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

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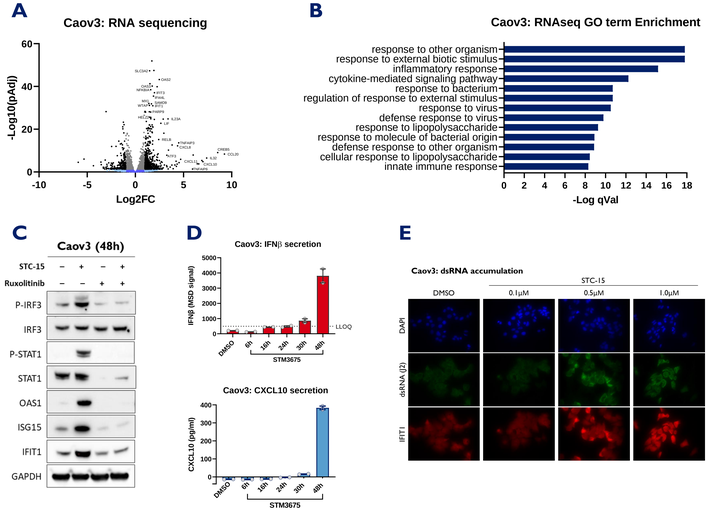
1 Introduction

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.

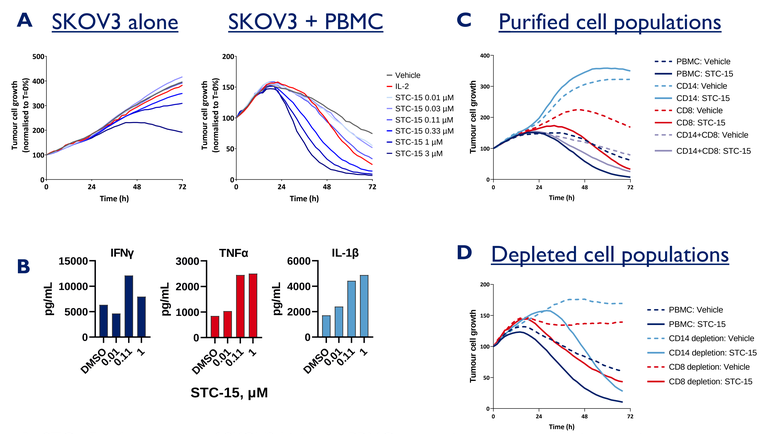
Here we present pre-clinical data showing that the orally bioavailable small molecule METTL3 inhibitor STC-15 inhibits cancer growth and induces anti-cancer immunity, by mechanisms involve the activation of CD8$^+$ cytotoxic T-cells.

2 STC-15 induces innate immune signalling



A. RNAseq: Differential Expression (DE) analysis of Caov3 ovarian cell line treated with STC-15 for 48 hours, compared with DMSO control. **B.** Gene Ontology (GO) Biological Process (BP) analysis of DE genes (Log2FC>1, pAdj<0.05), showing activation of innate immunity, including the interferon and NF-kB signalling pathways. **C.** Validation of the activation of the interferon signalling pathway by Western blot. The activation can be blocked by co-treatment with Ruxolitinib, a JAK1/2 inhibitor. **D.** Secretion of IFNβ (top) and the chemokine CXCL10 (bottom) from cells following treatment with STM3675, a METTL3 tool compound inhibitor. **E.** Double-strand RNA (dsRNA) accumulation is likely the cause of innate immunity activation.

