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Abstract

Background. Vincristine sulfate (VCR) is a lipophilic, cell-cycle specific, antineoplastic agent that inhibits cell division by specifically binding to tubulin in mitotic spindles. Marqibo[®] (Vincristine Sulfate Injection) is a proprietary sphingomyelin/cholesterol liposome (OPTISOME[™]) encapsulated formulation of VCR with an extended circulating half-life and the potential for enhanced tumor tissue targeting, exposure, and anti-tumor activity. This study evaluated and compared the accumulation of VCR in the tumor tissue and bone marrow of tumor-bearing mice following intravenous (IV) administration of Marqibo or VCR.

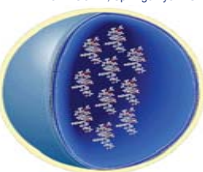
Methods. Mice were implanted subcutaneously with MX-1 human breast tumor tissue. When the tumor volume reached 160-485 mm³, mice received a single IV 1.5 mg/kg dose of [¹⁴C]-VCR or a Marqibo dose containing 1.5 mg/kg [¹⁴C]-VCR. The total radioactivity from parent compound and metabolites in tissue VCR_T was analyzed by the tissue digestion (TD) method and Quantitative Whole Body Autoradiography (QWBA). For TD, tumor tissue was obtained from 3 mice per group prior to infusion and at 4, 16, and 48 hours post-infusion. Samples were chemically digested and analyzed for radioactivity by liquid scintillation counting. QWBA analysis for tumor tissue and bone marrow analysis required 1 flash-frozen mouse per group at the same time points used for the TD method. Sagittal sections were examined histologically for radioactivity.

Results. The TD and QWBA methods of analysis provided comparable VCR_T accumulation results. Marqibo infusion resulted in tumor VCR_T concentrations of 1.22, 2.04, and 1.35 mcg drug per gram of tissue (mcg/g) at the 4, 16, and 48-hour time points, respectively, compared to 0.28, 0.57, and 0.31 mcg/g following VCR infusion. Marqibo infusions resulted in bone marrow VCR_B concentrations of 2.6, 2.75, and 0.99 mcg/g at the 4, 16, and 48-hour time points, respectively, compared to 2.1, 0.94, and 0.36 mcg/g following VCR infusions. TD indicated that tumors in Marqibo-treated mice had 3-times the C_{max} (2.08 vs. 0.65 mcg/g) and 6-times the AUC_{0-48h} (123 vs. 21 hr*mcg/g) compared to VCR-treated mice over a 48-hour period.

Conclusions. Optimal encapsulation of vincristine, Marqibo, results in targeted delivery of drug, increased concentration of drug in tumor tissue (4-fold) and bone marrow (3-fold) at 48 hours, and maintenance of significant tissue drug concentrations for several days compared to conventional VCR. These unique characteristics result in greater tumor drug exposure and the potential for enhanced anti-tumor activity without increased toxicity. The ability of Marqibo to target drug to the bone marrow makes it particularly attractive as a treatment for hematologic malignancies like myeloma, lymphoma, and acute lymphoblastic leukemia (ALL). Late-stage clinical development of Marqibo in adult ALL is underway.

Background

OPTISOME, Sphingomyelin/Cholesterol Liposome Encapsulated Drug

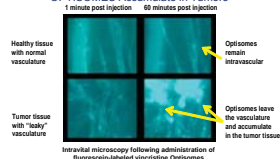


- Conventional Vincristine (VCR)
 - Cell-cycle specific (G2/M phase) antineoplastic agent
 - Limited cell-to-cell penetration through cell membrane
 - Approved for use in treatment of leukemia
- An amphiphilic liposome formulation of VCR
- The encapsulation results in a receptor delivery system with an extended circulating half-life

Vincristine Sulfate Liposome Injection (Marqibo)

- Optimizes how the patient is prepared to improve delivery of chemotherapeutic agents
- Superior pharmacokinetic/intracellular results in a highly targeted lipid bilayer
- 100 mg/ml maximum concentration
- > 1000 mg/ml maximum per liposome
- Aqueous pH of 6.0 ± 0.1 enables high drug concentration loading and prevents payload degradation
- Retains drug long enough to reach sites of tumor accumulation
- Predictable release and release rates (10-100 hrs)

OPTISOMES Accumulate in Tumors



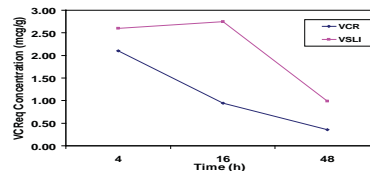
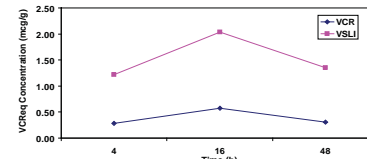
Methods

