Development of a human therapeutic L-Cyst(e)ine-degrading enzyme for the treatment of hematological malignancies

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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 Lymphocytic 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[a,b,c,d]. L-Cysteine (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 synthetize L-Cys via the transsulfuration 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-Cystine (CSSC), and because CSSC 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

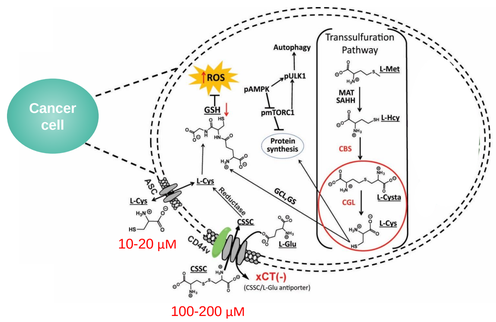


Figure 1. L-Cysteine biosynthesis and utilization in cancer cells. Abbreviations: MAT = methionine adenosyl transferase, SAHH = S-adenosylhomocysteine hydrolase, CBS= Cystathionine-β-synthase, CGL= Cystathionine-γ-lyase, GCL = glutamate-cysteine ligase, GS = glutathione synthase, ASC= alanine/serine/cysteine transporter, ROS= Reactive Oxygen Species, L-Met= L-Methionine, L-Hcy= L-Homocysteine, L-Cysta= L-Cystathionine, L-Cys= L-Cysteine, CSSC= L-Cystine, GSH= Glutahione, L-Glu= L-Glutamate

CLL and subsets of ALL cells have been reported to express low levels of xCT(-) and to rely on the stromal supply of cysteine[a,b] (Fig 2) 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

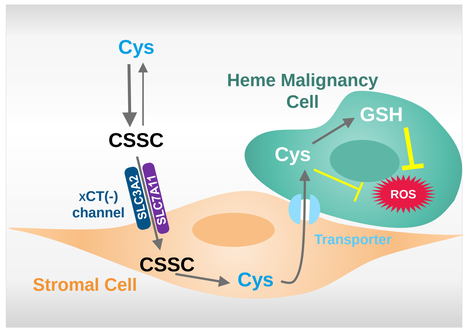


Figure 2. Stromal cells supply cysteine to heme malignancy cells promoting their survival[a].

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 CSSC degrading enzyme Cyst(e)inase (AEB3103)[e] that can effectively deplete L-Cys and CSSC in plasma to levels below 15 µM for 144 hours when administered at a dose of 40 mg/kg in cynomologus monkeys (Fig 3). Herein, we report the evaluation of AEB3103 therapeutic efficacy against hematological malignancies in *in vitro*, *ex vivo* and *in vivo* pre-clinical studies.

AEB3103 pharmacodynamic profile

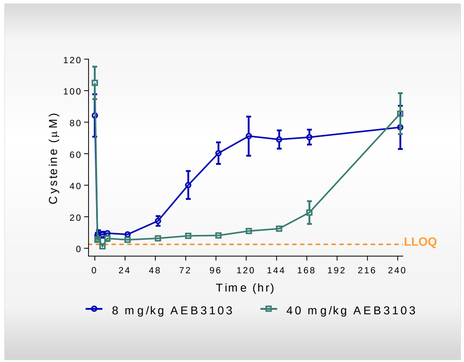


Figure 3. *In vivo* L-Cys depletion in cynomologous monkey plasma after administration of 8 mg/kg or 40 mg/kg AEB3103 (LLOQ = lower limit of quantitation).

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 PDx models. GSH levels after AEB3103 treatment were measured in CLL cells 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

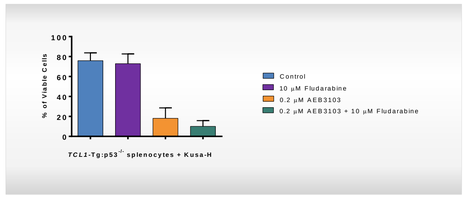
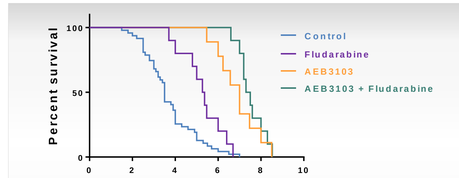


Figure 4. AEB3103 *in vitro* efficacy in splenocytes isolated from the TCL1-TG:p53 -/- mouse model.

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 NKTert to mimic the CLL microenvironment (Fig 6A). In contrast, the viability of normal lymphocytes from healthy donors co-cultured with NKtert was reduced only from 88% to 67% (Fig 6B). AEB3103 treatment reduced GSH levels from ~99% to ~9% in CLL cells derived from either CLL patients (with and without 17p deletions) or TCL1-TG:p53 -/- mice (Fig 6C), confirming the mechanistic rationale that cyst(e)ine depletion via AEB3103 treatment modulates GSH synthesis.

