A novel method to eliminate side effects of chemotherapy: Successful elimination of bone marrow & other toxicities of methotrexate and vinblastine

K. N. Matsumura, M.D., Christi L. Ober, B.S., M.D., Scott Hsu-Storaker, B.S., & Masa Rikimaru, Ph.D.*

Alin Foundation, Berkeley, CA (U.S.A.) 94704-2062

Summary. We have developed a method for eliminating toxic side effects of cancer drugs. Without side effects, we can use extremely high doses of the cancer drugs to eradicate cancer. In our method, we use a chemical which is an antidote to a cancer drug. Virtually all drugs have their antidotes. We have discovered a way to deliver the antidote to normal dividing cells without delivering the antidote to cancer cells. Normal dividing cells are usually harmed by cancer drugs, but if we continue to deliver the antidote to normal dividing cells, we can use high curative doses of cancer drugs without causing the usual side effects such as vomiting, diarrhea, and destruction of the blood-making bone marrow. To accomplish the selective delivery of antidote to only normal dividing cells, we place the antidote in specially-designed dendro-microspherules. The antidote-loaded microspherules are given intravenously about the time the cancer drug is administered.

DISABLING SIDE EFFECTS too often accompany cancer and AIDS chemotherapies and cause patients to give up hope. Some abandon their struggle and choose death. Cancer might be curable if side effects of anti-cancer drugs did not prevent their use in sufficient dose and duration. Similarly, viruses may finally become treatable by drugs if we can learn how to eliminate drug toxicity to bone marrow and other normal cells. Indeed, many effective drugs that are sitting abandoned on chemists' shelves because of their toxicity may be restored as valuable therapeutic agents when methods are found to reduce their side effects.

Side effects result from insufficient focus of drug effects. For example, while killing cancer cells, cancer drugs destroy other normal, dividing cells in the body, such as those in the bone marrow and the gastrointestinal tract. Cancer and viral drugs act by interfering with cell division; however, the drugs do not distinguish the cell division in cancer or viral cells from the cell division in normal cells; all dividing cells are affected.

*Address communications to Dr. Matsumura at Alin Foundation Institute, One Alin Plaza, 2107 Dwight, Berkeley, CA 94704-2062 (USA)

Until recently, cancer research, including work in our laboratory, has sought to achieve greater focus of drug action by attempting to selectively deliver the drug only to its target cells. Some spent many years attaching cancer drugs to monoclonal antibodies raised against elusive marker on cancer cells, all the while worrying that a few cancer cells lacking the marker would escape being killed and survive to kill the patient.

We have since developed a different way to focus drug effects. We decided to investigate the possibility that drug side effects could be suppressed if we developed a reliable, broadly applicable method for delivering the drug's antidote preferentially to any normal body cells that are undesirably affected by the drug. In work spanning much of the past two decades, we experimented with different means of delivering antidotes exclusively to the body cells needing protection. While the normal body cells are protected, the drugs would be able to destroy tumors and viruses, which are not protected.

We have successfully tested our approach for reducing bone marrow and other toxicities of methotrexate and vinblastine, in many animals, especially rats and rabbits. For example, we have been able to show that our method preserves erythrogenic marrow activity and reduces toxic effects of methotrexate on leukocytes even when methotrexate is used in high dosages that usually cause the virtual destruction of the erythrogenic marrow. We have also prevented the severe weight loss, diarrhea, and near total granulocytopenia that accompany the use of near-lethal doses of vinblastine. Maintaining the circulating granulocyte level is essential because low levels have been the primary reason for the termination of potentially lifesaving chemotherapy. Our studies show that while marrow damage and other undesirable effects are suppressed, our approach does not interfere with the killing of tumor cells by the cancer drugs. Huge tumors in rodents have been completely eradicated with a never-before tried protocol using doses of vinblastine so high that most animals receiving such dosages without protection of our microspherules become sick and must be euthanized. This paper deals mainly with experimental findings on work done with hundreds of rats. More recently, similar methods have been conducted with rabbits and mice, and human trials have started.

Generally, in our method, an antidote to a cancer drug, for example, folinic acid for methotrexate (MTX) or monosodium glutamate (MSG) for vinblastine, is encapsulated in dendro--microspherules. Dendro-microspherules are tiny spherules of lipids containing water and any drug dissolved in the water. Our microspherules are made from non-toxic chemicals which individually are already FDA-approved. We have succeeded in developing several ways to deliver the microspherules preferentially to normal dividing cells including specially-designed proprietary microspherules.

 © Copyright, 2010. All Rights Reserved

To allow time for the delivery of the antidote to targeted normal cells, antidote-containing microspherules are injected intravenously as much as 24 hours prior to the administration of a toxic cancer drug. When a cancer drug such as methotrexate is administered daily, or when a single high dose of an oncolytic such as vinblastine is used, a cytocidal concentration of the oncolytic is sustained in the extracellular fluid for many days. Microspherule administration is continued on a daily basis for as long as cytocidal blood levels of the oncolytic are maintained. Experiments were conducted at least in triplicates, and usually repeated a number of times varying the conditions of the experiments slightly.

