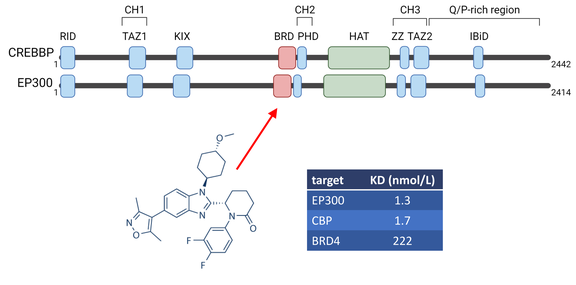
Therapeutic Targeting of EP300/CBP by Bromodomain Inhibition in Acute Myeloid Leukemia

Luciano Nicosia[1], Gary J Spencer[1], Nigel Brooks[2], Fabio Amaral[1], Naseer Basma[1], John Chadwick[1], Bradley Revell[1], Bettina Wingelhofer[1], Alba Maiques-Diaz[1], Filippo Ciceri[1], Daniel H Wiseman[3], Neil A Pegg[2], William West[2], Tomasz Knurowski[2], Kris Frese[2], Karen Clegg[2], Victoria Louise Campbell[4], Mhairi Copland[5], Emma Searle[6] and Tim C.P Somervaille[1]

1. Cancer Research UK Manchester Institute, The University of Manchester, Manchester, United Kingdom
2. CellCentric Ltd, Cambridge, United Kingdom
3. Division of Cancer Sciences, The University of Manchester, Manchester, United Kingdom
4. Western General Hospital, Edinburgh, United Kingdom
5. Paul O'Gorman Leukaemia Research Centre, Institute of Cancer Sciences, Gartnavel General Hospital, Glasgow, United Kingdom
6. The Christie Hospital, University of Manchester, Manchester, United Kingdom.

Introduction

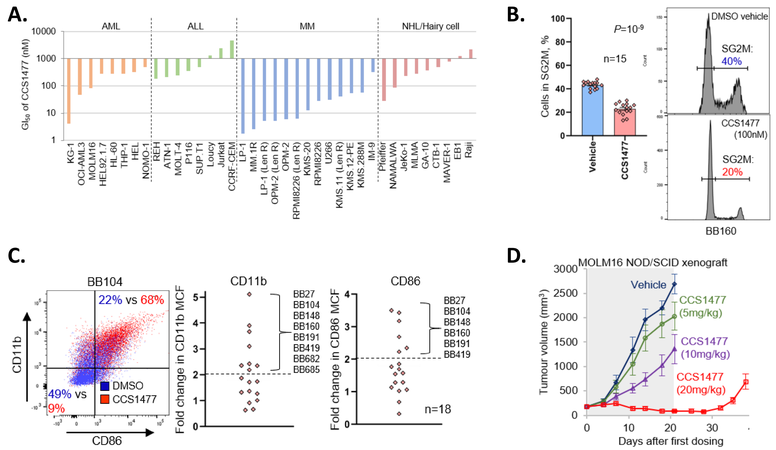
- EP300/CBP are two paralogous acetyltransferases involved in transcriptional activation.
- EP300/CBP act as co-activators of cancer associated transcription factors including MYC, MYB, IRF4, androgen receptor.
- EP300/CBP are attractive therapeutic targets in cancer in view of their critical role in promoting cellular growth and cell cycle progression.
- In haematological malignancies, there is ample evidence that pharmacologic targeting of the EP300/CBP bromodomain may be a useful therapeutic strategy.



