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CLINICAL AND PRECLINICAL PHARMACOLOGY OF LIPOSOMAL VINCRISTINE

Murray S. Webb,[1] Andreas H. Sarris,[2] Fernando Cabanillas,[2] Lawrence D. Mayer,[3] Marcel B. Bally,[3] Clive Burge,[1] and Pieter R. Cullis[1,4]

[1]Inex Pharmaceuticals Corporation, Burnaby, B.C., Canada
[2]MD Anderson Cancer Center, Houston, Texas, U.S.A.
[3]British Columbia Cancer Agency, Vancouver, B.C., Canada
[4]Department of Biochemistry, University of British Columbia

SUMMARY

Vincristine is one of the most commonly administered anticancer drugs and is active in a wide range of indications including non-Hodgkin's lymphomas, acute lymphocytic leukemias and lung cancer. Administration of vincristine in long-circulating liposomes may be expected to result in increased accumulation of drug at tumor sites due to "passive targeting" or "disease-site targeting" effects arising from the more permeable vasculature in these regions. Further, for liposomes with appropriate drug release characteristics, extended exposure of tumor cells to vincristine would result from liposomal delivery. The combination of increased drug delivery and extended duration of drug exposure may be expected to result in increased efficacy, particularly because vincristine is a cell-cycle specific drug. It is shown that vincristine can be encapsulated in large unilamellar vesicles (diameter ~ 100 nm) using a pH gradient (interior acidic) approach. Further, the efficacy of liposomal formulations of vincristine in animal models is highly sensitive to the drug release rate in vivo. A liposomal formulation with drug retention characteristics such that more than 50% of the vincristine is retained in the carrier 24 h following i.v. injection exhibits significantly improved antitumor efficacy in A431 xenograft and P388 murine tumor models in comparison to either free drug or leakier liposomal formulations. The clinical activity of liposomal vincristine has been investigated in relapsed or refractory non-Hodgkin's lymphoma patients

at a dose level of 2 mg/m^2 every two weeks. Of 83 registered patients, there were 24 responses in 68 evaluable patients. The responses according to histology are: Indolent-13%; Transformed-42%; Aggressive-45%. There were no serious cases of myelosuppression or any toxic deaths. It is concluded that liposomal vincristine can be given at high doses, is active and well tolerated and is rarely neurotoxic or myelosuppressive in these heavily pretreated patients. It appears that the benefits of low toxicity and enhanced efficacy noted in the tumor models are also observed in the clinical setting. A multicenter pivotal Phase II trial of liposomal vincristine in relapsed and refractory non-Hodgkin's lymphoma has been approved by the US FDA and is ongoing.

INTRODUCTION

Rationale for Liposomal Delivery of Vincristine

Since its initial approval by the FDA in 1963 for the treatment of acute leukemia in children, vincristine remains one of the most commonly prescribed anti-cancer drugs (Figure 1). Currently, approximately 50% of the use of vincristine is in the treatment of lymphomas. Moreover, the clinical use of vincristine in single-agent therapy is negligible. Instead, vincristine is almost exclusively used as a component of combination chemotherapy protocols. One of the most common of these is the CHOP combination (cyclophosphamide, doxorubicin, vincristine, prednisone) for the first-line treatment of lymphomas, including non-Hodgkin's lymphomas (1).

Vincristine is a vinca alkaloid and was initially purified from the periwinkle (*Catharanthus roseus*). The cytotoxic activity of vincristine is cell-cycle specific,

