The tissue targeted cytotoxic prodrug, AQ4N, is an effective single agent therapy in solid tumor and leukemia models

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ABSTRACT SUMMARY

There is a significant need for targeted therapies for cancer to achieve efficacy without the toxicity observed with traditional chemotherapeutics. AQ4N selectively targets lymphoid tissues and hypoxic tumor tissues, killing malignant lymphocytes and tumor cells, and causing dose dependent lymphoid tissue atrophy. It has demonstrated activity as a single agent in standard models of lymphocytic leukemia and solid tumors. The results presented here support the evaluation of AQ4N in human clinical trials in several oncology indications. AQ4N is currently under investigation in several human Phase I trials.

INTRODUCTION

AQ4N (banoxantrone; 1,4 bis[[2-(dimethylamino) ethylamino}-5,8-hydroxyanthracene-9,10-dione bis N-oxide) is a targeted cytotoxic prodrug that is bioreduced to AQ4 (reduced AQ4N), a highly potent DNA topoisomerase II inhibitor. AQ4N has been shown to be reduced by cytochrome P450 enzymes (CYPs), primarily CYP1A1, CYP1B1, and CYP3A4, under conditions of hypoxia (Patterson 2002; Raleigh et al., 1998).
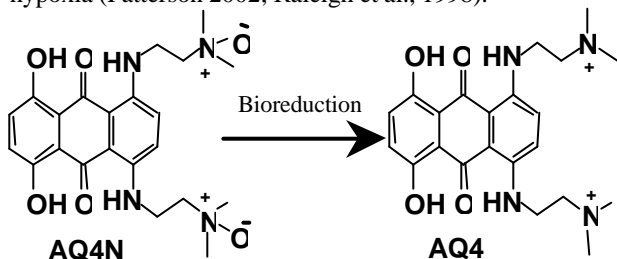
Hypoxia is a common characteristic of solid tumors and occurs due to inadequate or abnormal neovascularization and metabolic demands of tumor cells distal to the available blood supply, which in turn compromises the oxygen availability to the more distant tumor cells.

While tumors are not completely hypoxic, significant regions of hypoxia have been observed in a variety of human tumors through either in situ or ex vivo measurements. Tumors constantly undergo transient waves of hypoxia due the slow mass transfer throughout the solid tumor mass (Bennewith & Durand, 2004). Through the use of oxygen probes or chemical markers of oxygenation, several human tumors have been observed to have regions of hypoxia while adjacent normal tissues are fully oxygenated (Koong et al., 2000; Mueller-Klieser et al., 1991). Pancreatic (Kong et al., 2000), brain (Rampling et al., 1994; Evans et al., 2004; Doll et al., 2003), cervical (Nordsmark et al., 2003), prostate (Movsas et al., 2000), soft tissue sarcomas (Bentzen et al., 2003; Brizel et al., 1994), squamous cell carcinoma of the head and neck (Terris, 2000; Stadler et al., 1999), breast (Hohenberger et al., 1998), and bladder (Hoskin et al.,

2004) tumors all have significant regions of hypoxia. The extent of hypoxia in tumors has also been correlated to a poor prognostic outcome for survival with or without therapeutic or surgical intervention (breast, Hohenberger et al., 1998; head and neck, Stadler et al., 1999; cervix, Hockel et al., 1996). In some tumors, the region of hypoxia has been observed to be as large as 76% of the tumor mass (Brizel et al., 1994). Clearly, the treatment of hypoxic tumors is a major opportunity for clinical benefit through therapeutic intervention.

During studies of AQ4N toxicology and distribution in animals, it was noted that AQ4N selectively targets lymphatic system causing lymphoid tissue atrophy (spleen, thymus, and lymph nodes). This observation suggested the potential utility of AQ4N to treat lymphoid malignancies as well as solid tumors. A review of AQ4N activity in both solid tumor and leukemia models will be presented here along with information on the biodistribution of AQ4N in tumor bearing animals.

DISCUSSION

Solid Tumor Activity

Cell cytotoxicity assays were performed to assess the activity of AQ4N and AQ4 on cells from both solid tumors and hematological malignancies. Solid tumor cell lines were screened for sensitivity to AQ4N. To date, 15 cell lines from solid tumors were assessed and AQ4N had activity in 6 cell lines under normoxic conditions (BXPC-3, HCT 116, LoVo, LS174T, KB, and FaDu; IC50 = 3.6 – 59.4 μM). AQ4 had comparable or lower activity compared to standard of care agents used as positive controls for each cell line. An in vitro evaluation of AQ4N with gemcitabine in a pancreatic cancer cell line (BXPC-3) indicated that dosing gemcitabine prior to AQ4N results in increased cytotoxicity.

From these results, in vivo xenograft tumor studies were performed with human pancreatic (BXPC-3) and colon (HT-29) cancer cell lines. Tumor implants were performed subcutaneously in nude mice (8-12 mice/group). Animals were randomized to treatment groups after tumors reached 50-100 mm^3 and then AQ4N treatment was initiated. Three studies in each model (BXPC-3 and HT-29) were performed to refine the dose regimen for efficacy of AQ4N as monotherapy or in combination with standard agents (BXPC-3: gemcitabine; HT-29: irinotecan).