To test the efficacy of our side effect-reducing method, four weeks old Wistar male rats were given high doses of methotrexate, some with and some without protective microspherules, and were evaluated for evidence of toxicity suppression. Methotrexate (1 mg/kg intraperitoneally daily for four days) causes significant bone marrow suppression in rats, as evidenced by low peripheral blood leukocyte and granulocyte counts when measured 7 days after the first dose of methotrexate. This methotrexate-induced marrow suppression was virtually eliminated by the accompanying injections of microspherules that contained folinic acid. While animals receiving methotrexate alone exhibited significant granulocytopenia of 570/mcl ± 37 (normal value 1144/mcl ± 31), those animals also receiving the microspherules maintained near normal counts (1040/mcl ± 32), a significant difference ($p<0.01$). The microspherules also protected the total leukocyte, platelet and hemoglobin counts. The data are depicted in Figures 1, 2, 3, 4 and 5.

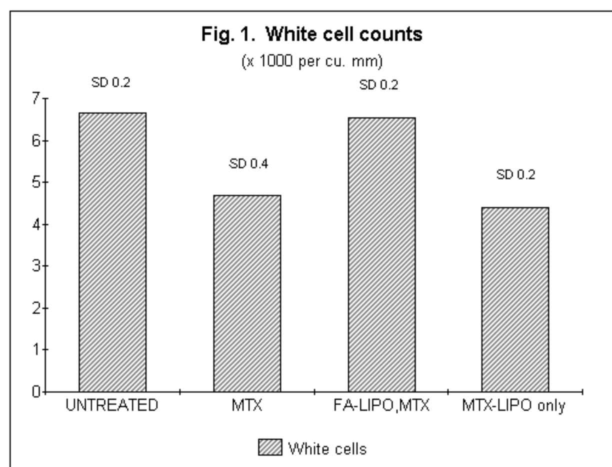
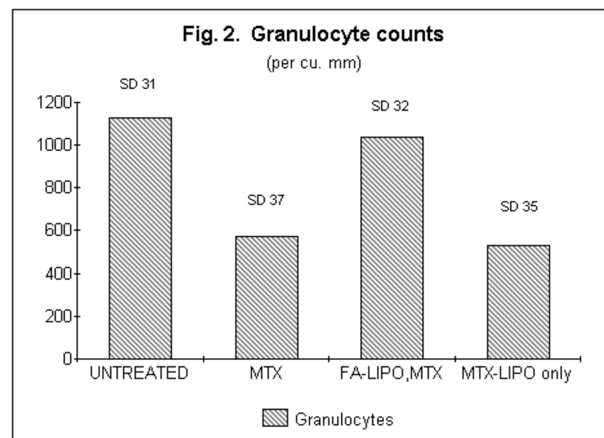
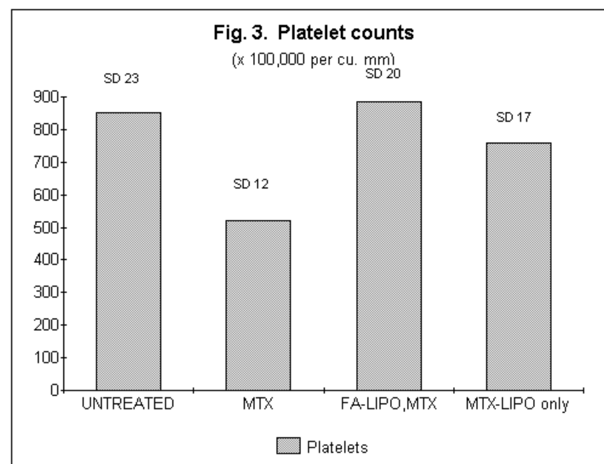
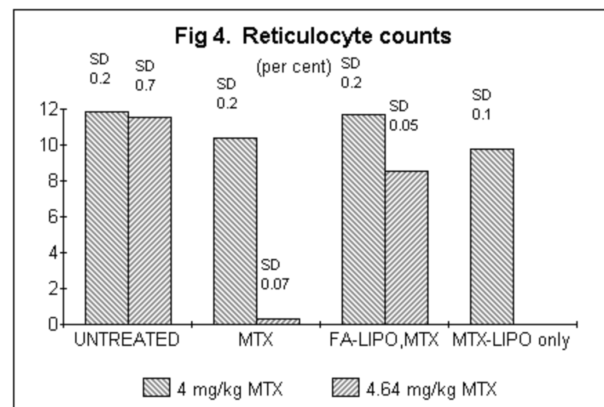


Figure 1. Leukocyte count in untreated rats, rats receiving 4 mg/kg methotrexte (MTX), rats receiving MTX along with toxicity-reducing microspherules (FA-LIPO, MTX), and rats receiving leukocyte-directed microspherules holding a minute quantity of MTX (MTX-LIPO).

