STC-15, a small molecule inhibitor of the RNA methyltransferase METTL3, activates anti-tumor immunity and reshapes the tumor microenvironment

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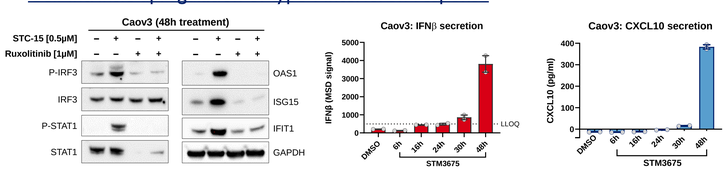
1 Introduction

METTL3 is an RNA methyltransferase responsible for the deposition of N-6-methyladenosine modification (m6A) on mRNA, regulating mRNA stability, splicing and protein translation. Small molecule inhibitors of METTL3 (METTL3i), such as STM3675 and STC-15, tested in various preclinical models[1,2] have demonstrated broad anti-tumor efficacy. Inhibition of METTL3 induces a cell-intrinsic innate immune response through activation of the double strand RNA (dsRNA) sensing machinery and interferon (IFN) signalling[2], supporting testing of STC-15 in the clinic.

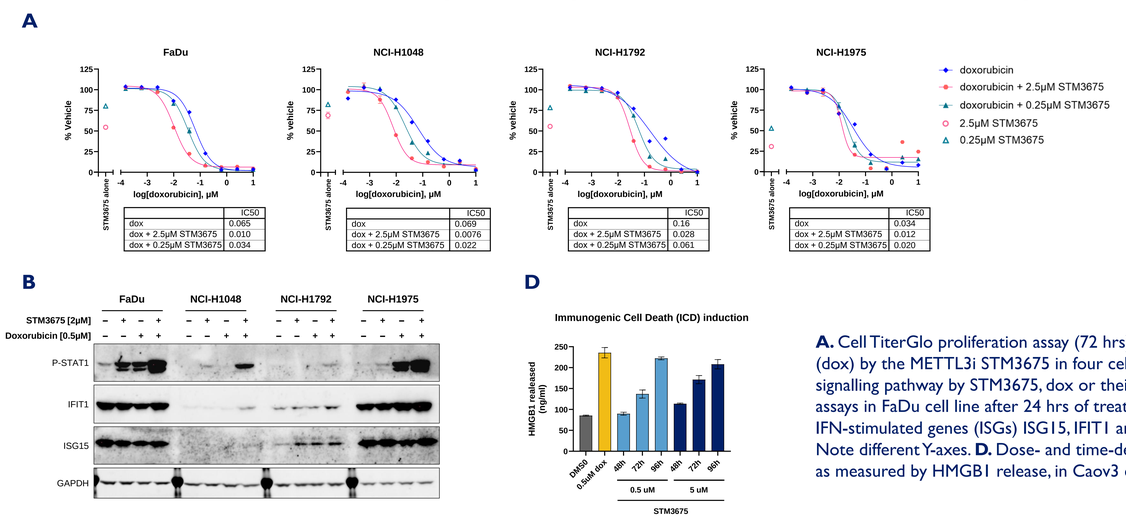
Here we present new preclinical data, investigating the combination of METTL3i with DNA damaging therapies. The combination of METTL3i with DNA damaging chemotherapies augments cytotoxicity and increases innate immune activation in vitro. In vivo, we show that STC-15 acts through an immune-based mechanism, and that STC-15 profoundly reshapes the tumor microenvironment (TME).

Cell-intrinsic upregulation of type-I interferon response



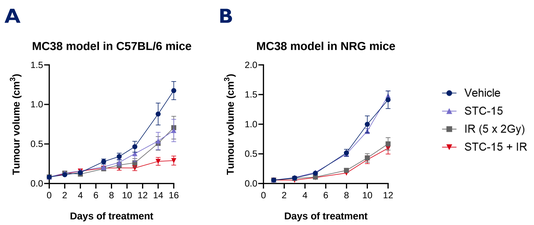
A-C. Caov3 ovarian cell line treated with METTL3i . **A.** STC-15 dependent induction of cell-intrinsic IFN signalling, that can be blocked by Ruxolitinib (a JAK1/2 inhibitor). **B-C.** Time dependent secretion of IFNβ (**B**) and CXCL10 (**C**) as measured by an MSD and ELISA, respectively.

