STC-15, an oral small molecule inhibitor of the RNA methyltransferase METTL3, inhibits tumour growth through activation of anti-cancer immune responses associated with increased interferon signalling, and synergizes with T cell checkpoint blockade

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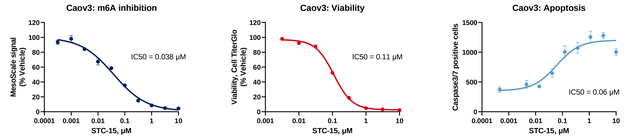
1 Introduction

METTL3 is an RNA methyltransferase responsible for the deposition of N-6-methyladenosine (m6A) modification on mRNA and long non-coding RNA (lncRNA) targets, to regulate their stability, splicing, transport and translation.

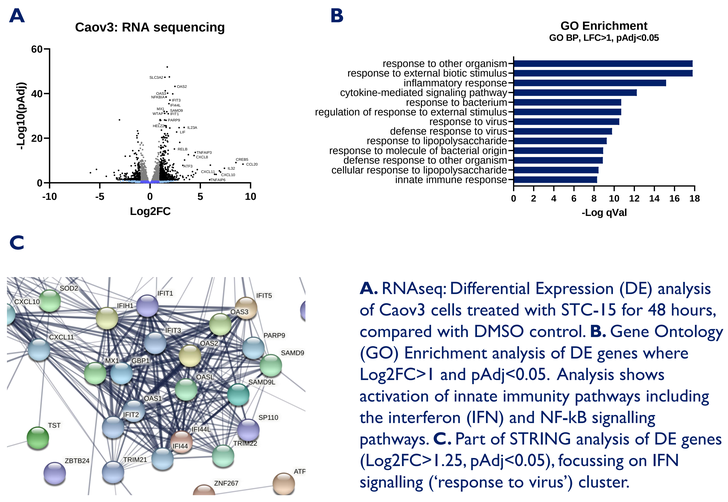
Small molecule inhibitors of METTL3 catalytic activity have previously demonstrated direct anti-tumour efficacy in models of acute myeloid leukemia (AML) (Yankova et al., *Nature*, 2021) and solid tumours.

Here we present pre-clinical data showing that the orally bioavailable small molecule METTL3 inhibitor STC-15 inhibits cancer growth and induces anti-cancer immunity.

STC-15 inhibition of m6A modification induces apoptosis in cancer cell lines

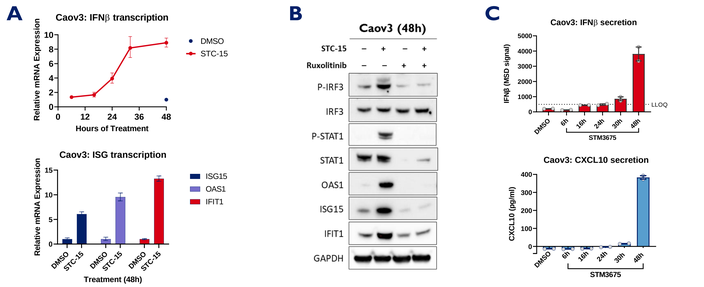


2 RNAseq: STC-15 activates innate immune signalling

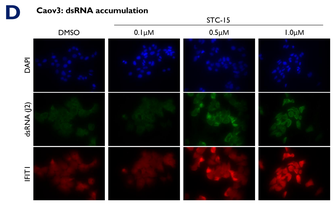


A. RNAseq: Differential Expression (DE) analysis of Caov3 cells treated with STC-15 for 48 hours, compared with DMSO control. **B.** Gene Ontology (GO) Enrichment analysis of DE genes where Log2FC>1 and pAdj<0.05. Analysis shows activation of innate immunity pathways including the interferon (IFN) and NF-kB signalling pathways. **C.** Part of STRING analysis of DE genes (Log2FC>1.25, pAdj<0.05), focussing on IFN signalling ('response to virus') cluster.