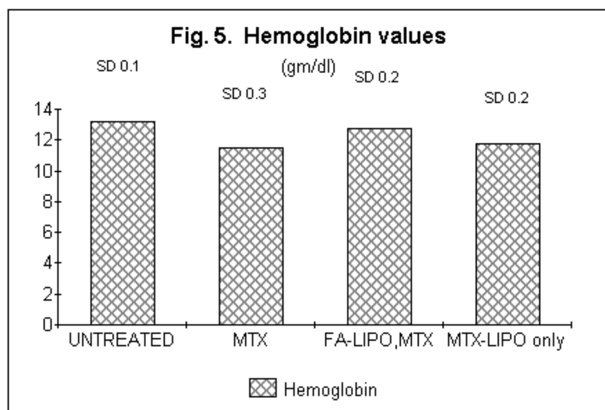






When animals received even higher doses of methotrexate (1.16 mg/kg i.p.daily for 4 days), the reticulocyte counts, which reflect the state of the erythrogenic marrow, dropped to near zero (0.2% ± 0.07) from a normal level of 11.9% ± 0.2 (Fig. 4). However, when the rats also received the antidote-containing microspherules, the reticulocyte count remained high (8.8% ±0.05), reflecting the protection of the erythrogenic marrow ($p<0.01$).

 © Copyright, 2010. All Rights Reserved



To further test the effectiveness of the drug delivery system, similar microspherules containing an oncolytic MTX (instead of an antidote) at a dosage of only 0.035 mg/kg body weight were injected intravenously into rats. Injected rats were sacrificed for analysis at the end of the seventh day after the injection. The total dosage of MTX each rat received in the encapsulated microspherule form was a mere 0.035 mg/kg body weight. A previous study had shown that MTX at 0.035 mg/kg, if freely injected into the blood stream, is too low a dosage to have any appreciable effect on the blood count. However, when this miniscule dosage was contained inside our special microspherules, it caused marrow destruction which was comparable to more than four daily injections of unencapsulated MTX at 1 mg/kg each. Five weeks old rats (i.e., the age at sacrifice) have reticulocyte indices of 11 to 13% (mean of 11.9% ± 0.2%). This reflects a high activity of the erythrogenic marrow. Rats receiving our microspherules containing MTX were found to have a mean reticulocyte index of only 9.1% (± 0.1), a difference from norm significant at $p<0.01$. Similarly, rats receiving MTX-microspherules showed depression in hemoglobin measurement (11.8 gm/dl ± 0.3; untreated, 13.2 ± 0.1) and counts of leukocytes (4.4 x 10^3/mcl ± 0.20; untreated, 6.7 x 10^3/mcl ± 0.20) and platelets (7.66 x 10^5/mcl ± 0.16; untreated, 8.49 x 10^3 /mcl ± 0.24) ($p<0.05$). The data are presented in Figures 1,2, and 4. The results suggest that a concentrated dosage of MTX was delivered to the interior of the target cells since MTX at a dosage of 0.035 mg/kg in free, unencapsulated form cannot cause the marrow suppression observed.

As an additional test of the effectiveness of our method for eliminating side effects, four weeks old Wistar rats were given a different oncolytic, vinblastine, some with and some without protective microspherules, and evaluated for evidence of toxicity elimination. A single injection of vinblastine at a dosage of 1.5 mg/kg body weight is so toxic that animals lose most of their circulating granulocytes (0.205 x 10^3/mcl ± 0.015). However, the accompanying administration of toxicity-eliminating microspherules containing the antidote MSG can substantially reduce marrow toxicity ($p<0.01$) as demonstrated by the near-normal granulocyte counts in the peripheral blood smear (1000/mcl ± 173).

Our method has also succeeded in eliminating the gastrointestinal side effects of oncolytic drugs. Juvenile rats are in their growth phase and normally gain on the average about 35 gms over 5 days. However, when they are given high doses of vinblastine, considerable growth retardation occurs, due primarily to anorexia and diarrhea; animals receiving vinblastine, lost an average of 4.5 gms (± 3.5) over 5 days, instead of gaining weight as usual. However, when toxicity-eliminating microspherules were administered with the vinblastine, animals gained an average 29.7 gms (± 1.31) over 7 days, showing a significant reduction ($p<0.01$) of gastrointestinal toxicity.

While our toxicity-eliminating microspherules protect the marrow and reduce gastrointestinal effects of the toxic oncolytics, the microspherules do not protect tumor cells from a cancer drug's tumoricidal action. Twelve 4 week old Sprague Dawley (Sp-Daw) rats (Harland Sprague-Dawley, Indianapolis, IN) were injected intraperitoneally with ascites Walker tumor cells (600,000 cells/animal).

