*Anti-Cancer Drugs 2000,* **11,** pp. 555–563

## Clinical report

# Inhalational interleukin-2 liposomes for pulmonary metastases: a phase I clinical trial

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**The lung is a common site of both metastases and primary neoplasia. This phase I study was designed to test the feasibility and toxicity of administering interleukin (IL)-2 liposomes by aerosol to patients with pulmonary metastases. The goal was to test whether IL-2 liposomes could be given by aerosol using biologically effective but non-toxic doses in an outpatient setting. Liposomes containing IL-2 or placebo (buffer) were synthesized and mixed to provide a constant lipid dose, and were nebulized using a Puritan twin jet nebulizer and a standard compressor. The liposome-containing mist was inhaled for about 20 min 3 times a day in order to selectively stimulate immune function within the lung and to avoid systemic toxicity. The dose chosen was based on canine efficacy and toxicity studies that used bronchoalveolar lavage to demonstrate increased cell numbers and activation of mononuclear cells after inhalation of nebulized IL-2 liposomes. Nine patients were treated in three cohorts of three patients at 1.5, 3.0 and 6.0 $\times 10^6$ IU of IL-2 3 times a day. No significant toxicity was observed. We conclude that the delivery of IL-2 liposomes by inhalation is well tolerated. Further studies of inhalational IL-2 liposomes to determine efficacy as an anti-cancer therapy are warranted. [© 2000 Lippincott Williams & Wilkins.]**

*Key words*: Aerosol, cancer, cytokine, immunology, lung.

## Introduction

Interleukin (IL)-2 has clearly documented anti-tumor activity *in vivo* in both experimental models and in humans.[1–8] IL-2 binds a specific $\alpha$, $\beta$, $\gamma$ trimeric high-affinity receptor, resulting in proliferation and activation of T lymphocytes, and a dimeric ($\alpha\beta$) receptor of lower affinity on monocytes and NK cells.[9,10] Eosinophilia is commonly observed with IL-2 administration and eosinophils may play a role in the biologic effects of IL-2.[11–13] The administration of i.v. or s.c. IL-2 is associated with toxicity in a dose-dependent manner.[14,15] Adverse effects, which can be debilitating, often include fever, chills, sweats, fatigue, malaise and a vascular leak syndrome.[6,12,14–16]

One approach to alter the therapeutic index of IL-2 is its incorporation into liposomes.[17–21] Liposomes, lipid vesicles with one or many bilayers, can markedly modify the toxicity and efficacy of incorporated drugs by altering their absorption, distribution, metabolism and elimination.[22] The particulate and lipid nature of liposomes modifies the biodistribution and promotes mononuclear phagocyte uptake and lymphatic absorption. Since liposome pharmacodynamics are different from those of the parent drug, an improved therapeutic index may occur. For example, a decrease in cardiotoxicity associated with doxorubicin has been documented using pegylated liposomal doxorubicin.[23,24] Also, the therapeutic index of the anti-fungal drug amphotericin B appears to be improved using a liposomal formulation.[25]

Liposomal formulations of IL-2 have been shown to provide a slow release of IL-2 and a different tissue distribution of the drug.[26] A phase I pharmacokinetic trial of i.v. IL-2 liposomes[27] found that patients treated with doses up to $60 \times 10^6$ IU/m$^2$/day qd $\times$ 5 had limited toxicity, that included only mild fever, malaise and flu-like symptoms. No evidence of capillary leak was observed. Nevertheless, pharmacokinetic data showed significantly prolonged serum clearance of IL-2 liposomes compared to bolus free cytokine. At 24 h, IL-2 levels of 15–30 IU/ml were obtained.

A second approach to improve the therapeutic index of IL-2 is to deliver relatively high concentrations

Supported in part by grant MO1-RR00400 from the National Center for Research Resources, the Cancer Treatment Research Fund, Children's Cancer Research Fund and the Hegman Foundation. Presented in part at the 90th Annual Meeting of the American Association for Cancer Research, Philadelphia, 10–14 April 1999.