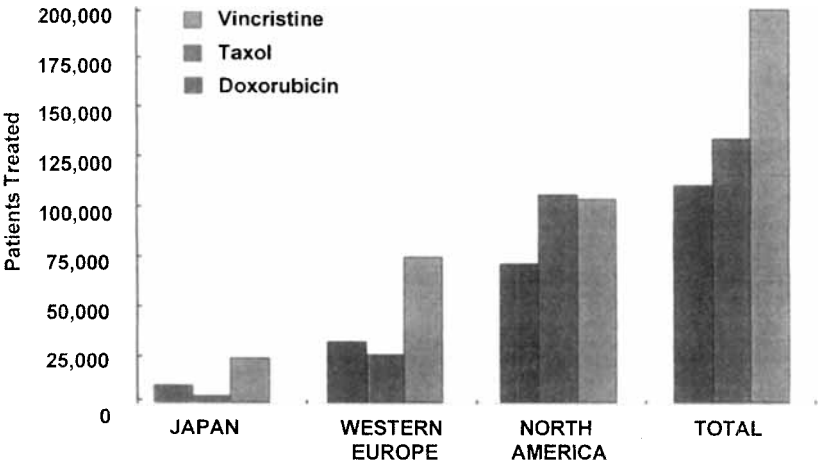

Figure 1. Summary of chemotherapeutic usage for vincristine, doxorubicin and taxol. Annual number of patients treated with each drug were estimated for Japan, Western Europe, and North America.

effected via inhibition of microtubule polymerization and depolymerization during mitosis (2). Based on this mechanism of action, it might be anticipated that increased duration of exposure to vincristine would substantially improve the cytotoxic activity of the drug. This expectation is supported by the *in vitro* observation that increasing the duration of exposure of L1210 leukemia cells to vincristine from 1 to 72 hours of exposure was associated with a 10^5-fold increase in cytotoxicity (Table 1). In contrast, the same increase in the duration of exposure of L1210 cells to doxorubicin, whose activity is believed to be less cell-cycle specific, was associated with only a 40-fold increase in cytotoxicity (3,4).

Several approaches have been used to increase the clinical activity of vincristine. Jackson *et al* (5,6) increased the duration of exposure to the drug by infusing patients with vincristine in Phase I and II studies. Substantial clinical activity was observed in patients with non-Hodgkin's lymphomas, as well as other neoplasms, but this activity was also associated with significant to severe toxicities, particularly neurotoxicities (6). This treatment protocol is not in current clinical use as single-agent therapy and multiple-day infusional vincristine is only used infrequently as part of the EPOCH (7) and VAD (8) combination chemotherapy protocols. The other approach, the subject of this review, is via liposomal delivery. The preclinical and clinical results summarized below demonstrate that vincristine, when encapsulated in an appropriately designed liposomal carrier, has significantly increased preclinical and clinical antitumor activity and is associated with similar or lower toxicities.

Design Features for Liposomal Delivery Systems

Liposomal delivery systems containing anticancer drugs must have several key properties to optimize their therapeutic potential (9). The most important of these properties are disease site targeting and regulated drug release (Figure 2). A wide variety of liposomal delivery systems, including liposomal vincristine, that lack an active targeting moiety (i.e. antibody) achieve their disease site targeting by a process referred to as passive targeting. The basis of passive targeting

Table 1. Effects of Exposure Time on the Cytotoxicity of Vincristine Against L1210 Cells in Vitro

EXPOSURE TIME (HOURS)	IC_{50} (nM)
1	12,000
6	2,400
24	7.3
72	0.12

The drug concentration required to achieve 50% inhibition of growth is presented at the IC_{50}. Data from (3).