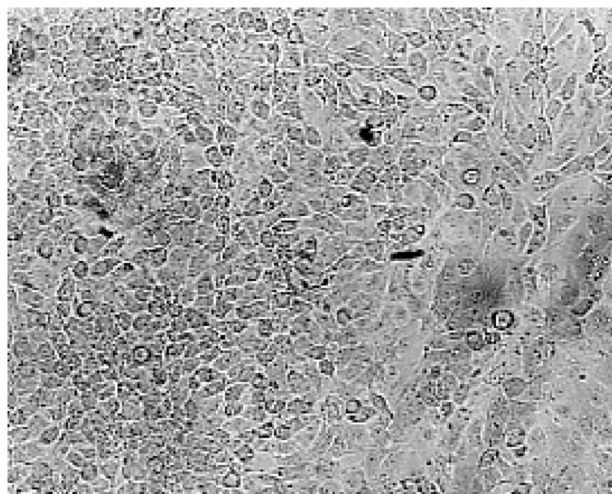


Plate 1. Walker rat tumor cells on flask, 100X.

Eight of the animals were given vinblastine intraperitoneally at a dose of 1.5 mg/kg 18 hours after the tumor injection. Five of the animals receiving vinblastine were also given protective microspherules intravenously on the day before and the day after the vinblastine injection. All animals receiving only tumor cells and no vinblastine died with tumorous ascites within a few hours of each other 10 days after the tumor inoculation. Of the animals receiving tumor inoculation and vinblastine, with or without microspherules, the only animal to develop ascites and die was that which received vinblastine alone without microspherules. None of the animals receiving vinblastine and microspherules developed ascites even though the

 © Copyright, 2010. All Rights Reserved

microspherules suppressed the bone marrow toxicity. Our study shows that vinblastine can kill the tumor cells regardless of the presence of MSG microspherules.

In another tumor study aimed at developing new strategies for completely eradicating cancer, eight 9 weeks old, 300 gm Sp-Daw rats were inoculated in the musculature at the anterior base of the thigh with 800,000 Walker tumor cells. A solid nodule became palpable at the injection site within 5 days of the inoculation. By 9 days after inoculation, a massive tumor approximately 5 cm in diameter formed and began to erode the stretched skin on the tumor (Plate 2). At this point, vinblastine was given at a high dose of 1.5 mg/kg intraperitoneally. Nine days later, another similar dose was given. By giving such a high dose twice over a short interval, we intended to maintain a tumoricidal level of vinblastine more continuously over a two week period than had ever before been attempted. In conventional chemotherapy, the toxicity of oncolytics to normal, dividing cells is so severe that tumoricidal levels can be maintained only for a brief period at a time (2 days, typically). This period is believed to be too short to eradicate all the tumor cells, since at any given time some of the tumor cells are in the resting phase of mitosis and thus less vulnerable to many phase-sensitive oncolytics. In control animals, just a single injection of this high dose of vinblastine (i.e., 1.5 mg/kg) given to animals weighing 300 grams killed half of them (a 50% mortality rate). A 1.5 mg/kg dose is nearly 50% stronger, in terms of a dosage calculated on basis of body surface area, when given to a 300 grams animal compared to a 100 grams animal.

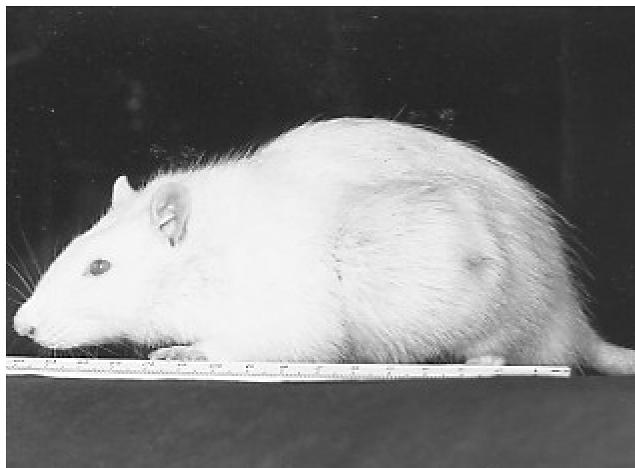


Plate 2. A typical SpDaw rat whose large Walker carcinoma was eradicated with two high dose vinblastine injections made possible by toxicity-reduction.

Therefore, in our protocol for tumor eradication, we injected a 50% lethal dose twice over a ten day period. Although we had expected that we might need to repeat this course of treatment of two high dose vinblastine injections, we were impressed by the rapid and continuing shrinkage of all the tumors over 3 weeks, by end of which the tumors were no longer visible but only palpable as tiny nodules of about 2 cu. mm or less. By the fourth week after the first vinblastine injection, no tumor was palpable in any animal, and additional treatments with vinblastine were cancelled as unnecessary. The animals were followed for over a year (comparable to a 35 year follow-up in humans) with no recurrence of the tumor.