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of the drug locally while minimizing systemic exposure. The administration of biologically active proteins by inhalation has been studied since at least 1925, when the absorption of inhaled insulin was documented. A variety of biologically active proteins have since been administered by inhalation, including granulocyte colony stimulating factor, α-interferon,[28,29] granulocyte macrophage colony stimulating factor[30] and DNAase.[31] Free IL-2 has been administered via inhalation and appears to have activity in the treatment of renal cell carcinoma.[13,32–36] Inhalation of IL-2 has been shown to increase immunocompetent cells in (BAL)[32] and to increase the accessory function of alveolar macrophages.[34] Canine studies of aerosolized IL-2 liposomes demonstrated low toxicity, immune activation in the lung and anti-tumor activity.[37–39] Two of six dogs with metastatic osteosarcoma obtained a complete response following 1 month of treatment with inhaled IL-2 liposomes.[38,40] Khanna *et al.* investigated the activation of immune cells within the lungs of dogs after nebulized IL-2 or IL-2 liposomes by using BAL to obtain effector cells from the lung.[37,38] In these studies, IL-2 liposomes had a significantly superior local effect compared to the free cytokine as documented by increases in BAL cell number and functional activation.

Based on these theoretical and preclinical observations, we hypothesized that the delivery of a liposomal preparation of IL-2 by inhalation would be feasible and could significantly increase the therapeutic index of IL-2 for treatment of cancers involving the lung. We therefore conducted a phase I trial of aerosolized IL-2 liposomes in patients with cancer metastatic to the lung.

## Materials and methods

### Preparation of IL-2 liposomes

IL-2 liposomes (IND #BB 3928) were prepared as previously described[40] using GMP procedures and components acceptable for clinical use, including human serum albumin (Baxter, Glendale CA), powdered dimyristoyl phosphatidyl choline (Avanti, Alabaster AL) and native sequence recombinant IL-2 [Roche IL-2 (Nutley, NJ) was a gift of OncoTherapeutics, Cranbury NJ]. Powdered lipid was sterilized using γ irradiation (1.3 MGy); HPLC analysis showed no difference between DMPC before and after sterilization. Bulk placebo (buffer containing) and IL-2 liposomes were stored at −20°C, thawed in the University of Minnesota Investigational Pharmacy and diluted with 0.9% NaCl USP. Liposomes were dispensed in 10 ml vials fitted with butyl rubber stoppers and flip-off, tear-off seals. This container/closure system was chosen to facilitate easy addition of additional preservative-free saline just before nebulization. Unit dose vials were dispensed in boxes containing both individual unit doses of IL-2 liposomes and unit doses of sterile saline for nebulization (Dey Laboratories, Napa, CA). Patients were instructed to store liposomes in their home refrigerator.

To document reliability of the manufacturing and to characterize the specific IL-2 liposome preparation used, IL-2 liposomes were analyzed for total IL-2 activity using the CTLL-2 bioassay, percent IL-2 associated with the liposomes (i.e. entrapment efficiency), particle size distribution (e.g. by FACS with latex beads as size standards), sterility and visual appearance.[17,20,40]

### Soluble IL-2 receptor assay

Soluble IL-2 receptors in serum were quantitated in duplicate using an immunoassay (R & D Systems, Minneapolis, MN) exactly as described by the manufacturer. Serum samples were stored at −80°C until use. Values agreed within 4%.

### Statistical analysis

Descriptive statistics and paired *t*-test *p* values were calculated using InStat (Graphpad Software, San Diego, CA).

### Nebulization instructions

IL-2 liposomes are in the solid/gel state when refrigerated. To mix for nebulization, the drug was briefly warmed in a hand or pocket until it became a freely flowing milky solution. The saline was similarly warmed. The seal of the vial and rubber stopper were removed and the entire contents of a unit dose vial of IL-2 liposomes (1 ml) was poured into the bowl of a Puritan Bennett twin jet nebulizer (Carlsbad, CA). Then 4 ml of sterile saline for nebulization was added to the IL-2 liposome vial, swirled around the walls of the vial, and then added to the nebulizer bowl. The solution was gently mixed, and compressed air from a nebulization apparatus (Bunn Compressor; Pediatric Home Respiratory Services, Roseville, MN; at 18 p.s.i.) was used to generate the IL-2 liposome aerosol mist. Each nebulization treatment took approximately 15–20 min.

