Introduction
Cancer cells experience higher intrinsic oxidative stress than their normal counterparts and acquire adaptive antioxidant mechanisms to maintain redox balance. This increased antioxidant capacity has been correlated to malignant transformation, metastasis and resistance to standard anticancer drugs. This enhanced antioxidant state also correlates with cancer cells being more vulnerable to additional oxidative insults, therefore disruption of adaptive antioxidant mechanisms may have significant therapeutic implications. Hematological malignancies including Chronic Lymphocytic Leukemia (CLL), Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML) and Multiple Myeloma (MM) are critically dependent on the cellular antioxidant glutathione (GSH), consistent with the higher intrinsic oxidative stress in these malignancies. L-Cyst(e)ine (L-Cys) is the rate-limiting substrate for GSH biosynthesis and adequate levels of L-Cys are critical to maintain the intracellular homeostasis of GSH. L-Cys is not considered an essential amino acid as many tissues can synthesize L-Cys via the transulfuration pathway (Fig. 1). However, endogenous L-Cys synthesis is often insufficient owing to markedly increased levels of intracellular reactive oxygen species (ROS), thus many tumors rely on an extracellular supply of L-Cys. Since the majority of extracellular L-Cys exists in the oxidized form, L-Cysteine (CSCC), and because CSCS is non-permeable to the cellular membrane, the specialized xCT(+) antiporter is required to maintain intracellular L-Cys levels and meet GSH production needs.

L-Cysteine biosynthesis and utilization

CLL and subsets of ALL cells have been reported to express low levels of xCT(+) and to rely on the stromal supply of cysteine64 for the synthesis of GSH in order to maintain redox balance, which in turn promotes cell survival and fosters drug resistance.

Trophic support of Heme malignancy

One approach to target this cancer specific dependency is by therapeutic depletion of amino acids via enzyme administration; a clinically validated strategy for the treatment of ALL. Aeglea BioTherapeutics Inc. has developed the bioengineered L-Cys and CSCS degrading enzyme Cyst(e)inase (AEB3103) that can efficiently deplete L-Cys and CSCS in plasma to levels below 15 μM for 144 hours when administered at a dose of 40 mg/kg in cynomolgus monkeys (Fig. 3). Herein, we report the evaluation of AEB3103 therapeutic efficacy against hematological malignancies in vitro, ex vivo and in vivo preclinical studies.

AEB3103 pharmacodynamic profile

Methods

The TCL1-Tg/p53/-/- mouse model exhibits a drug resistant phenotype resembling human CLL with unfavorable cytogenetic alterations (17p deletions) and highly aggressive disease progression. AEB3103 efficacy was tested in vivo in a long term survival experiment in TCL1-Tg/p53/-/- mice and compared to the CLL therapeutic fludarabine. Additionally, AEB3103 efficacy was evaluated ex vivo in CLL patient-derived cells and in 3D cultures of cells derived from ALL and AML models. GSH levels after AEB3103 treatment were measured in 3D cultures derived from either CLL patients or TCL1-Tg/p53/-/- mice. Lastly, AEB3103 efficacy was tested in vitro in 2D cultures of MM patient-derived cells.

Results

AEB3103 greatly decreased the viability of TCL1-Tg/p53/-/- cells cultured in vitro, whereas the CLL therapeutic fludarabine showed minimal cytotoxic effects (Fig. 4).

AEB3103 in vitro efficacy in CLL

In vivo treatment of TCL1-Tg/p53/-/- mice resulted in an increase in median survival time from 3.5 months in the control group to 5.3 months in the fludarabine treated group (p=0.05) and 7 months in the AEB3103 treated group (p=0.0005) (Fig. 5). These results indicate a superior therapeutic effect of AEB3103 compared to fludarabine.

Additionally, ex vivo AEB3103 treatment decreased the viability of CLL patient-derived cells (with 17p deletions) from 85% to 3% when cultured alone and from 94% to 19% when co-cultured with the human bone marrow stromal cell line NKiT22 to mimic the CLL microenvironment (Fig. 6A). In contrast, the viability of normal lymphocytes from healthy donors co-cultured with NKiT22 was reduced only by 35% to 67% (Fig. 6B). AEB3103 treatment reduced GSH levels from ~99% to ~9% in CLL cells derived from either CLL patients (Fig. 6D) or TCL1-Tg/p53/-/- mice (Fig. 6C), confirming the mechanistic rationale that cyst(e)ine depletion via AEB3103 treatment modulates GSH synthesis.

Conclusion

• AEB3103 effectively depletes Cyst(e)ine in plasma and xenograft cell lines derived from preclinical models.
• Hematological malignancies are sensitive to modulation of GSH levels via AEB3103-mediated cyst(e)ine depletion.
• AEB3103 represents a potentially safe and effective therapeutic modality against a variety of hematological malignancies.

References

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Disclosures