According to Cutts[1] and work in our laboratory, even huge amounts of MSG (e.g., 120 mg MSG/kg body weight/mg vinblastine) given as divided dosages daily with each vinblastine injection cannot reduce vinblastine's destructive effect on the bone marrow. Since we have found that even twice-daily injections of MSG, 8 mg/kg/day, i.v., has no protective effect on the bone marrow, it is especially remarkable that a tiny fraction of MSG/kg, when delivered more directly to the bone marrow cells via our microspherules, was sufficient to provide substantial protection to the marrow cells when given only twice near the time of vinblastine administration. This latter study also attests to the efficacy of our system for the delivery of antidote to the bone marrow cells.

There are numerous advantages in our approach to focusing the effect of drugs, as compared to those approaches which seek to deliver the cancer drug specifically to the tumor cells, using tumor-specific antibodies. Instead of having to produce antibodies customized to individual tumors, our technique can use "off the shelf" microspherules that can work with all tumors because the microspherules are designed to go to the normal dividing cells which are similar in all individuals.

Our method of toxicity suppression also has substantial advantages over the use of the marrow stimulators such as GM-CSF. First, unlike our method which protects also gastrointestinal cells, the GM-CSF method protects only blood counts. The typical image of a dying cancer patient undergoing chemotherapy is one of someone vomiting and retching. GM-CSF does nothing for such a patient. Second, GM-CSF permits only a modest increase in the dosages of oncolytics, because higher doses, that maintain prolonged cytocidal blood levels, will kill the immature marrow cells so that no immature cell remains for GM-CSF to stimulate. Our method allows indefinite maintainenance of cytocidal levels, since normal dividing cells will not be harmed by the oncolytics. Only with prolonged maintenance of cytocidal levels is there any possibility of eradicating all cancer cells, especially those that divide slowly. At this time, only our method has such a potential. Additionally, there are disturbing reports by Dedhar, S. et al.[2] & others suggesting that GM-CSF may also stimulate breast-carcinoma and sarcoma cells, which poses a real dilemma as to whether to use GM-CSF at all. Finally, it remains to be shown that using cytokines like GM-CSF does not eventually lead to premature exhaustion of the marrow by exposing to

 © Copyright, 2010. All Rights Reserved

oncolytic action the earlier progenitor marrow cells usually spared in chemotherapy. Then, a patient surviving cancer would face premature death from pan-hemocytopenia a decade or two later.

Until now, clinicians have had to rely on a large number of cancer drugs to treat various forms of the disease, because the different types of cancer cell vary in their absorbency and sensitivity to the available drugs. Effective treatment has been hampered because clinicians cannot simply choose the most effective drugs and dosages; they are limited by the need to avoid destroying marrow, gastrointestinal, and heart cells. If concerns about side effects were eliminated, clinicians would be free to use any drug at higher dosages than have ever been attempted. Cancers which are resistant to the currently standard dosages may be found to be treatable by the same drugs at higher dosages. Thus, it may become possible to treat all forms of cancer with just a few drugs, rather than the dozens currently required. Methotrexate and vinblastine themselves, which can now be used to treat only a few types of tumor, may become effective against many more when it becomes possible to use higher dosages.

While our approach may ultimately make it unnecessary to use more than two or three drugs in cancer treatment, we continue to explore other antidote-drug combinations. We have succeeded now in developing microspherule-antidotes to almost all known cancer drugs, including all in the class called alkylators such as cyclophosphamide, cisplatin, and doxorubicin, as well as such premier agents such as paclitaxel and vinorelbine. We have yet to encounter a drug for which an antidote is not known, and it is expected that more antidotes will be developed.

We chose to work with MTX and vinblastine for numerous reasons. MTX's antidote, folinic acid, is probably the best known antidote for a cancer drug and is already FDA approved. Vinblastine's antidote, MSG, is already well known as a food additive. The known spectrum of efficacy of MTX and vinblastine suggests that both drugs hold promise for improved therapy of many solid tumors, including breast and lung cancers.

In today's armamentarium against cancer, clinicians generally feel that drugs of the platinum and anthracycline class are stronger than methotrexate or vinblastine. However, drugs like methotrexate and vinblastine can be much stronger than any other drug being that now we can use methotrexate and vinblastine at such high dosages never before possible.

Initial clinical trials can be conducted relatively safely. In a first step, one gives to a patient antidote-containing microspherules together with a standard dose of such oncolytics as methotrexate or vinblastine. A week later, if the microspherules have successfully suppressed the usual bone marrow toxicity and there is no drop in the blood counts, increased dosages of the oncolytic is administered. The therapy is safe because it can be terminated before a patient suffers any irreversible harm.

Results from animal studies have now been corroborated in humans in early phase I clinical trials. Five patients with advanced stage IV breast cancer underwent collectively 14 cycles of weekly intravenous vinca chemo- therapy with varying test doses of toxicity-abating microspheric MSG.

