The cytotoxic prodrug, AQ4N, demonstrates tumor targeting and accumulation resulting in anti-tumor activity in the BxPC3 pancreatic xenograft model

**Susan E. Alters, Alvin Wong, Alshad S. Lalani and W.D. Henner**

Novacea, Inc., South San Francisco, CA

**Background**

- AQ4N (banoxantrone) is a prodrug of a potent topoisomerase II inhibitor, AQ4
- AQ4N is bioactive to AQ4 under hypoxic conditions as found in most solid tumors. In particular, human pancreatic tumors have significant regions of hypoxia (Koong et al. 2000, Int. J. Radiat. Oncol. Biol. Phys. 48:919)
- AQ4 has potent in vitro activity on a wide range of solid tumor lines (abstract #5445). AQ4N is relatively non-toxic under physiological/normoxic conditions
- AQ4N has significant efficacy as a single agent in pancreatic, colon, breast xenografts, and in combination with radiation and chemoradiation in bladder and lung xenografts (abstract #1130).
- Selective accumulation of AQ4 in hypoxic regions of tumors has now been demonstrated in a wide range of human solid cancers in a Phase I clinical study (abstract #2414).

**Methodology**

- **Efficacy in pancreatic xenografts (sc/Panc1):** Panc1 xenografts were implanted sc into nude mice (10/group) and treatment initiated when tumors reached ~150mm^3. Study endpoint was set at 2000 mm^3. Significance in tumor growth delay (difference in time to endpoint in treatment group compared to control) determined by log-rank test.
- **Efficacy in sc BxPC3:** Tumor cells were implanted sc into nude mice (15/group) and treatment initiated when tumors reached ~100mm^3. Outcome was determined as tumor growth inhibition (TGI), the change in mean treated tumor weight/the change in mean control tumor weight x 100 (ATCG). Significance was determined using ANOVA followed by the Dunnett multiple comparisons test.
- **Efficacy and metastases analysis in orthotopic (ot) BxPC3:** Tumor cells were implanted into the pancreas of nude mice (12/group + 5/group for histology). Treatment was initiated on day 14. Kaplan Meier analysis using Logrank statistics was performed to determine significance. Mice for histology were sacrificed on day 36, liver was paraffin embedded and H&E stained. Microscopy was performed independently by 2 individuals blinded with respect to treatment. To compute metastases index, the percentage of evaluated fields (5 per slide) occupied by the metastases versus the non-occupied areas (normal tissue) was calculated. Statistical analysis was done with the non-parametric Mann-Whitney U-test.
- **Biodistribution/PK:** BxPC3 cells were injected sc; tumors grew to ~300mm^3 prior to dosing. Mice received a single iv dose of AQ4N at 20 mg/kg, 60mg/kg, 120mg/kg, or 240mg/kg. Mice were sacrificed at 2,8,24 hours post AQ4N dose (3 mice/time); AQ4N and AQ4 levels were determined by LC/MS in human tumor and murine plasma. Limit of quantitation: Plasma: 50ng/ml; Tumor: 25ng/g

**Efficacy in Pancreatic Models**

- **Subcutaneous Panc1:** Treatment was initiated on day 14. Kaplan Meier analysis using Logrank statistics was followed by the Dunnett multiple comparisons test
- **Subcutaneous BxPC3:** Treatment was initiated on day 14. Kaplan Meier analysis using Logrank statistics was followed by the Dunnett multiple comparisons test
- **Orthotopic BxPC3:** Treatment was initiated when tumors reached ~50-100mm^3.

**Summary**

- AQ4N demonstrates significant efficacy in subcutaneous models of pancreatic carcinoma, Panc1 and BxPC3
- AQ4N also demonstrates significantly increased survival in the highly aggressive orthotopic BxPC3 model
- AQ4N treatment results in a decrease in the number and size of liver metastases in the OT BxPC3 model
- There is rapid conversion of AQ4N to AQ4 in tumor with near maximum AQ4 levels apparent by 8 hours
- Linear dose-dependent deposition of AQ4 occurs and is retained in the tumor microenvironment for at least 24 hours
- AQ4 is rapidly lost and AQ4 is undetectable in the plasma at all doses from 20-240mg/kg
- These data indicate that AQ4N is a unique hypoxia-activated cytotoxic that has minimal systemic cytotoxicity and selective anti-tumor and anti-metastatic activity as a single agent in preclinical models of pancreatic adenocarcinomas as well as other solid tumors

**Effect on Metastases Formation**

- Median Survival Time: MST
- Percent Survival
- Tumor Volume (mm^3)
- Efficacy and metastases analysis in orthotopic (ot) BxPC3:
- Treatment was initiated on day 14.
- Kaplan Meier analysis using Logrank statistics was followed by the Dunnett multiple comparisons test
- Tumor cells were implanted sc into nude mice (10/group) and
- treatment initiated when tumors reached ~150mm^3. Study endpoint was set at 2000 mm^3. Significance in tumor growth delay (difference in time to endpoint in treatment group compared to control) determined by log-rank test.
- Plasma and Tumor AQ4N/AQ4 levels as measured by LC/MS
- Plasma AQ4N and AQ4 levels were determined by LC/MS in human tumor and murine plasma. Limit of quantitation: Plasma: 50ng/ml; Tumor: 25ng/g

**Plasma and Tumor AQ4N/AQ4 levels as measured by LC/MS**

- Rapid loss of AQ4N from plasma. Mostly cleared after 8 hours for all doses
- AQ4 levels undetectable in plasma at all doses
- Tumor AQ4N levels decrease with time. Higher rate of clearance for higher doses
- Rapid generation of AQ4 in tumor over time. Levels at 2 hours are 55-85% of levels at 24 hours. Near maximum levels produced by 8 hours
- Previous data show measurable AQ4 levels in tumor up to 14 days post 20mg/kg dose and tumor AQ4 concentrations fairly stable for 7 days
- Activation of AQ4N to AQ4 has now been demonstrated in a wide range of human solid tumors in a phase I clinical study (Harris et al. abstract 2414).