AEB3103 *in vivo* efficacy in CLL



Vehicle (n=47)	G1		
Fludarabine (34mg/kg, QDx5 followed by three weeks respite until death, n=10)	G2	*	
AEB3103 (100mg/kg, BW until death), n=10)	G3	***	**
AEB3103 + Fludarabine (34mg/kg, QDx5 followed by three weeks respite until death + 100mg/kg, BW until death, n=10)	G4	***	***
		G1	G2

*p<0.05, **p<0.005, ***p<0.0005. The log-rank (Mantel-Cox) test was used to compare survival curves

Figure 5. AEB3103 *in vivo* efficacy in the TCL1-TG:p53 -/- mouse model.

AEB3103 effect on CLL & Control Cells

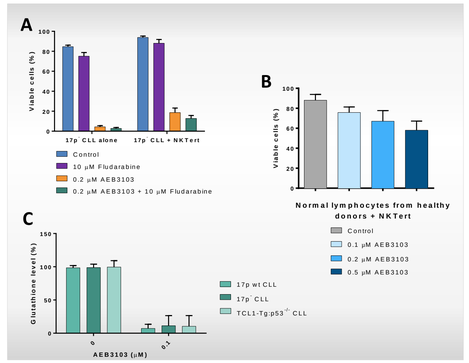


Figure 6. AEB3103 *ex vivo* efficacy in CLL cells (A) and normal lymphocytes (B). GSH levels after AEB3103 treatment (C).

AEB3103 treatment in *in vitro* 2D cultures of patient-derived MM cells (Fig 7), and in *ex vivo* 3D cultures of cells derived from ALL, AML and lymphoma PDx models (Fig 8) resulted in significant cell growth inhibition with therapeutically relevant IC_{50} values (1-30nM).

AEB3103 efficacy in Multiple Myeloma

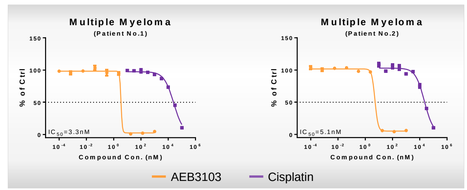


Figure 7. AEB3103 efficacy *in vitro* in multiple myeloma patient-derived samples.

AEB3103 efficacy in ALL, AML and Lymphoma

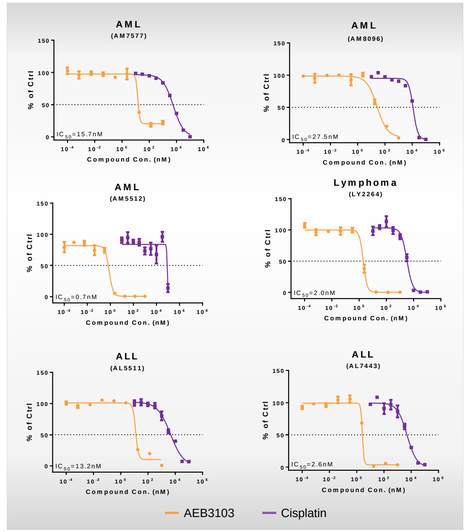


Figure 8. AEB3103 *ex vivo* efficacy in ALL, AML and lymphoma cells derived from PDx models.

Conclusion

- AEB3103 effectively depletes Cyst(e)ine in plasma and is very well tolerated for prolonged periods
- 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 [a] Zhang et al. (2012) "Stromal control of cystine metabolism promotes cancer cell survival in chronic lymphocytic leukaemia" Nat Cell Biol. 2012 Feb 19;14(3):276-86. [b] Boutter et al. (2014) "Image-based RNA interference screening reveals an individual dependence of acute lymphoblastic leukemia on stromal cysteine support" Oncotarget. 2014 Nov 30;5(22):11501-12. [c] Pei et al. (2013) "Targeting aberrant glutathione metabolism to eradicate human acute myelogenous leukemia cells" J Biol Chem. 2013 Nov 22;288(47):33542-58. [d] Tagde et al. (2014) "The glutathione synthesis inhibitor buthionine sulfoximine synergistically enhanced melphalan activity against preclinical models of multiple myeloma" Blood Cancer J. 2014 Jul 18;4:e229. [e] Cramer et al. (2016) "Systemic depletion of L-cyst(e)ine with cyst(e)inase increases reactive oxygen species and suppresses tumor growth" Nat Med. 2016 Nov 21.

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Disclosures G. Agnello, S. Alters, J. Tyler, A. Lowe, D. Lowe, and S. Rowlinson are employees of, and have an equity interest in Aeglea BioTherapeutics Inc.; E. Stone and G. Georgiou have an equity interest in Aeglea BioTherapeutics Inc.