The degree of granulocyte protection observed in patients was closely tied to the dosage of protective microspherules used. Complete protection was consistently achieved every time optimized dosage of protective microspherules was used. Tables I - III show some of the results observed in patients when adequate doses of microspheric MSG were administered to patients for up to four days after vinca injections. In the same patients where a perfect protection of granulocytes was achieved, if instead inadequate doses of microspheric MSG were employed, granulocyte counts fell dramatically showing that these patients are subject to bone marrow toxicity from vinca alkaloid unless specifically protected against it.

The objective of the Phase I trial is primarily to determine safe dosages of microspheric MSG, but because there was such a dramatic difference in granulocyte protection between instances where adequate dosages of microspherule were employed from when insufficient dosages were used, early clinical trial results have already shown with a high level of confidence ($p<0.01$) the effectiveness of microspheric MSG to protect circulating granulocyte count from bone marrow-damaging doses of vinca alkaloids. In addition, none of the patients experienced significant gastrointestinal symptoms except when receiving only a low dose of microspheric MSG.

Furthermore, most interestingly, we have made objective observations that we killed tumor cells in patients while microspheric MSG protected granulocytes, such as when a single dose (0.15 mg/kg) of vinblastine decimated the bulk of the cancer cells in the pleural fluid of a patient who had just five months before failed to respond to conventional therapy with continuous vinblastine infusion. The pathologist reported, in viewing the pleural fluid one week after the dose of vinblastine and microspheric MSG: "Prepared smears from submitted pleural effusion show: Blood, few polymorphonuclear leukocytes and few scattered small groups, as well as single atypical epithelial cells with enlarged nuclei and prominent nucleoli. The cytoplasm is abundant and vacuolated. This specimen when compared to her previous pleural specimen shows an obvious decrease in the number of tumor cells. The previous specimen was extremely hypercellular and showed very large cohesive clusters of tumor cells.

 © Copyright, 2010. All Rights Reserved

Table I. Protection of Neutrophil Count after vinorelbine (30 mg/m²) – Patient #1

	Before vinca	6 days after Vinca
White Cells (/cu.mm)	3600	3500
Neutrophils	1690	1900

Table II. Protection of Neutrophil Count after vinorelbine (30 mg/m²) – Patient GL

	Before vinca	6 days after Vinca
White Cells (/cu.mm)	8200	9300
Neutrophils	5248	6045

Table III. Protection of Neutrophil Count after vinblastine (20 mg/kg) – Patient PL

	Before vinca	6 days after Vinca
White Cells (/cu.mm)	3000	4200
Neutrophils	2130	4032

Mitotic figures were present in large numbers including numerous atypical mitotic figures. The present specimen is almost hypocellular and predominantly composed of few single cells and very small clusters (2-3 cells) of tumor cells. Mitotic figures are extremely rare. These differences between specimens probably represents (sic) chemotherapy effect."

Prior animal studies had revealed that a given dosage of vinblastine can kill more cancer cells when granulocyte count is protected using microspheric MSG than if granulocyte count is allowed to fall as in conventional chemotherapy. Granulocytes aid the body in completing the kill of cancer cells partially damaged by cancer drugs, by participating in surveillance of dying cells, delivering fatal puncturing of such cells, and digesting and removing dead cells. Without an adequate granulocyte presence, partially damaged cancer cells survive chemotherapy, self-repairing themselves, and live on to kill the host patients. For the first time, we have been able to perform chemotherapy with cytocidal anti-metabolic agents like vinca alkaloids without also lowering the count of circulating granulocytes. We could expect a higher rate of complete and partial responses when using antidotal microspherules even from conventional doses of chemotherapy by keeping the granulocytes healthy.

References

1. Cutts, J. H. Biochem. Pharmacol. 13: 421-431 (1964).
2. Dedhar, S., Gaboury, L, Galloway, P., and Eaves, C. Proc.Natl. Acad. Sci. USA 85: 9253-9257 (1988).
3. This report prepared in August 2009 but internally embargoed against release until preparations were complete to expand our clinical trial. Patient #6 died from subclavian syndrome resulting from recurrence, at 38 mon.

Notes Added to Manuscript

Clinical trial Phase II Patient #1 (Table I), a 58 year-old white female with advanced breast cancer (invasive ductal carcinoma treated with lumpectomy, radiation, and CMF 14 years before) presenting with metastases to femur, spine, and extensively to the liver) underwent alternating cycles of gemcitabine (180 mg/m^2) first week and carboplatin (100 mg/m^2) second week, both intravenously, along with microspheric mesna during the week of carboplatin. Table IV summarizes hematologic data from two paired cycles where adequate doses of microspheric mesna were given. Table V depicts data from one cycle where inadequate doses of microspheric mesna were given.

