concerns against which the oncologist-patient team effort must win over. There are specific drugs trying to block these pathways, particularly the NFKB (nuclear factor kappa B), which is one of the more important survival pathway that many drugs are designed to block it. But to his amazement, curcumin would reach and block all these pathways while it is benign to normal cellular functions. He is working with a medical group who tried 40 different pancreatic cell lines and found all 40 responded strongly to curcumin. Unfortunately, curcumin as a pill cannot readily be absorbed by the GI. They are developing an injectable version pending FDA approval. In the

meantime, the pill, curcumin with bioperine, can be obtained from "Life Extension" of Florida. I am not advocating here which chemoagent for what cancer treatment or certain group of kinases/pathways can best be dealt with by which compound, only trying to address a different aspect of tumor management that may drastically reduce the side effect.

While Larry treated patients, Christiana, directed an *invitro* lab and maintained a nude mice facility. This couple was the most productive team in oncology for over 40 years.

Blind Molecular Oncology

A recent hot issue is individualized gene therapy. Clinicians would ideally tailor the drugs to treatments according to our genes. I found it to be an unscientific wishful hope. To begin with, we have not, and are unlikely to find, an effective "vector" that could inject a desired gene into a tissue cell that could travel through the body fluid, go into the cell, and become functional. A typical gene has ~8,000 base pairs, and we can assume that 0.5% of these are "hot points" where a mutation would deform the protein the gene directs. Also assume that there are only 100 oncogenes, with some of them specific to cancers in certain tissues, and some of them common to most transformed cells, and allowing 40 mutations to each of them, then the total probability becomes $(100)^{40}$. This number unfortunately, is 100 times larger than the number of atoms in the universe, or ten times larger if the dark matter is included. Assume one atom can deal with one set of possible transformed cancer gene diseases; there are not enough atoms in the universe to deal with the job.

I love to see people work harder and try harder, now with this example of messy tech approach, try harder in messy technology of this scale is not good enough. The only solution to such a messy and difficult endeavor is to find a solution in simple tech.

Rationale to Use Cell Cycle and G_0 for Smart Chemo (2)

While it is not feasible to find all possible mutated genes and replace them base-by-base, it is possible to ask why the normal cells behave normally. Normal replicating cells evolve through a cell cycle which can usually be followed like the hands of a clock; going to G_1 stage at 3 o'clock, to Synthesis S stage at 6 o'clock, to G_2 stage at 9 o'clock and to Mitosis M stage at 12 o'clock where the daughter cells would emerge. When the chromosomes of the cells are assaulted,

by radicals from radiation or from foreign chemicals, the edges of the phosphorus spine of the DNA double helix would change in size and there are enzymes to sense such a change. Since the double helix is composed of complementary DNA bases, any damaged base can be cutout with its complement part remaining, and a new base inserted to fill the void in seconds. This repair is very effective for the double helix to maintain its integrity and stability. But if the damage is too large statistically, the repair mechanism will be overwhelmed if, for example, thousands of Rads are received in a short duration, and the cell will die. For a substantial repair, the cell would go to G_0 stage (1 o'clock at the cell cycle), there it will remain for 20-40 hours. During the stay at G_0, the cell is busy checking and repairing its possible chromosome damage and will not enter the DNA synthesis, the S state (6 o'clock), and prepare DNA material for its daughter cells. And this allows us a window of low uptake to apply the toxic DNA as chemoagent. A large majority of chemoagents are indeed analogs of DNA that can be used in this rationale. As cancer cells would replicate non-stop, they use more DNA material than the normal counterpart, and this higher DNA uptake gives the rationale for most chemoagents to be simply the toxic DNA. All cells will prefer existing DNA instead of synthesizing it. By sending normal cells to the G_0 stage and void the uptake of DNA-based chemoagents, the tumor cells, disobeying the cell cycle rules, would skip the G_0 stage to continue in S stage and be killed more exclusively.

