

# Liposomal Encapsulation of Flavopiridol for the Treatment of Chronic Lymphocytic Leukemia

Treatment of Chronic Lymphocytic Leukemia employs multiple chemotherapeutic agents, which show promising effects, but are subject to drug resistance. As has been proven to be the case with antibiotics, we will have to alter our drug's existing structure or continue to find new and innovative compounds to avoid resistance.

Flavopiridol is currently a Phase II drug for the treatment of Chronic Lymphocytic Leukemia (CLL), and is specifically of interest for CLL treatment because of tumor lysis by induced apoptosis, anti-resistance, and a curative effect that other therapies lack. Serious toxicities occur upon initiation of treatment and up to 24 hours after preliminary dosing. The most common adverse effect, severe dehydration, creates an essential and undesirable need to give additional medications to the patient.

Pharmacokinetics hypothesize that drugs entrapped in a liposome have the ability to lower plasma clearance, causing an increase in the AUC, half life, and circulation time. Applying this innovative idea to Flavopiridol should effectively lessen the adverse toxicities experienced while increasing the therapeutic outcome.

## Creation of a Liposome to Carry Flavopiridol

Creation of the liposome follows the design of the liposomal formulation of Doxorubicin, released as the first liposome formulation in the market.

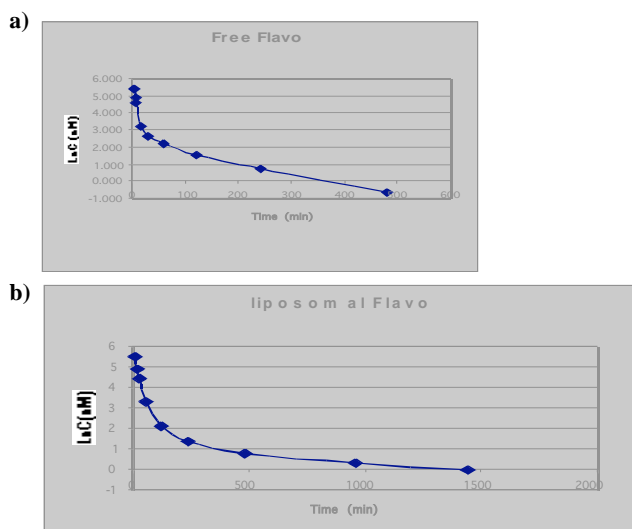
- Liposomes are comprised of hydrogenated soy phosphatidylcholine (HSPC), cholesterol, and Polyethelene glocol - distearoyl phosphatidylethanolamine (DSPE-PEG) in a molar ratio of 11:8:1.
- Entrapment via pH gradient and an ammonium sulfate chemical gradient
- Polyethylene glycol (PEG) adds protection against uptake by the reticuloendothelial system.
- Thin layer chromatography for even distribution of lipids during the dissolution of lipids.
- Extended circulation time. Area under the concentration-time curve (AUC) is increased
- Enhanced safety and heightened efficacy

For our formulation of Flavopiridol, we create a 100 nanometer diameter that allows for greater extravasation through tumor vasculature. PEGylation (polyethylene glycol) will help avoid clearance by RES (reticuloendothelial system), which clears drugs and unwanted compounds from the plasma. Flavopiridol is entrapped via pH and an ammonium sulfate chemical gradient in the aqueous interior of the liposome. The drug permeates through the bilayer to the interior, encounters the ammonium sulfate and crystallizes, becoming trapped. When the liposome bursts or enters its target cell, the bilayer is destroyed, and the crystallized drug returns to the solution phase as the ammonium sulfate is dispersed. This allows for a high concentration to be delivered at a

specific site, instead of circulating a great amount of drug throughout the system at once. The optimal proportions of lipid that have been chosen provide a unique balance of fluidity to ensure the right amount of stability in circulation, shelf life and the mechanistic release of the therapeutic compound into the bloodstream.

