BST-236, a Novel Cytarabine Prodrug, Demonstrates Superior Safety-Efficacy Outcome Compared to Cytarabine *In Vivo*

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BACKGROUND

AML is associated with poor outcomes in older and medically unfit patients, largely due to the severe toxicity associated with cytarabine treatment, which precludes the administration of effective cytarabine doses. BST-236 is a novel antimetabolite, a cytarabine prodrug, which delivers high cytarabine doses to leukemia cells with reduced systemic exposure to free cytarabine and therefore reduced undesired toxicity.

MECHANISM OF ACTION

- BST-236 is a novel antimetabolite, composed of cytarabine covalently bound to asparagine.
- The intact BST-236 is inactive and non-toxic, enabling safe high-dose administration.
- BST-236 activity is triggered by hydrolysis and release of cytarabine while avoiding peak toxic systemic exposure to free cytarabine.
- Until its release, cytarabine is protected from inactivation by deamination or activation by phosphorylation.

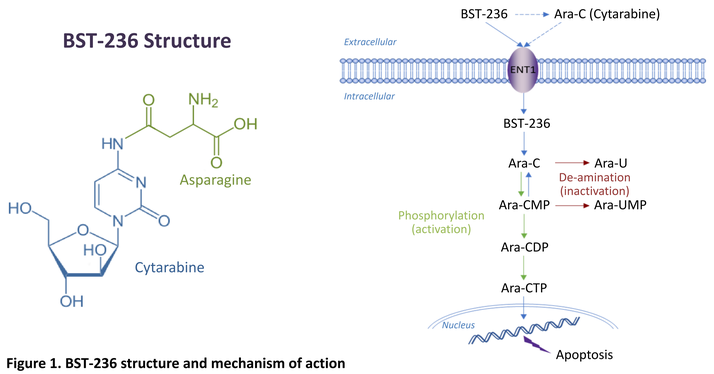


Figure 1. BST-236 structure and mechanism of action

RESULTS

In vitro studies demonstrate that BST-236 is effective in a variety of leukemia cells (**Figure 2**). BST-236 enters the leukemia cells, accompanied by cellular accumulation of free cytarabine, which is released from BST-236 (**Figure 3**).

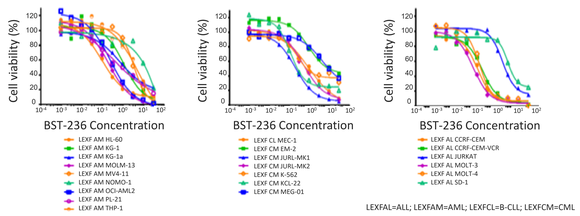


Figure 2. Induction of Cell Death by BST-236 in Various Leukemia Cell Lines

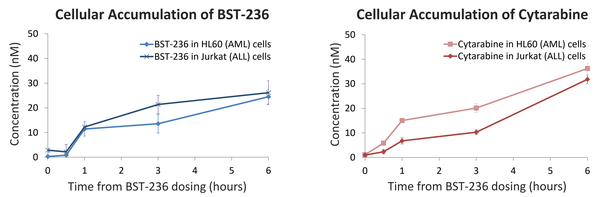


Figure 3. Accumulation of BST-236 and Cytarabine in Human Leukemia Cells Incubated With BST-236

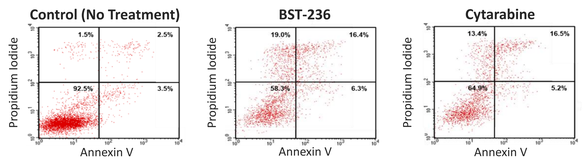


Figure 4. Induction of Apoptosis by BST-236 and Cytarabine

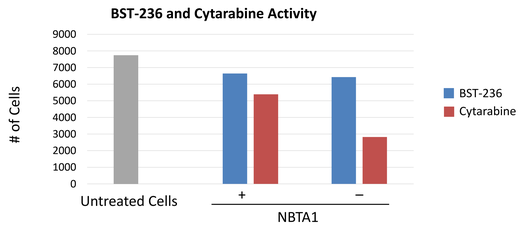


Figure 5. Inhibition of BST-236 and Cytarabine Activity by the ENT1 Inhibitor NBTA1

Like cytarabine, BST-236 induces apoptosis (**Figure 4**), an activity dependent on the human equilibrative nucleoside transporter 1 (hENT1) (**Figure 5**).

