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The targeted cytotoxic prodrug, AQ4N, has significant activity in the breast adenocarcinoma model MDA-MB-231

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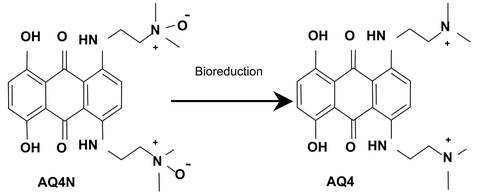
Background

- AQ4N (banoxantrone) is a N-oxide prodrug designed to be bioreduced under hypoxic conditions which are found in most solid tumors
- Previous studies have shown that AQ4N undergoes two consecutive 2 electron reductions to yield AQ4, a potent topoisomerase II inhibitor (see Figure; Patterson, Cancer Metastasis Rev 1993; 12:119-34)



- Bioreduction of AQ4N is catalyzed by cytochrome P450 enzymes 1A1, 1B1, and 3A4 (Patterson, Drug Metab Rev 2002; 34: 581-92)
- AQ4 shows in vitro cytotoxicity on a wide range of human solid tumor cell lines. AQ4N is relatively non-toxic under physiologic/oxic conditions
- AQ4N has shown efficacy in preclinical models of colon and pancreatic carcinoma
- AQ4N is currently being studied in several human Phase I studies in the US and Europe (see Harris et al. abstract 2414)

Methodology

In Vitro Cytotoxicity

- Tumor cells were incubated with a range of concentrations of AQ4, AQ4N, and a standard agent for 24 hours under normoxic conditions
- Drugs were washed out, replaced with drug-free media and incubated for 72 hours
- Cytotoxicity was assessed by MTT assay and results expressed as IC50, the drug concentration needed to obtain a 50% inhibition of cell proliferation

In Vivo Efficacy

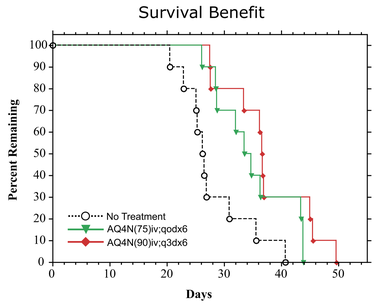
- Tumor cells were implanted subcutaneously in Nu/Nu mice and tumors allowed to grow to a mean size of 80-120mm^3 prior to treatment
- Mice (10/group) were matched to assure comparable inter-group variability at the start of treatment
- Drugs were injected iv at the dose-schedules indicated
- Endpoint of the study was defined as 1500mm^3 or 60 days
- Outcome was assessed by Tumor Growth Delay (TGD), the increase in median time to endpoint (TTE) in treatment compared to control group. Logrank test was used to analyze significance of differences in TGD

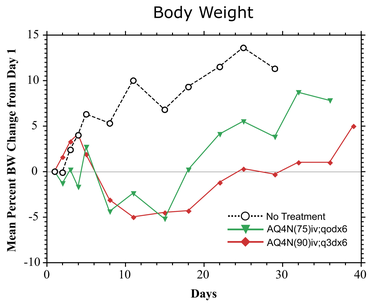
Cytotoxicity Assessment

Tumor Line	Tumor Type	AQ4 (μM)	AQ4N (μM)	Standard (μM)
MCF-7 **MDA-MB-231**	**Breast** **Breast**	**0.3** **2.7**	**NA** **NA**	**0.002 (paclitaxel)** **NA (paclitaxel)**
BxPC-3 Panc-1 Mia PaCa-2	Pancreatic Pancreatic Pancreatic	1.6 0.4 1.6	NA NA NA	0.060 (gemcitabine) 1.70 (gemcitabine) 0.020 (gemcitabine)
HT-29 HCT-116 LoVo LS174T	Colon Colon Colon Colon	0.7 3.9 0.2 0.7	101.5 NA 49.9 14.4	0.087 (SN38) 0.900 (SN38) 0.024 (SN38) 0.106 (SN38)
A-498 Caki-1	Renal cell Renal cell	2.5 1.8	NA NA	NA (paclitaxel) NA (paclitaxel)
A2780 OVCAR-3	Ovarian Ovarian	0.05 2.7	12.3 NA	0.001 (paclitaxel) 0.015 (paclitaxel)
A549 H460	NSCLC NSCLC	0.1 1.4	85.4 103.0	0.010 (paclitaxel) 0.006 (paclitaxel)
PC-3 LNCa-FGC	Prostate Prostate	11.2 0.8	NA 43.0	0.007 (paclitaxel) 0.002 (paclitaxel)
FaDu KB	Pharynx SCC Mouth carcinoma	0.3 0.6	64.7 13.4	0.007 (paclitaxel) 0.002 (paclitaxel)
Hep3B2,1-7	Hepatocellular	0.8	NA	0.014 (paclitaxel)
A375	Human melanoma	3.3	NA	0.006 (paclitaxel)

NA: not achievable

Efficacy in Breast Model





Treatment Response Summary

Treatment Regimen			Efficacy Parameters				
Agent	mg/kg	schedule	median TTE	T-C	%TGD	P value	Mean BW nadir
None	--	--	26.4	--	--	--	-0.1% day 2
Paclitaxel	30	qod x5	60	33.6	128%	0.0001	-6.5% day 11
AQ4N	75	qod x6	34.1	7.7	29%	0.02	-5.2% day 15
AQ4N	90	q3d x6	36.7	10.3	39%	0.005	-5.0% day 11

Study Endpoint = 1500 mm^3
TTE = time to endpoint
T-C = difference between median TTE (days) of treated versus control group
%Tumor Growth Delay (TGD) = [(T-C)/C] x 100
Statistical Significance = Logrank test as compared to Untreated
Mean BW Nadir = lowest group mean body weight, as % change from Day 1

Summary

- **AQ4N is selectively bioreduced under hypoxic conditions in the tumor microenvironment to generate AQ4, a potent cytotoxic metabolite that intercalates in DNA and functions as a topoisomerase II poison**
- **The bioreduced topoisomerase II poison, AQ4, but not its prodrug form, AQ4N, demonstrates potent inhibition on a wide spectrum of tumor cell lines when tested under physiological conditions in vitro**
- **Two AQ4N monotherapy regimens demonstrate significant efficacy when evaluated for tumor growth delay in the MDA-MB-231 breast model**
- **AQ4N at 75mg/kg qod x 6 results in 29% tumor growth delay**
- **AQ4N at 90mg/kg q3d x 6 results in 39% tumor growth delay**
- **Both regimens are well tolerated with maximum weight loss of approximately 5%**
- **Taken together with previous results showing comparable efficacy to standard agents in pancreatic and colon carcinoma models these data indicate that AQ4N may have utility in the treatment of a variety of solid tumor malignancies**

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