## Pharmacokinetic Parameters and Protein Binding

To ensure that our liposome performs as it should, a pharmacokinetic study is performed using 6 B6C3 mice. One dose is given to each mouse at 2.5mg/kg, or 125nmol/mouse. Mice are randomly assigned to one of two groups, Free Flavopiridol delivery or Liposomal Flavopiridol delivery. Blood samples are collected for analysis of drug concentration at time points shown below, and PK curves are created as follows;



**Figure 1.** Pharmacokinetic concentration curves, representative of all Flavopiridol in the bloodstream at time of collection. Figure a) has collection points 0-480min, while Figure b) for liposomal Flavopiridol ranges from 0-1480min.

Calculations using WinNonlin analysis confirm the AUC of free Flavopiridol to be 2927nmol\*min/L, as compared to that of the liposomal formulation of 8716nmol\*min/L. It is also confirmed that the half life and clearance are 31.8min and 9.02nmol/L respectively for free Flavopiridol, compared to 41.0min and 4.17nmol/L for the liposomal drug.

An issue discussed was whether or not the AUC was correct, since the method of Flavopiridol analysis does not account for the difference in liposomally entrapped drug that remains in circulation at the time of collection, which may not be effective while still entrapped. This shows a need to develop a method that will effectively separate all of the free, available active form of the drug from that which is still entrapped. To do this, we use ultrafiltration to separate the liposome and other blood plasma proteins from the free

drug and other small molecules. After this separation, we can quantify the amounts that have passed through the filter (free drug) and the amount of Flavopiridol left on the filter that would not pass (liposomally entrapped drug). Data for our control group of only free flavopiridol passed through the filter in neat solution vs. plasma did not show results as expected. While 100% of the drug should have been recovered, as none of it is entrapped in liposome, there was only an average of 50% recovery for each of the concentrations in the standard curve, ranging from 30nM to 30uM. The relevant charted data for this discovery is shown below.

<b>Free Drug Recovery Using Ultrafiltration</b>			
Concentration(nM)	Neat	Plasma	Recovery(%)
30	46	25	53.47
100	232	139	59.81
300	638	484	75.87
1000	2430	1107	45.55
3000	6484	3309	51.03
10000	26432	11483	43.44
30000	66078	31851	48.20

**Figure 2.** Organization of two standard curves expressing an average for triplicates at each concentration detected by mass spectroscopy. Recovery is represented as a percentage of plasma over neat concentration.

It is concluded that this result is due to plasma protein binding. Molecules with certain properties are able to stick to albumin protein freely circulating in the blood. The drug is still active while bound and has the ability to detach again, but since the affinity for the binding is high, a large portion of the drug remains bound until being cleared from the system. These results show us that ultrafiltration is not a sufficient method to separate all of the free drug, and that by using these methods of detection to quantify the drug, we are not able to get an accurate measurement of the proportion of liposomally entrapped drug in a blood sample.

Although this seems detrimental to our experiment, it is still possible for us to see the effects of the free drug that is available in the bloodstream, and these effects can be used to compare formulations.

### **Efficacy**

To do this, we set up an *in vivo* xenograft study where Leukemia tumors will be created and then treated. 24 female nu/nu mice will be injected subcutaneously in the left flank with Human Promyelocytic cell line HL-60. Mice will be randomly assigned to one of three groups, control, free Flavopiridol and liposomal Flavopiridol. Dosing will last 5 consecutive days, administering 5mg/kg of the respected formulation via IV bolus tail vein injections. The control group will receive the same mL amount of saline solution via IV bolus tail vein injection. Additionally, the administration of Cephalexin (antibiotic) as 125mg/250mL drinking water 24 hours before to 48 hours after Flavopiridol treatment is essential to the

survival of the mice to protect against lethal bacterial levels in the lower GI tract.

Future work will be comprised of more xenograft studies of different cell lines, where the efficacy can surpass that of the free drug. Combination therapies and targeted delivery via folate receptors are possibilities as well.