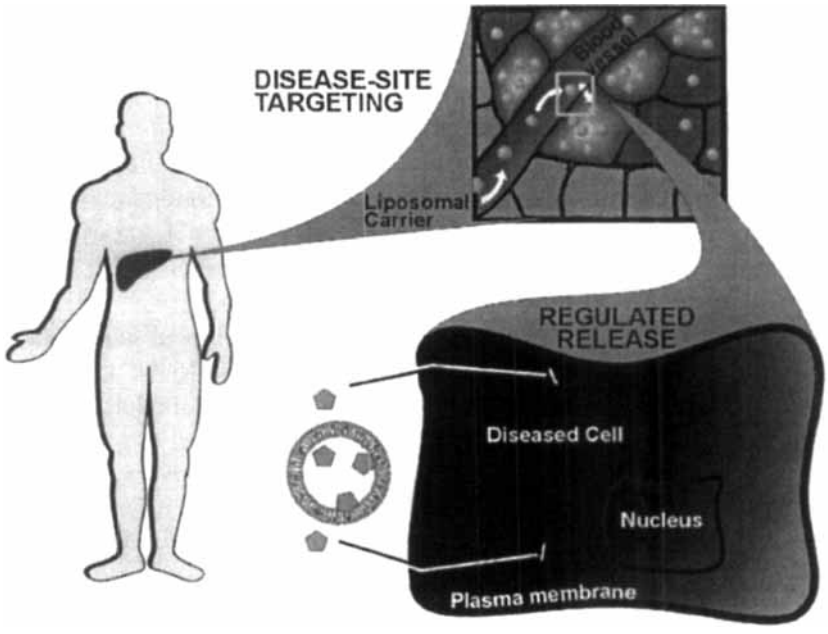

Figure 2. Key design features of liposomal anticancer systems include disease site targeting and regulated drug release. Disease site targeting is achieved by extravasation from liposomal carriers from the circulation to the diseased tissue via discontinuities in the vascular endothelium. Regulated drug release is achieved by optimization of the liposomal formulation for the encapsulated drug and the disease characteristics.

lies in the fact that the vasculature at tumor sites is often discontinuous and more permeable than normal vasculature (10). Consequently, liposomal systems can extravasate from the circulation and preferentially accumulate at tumor sites, resulting in significant increases in anticancer drug accumulation. For liposomal vincristine, this process has been demonstrated in both ascitic and solid tumors (see Preclinical Pharmacology Section below). To achieve passive targeting, liposomal delivery systems must be designed to achieve extended circulation times ($T_{1/2} > 6$ hours) and a diameter sufficiently small (about 100 nm) to facilitate extravasation from the circulation to the intercellular spaces in the tumor mass.

Formulation of Vincristine in Liposomes

Vincristine is readily loaded into liposomes bearing a transmembrane pH gradient in a manner identical to that used for doxorubicin (11). Liposomes prepared with an interior volume that is buffered to pH 4.0 are equilibrated so that the external solution reaches a pH of approximately 7.5 (Figure 3A). In response to this pH gradient of 3–3.5 pH units, the vincristine redistributes to the liposome interior as described by the Henderson-Hasselbach equation (11). Experimentally, this is observed as the encapsulation of >95% of vincristine in 5–10 minutes (Figure 3B) at temperatures above the gel-liquid crystal phase transition temperature for the phospholipid component of the liposome.

