



A Novel N-oxide Drug, AQ4N, Has *in vitro* Activity in Lymphoma and Leukemia Cell Lines and Selectively Targets Lymphocytes and Lymphatic Tissues *in vivo*.

Jeffrey L. Cleland, Alvin Wong, Susan E. Alters, John G. Curd, Robert L. Capizzi and William D. Henner*.

Novacea., Inc., South San Francisco, CA

Novel Therapies Improving Lives

Objective

Evaluate the potential for a targeted cytotoxic prodrug, AQ4N, to selectively kill malignant lymphoid cells

Background

Banoxantrone (AQ4N; 1,4 bis(2-(dimethylamino)ethylamino)-5,8-hydroxyanthracene-9,10-dione N-oxide) is selectively bioreduced to AQ4

AQ4 binds to DNA and inhibits topoisomerase II activity
AQ4N is bioreduced in hypoxic tumors by cytochrome P450 enzymes (primarily 3A4)
AQ4N (banoxantrone) is in the same class of anthracenediones as mitoxantrone
Human dosing up to 447 mg/m² without DLT

In Vitro Cytotoxicity Assays

Cells (from ATCC; 5x10³-5x10⁴ cells/well) treated 24 hrs after plating with AQ4, AQ4N, or doxorubicin
4 hrs exposure to each agent, washed with media and incubated for an additional 48 hrs.
Number of proliferating cells was measured by an MTS assay and IC₅₀ values from % Growth Inhibition values
Studies done in duplicate.

Tumor Line	Type	AQ4	AQ4N*	Dox
audi	Burkitt Lymph	4.6 nM	NA**	0.5 nM
aji	"	200 nM	NA	0.9 nM
amos	"	8.0 nM	NA	1.8 nM
amalwa	"	0.2 nM	400 nM	7.4 nM
DLT-4	ALL (human)	1.5 nM	700 nM	7.5 nM
60	AML (human)	10 nM	NA	0.1 mM
1a	AML (human)	50 nM	43 mM	0.6 mM
62	CML (human)	400 nM	1.0 mM	0.2 mM
88	CLL (mouse)	10 nM	31.5 mM	0.1 mM
210	ALL (mouse)	1.2 nM	0.6 mM	45 nM
178Y	mouse lymph	26 nM	0.3 mM	50 nM
MI8226	multiple myel.	0.2 mM	NA	0.1 mM
MI8226/Dox	"	1.1 mM	NA	54.7 mM
H177	"	0.2 mM	NA	--

All studies done under normoxic conditions only
NA = not active; IC₅₀ > 100 mM

AQ4N is active in lymphoma & leukemia cell lines

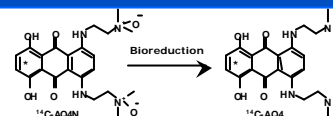
Rat Toxicology Study

- Study Design**
 - 20 SD rats (10 male/10 female)
 - AQ4N dosed IV at MTD of 20 mg/kg (120 mg/m²) per wk for 6 wks
 - Sacrificed one wk after last dose or 4 wks after last dose (recovery period)
 - Gross and microscopic histology was performed on all animals
- Results**
 - 22-24% less body weight gain compared to controls
 - ~50% reduction in WBCs, decreased lymphocytes, and occasional neutropenia
 - 18-22% reduction in group mean spleen weight
 - Minor atrophy of thymus, mesenteric and mandibular lymph nodes, and bone marrow

Monkey Toxicology Study

- Study Design**
 - 6 cynomolgus monkeys (3 male/3 female)
 - AQ4N dosed IV at MTD of 6 mg/kg (72 mg/m²) per wk for 6 wks and 4 wk recovery phase
 - Sacrificed one wk after last dose or 4 wks after last dose (recovery period)
 - Gross and microscopic histology was performed on all animals
- Results**
 - Atrophy of spleen (lymphoid), mandibular and mesenteric lymph nodes, and marrow (sternum), all of which reversed after the 4 wk recovery phase
 - Microscopy findings also included urothelial degeneration in kidney and urinary bladder, and tubular nephropathy in the kidney
 - ~50% decrease in WBC, decrease in lymphocytes, and occasional neutropenia which reversed after 4 wk recovery phase

Toxicity primarily minor lymphoid atrophy & lymphopenia



Biodistribution in Rats

- Single dose 20 mg/kg AQ4N (130-140 mCi/kg)
- 1 pigmented rat per time point (Day 1, 3, 5, 7, & 14) sacrificed for whole body autoradiography
- Spiked blood samples used for quantitation (0.04 to 236 mg equivalents/g tissue)

Tissue	t _{1/2} (hrs)	168 hrs (mg equiv./g tissue)
Blood (mg/mL)	8.63	0.000
Spleen	537.9	0.865
Harderian gland	621.2	1.067
Liver	82.8	1.472
Kidney (cortex)	318.6	0.496
Small Intestine (mucosa)	107.7	9.948
Testes	252.3	0.087
Bone marrow	81.3	0.240
Adrenal medulla	45.1	0.183
Non-pigmented skin	65.4	0.052

Biodistribution in Monkeys

- Cynomolgus - 10 mg/kg AQ4N (135 mCi/kg)
- One male and one female animal sacrificed at 168 hrs for whole body autoradiography
- Spiked blood samples for quantitation

Tissue	mg equiv./g tissue
Blood (mg/mL)	0.27 - 1.54
Spleen	76.6 - 86.6
Thymus	19.5 - 27.4
Mandibular Lymph Nodes	29.1 - 40.2
Liver	67.9 - 78.6
Myocardium	15.1 - 36.1
Large Intestine	9.48 - 23.4
Small Intestine	8.77 - 45.4
Gall Bladder Contents	35.4 - 58.6
Lung	39.2 - 52.2
Adrenal Cortex	78.7 - 86.6
Bone Marrow	3.20 - 16.8

Rapid clearance from blood, long half-life in spleen

Summary

- AQ4N and the bioreduced form, AQ4, have potent cytotoxicity against lymphoma and leukemia cell lines
- AQ4N toxicity in rats and monkeys occurs primarily in lymphoid organs
- ¹⁴C-AQ4N radioactivity persists in lymphatic tissues
- Results indicate the potential for AQ4N to have selective activity against lymphoid malignancies

Ongoing Studies

- Mechanism of action studies to determine the activity of AQ4N in lymphoma and leukemia cell lines
- In vivo efficacy studies of AQ4N in lymphoma and leukemia models (survival and xenograft)
- Solid tumor efficacy studies and biodistribution studies (solid tumor) to demonstrate activity as monotherapy and combination therapy