Thanks to the researches in cell biology, we now begin to understand which gene could be triggered by what chemical or gene and send the cell to G_0. Similarly a light dose of radiation will force the G_0 entry. The key here is that 'to kill" is relatively easy, but to kill without harming the normal population is most important. Since the normal cells can be "primed", so to speak, therefore, the therapeutic research should focus on priming the normal replicating cell population as a vital aspect of the treatment procedure, and not just focus on killing the tumor cells.

Protocol with G_0

More specifically, the procedure of cancer therapy for solid tumors has been historically first surgery, followed by radiation and chemo if the surgeon deem necessary. But after surgical removal, the rapid growth of tumor in the surgical cavity often render the subsequent chemo and/or radiation un-effective. This was due to an inhibitive "tumor factor". As a result, the sequence of treatment becomes altered, using chemo to first shrink the tumor mass prior to surgery, and if necessary, then radiation and additional chemo. In the use of the above

mentioned "G_0 forced entry strategy", the sequence should include the priming of the healthy cells first in order to reduce the common side effects of chemoagents, then the chemo under the window of the G_0-stage with appropriate *in vivo* duration of the particular chemoagent to allow it leaving the body promptly so that as normal cells exit from the G_0 stage, the harsh chemo agents have largely departed and will exert minimal effects to the treatment. Note that while normal replicating populations under radiation assault would move to the G_0 stage, most transformed populations would get hanged at the G_2/S position and become particularly sensitive to those chemo agents directed at the S-phase uptake. This is an added therapeutic enhancement in this procedure. Under this procedure for minimal side effects, the chemo-dose and frequency could be much increased, and thereby delivering an enhanced therapy.

How to "prime" the normal replicating cell populations to enter the G_0 stage? There are two strategies, external and internal. The external one is to seek out genes such as P53, P22, P76 etc. that are known to control the cell cycles and the precursor genes or compounds necessary to force the activations of the key genes. Any drug that would induce the normal replicating populations to enter the G_0 stage will most likely be linked with these precursor genes or compounds. The internal strategy is to simply do a low dose total body radiation and make use of the known cellular repair functions – being damaged under ionizing radiation, the cells would enter G_0 for a duration to repair and recuperate. Time duration of staying in this G_0 period, however, would vary, and must be determined empirically organ-by-organ as a function of radiation dose, and then correlate the useful G_0 window with the list of biological time of various chemoagents in the body. In fact, such a well focused clinical trial can probably be carried out fairly easily and in a short time because we do have a great wealth of radiological dose/damage/repair data already in the literatures.

Nuclear Chemotherapy (1), a Silver Bullet Reaching the Transformed Gene

Now the story on nuclear chemotherapy. There are 4 DNA bases in two similar pairings, A/T and G/C. One of the oldest chemoagent is 5FU, which is the replacement of hydrogen of the T at the 5^{th} position with fluorine, making the analog of T far more reactive so that when the kinase takes it to assemble as part of a string, this base would not decouple with the kinase, will exhaust the kinase and sometimes kill the cell in the process. Similar to F, bromine Br can also be coupled to the U at its 5^{th} position. But Br is a larger atom, and has almost exactly the same ionic radius of a methyl (CH_3). Unlike 5FU, BrdU is absolutely non-toxic; it can be inserted massively into the DNA structure without "harm".

In fact, certain bacteria can be so used to BrdU that they will die when fed by real DNA. Also because bromine is a heavy atom, BrdU has been tagged for its heavy weight to separate the DNA from other cellular components under centrifugation.

Next we describe a few schemes in molecular engineering. First, instead of BrdU, we use BrdC where its top position of the base is oxygen instead of hydrogen and allow Br to remain in the 5^{th} position like a T or U while the 5^{th} position of C is a hydrogen atom. In short, it becomes neither a T nor C so that the cellular apparatus of mammalian cells would unlikely take it up. But some primitive genes such as those of Herpes virus are less discriminating and will incorporate it.