target	KD (nmol/L)
EP300	1.3
CBP	1.7
BRD4	222

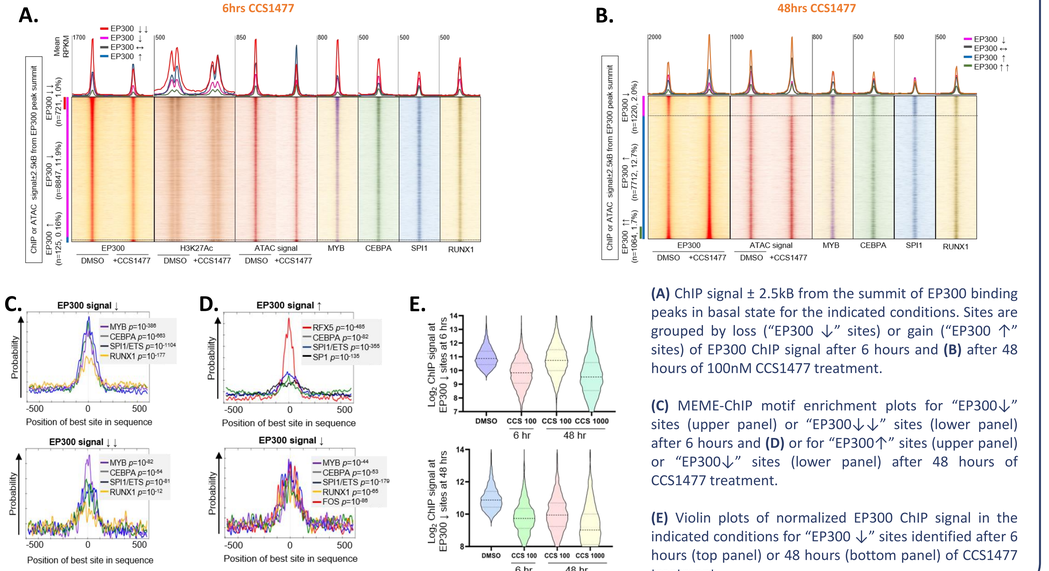
- CCS1477 (also known as inobrodib) is a first-in-class oral EP300/CBP bromodomain inhibitor currently in early phase clinical trials.
- Potent and highly selective versus other bromodomains.
- No biochemical inhibitory activity in a screen of 97 kinases and a safety screen of 44 receptors, enzymes, and ion channels at 10 µM.

Result 1. Sensitivity of diverse haematological models to CCS1477



(A) CellTiter-Glo GI_{50} values for the indicated cell lines exposed to CCS1477 in duplicate for 5 days. AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; MM, multiple myeloma; NHL, non-Hodgkin lymphoma. **(B)** Mean +/- SEM percentage of primary AML cells in S/G2/M. **(C)** Graphs show fold change in mean cell fluorescence (MCF) for CD11b and CD86 for each of 18 primary AML samples after four days of treatment in stromal coculture with DMSO vehicle or 100nM CCS1477. **(D)** Growth curves show mean +/- SEM tumour volume for MOLM16 AML cells at the indicated times, and at the indicated doses of CCS1477 (n=5 mice/cohort). Grey boxes indicate the duration of the treatment.

Result 2. CCS1477 induces rapid redistribution of EP300 from MYB bound enhancers to alternative sites

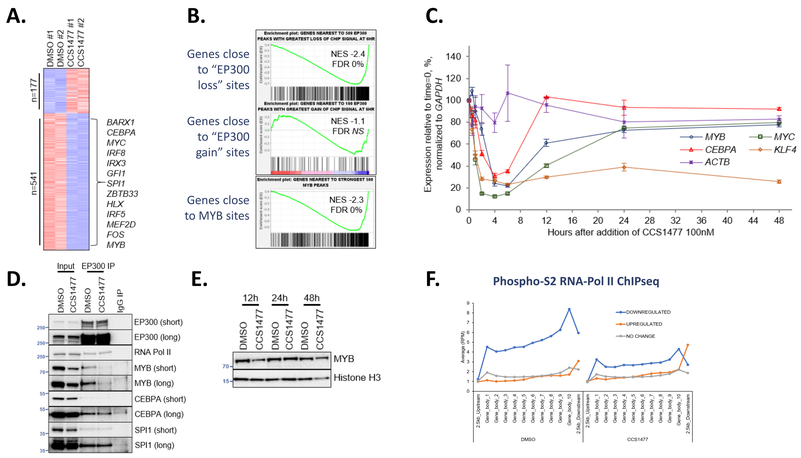


(A) ChIP signal ± 2.5kB from the summit of EP300 binding peaks in basal state for the indicated conditions. Sites are grouped by loss ("EP300 ↓" sites) or gain ("EP300 ↑" sites) of EP300 ChIP signal after 6 hours and **(B)** after 48 hours of 100nM CCS1477 treatment.

