Targeting Chronic Lymphocytic Leukemia by Interfering Glutathione Synthesis Using a Novel Therapeutic Enzyme Cyst(e)inase (AEB3103)

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Introduction

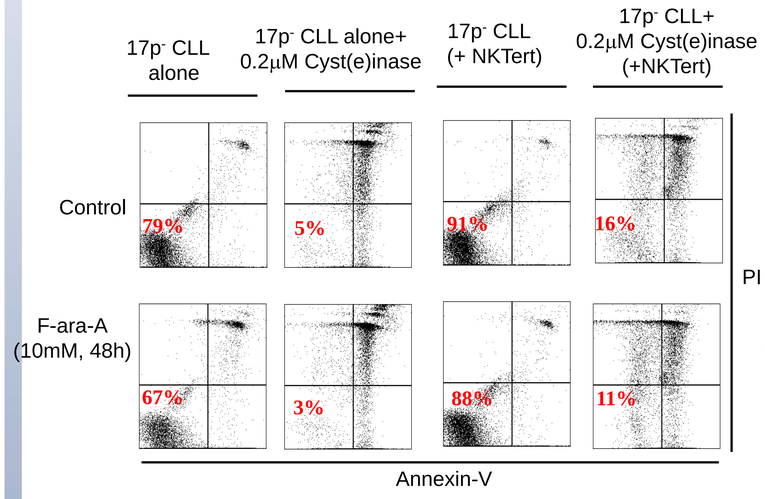
Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in the Western countries. Despite recent advance in new therapeutic agents that have improved treatment outcomes, CLL remains incurable due in part to the inability to completely eradicate all leukemia cells. Previous studies showed that CLL cells have high intrinsic oxidative stress and are highly dependent on the cellular antioxidant glutathione (GSH) to maintain redox balance and cell viability. One logical strategy to impact CLL cells would be to abrogate the glutathione protection of CLL cells *in vivo*. Recently we discovered that primary leukemia cells isolated from CLL patients were unable to effectively utilize cystine for GSH synthesis due to low expression of the cystine transporter Xc-, and that bone marrow stromal cells highly express Xc- and effectively take up cystine for conversion to cysteine. Cysteine is then released into the microenvironment and utilized by CLL cells for GSH synthesis, thus enhancing cell viability and drug resistance (Zhang et al: *Nature Cell Biology*, 2012). These findings provide a biochemical basis to develop novel strategies to effectively target leukemia cells in the stromal microenvironment and improve *in vivo* therapeutic activity. In this study, we hypothesize that the depletion of extracellular cystine and cysteine using a novel therapeutic enzyme-cyst(e)inase (AEB3103) would be a potential way to block GSH synthesis in CLL cells and abolish the stromal protection of the leukemia cells.

Experimental Approaches

1. Efficacy of Cyst(e)inase in primary CLL patient samples
2. Efficacy of Cyst(e)inase inTCL1-Tg:p53$^{-/-}$ CLL mouse model both *in vitro* and *in vivo*
3. Identification of oxidative stress accumulation and GSH depletion as a key cytotoxic mechanism of Cyst(e)inase against p53 deficient CLL

Results

Figure 1 Cytotoxic effect of Cyst(e)inase in primary CLL cells with 17p deletion



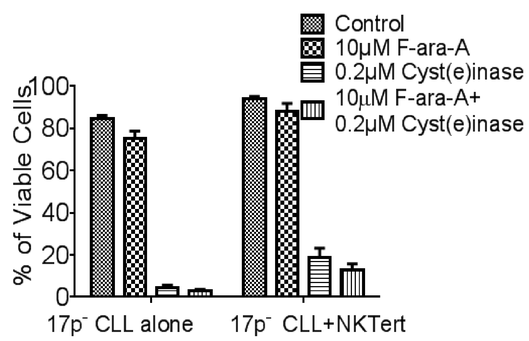


Figure 2 Cytotoxic effect of Cyst(e)inase in TCL1-Tg:p53$^{-/-}$ leukemic cells *in vitro*