In the BXPC-3 model, 60 mg/kg q3d x 6 provided the greatest tumor growth inhibition with a comparable response to gemcitabine (40 mg/kg q3d x 4). Combining gemcitabine (40 mg/kg q3d x 4) with AQ4N (30 mg/kg q3d x 4 or 90 mg/kg q wk x 2) provided an increased tumor growth inhibition, but was not significantly different from single agent therapy. In the HT-29 colon xenograft model, 60 mg/kg AQ4N qod x 6 resulted in a significant tumor growth inhibition compared to vehicle

control (p=0.021) and irinotecan at 40 mg/kg dosed on Days 2 and 10 (p=0.048; Day 1 = start of AQ4N treatment). The combination of AQ4N and irinotecan caused slightly greater tumor inhibition than irinotecan alone (40 mg/kg Days 2 and 10). The optimal tumor growth inhibition was 90 mg/kg AQ4N (Days 2, 9, 16 & 23) combined with 40 mg/kg irinotecan (Days 1, 8 & 15), although the inhibition was not significantly different from either single agent treatment. Overall, these results demonstrated that AQ4N has significant activity as a single agent.

Lymphoma and Leukemia Activity

In addition to the solid tumors, 14 cell lines from hematological malignancies including lymphoma, leukemia, and myeloma were used and IC50 values were determined after 24 hr drug exposure and viable cell count was determined using MTS dye. AQ4N was observed to have activity in 6 of the cell lines (Namalwa, MOLT-4, KG1a, K562, P388 & L1210; IC50 = 0.2-310 μM) and AQ4 had IC50 values comparable to doxorubicin in most of the cell lines tested. These results suggested that AQ4N has activity in hematological malignancies under normoxic conditions.

To further evaluate AQ4N activity in lymphoma and leukemia, three in vivo models were selected: P388 (murine chronic lymphoblastic leukemia), L1210 (murine acute lymphoblastic leukemia), and Namalwa (human Burkitt lymphoma). For the P388 and L1210 models, mice (10-11 DBA/2 mice/group) were injected ip with tumor cells (10^6 and 10^5, respectively) on Day 0 and treatment was initiated on Day 1. Survival (after 60 days) was the primary endpoint of each study. In the P388 model, mice treated with 60 mg/kg AQ4N qod x 3 had a 236% increase in survival (25.1 $\pm$ 4.3 days vs 11.1 $\pm$ 0.3 days, vehicle), while 2 mg/kg mitoxantrone q4d x 3 produced a comparable survival benefit (24.2 $\pm$ 13.7 days). 90 mg/kg AQ4N qod x 2 in the L1210 model yielded a 575% survival increase (42.0 $\pm$ 17.2 days vs 8.1 $\pm$ 1.2 days, vehicle) with 5/10 long-term survivors, which was comparable to 2 mg/kg q4dx3 mitoxantrone (525%; 38.0 $\pm$ 21.3 days). The survival benefit was observed in repeated studies of AQ4N in these models. The Namalwa model was performed as a subcutaneous implant of tumor cells (xenograft model). Tumors were allowed to grow to 50-100 mm^3, animals were randomized to allow comparable mean tumor sizes across groups, and then treatment was initiated with AQ4N. AQ4N dosed iv at 30 mg/kg qod x 4 yielded a significant anti-tumor effect (p<0.05) on day 15, with 58% tumor growth inhibition.

Biodistribution

To further evaluate potential indications for AQ4N treatment, two biodistribution studies were performed with ^{14}C labeled AQ4N (Study 1: ^{14}C in first benzene ring; Study 2: ^{14}C in tertiary methyl groups). Mice (nu/nu) bearing subcutaneous BXPC-3 tumors (~ 60 mm^3) were administered 20 mg/kg AQ4N (120 μCi/kg) and tissues were harvested from sacrificed animals (3 mice/time point) over time up to 2 weeks. The concentration-time curves were generated for each compound and each tissue. The differential exposure of radioactivity was the greatest in the liver (Study 1: 2089 μg/g.hr; Study 2: 530.0 μg/g.hr) and kidney (Study 1: 632.5 μg/g.hr; Study 2: 249.0 μg/g.hr) suggesting metabolism of AQ4N to a less cytotoxic form based upon the lack of liver toxicity. In contrast, the spleen and large intestine had comparable and high exposures to both compounds. AQ4N radioactivity increased significantly over time (Tmax ~ 72 hrs) in the spleen and was sustained for 2 weeks for both compounds. The exposure in the large intestine is likely due to biliary recirculation of AQ4N that was observed in toxicology and other distribution studies. AQ4N radioactivity in the tumors persisted for 2 weeks and the exposure was 5-10 times greater in tumors than in plasma suggesting tumor targeting and accumulation.

Conclusions

AQ4N has activity as a single agent in lymphoid and solid tumor malignancies. The doses required for activity in animal models are comparable to well tolerated doses in humans (current dose of 447 mg/m^2 well tolerated). Distribution of AQ4N supports the targeting of tumors especially those associated with the large intestine (colon cancer) and the spleen (lymphoma) as well as the bladder (urinary excretion of AQ4N). Current preclinical studies are focused on understanding the mechanism of action of AQ4N in hematological malignancies under normoxic conditions and further evaluation of AQ4N in additional models of lymphoma, myeloma and solid tumors.

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