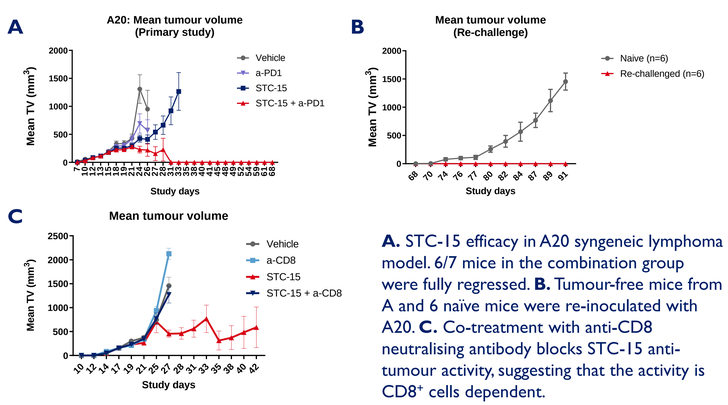
3 STC-15 enhances PBMC-mediated killing of cancer cells in a co-culture system



A. STC-15 enhancement of SKOV3 killing by PBMC occurs in low concentrations, which do not affect SKOV3 viability directly. **B.** Dose-dependent secretion of pro-inflammatory cytokines in the co-culture. **C.** Co-culture assay comparing PBMC to purified cell populations of CD14$^+$ (myeloid cells), CD8$^+$ (cytotoxic T cells) or CD14$^+$ + CD8$^+$, treated with either 0.3 μM STC-15 or DMSO control. **D.** As above, but using either CD14$^+$ or CD8$^+$ depleted PBMC.

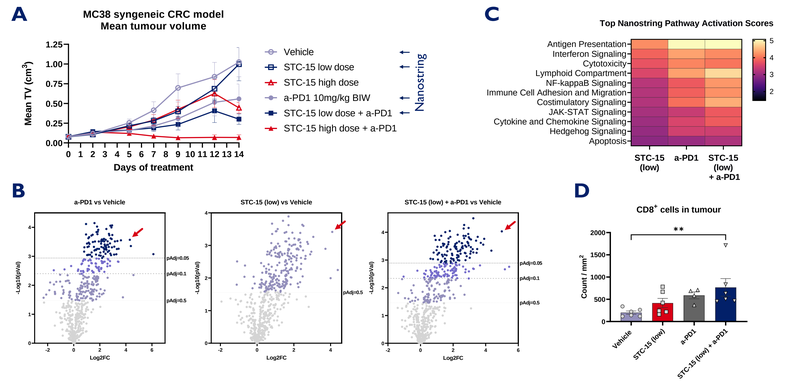
The data suggest that both CD14$^+$ and CD8$^+$ populations are affected by STC-15 treatment, and both populations contribute to effective cancer cell killing in the co-culture.

4 The in-vivo activity of STC-15 in A20 syngeneic lymphoma model is dependent on CD8$^+$ cells



A. STC-15 efficacy in A20 syngeneic lymphoma model. 6/7 mice in the combination group were fully regressed. **B.** Tumour-free mice from A and 6 naïve mice were re-inoculated with A20. **C.** Co-treatment with anti-CD8 neutralising antibody blocks STC-15 anti-tumour activity, suggesting that the activity is CD8$^+$ cells dependent.

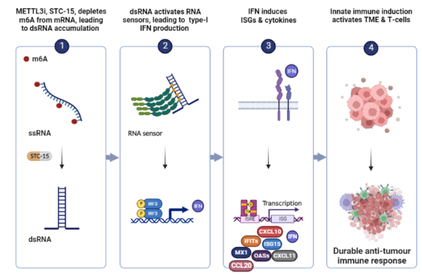
5 Increased CD8$^+$ tumour infiltration following STC-15 treatment, alone or in combination with anti-PD1



A. Dose dependent efficacy of STC-15 in MC38 syngeneic colon cancer model. Multiple regressions in high dose group combined with a-PD1. **B.** Nanostring gene expression analysis using the IO-360 panel was performed on samples from the 4 groups indicated by arrows in A. DE was calculated for each treatment group compared to vehicle control. Note that the Cd8a gene was among the top upregulated genes in all treatment groups (red arrows) . **C.** Nanostring pathway activation score for the most upregulated pathways. **D.** CD8$^+$ tumour infiltration was assessed by IHC.

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.

The anti-tumour effect of STC-15 is mediated via CD8$^+$ T-cell recruitment and activation. 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 started in Q4 2022 (NCT05584111).

Harnessing the Power of RNA Modification