Nine patients at the University of Minnesota entered this study between July 1996 and August 1998. All patients had biopsy proven locally advanced or metastatic sarcomas or other refractory solid tumors

(Table 1). All patients had a Karnofsky performance status of $\geqslant$70%. All patients met the following criteria: estimated life expectancy $\geqslant$3 months; age $\geqslant$18 years; neutrophil count $\geqslant$1500/$\mu$l; platelet count $\geqslant$100 000/$\mu$l; creatinine $\leqslant$2.0 mg/dl; serum bilirubin less than twice normal; AST$\leqslant$3 times normal; no concurrent infection; HIV negative; no significant abnormality on EKG; measurable disease (defined as any mass reproducibly measured in two perpendicular dimensions by physical or radiological means); $FEV_1$ and FVC $\geqslant$70% predicted, $DL_{CO}\geqslant$60% predicted; discontinuation of cytotoxic chemotherapy, hormonal therapy, corticosteroids, radiation therapy and/or other biologic therapy, including the administration of other cytokines or retinoids, for at least 4 weeks before entering the trial; negative pregnancy test in females of childbearing age; no central nervous system disease as evidenced by history and physical examination; and no history of reactive airway disease.

At the time of entry, all patients had the following studies performed: history and physical examination, complete blood counts (CBC), differential, electrolytes, BUN, creatinine, AST, alkaline phosphatase, bilirubin, PT, PTT, urinalysis, $DL_{CO}$, spirometry, EKG, chest X-ray and appropriate radiographic imaging as indicated. History and physical examination, blood counts, $DL_{CO}$ and spirometry were repeated on day 7 and 28. All patients gave written informed consent. The trial was approved by the Institutional Review Board and the Clinical Research Center of the University of Minnesota, and conducted using an investigator initiated IND (#BB 3928).

## Treatment plan

At the initial visit to the Clinical Research Center (CRC), baseline studies were recorded and patients were taught to use a remote spirometry apparatus (Asthmalog; Datalog, Stillwater, MN) capable of measuring $FEV_1$, $FEF_{25-75}$, peak flow and FVC. Patients also learned how to nebulize 5 $cm^3$ saline. Patients then performed saline nebulizations at 8 a.m., 2 p.m., and 8 p.m. for 3–7 days to establish 'baseline' PFT values with the remote spirometry device before and after nebulization. Remote spirometry was done just before and 5 min after each nebulization.

On the patients return admission to the CRC, baseline PFT parameters were evaluated. The normal variance in $FEV_1$ and FVC measurements has been reported to be about 3%. Each patient received a test dose of empty liposomes at 2 p.m. the day before IL-2 liposome treatment began, and was observed closely with spirometry post nebulization, and at 8 p.m., 8 a.m. and 2 p.m. following the treatment. The first treatment of nebulized IL-2 liposomes was given 4 times over 24 h, and patients were observed after each treatment for any adverse effects. Pre- and post-(5 min.) nebulization PFT tests were obtained using the remote PFT device with each treatment. Patients were discharged no sooner than 1 h after the fourth IL-2 liposome treatment.

Patients then self administered IL-2 liposomes by inhalation for 1 month (t.i.d. $\times$ 28 days at 8 a.m., 2 p.m., and 8 p.m.). PFT data before and after (5 min.) each inhalation treatment was obtained using the remote spirometry device and sent automatically to the CRC. Any deterioration in pulmonary function (i.e. $FEV_1$ or FVC $<$ 90% baseline or dyspnea) was reported to the principal investigator. The expired air from the nebulizer was vented to the outside air. One week after IL-2 liposome treatment began, each patient was seen in follow up as an outpatient for history and physical and PFTs via Asthmalog in the CRC, and also a $DL_{CO}$ and CBC. On days 14 and 21 after IL-2 liposome treatment began, the patient was also seen in follow up as an outpatient for history and physical and PFTs. Four weeks after starting IL-2 liposome treatment, the patient was seen in follow up for a detailed history and physical, CXR, CBC, differential, electrolytes, BUN, creatinine, AST, alkaline phosphatase, bilirubin, LDH, $DL_{CO}$, and PFTs. Additional treatment was planned for patients with stable disease or response for up to 3 months as indicated in Figure 1.