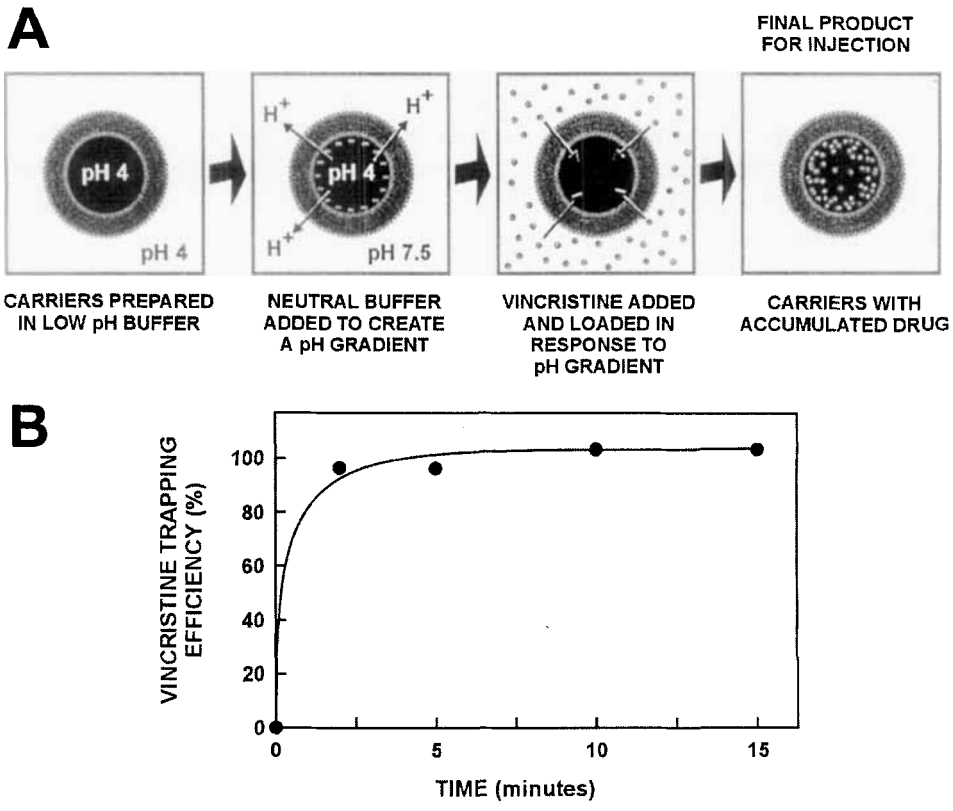

Figure 3. Liposomal loading of vincristine in response to a transmembrane pH gradient. A: Diagrammatic representation of the preparation of liposomal carriers with a transmembrane pH gradient and the loading of vincristine in response to the pH gradient. Details are provided in the text. B: Vincristine uptake into DSPC/Chol liposomes at 60°C in response to a transmembrane pH gradient.

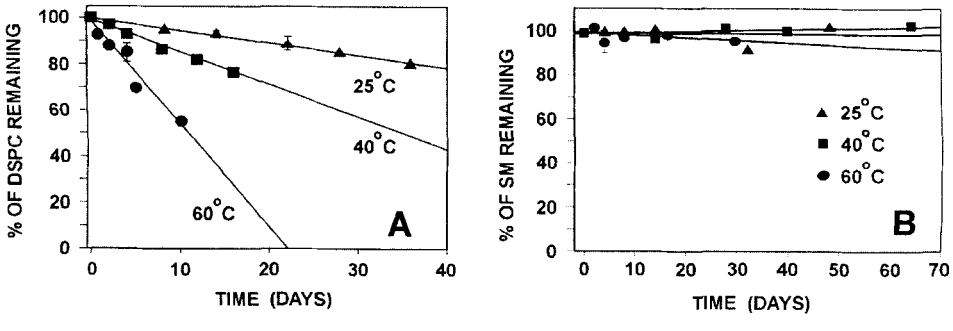

Figure 4. Temperature and time dependencies of the *in vitro* stability of DSPC/Chol (A) and SM/Chol (B) liposomes in buffer at pH 4.0.

For the ongoing clinical evaluation of liposomal vincristine, a liposome drug loading kit is provided to the hospital pharmacies for on-site constitution of liposomal drug. This kit is comprised of three principle components: (1) 100 nm sphingomyelin/cholesterol (SM/Chol) liposomes in buffer at pH 4.0; (2) pH 9 buffer to establish the transmembrane pH gradient and; (3) vincristine sulfate. The pharmaceutical stability of this kit has been significantly enhanced by the use of sphingomyelin as the phospholipid component of the liposomal carriers. The stability of sphingomyelin to acid hydrolysis that occurs during the storage of liposomal preparations at pH 4.0 for extended periods is significantly greater than the stability of conventional phosphatidylcholine/cholesterol preparations (Figure 4) (12).

PRECLINICAL PHARMACOLOGY OF LIPOSOMAL VINCRISTINE

It is well known that liposomal encapsulation confers significant increases in the plasma concentrations of encapsulated drugs and substantially longer drug circulation lifetimes. In SM/Chol liposomes that have been optimized for retention of vincristine, plasma drug concentrations are increased by up to 10^3-fold compared to free drug and the circulation half-life of the drug is increased from several minutes to approximately 12 hours (Figure 5) (12). In a variety of preclinical tumor models, it has been demonstrated that liposomes extravasate from the circulation to the tumor site intact with encapsulated drug (12). Consequently, a key characteristic of an optimized liposomal carrier is that the drug retention is sufficient to ensure that liposomes extravasating to the tumor site contain maximum amounts of encapsulated drug. In early preclinical experiments, it was established that DSPC/Chol liposomes with an internal pH (pHi) of 2.0 had improved drug

