STC-15, a novel METTL3 inhibitor, and its combination with Venetoclax confer anti-tumour activity in AML models

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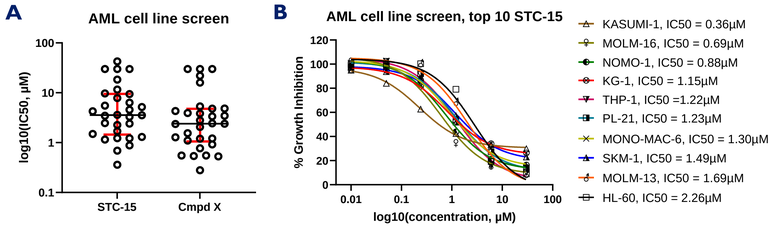
1 Introduction

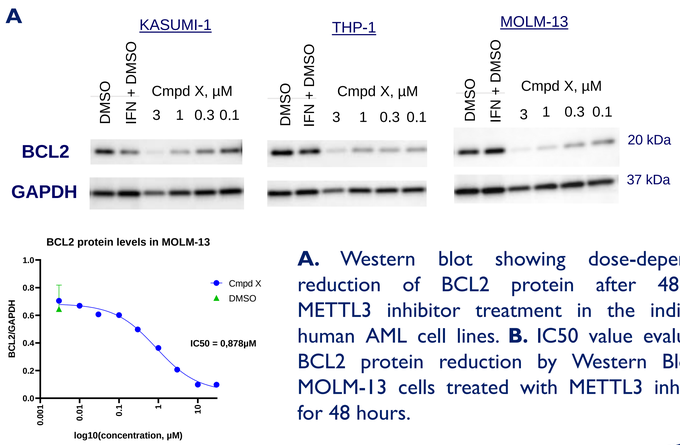
METTL3 is an RNA methyltransferase responsible for the deposition of N-6-methyladenosine (m^6A) modification on mRNA and lncRNA targets to regulate their stability, splicing, transport and translation. To date, METTL3 has been implicated in the initiation and progression of multiple cancer types, with the highest expression of METTL3 mRNA observed in acute myeloid leukemia (AML). Currently, one line of standard of care therapy for AML patients is Venetoclax, which targets the anti-apoptotic protein BCL2. It was shown that m^6A, deposited by METTL3 on BCL2 transcript, affects BCL2 mRNA stability and translation. Storm Therapeutics has developed potent and selective METTL3 inhibitors, including STC-15, which is in clinical development (clinical trial NCT05584111). Here, we explore pharmacological inhibition of METTL3 as monotherapy or in combination with Venetoclax in AML models *in vitro* and *in vivo*.

2 Multiple human AML cell lines and PDXs are sensitive to METTL3 inhibition



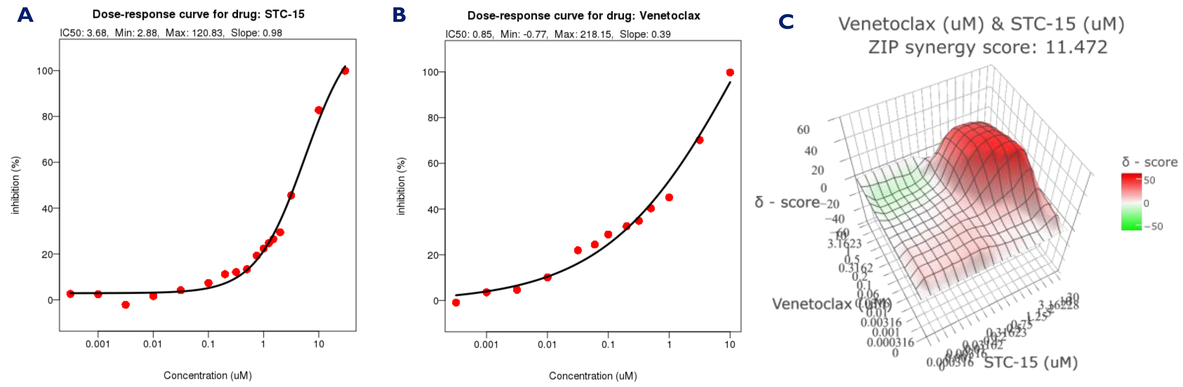
A. IC50 values of STC-15 in 30 AML cell lines, where cells were treated for 5 days and cell viability assessed by SRB assay. **B.** Growth inhibition curves of the top 10 most sensitive cell lines as defined in A. **C.** IC50 values of 12 AML PDXs, where cells were treated *in vitro* with METTL3 inhibitor for 6 days and cell viability assessed by CellTiter-Glo assay.