Nine patients received a fixed dose of lipid [40 mg dimyristoyl phosphatidyl choline (DMPC) per dose] in liposomes in normal saline (total volume=5 $cm^3$) via a nebulization apparatus equipped with a Puritan Bennett twin-jet nebulizer bowl; empty liposomes were mixed with active IL-2-containing liposomes in the University of Minnesota Investigational Pharmacy and then dispensed in unit dose vials with flip-off seals. The first three patients received $1.5\times10^6$ IU of IL-2 liposomes t.i.d. with subsequent doses for each cohort of 3 and $6\times10^6$ IU, respectively. The duration of each treatment was about 15–20 min t.i.d. for 28 days per treatment course. No patient entered the next cohort until the third patient from the previous cohort finished their 28 day treatment.

Dose modifications were planned based on toxicity, but were not necessary in this study. Toxicities were graded according to standard CALGB criteria. Standard criteria for objective response to treatment were used.

Three questions were addressed in this study. (i) What are the toxicities of IL-2 liposomes given by inhalation? (ii) is outpatient ambulatory inhalation of IL-2 liposomes feasible? We chose an operational definition of feasibility to be 'if seven or more of nine patients complete the study without significant

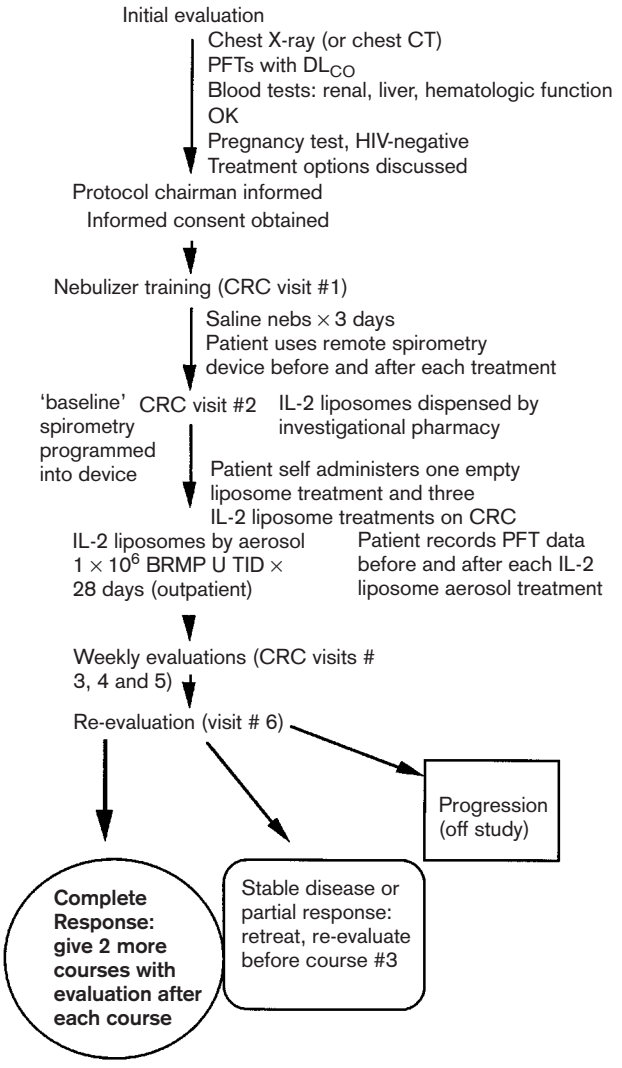


**Figure 1.** Design of aerosol IL-2 liposome feasibility study.

toxicity, the use of IL-2 liposomes at this dose and schedule will be considered feasible'. (iii) At the doses used, is safety monitoring using remote spirometry necessary in future trials?

## Results

### Patient characteristics

The ages of the nine patients in this study ranged from 33 to 61 years (median 55 years). There were seven men and two women. Five patients had sarcoma (one Ewing, two osteogenic and two alveolar soft part), three had renal cell cancer and one had melanoma. All patients had a Karnofsky score $\geqslant 70$. All but one (alveolar soft part) of the patients with sarcoma had received previous chemotherapy for metastatic disease.

Treatment was begun in the CRC to monitor for toxicity. Subsequent treatments were given on an ambulatory basis at home as described in Materials and methods. The number of courses given per patient ranged from 8 days to 3 months [four patients received 1 month, 1 patient received 2 months, two patients received 3 months, one received 15 days (developed brain metastases) and one received 8 days (drug supply exhausted) (see Table 1)].