In a typical viral disease, such as having a cold, the virus enters the cells of certain tissues, kills the cells and produces tens of thousands of viral particles. For a virus to take command of the cellular apparatus and make massive numbers of copies using the cellular material is a very complicated procedure. Often this viral infection is aborted before it can kill the cell. Now if the viral DNA has already been integrated with our genome but unexpressed, there is some chance this DNA would enter our gene pool and become the random DNA material that constitutes a major fraction of our chromosomes.

In a transformed cell, many of the unexpressed background genes, sometimes including the well known Herpes genes, for example, become active. This also explains that once the cell becomes transformed and out of control of its expressing, the cellular characteristics or even morphology can undergo drastic alterations under chemotherapy, because often certain chemoagents would kill cells with only a certain set of parameters, and rarely could the transformed cells evolve back to normal.

Now that BrdC can possibly be incorporated with certain transformed cells, we need to make this BrdC molecule terribly toxic, which is not difficult. We make the Br isotope radioactive with an inner shell ionization. (A k-electron captured by an excited proton of its nucleus, and such a bromine atom can be made from Arsenic with a proton insertion under a cyclotron, leading to an Auger cascade.)

Under an Auger cascade, the inner most k-void (ionization) will be filled by a L-electron, with the L/K energy difference absorbed by a L-electron,, which will leave the atom. In the process there are now two L-voids, as two M-electrons could fills these voids, and the M/L energy difference is absorbed by two M-

electrons, which will also leave the atom thereby creating 4 M-voids, etc. Under such a cascade, all orbital electrons will leave the atom with Auger electrons at 12-18eV each. Summed over all these soft ionizing electron energies over a very small sphere, the dose calculated in Rad (or Gray that equals to 100Rad) per cm^3 becomes 10^8Rad, or 5 orders higher than the kilorad range for cell kill. But the usual radiation dose covers the whole cell for a kill. In this Auger dose, it is so localized, having an effective range of only a fraction of the thickness of a cellular membrane, and therefore cannot even cause the cell to be leaky, much less to kill. But if this dose occurs in the midst of a DNA double helix, it will most likely damage the paired DNA base locally, and as both the complementary DNA units are damaged, the replication of the DNA helix in this section will be stopped.

Dr. Lawrence Helson, an attending oncologist at Sloan specialized in neuroblastoma. He had a nude mouse lab as well as extensive neuroblastoma cell lines. We tried the ^{77}BrdC on 10 of his blastoma lines, and found two of the 10 lines would stop replicating. The effected cells, however, were not killed, as they still stuck to the flask surface, only their replication process is terminated, as anticipated.

My Discovery at Sloan-Kettering

I like to tell a story that was perhaps my biggest discovery while working at Sloan. The radioactive Bromine ^{77}Br was made from Arsenic by a cyclotron facility in UK. ^{77}Br has a "half-life" of only sixty some hours. That is, half of this material will be lost in this half-life and so every hour counts. Each Friday, I would drive to JFK airport to a special terminal to get a small lead enclosed glass jar after showing my identification, come back to Sloan and typically work through the weekend to prepare the ^{77}BrdC for *in vitro* and mouse injection during the following week. Radioactive materials are highly controlled substances regardless of how small an amount and how quickly it decays away. Usually when I returned to Sloan, the registration office for incoming material was already closed, and during the follow week, the material is already in use biologically and is not registered either. On one Saturday night while we were intensely synthesizing the ^{77}BrdC, Helen Woodward appeared. Dr. Woodward was a very dedicated medical physicist. Born in 1900 from a wealthy family, she had never drawn a salary and was above any administrative management. She was in charge of nuclear materials and had the power to close down any laboratory. When learning of what we were doing, she was extremely supportive of what we intended to achieve, but warned us that we must register our nuclear

material from the UK in her office even though the quantity was very small and all material had decayed and disappeared. Since that visit, she came almost every weekend night inquiring about our experiments and asked us to makeup all the registration paper work. I did not follow her order and finally she wanted to close our lab.