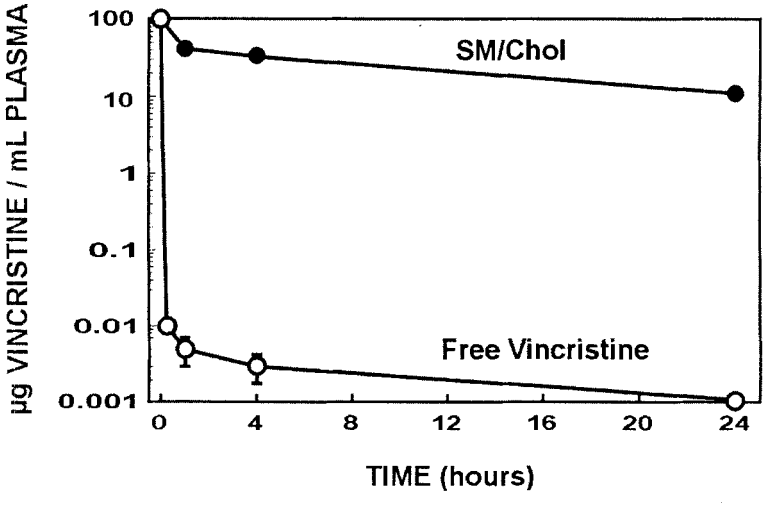

Figure 5. Plasma concentrations of vincristine in mice after the i.v. administration of either free vincristine or vincristine encapsulated in SM/Chol liposomes.

retention, compared to DSPC/Chol liposomes with a pHi of 4.0, and greater antitumor activity against the P388 ascitic leukemia model (13,14,15). However, these liposomal formulations having an internal pH of 2.0 were found to have insufficient stability to acid hydrolysis to be acceptable for pharmaceutical development (Figure 4A). Subsequent development of liposomal vincristine was based on the SM/Chol formulation due to its greater stability to acid hydrolysis (Figure 4B). Importantly, it was also demonstrated that SM/Chol liposomal vincristine formulations had significantly improved *in vivo* drug retention properties compared to that for DSPC/Chol liposomal formulations of vincristine (Figure 6A) (12). The increase in drug associated with circulating liposomes in the SM/Chol formulation is also reflected as increased delivery of vincristine to both murine ascitic P388 (12) and human A431 carcinoma xenograft models (Figure 6B). Importantly, the

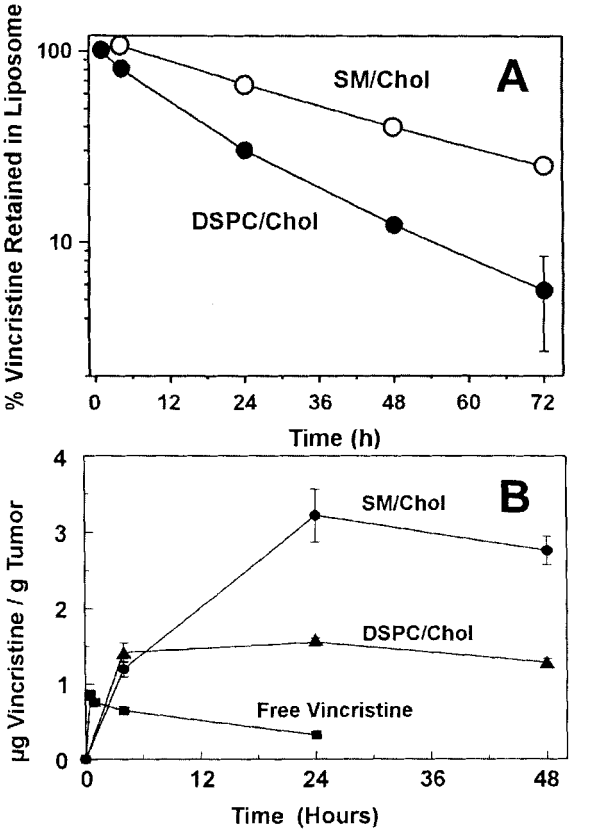

Figure 6. A; Regulated vincristine release *in vivo*. Enhanced retention of vincristine is observed in SM/Chol liposomes, compared to DSPC/Chol liposomes, is observed in the plasma of mice after i.v. administration. B; Enhanced tumor delivery of vincristine in SM/Chol liposomal carriers. Vincristine accumulates to higher concentrations in the A431 human solid tumor xenograft and remains over longer durations after encapsulation in SM/Chol liposomes, compared to DSPC/Chol liposomes. Both SM/Chol and DSPC/Chol liposomal delivery systems substantially increase vincristine accumulation at the tumor site compared to free drug.

