The differentiated CD47 monoclonal antibody AO-176 exhibits significant in vivo activity against xenograft models of pediatric acute lymphoblastic leukemia (ALL)

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1. Introduction

Acute Lymphoblastic Leukemia (ALL) and CD47

- The 5-year survival rate for children with ALL exceeds 90% (Siegel et al., 2019), although high-risk subtypes confers an unfavorable prognosis.
- Pediatric T-cell ALL is stratified into risk treatment regimens with event-free survival rates approaching 85% in recent clinical trials (Dumitru et al., 2020), but with relapsing cases requiring further treatment.
- CD47 is a cell surface marker expressed by macrophages and dendritic cells that is involved in the negative regulation of phagocytosis. This regulation is brought about by the interaction of CD47 with signal regulatory protein alpha (SIRPα), eliciting a ‘don’t eat me’ signal that prevents phagocytosis (Onderberg et al., 2005).
- CD47 is expressed on tumor cells, including acute leukemia cells and has been identified as a prognostic factor whereby high expression of the CD47 protein is associated with shorter overall survival and increased relapse rates.

Development of AO-176 by Arch Oncology Inc

Arch Oncology Inc developed a next-generation monoclonal antibody against CD47 that binds to human CD47 with high affinity effectively blocking the CD47: SIRPα interaction. AO-176 preferentially binds to tumour cells, sparing normal hematopoietic cells (Puro et al., 2020).

AO-176 is currently in phase III clinical trials for adults with solid tumors and hematological malignancies (NCT04420723).

This study aimed to evaluate the in vivo activity of AO-176 against a select panel of the PPTC preclinical models of pediatric ALL patient-derived xenografts (PDx).

2. Study Methods

2.1 Study design and analysis

- CD47 mRNA expression was quantified by RT-qPCR (https://pedontvornet.org).
- Cell surface CD47 expression was assessed for a panel of eight T-ALL PDx by flow cytometry and shown as relative fluorescence intensity (RFI).
- A panel of PDx was previously evaluated in xenografts at early exponential phase via tail vein injection of NOD/SCID or NSG mice. The PDx models develop as models of systemic disease (Lock et al., 2002; Sury et al., 2019).

- 21 NSG mice per PDx were inoculated via tail vein injection and treatment began when the % human CD45+ cells (%huCD45+) in the murine peripheral blood (PB) reached a median ± 2% for the cohort. A subset of 8 mice per PDx were allocated to timepoints (n = 8) to Day 0, n = 8 to Day 28 or whenever occurred first (per treatment group) to assess toxicity and relevant patient outcomes.

- Prior to dosing with AO-176, or the IgG2 control, each mouse was treated with a platelet activating factor inhibitor (CV) to avoid any CV induced hypoperfusion reaction to the antibody treatment.
- AO-176 (or the IgG2 control) was dosed at 25 mg/kg, via intraperitoneal injection, once per week for 4 weeks.

- Events were defined when the %huCD45+ in the PB exceeded 25%, or the animal exhibited lethargy, which was then closely associated with high-level leukemia (≥90%) of at least 2 major organs.

- Representative response measures (RRMs) are as described by Houghton et al. 2007.
- PDI (when the %huCD45+ never drops below 1% and reaches event before the end of the study in the PB (Pudo et al., 2010).
- PO2 (when the %huCD45+ never drops below 1% and reaches event before the end of the study in the PB and/or PB and spleen (Pudo et al., 2010).
- SD = stable disease, %huCD45+ in PB < 1% and mouse never reaches event during the study period (42 days from start of drug treatment).
- PR = partial response, %huCD45+ in PB < 1% since the study period.
- CR = complete response, %huCD45+ in PB < 1% for at least 1 consecutive weekly readings during the study period.
- MCR = maintained complete response, %huCD45+ in PB < 1% for at least 3 consecutive weekly readings at any time after treatment has been completed.

The Kaplan-Meier method compares event-free survival (EFS) between the control (C) and treated (T) groups per PDx.

The differences in organ infiltration of Day 0 or Day 28 event mice were assessed by two-way ANOVA.

3. Results

3.1 CD47 mRNA expression

- CD47 mRNA expression was determined to be low, moderate, or high, based on the quantile range of the mRNA expression across all 9 PPTC PDx (Figure 1A).
- Low <55, moderate=55-108.3, high >108.4 expressed as FPKM.
- The significantly higher overall mRNA expression in T-ALL PDx (n=25; mean ± SD: 42.5 ± 17.87) compared to B-ALL (n=4; 6.5 ± 1.7 FPKM) is shown in Figure 1B (P<0.001, unpaired t-test).
- CD47 expression was highly expressed in a subset of T-ALL PDx with a median of 40.2 (RFI range 17.8–81.9) (Figure 2).
- AO-176 was well tolerated in vivo, exhibiting a maximum mean weight loss of less than 1% to any AO-176 treated cohort of the 4 PDx (T, 3 ALL; ETP, ALL) (Table 1).
- The EFS (T) range from 12.7-56.9 days from treatment initiation and AO-176 signifying a mean EFS of 4 days (Figure 3). EFS was measured from the first event until the last event during the study period (42 days from start of drug treatment).
- No significant toxicity was observed for the control group for all PDx except ALL-121 (Table 1).
- An objective response was observed for one PDx (Partial Response, ETP-2) (Table 1).

3.2 AO-176 efficacy

- AO-176, a monoclonal antibody, exhibited impressive single-agent in vivo activity against a panel of 4 T-ALL PDx, eliciting significant promising delays in 3 PDx.

- AO-176 was well tolerated to all mice of the 3 PDx following treatment, and markedly reduced splenectomy incidence in a third PDx.

- Additional studies investigating the activity of AO-176 in combination with standard-of-care drugs are warranted.

4. Discussion and Conclusions

- AO-176, a monoclonal antibody, exhibited impressive single-agent in vivo activity against a panel of 4 T-ALL PDx, eliciting significant promising delays in 3 PDx.
- AO-176 was well tolerated to all mice of the 3 PDx following treatment, and markedly reduced splenectomy incidence in a third PDx.
- Additional studies investigating the activity of AO-176 in combination with standard-of-care drugs are warranted.

5. References


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