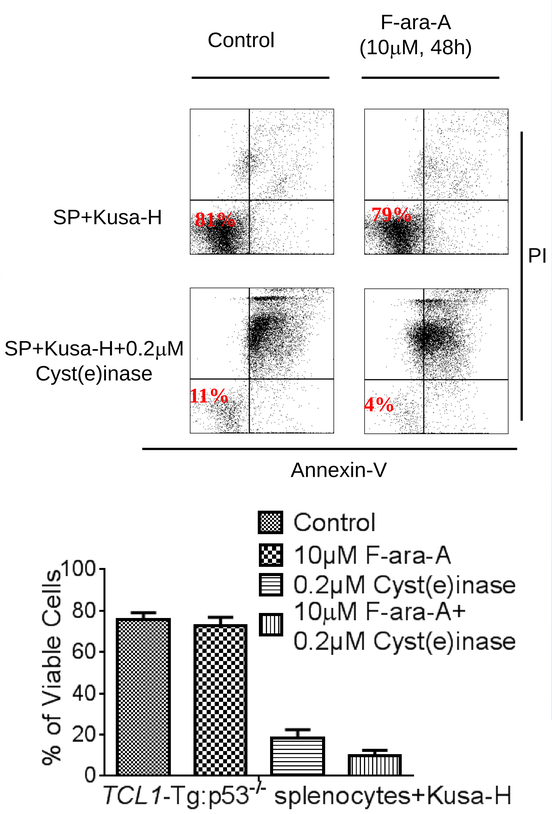


Figure 3 Glutathione depletion in p53 deficient CLL cells treated with Cyst(e)inase

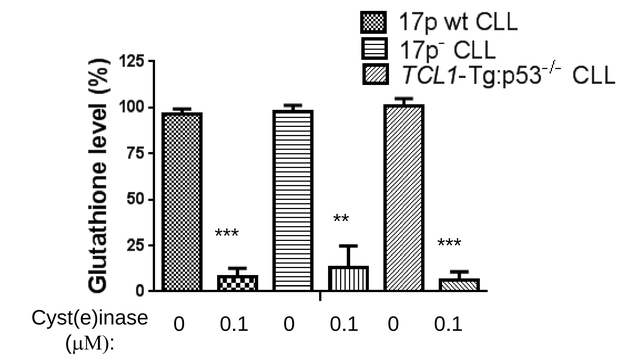
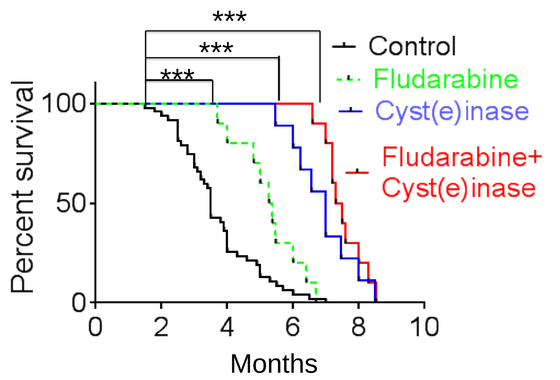
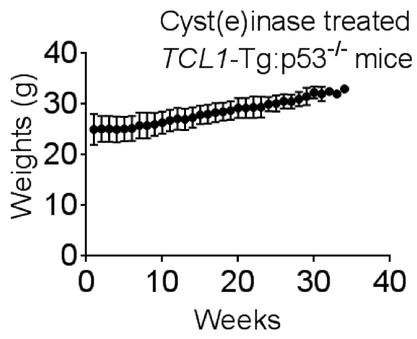


Figure 4 *In vivo* therapeutic activity of Cyst(e)inase in CLL mice with p53 deletion





Summary

Our study showed that AEB3103 was very effective in depleting GSH in CLL cells and caused massive CLL cell death even in the presence of stromal cells. Importantly, this enzyme was effective against CLL cells with p53 deficiency which are usually resistant to standard anti-CLL agents. *In vivo* study showed that AEB3103 significantly prolonged the survival time of CLL mice with *TCL1*-Tg:p53-/- genotype without observable toxic side-effect. Our study suggests that AEB3103 and its combination with standard anti-CLL drugs may potentially be useful for clinical treatment of CLL

Acknowledgments

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References

1. Zhang W.; Trachootham D.; Liu J.; et al., ***Nature Cell Biology***, 2012, 14(3); 276-286.
2. Liu, J.; Chen, G.; Feng, L.; et al., ***Leukemia***, 2014, 28(1):118-28.