### Toxicity

All patients completed at least 8 days of treatment, eight completed 15 days, seven completed 28 or more days of treatment, three completed 56 or more days of treatment and two completed 84 days of treatment. All patients were evaluable for toxicity. All patients

remained ambulatory and performed normal activities during treatment. No significant toxicity was observed (Tables 1 and 2).

In the first cohort ($1.5 \times 10^6$ IU/dose) there was one episode of upper respiratory symptoms lasting about 3 days and one episode of a decrease in $FEV_1$ >10% resulting in cessation of treatment (both in the same patient). As per the protocol, no further treatment was given for 3 days (days 18–20) and treatment was re-instituted without evident toxicity. This patient had significant variability of his remote spirometry before initiating treatment and received two more 28 day courses without evident toxicity.

In the second cohort ($3 \times 10^6$ IU/dose) there was no evident toxicity. In the third cohort ($6 \times 10^6$ IU/dose) one patient completed 28 days without evident toxicity. One patient developed slowed mentation after 15 days of treatment. An MRI revealed a new brain metastasis and the patient was removed from study with no evident toxicity of IL-2 treatment (stable $FEV_1$, FVC, $DL_{CO}$, CBC and no symptoms). An accidental loss of study drug allowed only 8 days of treatment for the last patient and he had no evident toxicity during this truncated course.

Analysis of the group data revealed no significant differences in $FEV_1$, FVC or $DL_{CO}$ between day 0 and day 8 (paired *t*-test $p$=0.16, 0.68 and 0.79, respectively). $FEV_1$ was measurably decreased on day 28 compared with day 1 ($107 \pm 14.5$ versus $95 \pm 16.3$% predicted, paired *t*-test $p$=0.003); however, this small change was of no clinical significance. No significant differences in FVC or $DL_{CO}$ between day 0 and day 28 were detected (paired *t*-test $p$=0.44 and 0.37, respectively).

## Soluble IL-2 receptors

Soluble IL-2 receptors were measured in serum samples obtained at the start and end of the first 28 day treatment cycle in seven patients (Table 3). The level of soluble IL-2 receptors was higher at the end of 28 days of treatment than at baseline in six of the seven patients, thus demonstrating a biological effect of the treatment. The mean soluble IL-2 receptor concentration in these patients on day 0 was $1603 \pm 616$ pg/ml and on day 28 was $1833 \pm 667$ pg/ml; this group difference was statistically significant (paired *t*-test $p$=0.048).

## Response

Seven patients were evaluable for anti-tumor response (Table 1). Of four evaluable patients with a sarcoma, two had progressive disease (PD), and two had stable

**Table 1**. Phase I trial of inhalational IL-2 liposomes

| Patient | Age | Sex | Diagnosis | Dose[a] | Days[b] | Toxicity | Biotherapy[c] | Extent[d] |
|---|---|---|---|---|---|---|---|---|
| 1 | 61 | M | renal cell carcinoma | $1.5 \times 10^6$ | 84 | none | IL-2/IFN | multiple largest 3 cm |
| 2 | 46 | M | Ewing sarcoma | $1.5 \times 10^6$ | 28 | none | no | multiple largest 5 cm |
| 3 | 55 | M | renal cell carcinoma | $1.5 \times 10^6$ | 84 | minor[e] | IL-2 | TNTC largest 2.9 cm |
| 4 | 54 | M | osteosarcoma | $3 \times 10^6$ | 28 | none | no | multiple largest 4 cm |
| 5 | 38 | M | ASPS[f] | $3 \times 10^6$ | 28 | none | no | multiple largest >8 mm |
| 6 | 33 | F | ASPS[f] | $3 \times 10^6$ | 28 | none | no | TNTC largest 1.5 cm |
| 7 | 39 | M | melanoma | $6 \times 10^6$ | 28 | none | IFN | 6 nodules largest 1 cm |
| 8 | 34 | F | osteosarcoma | $6 \times 10^6$ | 15 | none[g] | no | >10 nodules largest 2 cm |
| 9 | 59 | M | renal cell carcinoma | $6 \times 10^6$ | 8 | none[h] | no | TNTC largest >4 cm |

[a]IL-2 liposome dose/treatment (IU).
[b]Number of days of treatment.
[c]Prior IL-2 or IFN treatment.
[d]Extent of pulmonary disease (TNTC = too numerous to count).
[e]Transient decrease in $FEV_1$, 3 days of upper respiratory symptoms.
[f]Alveolar soft part sarcoma.
[g]Developed brain metastasis.
[h]Limited drug supply.

