The prodrug AQ4N displays potent anti-tumor activity in a xenotransplantation model of primary human acute lymphoblastic leukemia.

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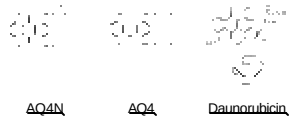
Introduction

- Approximately 50% of patients with adult acute lymphoblastic leukemia (ALL) relapse after initial chemotherapy.
- The introduction of novel chemotherapeutics that target leukemia or leukemia-harboring tissues could improve response rates.
- AQ4N (banoxantrone) is designed to have anti-tumor activity following a bioreduction step to AQ4, a DNA topoisomerase II inhibitor.
- Although well tolerated, AQ4N induced lymphopenia and atrophy of lymph nodes and spleen in monkeys and rats, suggesting selective activity in lymphoid cells.
- We postulated that AQ4N might have therapeutic potential in ALL.
- Therefore we compared the efficacy and toxicity profile of AQ4N with that of daunorubicin, a commonly used component of induction therapy.

Materials & Methods (in vitro)



In vitro anti-leukemic efficacy of AQ4N, AQ4 and daunorubicin was assessed in an 18hr 3H-thymidine incorporation assay after five days of culture in the presence of either agent, using ALL cell lines recently generated from primary ALL cells[1].

1) See abstract #857

Results (in vitro)

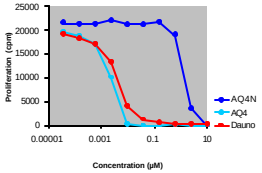


Figure 1: In vitro cytotoxicity of AQ4N, AQ4 and daunorubicin on LeidenALL-CM cells. Topoisomerase II inhibitors AQ4 and daunorubicin displayed similar in vitro activity with IC50 values of 2-9 nM. The prodrug AQ4N displayed little activity with IC50 values of 600- 2,500nM.

Materials & Methods (in vivo)

- Female NOD/scid mice were inoculated intravenously with 10^7 primary human ALL cells. Engraftment and progression were monitored by weekly flowcytometric determination of leukemic cell counts (LCC) in the peripheral blood.
- Leukemic animals received 3 dosings of daunorubicin or AQ4N, 7 days apart. LCC monitoring was continued througout the dosing period. Designed as a dose-range study, all animals in the study received a different dose. The study was concluded 7 days after the last dosing.
- Overall treatment efficacy was determined for each dose by calculation of the ratio between LCC 7 days after the last dosing (or at the moment of death), and LCC directly before the first dose (e.g. 10: progression, 1: stable disease, 0.5: regression, 0: remission).
- Murine hemoglobin levels and murine leukocyte counts were monitored to assess toxicity profiles of daunorubicin and AQ4N.

Results (in vivo)

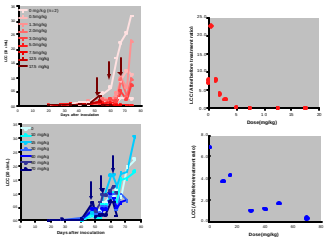


Figure 2: In vivo activity of daunorubicin (red) and AQ4N (blue). Left panels display leukemic cell counts in individually dosed animals. Dosings are indicated by arrows. Right panels display overall treatment efficacy

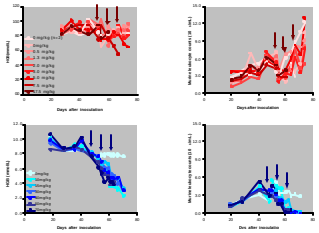


Figure 3: Hematologic toxicity of daunorubicin (red) AQ4N (blue). Left panels display hemobglobin levels in individually dosed animals. Right panels display corresponding murine leukocyte counts.

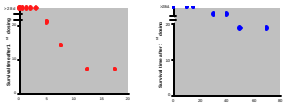


Figure 4: Lethality of daunorubicin (red) and AQ4N (blue). Lethal dosing of daunorubicin resulted in deaths 7-21 days after first administration. Lethal dosing of AQ4N resulted in deaths 19-23 days after first administration.

Strain	Daunorubicin (3x5.0 mg/kg)	AQ4N (3x120 mg/kg)
NOD/scid	Diarrhea, death	Anemia, pan-cytopenia, death
BNX	no effects	no effects
Balb/c	no effects	no effects

Table 1: Lethality was strain dependent. Non-pkrdc/scid mice tolerated high dosings of daunorubicin or AQ4N without anemia, cytopenia or death (table).

Conclusions

- AQ4N displays potent anti-leukemic activity against human acute lymphoblastic leukemia cells in vivo.
- Toxicity of daunorubicin appears to be exerted systemically and presents as gut toxicity. Toxicity of AQ4N appears to be hematologic and presents as anemia and cytopenia.
- AQ4N therefore appears to be specifically active on hematopoietic cells, suggesting that either bioreduction of AQ4N to AQ4 efficiently takes place in hematopoietic cells c.q. in the hematopoietic environment, or that hematopoietic cells are particularly sensitive to AQ4.
- NOD/scid mice are an unsuitable model for toxicity studies of DNA-interfering drugs because of their intrinsic hypersensitivity to these compounds.
- Further studies will be performed using less sensitive strains as hosts