Table IV. Protection of Blood Elements
after carboplatin – with microspheric mesna

DTRA Dose 9.0 units	Before carbo	6 days after carbo
White Cells (/cu.mm)	4800	7700
Neutrophils	3400	6230
Hemoglobin (g/dl)	10.1	11.2
Red Cells (/cu.mm)	3.36×10^6	3.58×10^6
Platelets	134×10^3	207×10^3

DTRA Dose 9.0 units	Before carbo	6 days after carbo
White Cells (/cu.mm)	2800	5800
Neutrophils	2160	4470
Hemoglobin (g/dl)	11.0	11.1
Red Cells (/cu.mm)	3.29×10^6	3.47×10^6
Platelets	124×10^3	105×10^3

Table V. Reduced Protection of Blood Elements after carboplatin by Inadequate Dose of microspheric mesna

DTRA Dose 7.0 units	Before carbo	6 days after carbo
White Cells (/cu.mm)	5600	3200
Neutrophils	3370	2400
Hemoglobin (g/dl)	10.0	8.7
Red Cells (/cu.mm)	3.09×10^6	2.62×10^6
Platelets	78×10^3	61×10^3

Above data present a dramatic showing of efficacy of microspheric mesna to protect the bone marrow when an adequate dose is employed. Since the carboplatin week always followed a gemcitabine week for which we did not employ any microspheric protective agent, cell counts tended to be low at the beginning of carboplatin therapy. Rather than the typical experience in conventional carboplatin therapy when blood counts fall, blood counts actually rose. It is noteworthy that platelets and red blood cell counts also rose. Further, the microspheric mesna also protected the patient from the nausea and vomiting which are so typical of the harsh agent carboplatin. Although this study was limited to only three cycles, the results were consistent and closely reflected the adequacy of the dose of microspheric mesna,

 © Copyright, 2010. All Rights Reserved

thereby further validating the data. The patient showed dramatic improvements in her hepatic metastases as determined by CT scans. The liver showed multiple lacunae where islands of metastases had been. She also reported a considerable improvement in her bone pain that had been due to extensive bony metastases. What is most remarkable is that these results occurred so rapidly after only a few weeks of chemotherapy. For islands of metastases to have lysed so completely suggests that protecting neutrophils with microspheric antidotes preserve the natural neutrophil cascade (described above) for lysis of chemotherapy-damaged, sick cancer cells that does not occur with conventional chemotherapy. In conventional chemotherapy, usually only cells which are outright killed immediately by the drugs are removed, allowing remainder of the tumor to survive and self-repair because the neutrophil cascade has been disabled by the chemotherapy. Consequently, one sees only a slow shrinkage of the tumor in a CT scan, not the rapid and more complete liquefying effect we have seen with microspheric-antidote protected chemotherapy.

Carboplatin is one of the most versatile cancer drugs with a very broad spectrum of activity against many types of cancer. The dramatic eradication seen in this patient suggests that it may not be necessary to use high doses of an oncolytic, but instead use frequent lower doses, which would also promote anti-angiogenesis.

Microspheric mesna has been shown in animal studies to reduce side effects of cyclophosphamide and thioTEPA. It is expected that microspheric mesna is active against all alkylating agents, which is the largest class of anti-neoplastic agents in our armamentarium against cancer.

The following is a report on consecutive patients #3, 4, 5, and 6 of the early Phase II clinical trial:

Patient #3: A 62 year-old white male with Stage IIIB non-small cell lung cancer (5.7 cm well-differentiated adenocarcinoma mass in superior lobe of the right lung) was given palliative radiotherapy (20 Gy/15 fr.) for severe pain and then treated with eight cycles of carboplatin AUC 5 and gemcitabine 1000 mg/m^2 on day 1 of the 21 day-cycle. Carboplatin was accompanied by microspheric mesna. His tumor became non-visible on his CT scans after five cycles and he remained free of cancer at 52 months from the start of chemotherapy. He experienced no side effect at all during his chemotherapy. A representative cell count taken on day 10 is depicted in Table VI.

Patient #4: A 53 year-old white female with invasive lobular carcinoma of the left breast measuring 2.5 cm, ER/PR negative, was found to have a single 21mm liver metastasis. She was initially treated with conventional chemotherapy of taxane and capecitabine for four cycles and then underwent lumpectomy plus axillary radical node dissection (all nodes negative). Her liver metastasis persisted and she was treated with 8 cycles of weekly carboplatin AUC 1.5, together with microspheric mesna. Her liver metastasis became non-visible after five cycles. After her 8 cycles, she was placed on anastrozole and she remained free of cancer at 44 months from start of her carboplatin therapy. She experienced no side effect during her 8 cycles and Table VII depicts a representative cell count at 6 days after carboplatin.