**Table 2**. Phase I trial of aerosol IL-2 liposomes: pulmonary function studies

| Patient | Day | Percent predicted | | |
|---|---|---|---|---|
| | | $FEV_1$ | FVC | $DL_{CO}$ |
| 1 | 1 | 83 | 85 | 77 |
| | 8 | 81 | 92 | 60 |
| | 28 | 82 | 88 | 71 |
| | 56 | 82 | 88 | 74 |
| | 84 | 82 | 90 | 75 |
| 2 | 1 | 93 | 96 | 118 |
| | 8 | 98 | 106 | 120 |
| | 28 | 87 | 100 | 115 |
| 3 | 1 | 106 | 110 | 121 |
| | 8 | 102 | 106 | 132 |
| | 28 | 105 | 115 | 125 |
| | 56 | 102 | 110 | 126 |
| | 84 | 94 | 105 | 139 |
| 4 | 1 | 132 | 123 | 92 |
| | 8 | 100 | 106 | 96 |
| | 28 | 127 | 117 | 77 |
| 5 | 1 | 106 | 108 | 105 |
| | 8 | 101 | 108 | 93 |
| | 28 | 99 | 105 | 100 |
| 6 | 1 | 106 | 101 | 95 |
| | 8 | 99 | 101 | ND |
| | 28 | 102 | 102 | 96 |
| | 56 | 100 | 102 | 87 |
| 7 | 1 | 90 | 96 | 78 |
| | 8 | 81 | 86 | 84 |
| | 28 | 83 | 89 | 86 |
| 8 | 1 | 89 | 86 | 69 |
| | 8 | 85 | 83 | 76 |
| | 15[a] | 78 | 76 | 66 |
| 9 | 1 | 101 | 95 | 95 |
| | 8 | 107 | 91 | 86 |

Pulmonary function tests during inhalational IL-2 liposome treatment. ND, not determined.
[a]Due to brain metastases patient effort was suboptimal.

**Table 3**. Serum soluble IL-2 receptors

| Patient | Day 0 | Day 28 |
|---|---|---|
| 1 | 1813 | 2172 |
| 2 | 2692 | 3163 |
| 3 | 1224 | 1472 |
| 4 | 2014 | 1739 |
| 5 | 985 | 1149 |
| 6 | 1027 | 1425 |
| 7 | 1463 | 1709 |

Serum soluble IL-2 receptor concentration in plasma before and after 28 days of treatment. Values are expressed as pg/ml and represent the means of duplicate samples that agreed within 4%. The normal control value was 742 pg/ml.

disease (SD) (the latter both had alveolar soft part sarcoma which had been growing very slowly before treatment). One patient with progressive renal cell cancer had disease stabilization for 3 months of treatment and progressed off treatment, and a second had PD after 3 months of treatment. One patient with slowly growing melanoma with six approximately 1 cm lung nodules had SD at the end of 1 month of treatment, but developed a complete remission (CR) thereafter, although no further IL-2 was given. This 39-year-old male had an excellent performance status 1 year after therapy with no evidence of metastatic disease.

## Discussion

No significant toxicity was observed in this phase I trial of inhalational IL-2 liposomes. At the doses used in this study, chronic intermittent treatment appears feasible. Toxicity would have allowed continued dose escalation in these patients.

This study was a dose escalation trial with three cohorts of three patients each, $n$=9. The number of subjects in this initial study was chosen to permit feasibility testing of the concept that nebulized IL-2 liposomes in patients with cancer involving the lung is non-toxic. Because of difficulty obtaining IL-2 for this study, the study was of limited duration. While we expected $3 \times 10^6$ IU TID to be well tolerated and have anti-tumor activity based on canine studies, we started at half this dose for the first cohort, and then increased subsequent cohorts to 3 and $6 \times 10^6$ IU TID, respectively. With three patients there is a 50% power to find an incidence of a given toxicity of 20% and an 80% power to find a rate of 45% in that cohort. Due to a limited supply of drug, the last patient could only be treated for 8 days.