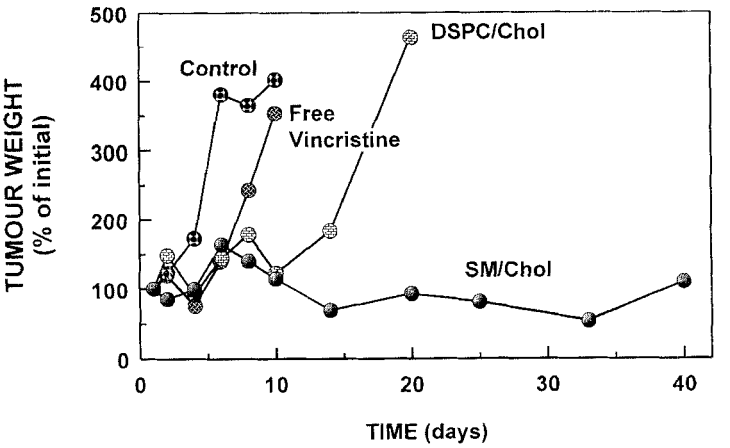

Figure 7. Antitumor efficacy of free vincristine and vincristine encapsulated in either DSPC/Chol or SM/Chol formulations of liposomal vincristine. The growth of the A431 human xenograft tumor is shown after a single administration of drug on Day 0. Tumors were approximately 100 mg at treatment. Vincristine dose was 2 mg/kg. Data from (12).

accumulation of liposomal carriers at the tumor site, coupled to the regulated drug release properties of optimized liposomal formulations, achieves both increased amount of drug at the tumor and increased duration of exposure of the tumor to high concentrations of the drug (Figure 6B). These characteristics result in significantly improved antitumor activity for liposomal vincristine formulations against both murine (12,15) and human xenografts (Figure 7). In all tumor models evaluated to date with various liposomal vincristine formulations, enhanced drug retention is associated with increased antitumor activity (15).

Taken together, these data demonstrate that optimized liposomal formulations of vincristine have several desirable properties including appropriate carrier size, extended circulation lifetimes, disease site targeting and regulated drug release rates. In formulations in which all of these parameters have been optimized, as in the SM/Chol liposomal formulations of vincristine, dramatic improvements in antitumor activity have been observed in animal tumor models (Figure 7). Interestingly, the duration of tumor exposure to drug observed after treatment with liposomal vincristine (Figure 6B) is similar to what might be expected from continuous infusion of free vincristine, but was achieved by bolus injection.

CLINICAL PHARMACOLOGY OF LIPOSOMAL VINCRISTINE

A dose-escalating Phase I safety evaluation of liposomal vincristine (DSPC/Chol formulation) was carried out at the British Columbia Cancer Agency. A total of 25 patients with advanced malignancies that were unresponsive to conventional treatment received 1–5 (median 2) 1-hour intravenous infusions of liposomal vincristine every three weeks at doses between 0.5–2.8 mg/m^2. This study established

the recommended dose for Phase II evaluation as 2.0 mg/m^2 based on the occurrences of the most frequent toxicities (neurotoxicity). This dose represents a substantial increase above the standard adult dose for free vincristine of 1.4 mg/m^2 with a dose cap of 2 mg for adults. Objective biological responses were observed in 3 patients at the higher dose levels (16). Pharmacokinetic evaluation of liposomal vincristine in these patients demonstrated significantly higher concentrations of vincristine in the plasma than would be expected after the administration of free vincristine. These data suggested that encapsulation of the drug in the liposomes eliminated the early phase of drug elimination seen after the administration of free vincristine (17).