- MX-1 tumors from four in vivo passages in mice were harvested when tumor diameter reached 10-15 mm or tumor volume reached 300-600mm³
- Total pool of 156 female C57BL/6J (CD-1[™])BR nude mice
- 130 mice were implanted subcutaneously with MX-1 human breast tumor tissue
- 26 mice were not implanted with tumor (tumor-free)
- On the day of treatment, tumors were measured and mice with tumor volumes ranging from 160-485 mm³ were selected for inclusion into the study
- Treatments were administered as a single bolus injection of 1.5 mg/kg of [¹⁴C]-VCR or Marqibo dose on Days 18, 19 or 20 post-implantation
- Following randomization, mean tumor volumes for all treatment groups ranged from 223-255 mm³
- VCR was analyzed using TD and QWBA
- TD: tumor tissue was obtained from 3 mice/group prior to infusion, 4, 16 and 48 hours post-infusion. Samples were analyzed by liquid scintillation counting
- QWBA: 1 flash-frozen mouse/group prior to infusion, 4, 16 and 48 hours post-infusion. Samples were analyzed histologically for radioactivity.

Results

Summary of QWBA VCR_T Distribution in Tumor-Bearing Mice Following Administration of Either 1.5 mg/kg VCR or Marqibo (VSLI)

Tissue	VCR _T Concentration (mcg/g)					
	VCR Tumor-Bearing			VSLI Tumor-Bearing		
	4 h	16 h	48 h	4 h	16 h	48 h
Bone Marrow	2.10	0.94	0.36	2.60	2.75	0.99
Liver	0.53	0.16	0.13	3.67	1.06	0.47
Lymph Nodes	-	-	-	-	-	1.98
Spleen	2.22	1.21	0.76	8.56	6.45	6.08
Tumor	0.28	0.57	0.31	1.22	2.04	1.35

VCR_T Concentration in Bone MarrowVCR_T Concentration in Tumor Tissue

Summary of TD VCR_T Distribution in Tumor-Bearing Mice Following Administration of 1.5 mg/kg VCR

Tissue	C _{max} (mcg/g)	T _{max} (h)	AUC _{0-48h} (hr*mcg/g)	MRT _{0-48h} (h)	A ₀ (dpm)	t _{1/2α} (h)
Spleen	25.9	16	620	16.2	0.0335	20.7
Cell Bladder	45.3	0.35	620	6.50	0.0254	27.1
Tumor	0.65	1	21.0	22.3	0.0057	122
Lymph Nodes	1.49	1	16.5	12.2	0.0280	24.8
Liver	3.15	0.25	12.0	11.0	0.0069	69.8
Kidney	0.33	0.25	12.2	6.27	0.0410	16.9
Skin	0.334	0.25	2.13	7.86	0.0161	43.1
Lung	3.12	0.25	21.9	7.00	0.0465	11.5
Heart	1.85	0.25	8.82	5.54	0.0483	14.3
Muscle	0.743	0.25	5.28	5.79	0.008	7.63
Fat	0.296	0.25	0.85	3.14	0.159	6.00

Summary of TD VCR_T Distribution in Tumor-Bearing Mice Following Administration of 1.5 mg/kg Marqibo

Tissue	C _{max} (mcg/g)	T _{max} (h)	AUC _{0-48h} (hr*mcg/g)	MRT _{0-48h} (h)	A ₀ (dpm)	t _{1/2α} (h)
Spleen	10.8	16	612	35.4	0.0229	30.3
Cell Bladder	0.35	4	208	27.8	0.0055	26.2
Tumor	2.08	16	123	39.9	0.0147	47.1
Lymph Nodes	2.77	16	121	25.0	0.0223	21.4
Liver	2.49	4	68.8	29.4	0.0220	31.5
Kidney	2.26	16	66.2	25.0	0.0225	25.5
Skin	0.882	34	42.5	34.6	0.0724	9.57
Lung	0.696	34	33.1	30.6	0.0245	26.3
Heart	0.580	16	19.4	26.4	0.0156	35.6
Muscle	0.256	16	6.11	30.8	0.0214	32.4
Fat	0.127	16	3.23	16.4	0.0576	12.1

Conclusions

- Tissue vincristine exposures (AUC) were greater in Marqibo-treated than in conventional vincristine-treated mice
- Intra-tumor vincristine concentrations were greater in Marqibo-treated mice compared to conventional vincristine-treated mice at all time points (4, 16, and 48 h) resulting in greater vincristine exposure
- Marqibo administration resulted in a 3-fold increased concentration of vincristine in bone marrow at 48 hours and maintained significant tissue concentrations for several days compared to conventional vincristine
- The unique characteristics of Marqibo (Optisome-encapsulated vincristine) resulted in greater targeted drug exposure and the potential for enhanced anti-tumor activity without increased toxicity
- Late-stage clinical development of Marqibo in adult ALL is underway