The optimal dose and timing of the administration of IL-2 liposomes by inhalation is unclear, but this study supports the safety of using $3–6 \times 10^6$ IU 3 times a day. Canine radioscintigraphy studies indicated that twice a day dosing may also be feasible.[39] Since the peak effects of some cytokine therapy occurs 3–5 days after starting treatment,[41] it is possible that 1 week on/1 week off schedules could be more effective in terms of cellular recruitment.

The size of aerosol particles is a critical determinant of their utility for administration by inhalation. Respirable particles have a maximum diameter of around 5 $\mu$m, with larger particles depositing in the mouth and oropharynx or large conducting airways.[42–45] Particles significantly smaller than around 1 $\mu$m may potentially be exhaled without deposition in the lung.[46] Analysis of the IL-2 liposomes used in this study has demonstrated that the aerosol particles generated with this nebulization procedure have a mass median aerodynamic diameter (MMAD) of about

2.0 $\mu$m and a mode size of about 1.0 $\mu$m,[39] suggesting good pulmonary delivery via inhalation. This was confirmed in studies of radiolabeled IL-2 liposomes in dogs that showed deposition of the inhaled drug in all ventilated parts of the lung, with a central to peripheral deposition ratio of about 1.1,[39] suggesting uniform particle deposition in the peripheral airways and alveoli.[47]

Free IL-2 has also been administered by inhalation.[32–36] Studies by Huland *et al*. in more than 150 patients found a non-productive cough to be the limiting toxicity of inhalational free IL-2.[32,36] These studies suggested clinical benefit from the addition of this treatment to s.c. IL-2 in patients with renal cell cancer metastatic to the lung.[36] However, many of these patients have side effects from systemic and inhaled IL-2 therapy that may limit performance and compliance.

The lack of toxicity of aerosolized IL-2 liposomes may relate to local effects in the lung and lymphatics, but not in blood. Studies of the inhalation of this IL-2 preparation in dogs demonstrated significant local immune activation, as assessed by examination of cells obtained by BAL.[37] The BAL leukocyte count was higher following inhalation of IL-2 liposomes compared to free IL-2, and both treatments resulted in a higher percentage of lymphocytes and eosinophils in the BAL.[37] BAL leukocytes from IL-2 liposome-treated subjects had significant cytotoxicity *in vitro* against tumor cells, while blood leukocytes had little activity.

Despite the lack of toxicity, biologic effects of the treatment were observed. The level of soluble IL-2 receptors in serum was higher at the end of 1 month of treatment than at the start of therapy in six of the seven patients who completed 1 month of treatment. In addition, two patients appeared to experience an anti-tumor effect of the treatment. Patient #1 had renal cell carcinoma that had been clearly progressing before therapy and had disease stabilization during 3 months of treatment, and disease progression after therapy was stopped. Patient #7 had melanoma metastatic to the lung that had been progressing, although slowly, before treatment; while characterized as stable disease at the end of one month of therapy, subsequent CT scans demonstrated a CR at 1 year. Thus, responses may take longer than one month to be observed.

Thus, this study answered the three questions posed for evaluation of this novel formulation and route of administration of IL-2: (i) side effects were not serious, (ii) ambulatory inhalation of IL-2 liposomes is possible on a chronic basis (i.e. weeks to months) while maintaining a good performance status and (iii) at the doses used in this study, additional spirometric testing with each dose is not warranted in future clinical trials. We conclude that the delivery of IL-2 liposomes by inhalation is well tolerated and feasible at doses that are predicted to have anti-tumor activity. Further studies of aerosolized IL-2 liposomes to determine efficacy as anti-cancer therapy are appropriate.

## Conclusion

We conclude that the delivery of IL-2 liposomes by inhalation is well tolerated and feasible at doses that are predicted to have anti-tumor activity. Further studies of inhalational IL-2 liposomes to determine efficacy as an anti-cancer therapy are warranted.

## Acknowledgments

We thank the staff of the University of Minnesota General Clinical Research Center and the Masonic Oncology Clinic for assistance with the study and excellent patient care, Investigational Pharmacy (G Carlson, D Landrith and D Luke), Pediatric Home Respiratory Services for providing air compressors, the Datalog Corp for ambulatory PFT monitoring support, Drs J Little and D Northfelt for a critical review of the manuscript, and Chris Stocke for manuscript preparation.

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*(Received 16 May 2000; accepted 23 May 2000)*