# The tissue targeted prodrug, AQ4N, is activated to AQ4 in murine tissues and has anti-tumor efficacy in HCT116 colon and Panc1 pancreatic carcinoma xenograft models.

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## Background

- AQ4N (banoxantrone) is a prodrug of a potent topoisomerase II inhibitor, AQ4
- Systemic toxicity of AQ4N is minimized due to lack of systemic bioreduction to AQ4
- AQ4N selectively accumulates in xenografts and murine intestines, pancreas and lymphoid tissues where it is bioreduced to AQ4
- AQ4N has significant efficacy in several pre-clinical xenograft models including BXPC3 (pancreatic) and HT29 (colon carcinoma)
- AQ4N is being studied in human Phase I studies in the US and Europe

## Methodology

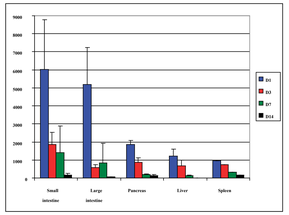
Biodistribution:

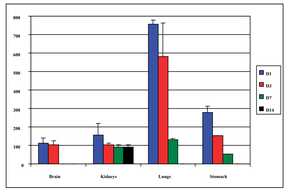
- $10^7$ BXPC3 (human pancreatic cancer) cells were injected sc; tumors grew to 200-300mm$^3$ prior to dosing
- Single IV injection of AQ4N at 20 mg/kg
- Mice were sacrificed at 24, 72, 168, 336 hours post AQ4N dose (3 mice/time point)
- AQ4N and AQ4 levels were determined by HPLC/MS-MS in human tumor and murine plasma and solid tissue
- Limit of quantitation: 1) Plasma: 50ng/ml
  2) Tissue: 25ng/g

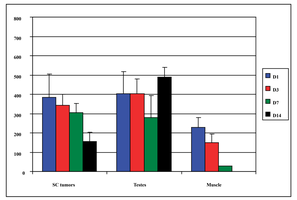
Efficacy:

- Panc1 or HCT116 xenografts were implanted sc into nude mice (10/group)
- Treatment was initiated when tumors reached ~100mm$^3$
- Treatment outcome determined as time to endpoint; endpoints were defined as 1200mm$^3$ for Panc1 and 2000mm$^3$ for HCT116
- Significance in tumor growth delay (difference in time to endpoint in treatment group compared to control) determined by log-rank test

## AQ4 Levels in Tissues

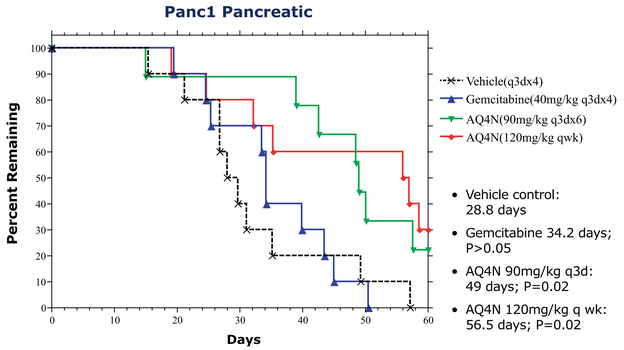


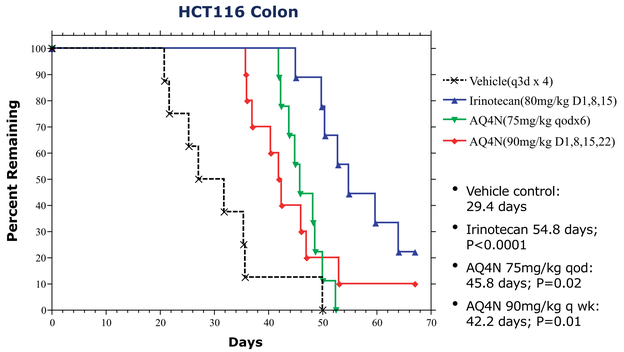




- Injected AQ4N no longer detected at 24 hours
- Highest levels of AQ4 detected in small, large intestine; elimination seen after 24 hours
- AQ4 measured in sc tumor at all sampling times; concentrations fairly stable for 7 days
- AQ4 no longer detected in plasma at 24 hours

## Efficacy in Xenograft Models




- Vehicle control: 28.8 days
- Gemcitabine 34.2 days; P>0.05
- AQ4N 90mg/kg q3d: 49 days; P=0.02
- AQ4N 120mg/kg q wk: 56.5 days; P=0.02




- Vehicle control: 29.4 days
- Irinotecan 54.8 days; P<0.0001
- AQ4N 75mg/kg qod: 45.8 days; P=0.02
- AQ4N 90mg/kg q wk: 42.2 days; P=0.01

## Summary

- AQ4N is converted to AQ4 within 24 hours following a single 20mg/kg dose.
- The highest levels of AQ4 are detected in the small and large intestine at 24 hours. Rapid elimination is seen in these organs.
- Levels of AQ4 can be measured in the sc tumor at all sampling times from 24 to 336 hours following a single 20mg/kg dose of AQ4N. AQ4 concentrations are fairly stable for 7 days in the tumor.
- AQ4 is no longer detectable in plasma post 24 hours.
- AQ4N demonstrates significant efficacy in colon and pancreatic tumor xenograft models at dose levels below the MTD.
- In the Panc1 model, weekly doses of AQ4N at 120mg/kg result in a significant increase in time to endpoint of 56.5 days as compared to 28.8 for vehicle and 34.2 for gemcitabine.
- In the HCT116 colon model AQ4N given at 75mg/kg qod results in a significant increase in time to endpoint of 45.8 days as compared to 29.4 for vehicle and 54.8 for irinotecan. Weekly doses of AQ4N at 90mg/kg also give a significant response.
- These data, taken together with our previous data showing a significant effect of AQ4N in BXPC3 pancreatic and HT29 colon carcinoma models, indicate the potential use of AQ4N as a single agent in these malignancies.

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