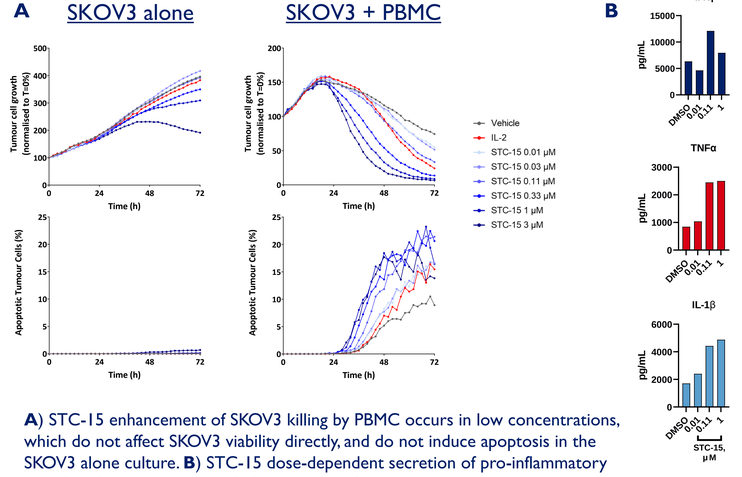
3 In-vitro validation of innate immunity activation



A. Induction of interferon β (IFNβ) (top) and Interferon Stimulated Genes (ISGs) (bottom) transcripts. **B.** Western blot showing the activation of interferon signalling by STC-15. The activation can be blocked by co-treatment with Ruxolitinib, a JAK1/2 inhibitor. **C.** Secretion of IFNβ (top) and the chemokine CXCL10 (bottom) from cells following treatment with STM3675, a METTL3 tool compound inhibitor similar to STC-15. **D.** Double-strand RNA (dsRNA) accumulation is likely the cause of innate immunity activation.



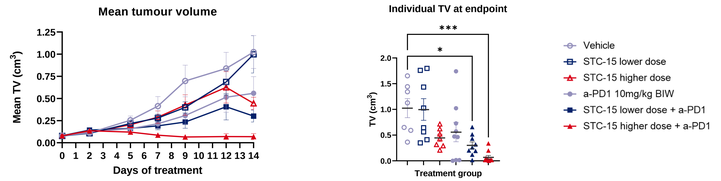
4 METTL3i enhances PBMC-mediated killing of cancer cells in a co-culture system



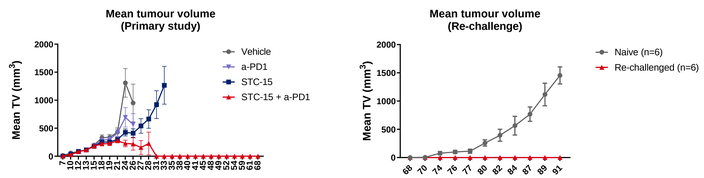
A) STC-15 enhancement of SKOV3 killing by PBMC occurs in low concentrations, which do not affect SKOV3 viability directly, and do not induce apoptosis in the SKOV3 alone culture. **B**) STC-15 dose-dependent secretion of pro-inflammatory cytokines in the co-culture. Similar data for 3 independent donors.

5 Anti-tumour activity of STC-15 in-vivo, alone or combined with anti-PD1 therapy

MC38 syngeneic CRC model

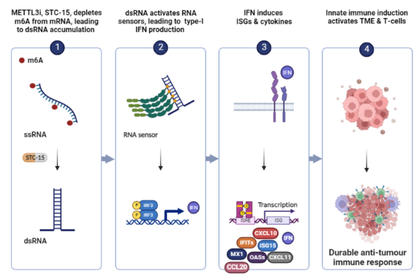


A20 syngeneic lymphoma model



6 Summary

STC-15 Mechanism of Action



In pre-clinical cancer models, STC-15 treatment inhibits tumour growth, activates innate immune pathways, and enhances the anti-tumour properties of anti-PD1 therapy, to generate a durable anti-tumour immune response. These data provide a rationale for the development of STC-15 as a novel treatment for solid tumour malignancies, as well as in combination with checkpoint inhibition.

A Phase I, First-in-Human clinical trial starts in Q4 2022.

Harnessing the Power of RNA Modification