2 The combination of METTL3i with DNA damaging chemotherapy augments cytotoxicity and activation of innate immunity in vitro



A. Cell TiterGlo proliferation assay (72 hrs) showing dose-dependent sensitisation of doxorubicin (dox) by the METTL3i STM3675 in four cell lines. **B.** Western blot showing induction of the IFN signalling pathway by STM3675, dox or their combination in four cell lines at 24 hrs. **C.** qPCR assays in FaDu cell line after 24 hrs of treatment, showing synergistic induction of IFNβ and the IFN-stimulated genes (ISGs) ISG15, IFIT1 and CXCL10 in cells treated with STM3675 and dox. Note different *Y*-axes. **D.** Dose- and time-dependent induction of Immunogenic Cell Death (ICD)[3], as measured by HMGB1 release, in Caov3 cell line.

3 METTL3i synergises with Radiation Treatment in vivo, in an immune based mechanism



A-B. MC38 colorectal syngeneic model grown in C57BL/6 mice (immune competent, **A**) or NRG mice (immune compromised, **B**), and treated by either STC-15, focal radiation treatment (IR) or the combination of STC-15 and radiation. STC-15 efficacy and combination effect are only observed in immune competent mice, in line with its immune-based mechanism of action.

4 STC-15 shifts the TME from immunosuppressive to immunostimulatory state

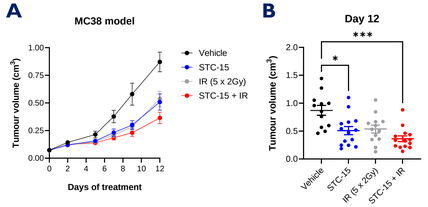
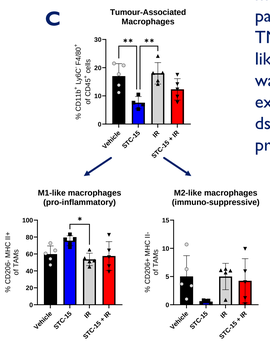
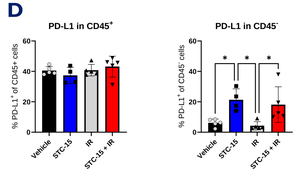


Table 1

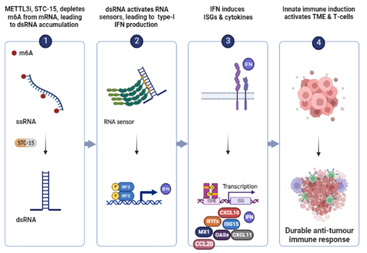
	STC-15	IR	STC-15 + IR
CD45+	↑	=	↑↑
Lymphoid	↑↑	=	↑↑
Myeloid	↑	=	↑↑
CD8+	↑	=	↑
CD4+	=	↓	↓↓
Treg	↓	↑↑	↓
Monocytes	=	↑↑	=
Macrophages	↓↓	=	↓
M2	↓↓↓	=	=
M1	↑	=	=
pDCs	↓↓	=	↓↓
MoDC	↑	=	↑↑
Neutrophils	↓↓↓	=	↓
B cells	↓	↓	↓↓





MC38 colorectal syngeneic model bearing mice were treated with either STC-15, focal radiation (IR) or the combination of STC-15 and radiation. **A.** Tumor growth during the treatment period, presented as a group average. **B.** Tumor volume at endpoint (day 12) for individual mice.
Upon study termination on day 12, tumors were harvested and subjected to flow cytometric analysis to characterise the TME. The results are summarised in **Table 1**. **C.** The effect on macrophage polarisation as measured by the flow cytometry panel. STC-15 reduces the overall number of macrophages in the TME. A trend for increased pro-inflammatory macrophages (M1-like) and reduced immuno-suppressive macrophages (M2-like) was observed. **D.** STC-15-mediated increase in PD-L1 expression is only seen on CD45⁻ cells, suggesting that the dsRNA-dependent induction of type-I IFN signalling is more prominent in tumor cells (in line with ref 2).

5 Summary



- METTL3i enhances cell cytotoxicity of DNA damaging chemotherapies such as doxorubicin
- Combination of STC-15 with chemotherapies activates cancer cell intrinsic interferon signalling that contributes to the induction of immunogenic cell death (ICD). ICD was shown to elicit an adaptive anti-tumor immune response and general changes to the TME[3]
- STC-15 synergises with radiation therapy in vivo
- STC-15 remodulates the TME, shifting it from immuno-suppressive, pro-tumorigenic state to immune-stimulatory, anti-tumor state.

A Phase I, First-in-Human clinical trial is ongoing (NCT05584111).

References
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