Table VI. Representative Cell Count
Patient #3 on 8th day after carboplatin/gem

WhiteCells(/cu.mm)	8200
Neutrophils	4920
Hemoglobin(g/dl)	10.5
Platelets	196x10^3

Table VII. Representative Cell Count
Patient #4 on 6th day after carboplatin

WhiteCells(/cu.mm)	3700
Neutrophils	1998
Hemoglobin(g/dl)	13.0
Platelets	195x10^3

Patient #5: A 61 year-old white female with seven years history of myeloproliferative disorder/chronic myelogenous leukemia (Normal chromosomes and *BCR/ABL* oncogene-negative) treated with interferon, then with thalidomide, was deteriorating requiring weekly transfusions for severe anemia in the range of hemoglobin 7 – 9gm/dl. She was treated with weekly carboplatin AUC 1.6 for three doses, with microspheric mesna. Subsequently, her hemoglobin stabilized at 10.5 gm/dl without transfusion and the rest of her cell count remained normal without further hematologic treatment for 18 months when she passed away from an unrelated cause. A representative cell count at 16 month after our treatment is depicted in Table VIII.

Table VIII. Cell Count
Patient #5 at 16th month after therapy

WhiteCells(/cu.mm)	5300
Neutrophils	4176
Hemoglobin(g/dl)	10.7
Platelets	273x10^3

Patient #6: Because decisive results on consecutive patients #3, 4, and 5 were so startling, we realized the need to finalize expanding our production capability for the microspherules. We had, however, committed to treat patient #6 during this change-over in production. We did not anticipate any problem because there were no fundamental changes in our production technique and our standard quality control tests revealed no difference between our earlier microspherules and the expanded-production microspherules. However, we did encounter some

 © Copyright, 2010. All Rights Reserved

differences in results with this patient, not all of which may be related to the change-over in production.

A 64 year-old white male patient presenting with Stage IIIB pulmonary adenocarcinoma had been treated four months before with four cycles of conventional carboplatin AUC 5/gemcitabine 1000 mg/m^2 day 1, gemcitabine 1000 mg/ m^2 day 8 on 21 day-cycle. He was started on carboplatin AUC 5/gemcitabine 1000 mg/m^2 day 1 with microspheric mesna on 21 day-cycle. When his CT scan taken after four cycles still revealed some residual tumor, it was decided that day 8 gemcitabine 1000 mg/m^2 be used and his treatment continued through cycle 9. The patient experienced mild nausea on cycle 2, something never seen before with microspherule-assisted chemotherapy, and in addition to some adjustment in dosing of microspherules ondansetron was added to our protocol for this patient. This patient's neutrophil counts were well-protected with microspheric mesna, but he experienced some problem with thrombocytopenia below 100,000 and low hemoglobin around 8.0 gm/dl requiring transfusions while we attempted to make fine adjustments with our production. The blood count taken the day before his final ninth cycle commenced shows that our expanded production was successfully optimized (Table IX). The CT scan taken after his ninth cycle showed clearing of his tumor in the right lung and from his ipsilateral and contralateral lymph nodes. He remained free of cancer at 36 month after his ninth cycle[3].

We believe based on our other patients receiving high dose chemotherapy together with microspheric antidotes that patients who have had previous chemotherapy are more challenging in protecting their bone marrow and their gastrointestinal tract, compared to chemotherapy-naïve patients. We are not certain how much of the trouble with cell counts Patient #6 had was due to his previous high dose chemotherapy instead of due to our production change-over.

It is interesting that while in conventional chemotherapy one cannot achieve a good response to a set of oncolytics previously used, we were able to achieve a complete and long-term response despite the fact that Patient #6 had been treated with the same oncolytics (carboplatin and gemcitabine) before, but without microspheric antidote. The reason why in conventional chemotherapy one would be ill-advised to use the same oncolytics is that tumor cells that survive the first treatment are those more resistant to the said oncolytics. However, when using microspheric antidotes that can enable cancer cells that are merely damaged (not outright killed) to also be killed, it is reasonable to choose the same oncolytics.

Probably of all findings over the years in this project, the truly remarkable and the most important discovery is the potentiation of chemotherapy agents by protecting and maintaining high neutrophil counst so that cancer cells not outright killed by an oncolytic but damaged could be eliminated via the neutrophil cascade described above. There is no other explanation how carboplatin at such low doses as AUC 1.6 can so thoroughly eradicate cancer cells after as few as three weekly injections.

Since the writing of the original manuscript to which these notes are appended, we have completed hundreds of patient-treatment cycles that demonstrate the same findings of efficacy in respect to marrow and gastrointestinal protection.

Because patients in advanced stage IV may not have time for a slower therapy to effectuate eradication, we have recently been taking volunteer patients to try more aggressive dose-intense therapies in the hope of eventually working up to the type of "lethal" doses that cured advanced cancers in the rat with just two injections given nine days apart. Our preliminary and consistent finding is that when microspheric antidotes are used, chemotherapy produces much faster responses, which means that the cost of treating cancer may come down drastically in the future.

If you qualify to write on Metprex and have registered, please click here if you wish to add a peer-review comment on this article. :

REVIEW/DISCUSS THIS ARTICLE

 © Copyright, 2010. All Rights Reserved