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BACKGROUND

NOTCH signaling is a developmental pathway known to play critical roles during embryonic development as well as for the regulation of self-renewing tissues. Aberrant activation of NOTCH signaling leads to deregulation of the self-renewal process resulting in sustained proliferation, evasion of cell death, loss of differentiation capacity, invasion and metastasis, all of which are hallmarks of cancer. Over activation of NOTCH in human cancers can be a consequence of over expression of NOTCH ligands/receptors, GOF mutations in NOTCH receptors as well as chromosomal translocations leading to constitutive activation of the pathway.

OBJECTIVES

Two different therapeutic approaches have been utilized to block NOTCH; a) monoclonal blocking antibodies (Mabs) against NOTCH receptors/ligands and b) small molecule γ -secretase inhibitors (GSIs). However, these approaches can only be effective if cancer cells express full length NOTCH ligands/receptors. In human cancers harbouring NOTCH gene fusions due to chromosomal translocations, the use of Mabs and GSIs will have limited clinical benefit. A third, yet not fully explored approach could be the direct targeting of NOTCH transcriptional activation complex using small molecule inhibitors. The aim of this study was to discover and develop small molecule inhibitors of the NOTCH pathway able to block NOTCH signalling by targeting NOTCH transcription complex

MATERIALS & METHODS

We have developed a HTS compatible DL4-N1 coculture and NICD-driven NOTCH responsive reporter system that can be utilized to screen libraries of small molecules. Using this platform we have discovered and identified a novel, orally-active small molecule inhibitor, named CB-103, of the NOTCH pathway. CB-103 blocks NOTCH signaling by directly targeting the NOTCH transcriptional activation complex.

We have utilized several biochemical and cell-based assays to confirm its novel mechanism of action in blocking NOTCH signaling. In addition, its anti-tumor activity was confirmed using xenograft models of NOTCH dependent human leukemias, breast cancer and PDX models of human T-ALL.

SUMMARY

CB-103 has shown the ability to block NOTCH signaling in human T cell acute lymphoblastic leukemia cancer cell lines and inhibits NOTCH dependent cellular processes in mice. Due to its novel mechanism of action, CB-103 exhibits anti-tumor efficacy in GSI resistant T-ALL cell lines. CB-103 also induces anti-tumor effect in a xenograft model of human triple negative breast cancer resistant to GSIs and Mabs against NOTCH ligands/receptors.

Furthermore, CB-103 has shown remarkable activity in *ex vivo* and *in vivo* patient derived models of human T-ALL harbouring activation of the NOTCH pathway.

RESULTS

Figure 1: CB-103 acts as a Pan-NOTCH inhibitor by targeting NOTCH transcriptional activation complex.

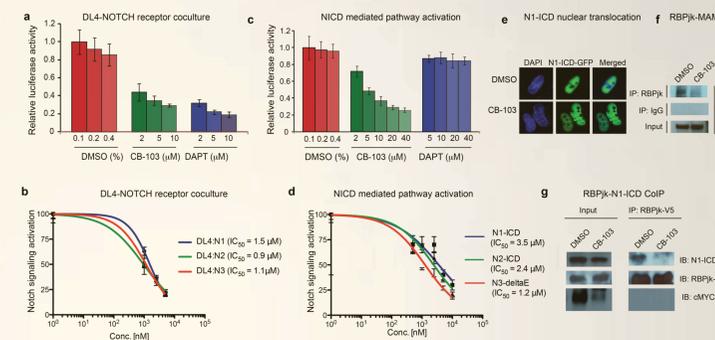


Figure 2: CB-103 blocks NOTCH signaling in human T-ALL leukemic cell lines.

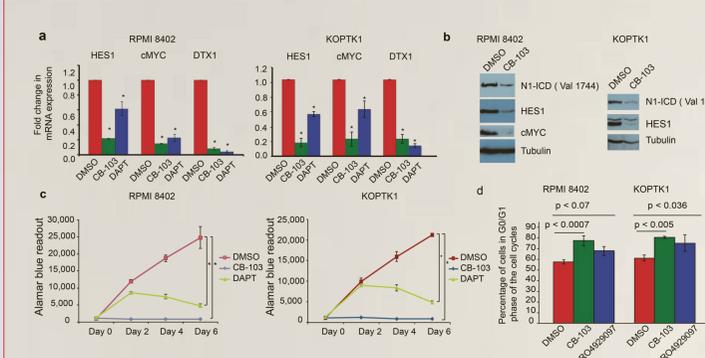


Figure 3: CB-103 inhibits NOTCH in GSI/Mabs resistant human cancer cell lines.

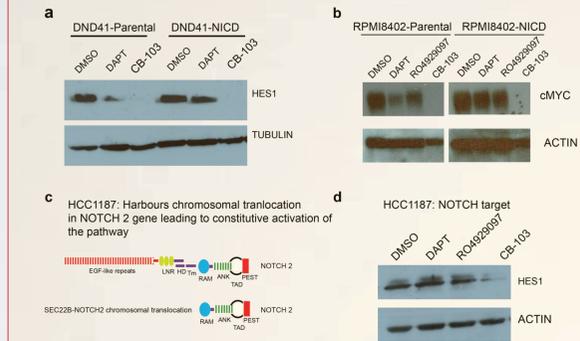


Figure 4: CB-103 exhibits *in vivo* activity and blocks NOTCH dependent cellular processes.

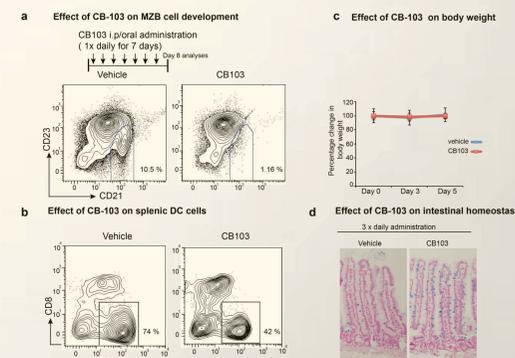


Figure 5: CB-103 impedes progression of NOTCH dependent xenograft model of human leukemia.

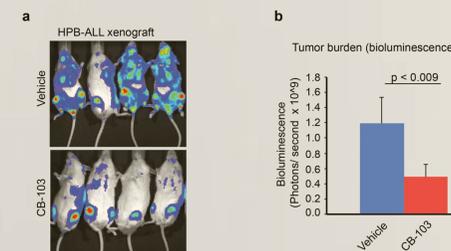
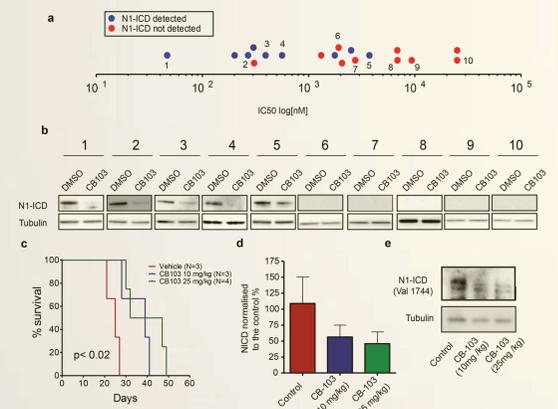


Figure 6: CB-103 blocks *in vivo* growth of PDX models of T-ALL



CONCLUSIONS

Based on SAR and *in vivo* pharmacokinetic/ADME data, CB-103 and its analogs are undergoing additional preclinical studies to nominate a development candidate for clinical development.

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