Abstract: P320

Title: A NOVEL MAMMALIAN L-ASPARAGINASE WITH NO L-GLUTAMINASE ACTIVITY IS HIGHLY EFFICACIOUS AGAINST T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA IN VIVO

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Background:

T-lineage acute lymphoblastic leukemia (T-ALL) is an aggressive hematologic malignancy that accounts for 10%–15% of pediatric and 25% of adult ALL cases. In contrast to normal cells, ALL cells lack the expression of asparagine synthetase. As such, ALL cells are unable to synthesize their own asparagine (Asn) and depend on an exogenous source. Depletion of blood Asn levels by L-asparaginase (L-ASNase) represents a critical component of the multi-drug therapy that is currently used for ALL. However, a major concern is failure to achieve sufficient long-term L-ASNase activity due to clearance of these bacterial-derived enzymes by the immune system, often associated with clinical hypersensitivity. The conjugation of bacterial L-ASNase enzymes with polyethylene glycol (PEG) has been used in the first-line treatment of ALL. However, the presence of anti-PEG antibodies is often observed and associated with rapid clearance of PEG-L-ASNase. On top of the immunogenic responses, additional unacceptable side-effects, like liver toxicity and pancreatitis are reported, resulting in significant lower cure rates.

Aims:

In this study we aim to develop a less toxic and non-immunogenic L-ASNase with similar L-ASNase activity as FDA-approved L-ASNases, but without the toxic glutaminase co-activity, for the treatment of T-ALL.

Methods:

In this study, *in vivo* pharmacokinetic (PK), pharmacodynamic (PD) and toxicity profiling of a novel, mammalian L-ASNase in comparison to PEG-L-ASNase was performed. Also, we evaluated the therapeutic efficacy in a T-ALL patient derived xenograft (PDX) model that was established in NOD scid gamma mice.

Results:

Here we show the development of a less-immunogenic mammalian L-ASNase by introducing a stabilization tag that dramatically increases its *in vivo* persistence. First, PK/PD properties were determined. Results show that the administration of 50IU/mouse stabilized mammalian L-ASNase every 4 days and PEG-L-ASNase every 8 days provides a continuous Asn depletion.

In addition, toxicity profiling showed a significant decrease in toxicity using our novel L-ASNase variants with no glutaminase co-activity. The body weight loss is drastically reduced and the general health/activity is significantly better. Amylase and bilirubin levels, markers for pancreas and liver toxicity, were significantly increased at day 14 of treatment in mice treated with PEG-L-ASNase but not in the mammalian L-ASNase treated mice.

Finally, we evaluated the therapeutic efficacy of our mammalian L-ASNase for the treatment of T-ALL. Therefore, a T-ALL PDX model was established in immunodeficient mice. The tumor burden was quantified via flowcytometric analysis of human CD45+ cells in peripheral blood. Mice were either treated with vehicle, our mammalian L-ASNase variant or PEG-L-ASNase. Mice received in total 9 shots of mammalian L-ASNase (25IU/4days) or 5 shots of PEG-L-ASNase (25IU/8days). During treatment, PEG-L-ASNase mice had lost more body weight compared to the mammalian L-ASNase. After treatment, no significant difference in survival was observed.

Summary/Conclusion:

In conclusion, we present a novel, mammalian L-ASNase derived from guinea pig for the treatment of T-ALL.

Besides its potentially reduced immunogenicity, this mammalian enzyme is also intrinsically L-glutaminase free, which results in a favorable toxicity profile. Since the toxic side effects of current L-ASNases often result in treatment discontinuation in ALL, this no L-glutaminase variant, as presented in this study, may have great clinical potential.

Keywords: T cell acute lymphoblastic leukemia, Acute lymphoblastic leukemia