Unlike free cytarabine, BST-236 is not phosphorylated by deoxycytidine kinase (dCK) (**Figure 6**) and remains inactive in its prodrug form until gradual release of cytarabine, thus avoiding its high levels associated with systemic toxicity

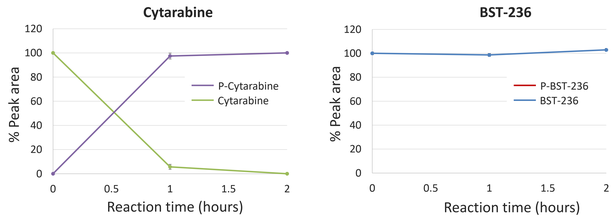
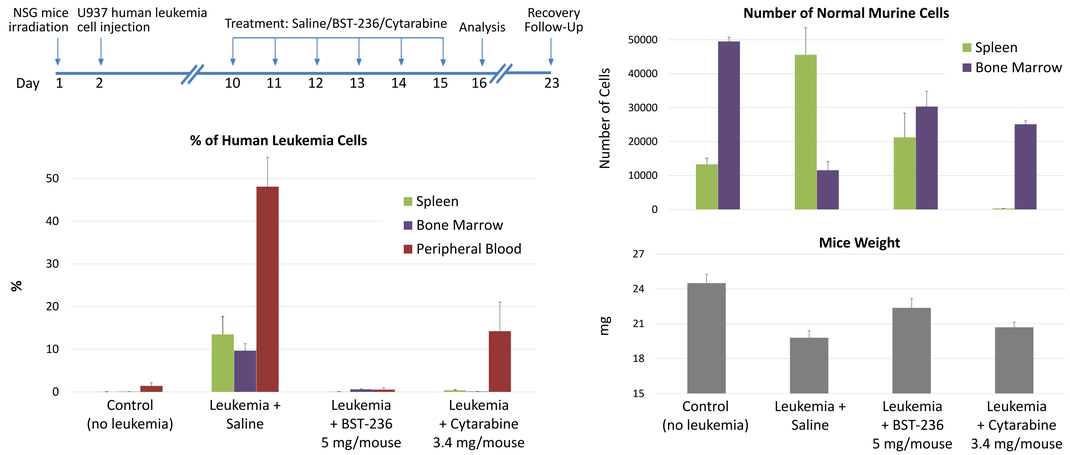


Figure 6. Phosphorylation by dCK

BST-236 Demonstrates Superior Safety-Efficacy Outcome Compared to Cytarabine in a Human Leukemia Model



Note: 5 mg of BST-236 contain 3.4 mg of cytarabine

At 1 week recovery follow up: All BST-236 mice were alive with no clinical signs and all cytarabine mice were dead

Figure 7. Comparison of BST-236 and Cytarabine in a Murine Model of Human Leukemia

BST-236 and cytarabine demonstrate similar efficacy of complete elimination of the leukemia cells in the bone marrow, spleen, and peripheral blood of NSG mice injected with U937 human leukemia cells (**Figure 7**).

However, while cytarabine treatment was associated with significant toxicity including weight loss, dramatic reduction in spleen size and number of normal mouse spleen cells, delayed normal murine white blood cell recovery, and post treatment death, BST-236 enabled spleen and BM recovery with minimal weight loss and no observed clinical signs.

CONCLUSIONS

- BST-236 enables the delivery of high-dose cytarabine with reduced systemic toxicity.
- These nonclinical findings are in line with the clinical results of the BST-236 Phase 1/2 study (ASH 2017 Abstract No. 893; manuscript in preparation) and suggest that **BST-236 may enable delivery of high cytarabine doses to older and medically-unfit patients who currently cannot benefit from an effective cytarabine therapy.**
- BST-236 demonstrates promising efficacy-safety profile, superior to cytarabine and hypomethylating agents, and may therefore replace them as the standard of care of AML and high-risk MDS.
- **A Phase 2b for front-line single-agent treatment for AML patients unfit for standard therapy is ongoing (NCT03435848).**

ST, LF, SG, RBY are employees of BioSight Ltd. IM, RPN, RM, AP have no conflict

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