The initial strategy for Phase II clinical development was to assess the single-agent activity of liposomal vincristine in tumor types expected to be insensitive to conventional (i.e., non-liposomal) vincristine. A multicenter Phase II study at the British Columbia Cancer Agency in Vancouver and Jewish General Hospital in Montreal was carried out in patients having carcinoma of the pancreas or metastatic colorectal carcinoma. This approach was taken, in part, based on observations in the Phase I study that included a partial response in a pancreatic cancer patient and a biological response in a patient with adrenocortical carcinoma (16). A total of 46 patients with pancreatic or colorectal carcinoma (16 and 30, respectively) were treated with SM/Chol liposomal vincristine at 2.0 mg/m^2 once every 3 weeks. This study established that liposomal vincristine was well tolerated in these patients at this dose and schedule, but did not establish efficacy against these tumor types. Pharmacokinetic evaluation of liposomal vincristine was also evaluated in a total of 13 patients and confirmed the preliminary observations from the Phase I study (17) that administration of vincristine in this liposomal formulation results in substantially higher plasma levels of the drug than would be anticipated from free vincristine. Pharmacokinetic analysis of the data also confirmed the Phase I data (17) by suggesting that liposomal encapsulation eliminated the early phase of rapid elimination seen after the administration of free vincristine (Mayer, L.D., Denyssevych, T. and St.-Onge, G., unpublished data).

The recent Phase II clinical development strategy has assessed the single-agent activity of liposomal vincristine in tumor types known to be sensitive to this drug. A Phase IIa study of SM/Chol liposomal vincristine in patients with relapsed non-Hodgkin's lymphoma (NHL) has been completed at the M.D. Anderson Cancer Center in Houston and preliminary data were recently published (18,19). Heavily pretreated patients with indolent (low grade), aggressive (intermediate grade) or transformed NHL have been treated with liposomal vincristine at 2 mg/m^2 every two weeks by one-hour intravenous infusion with no dose capping. Response results are presented in Table 2. The overall response rate in patients having indolent NHL was low (13%) and accrual for these patients was subsequently closed. In contrast, patients with aggressive or transformed histology NHL had good responses to treatment with liposomal vincristine, with overall response rates of 45% and 42% respectively. Furthermore, these response rates were achieved with relatively low toxicities. Specifically, the primary toxicity was

Table 2. Responses of Relapsed NHL Patients to Treatment with Liposomal Vincristine

	NHL HISTOLOGY			
	INDOLENT	AGGRESSIVE	TRANSFORMED	POST-BMT
Total number of patients	15	38	12	3
Number of CRs or PRs	2	17	5	0
Overall response rate (%)	13	45	42	0
95% Confidence interval	2–41	29–62	15–72	0–71

Complete Response (CR) is the complete elimination of all signs of the tumor; Partial Response (PR) is a > 50% reduction in overall tumor size. BMT: bone marrow transplantation. Data from (18).

motor or sensory neuropathy and was dose-limiting in approximately 10% of patients (18,19). Significant myelotoxicity (grade 3 or 4), the dose-limiting toxicity associated with the majority of other cytotoxic agents for the treatment of NHL, was not observed in patients treated with liposomal vincristine (18,19).

These preliminary results from the evaluation of liposomal vincristine in the treatment of relapsed NHL compare well with known literature on single-agent therapy in this patient population. A comprehensive review of the clinical literature (Webb, Saltman & Goldie, in preparation) has shown that the 45% response rate observed in heavily pretreated patients with aggressive histology NHL is competitive with the best response rates previously reported from reliable studies. Furthermore, this activity is associated with low (10%) dose-limiting neurotoxicity and no myelosuppression that contrasts sharply with the higher frequency of significant, and sometimes severe, myelotoxicity occurring with the administration other active single-agents (etoposide, paclitaxel, vinorelbine) in this patient population.

CONCLUSIONS

Vincristine is an anticancer agent that, by virtue of its cell-cycle specific mechanism of action, is a rational choice for significantly increased antitumor activity effected by liposomal delivery. This expectation has been confirmed in a variety of murine tumor models, human xenograft tumors in rodents and, most recently, in clinical evaluation in relapsed non-Hodgkin's lymphoma patients. In both preclinical and clinical settings, the increased antitumor activity is achieved with toxicities that are similar, or reduced, compared to those observed with free vincristine.

The successful preclinical and clinical development of the liposomal vincristine formulation has required: (1) optimization of the drug loading process; (2) solving both liposome stability and manufacturing issues; (3) optimization of the physical characteristics of the liposomes that determine disease-site targeting and regulated *in vivo* drug release.

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