(C) MEME-ChIP motif enrichment plots for "EP300↓" sites (upper panel) or "EP300↓↓" sites (lower panel) after 6 hours and **(D)** or for "EP300↑" sites (upper panel) or "EP300↓" sites (lower panel) after 48 hours of CCS1477 treatment.

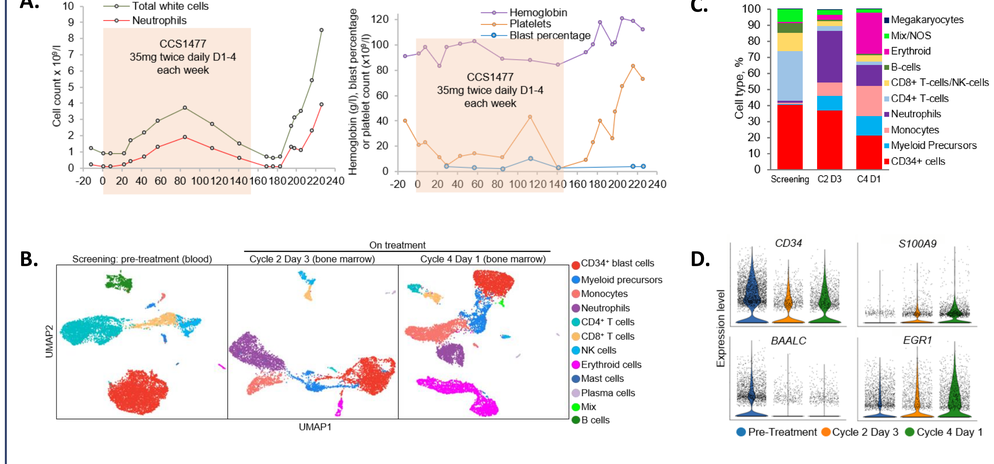
(E) Violin plots of normalized EP300 ChIP signal in the indicated conditions for "EP300 ↓" sites identified after 6 hours (top panel) or 48 hours (bottom panel) of CCS1477 treatment.

Result 3. CCS1477 induces selective but transient depletion of MYB protein and its associated transcriptional program



(A) Differentially-expressed genes after 6 hours of treatment with 100nM CCS1477 and **(B)** corresponding GSEA plots. **(C)** Expression of indicated genes over time in THP1 cells treated with CCS1477. **(D)** Western blots show the indicated proteins following EP300 immunoprecipitation and **(E)** prolonged treatment to CCS1477. **(F)** Line plot shows mean phospho-S2 RNA-Pol II ChIPseq signal (RPM) across gene bodies.

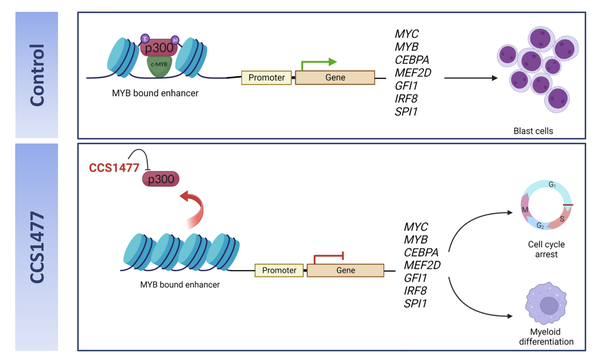
Result 4. Evidence of early phase trial activity of CCS1477 in MDS/AML



(A) Disease and treatment response trajectories for a patient with AML containing a normal karyotype in second relapse. **(B)** UMAP visualizations with cell type annotations of single cell RNA sequencing (scRNAseq) of the indicated samples collected at the indicated time points from a patient with myelodysplasia. **(C)** Proportional cell type composition of longitudinal samples in (B). **(D)** Violin plots show expression of the indicated genes at the indicated time points from samples in (B).

Conclusions and next steps

- CCS1477 treatment elicits a cell cycle arrest and induces differentiation in AML cells.
- CCS1477 promotes the redistribution of EP300 away from MYB binding sites and impinges on MYB expression.
- In patients with relapsed or refractory AML, CCS1477 induces therapeutically significant granulocytic responses and up regulation of MYB-regulated differentiation genes in blast cells.
- These data serve as the preclinical basis for an ongoing clinical trial in MDS/AML (NCT04068597) evaluating CCS1477 alone and in combination with standard-of-care agents azacitidine and venetoclax.



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