"Indeed, your experiments are most interesting. But I simply cannot allow you to carry on without registration. You have promised me many times to make up the paperwork and this is absolutely the last warning." She said.

"I need only two more weeks." I begged, as we were working around the clock.

"I don't care. No registration, no experiment." She insisted.

I ran out of excuses, did not have time, and could not shake her loose. All her attention was for me to makeup the paper work. Suddenly, I saw some roaches crawling in the chemical ventilation hood. There was water running in the hood and it had various radioactive materials stored behind lead bricks. This is common to all the ventilation hoods in the research labs, and roaches simply make a good use of this human-free environment. They could follow the water lines, moving from one lab to the next, from one floor to another, and even from one building to the next building.

"Helen, I bet you those reaches are hot." Hot implies radioactive here.

"Nonsense." She answered.

"Helen, I am serious, I bet you those roaches are hot." I insisted.

Being a good scientist, she had to verify. She caught a few roaches and measured them. The meter went off the scale. She caught more roaches in the hospital, they were all hot. She went to another hospital, they were also hot. Now she wanted to shut off all the hospitals, a mission far more interesting than our little experiment. But that battle was well beyond the power of the person responsible for registration radioactive materials. We did finish our experiments without being shut down. Hot roaches were my biggest discovery during the period.

With some limited success to deliver the dose to only certain specific transformed genes and stop the cancer behavior without killing them, we felt we

could move to Phase II, the animal studies. Unfortunately the liver of the mice kept on stealing the deoxy-sugar of our compound BrdC and render the compound non-specific to cells transformed or not. We wanted to by-pass the liver, but the blood supply to the liver of mice is too small to be by-passed, so we needed to treat dogs with a liver bypass. But the surgeons for dogs wanted $50k per dog, a budget well beyond what we could afford.

Lysosome as Target for Smart Chemotherapy (3)

Instead of using Auger cascade of BrdC to stop certain DNA from replicating, the same rationale can also be applied in lysosomes. There are certain non-toxic compounds like Rose Bengal, for example, a compound for red food coloring. It contains 4 Iodine atoms, and this compound would join the body fluid and enter cells. The uptake has a higher rate for tumor cells perhaps because of their relatively more leaky plasma membrane. Upon entering the cell, the molecules will be channeled to the lysosomes that contain mainly HCl acid. As such normal cellular routine could easily deal with this unwelcome foreign chemical. Now with the I-containing molecules in the lysosomes, we can shine a special X-ray to induce a resonant absorption of the iodine atoms, using the enhanced absorption at the K-edge energy level to create the K-shell ionization and the subsequent Auger cascade for a very high but localized dose. Such a dose would disrupt the small apparatus such as lysosome membranes, disrupt them all at once and kill the cell. Most chemo cannot be delivered locally. In this case, we use the cellular apparatus to do the chemo and deliver it with an aimed special X-ray beam. I once described this rationale to a venture investor who showed much interest and had a MD training. He preferred my X-ray induced chemo far more than the possible cure with the G_0 arrest because "there is no money in the cure". I was horrified by such a statement and could not let him be involved in any of my inventions, money or no money. Our special X-rays have been described in Chapter 5.

CHAPTER TEN REFERENCES

Dr. Lewis Thomas, in a series of poems for the New England Journal of Medicine, compiled into a book "Life of a Cell, Notes of a Biology Watcher" Penguin, Publisher. This book won the 1975 National Book Award in Sciences and Letters.

CHAPTER TEN KEYWORDS

Auger *in situ* Radiation
Cell Cycle
Chemo Therapy
G_0-Gate Chemotherapy
Localized Chemotherapy
Lysosome-based Chemotherapy
Nuclear Chemotherapy
Radiation-induced G_0 Arrest