3 METTL3 inhibition leads to downregulation of BCL2 protein in AML cell lines



A. Western blot showing dose-dependent reduction of BCL2 protein after 48-hour METTL3 inhibitor treatment in the indicated human AML cell lines. **B.** IC50 value evaluating BCL2 protein reduction by Western Blot in MOLM-13 cells treated with METTL3 inhibitor for 48 hours.

4 STC-15 shows synergy with Venetoclax *in vitro*



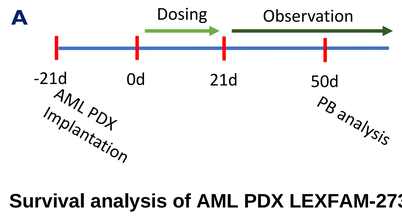
A. A dose-response curve of STC-15 in MOLM-13 cells treated for 72 hours. **B.** A dose-response curve of Venetoclax in MOLM-13 cells treated for 72 hours. **C.** A synergy plot generated in MOLM-13 cells treated with combination of STC-15 and Venetoclax for 72 hours.

Synergy score at the **highest synergistic area is 51**.

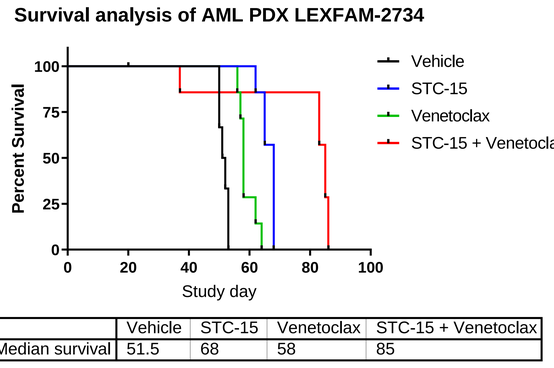
Data presented in figures A-C is a representative example of 3 independent experiments.

Plots and synergy analysis performed with www.synergyfinder.fimm.fi

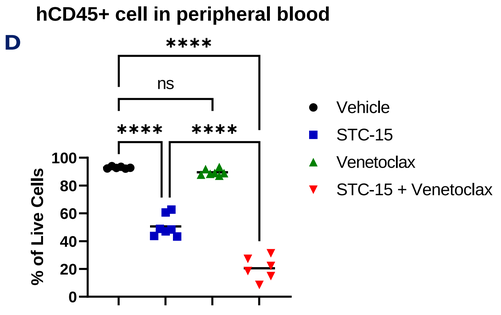
5 STC-15 and Venetoclax combination treatment enhances survival in an AML PDX model *in vivo*

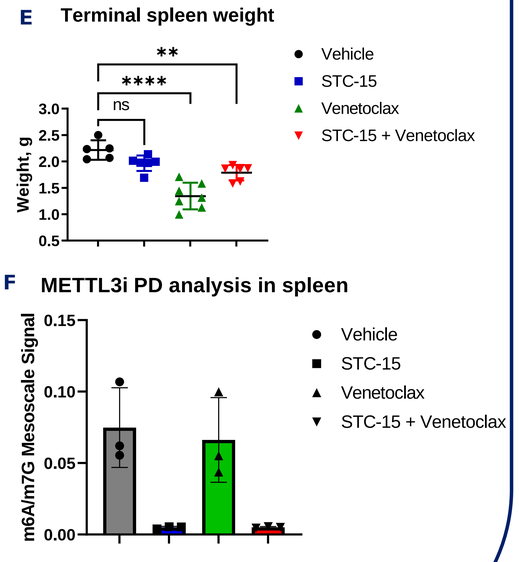


Compound	Dose	Schedule	Route
Vehicle	-	QD*21	PO
STC-15	100 mg/kg	QD*21	PO
Venetoclax	25 mg/kg		
STC-15 Venetoclax	100 mg/kg 25mg/kg	QD*21	PO



	Vehicle	STC-15	Venetoclax	STC-15 + Venetoclax
Median survival	51.5	68	58	85





A. Scheme representing the outline of the study (PB – peripheral blood). **B.** Table showing group assignment and therapy schedule. **C.** Survival analysis of all treatment groups in the study. **D.** Flow cytometry analysis of human CD45+ cells in mouse peripheral blood on study day 50. **E.** Spleen weight recorded in mice post termination. **F.** Measurement of m^6A levels in spleen samples (study day 21) as a direct readout of METTL3 enzymatic inhibition.

6 Summary

Here we have demonstrated that multiple AML cell lines and AML PDX models were sensitive to pharmacological inhibition of METTL3 by STC-15 or other METTL3 tool inhibitors. In addition, METTL3 inhibition led to a dose-dependent downregulation of BCL2 protein. Matrix-combination experiments have shown a high degree of synergy between STC-15 and Venetoclax: in MOLM-13 cells the most synergistic area had a score of 51, which indicates a 51% higher degree of inhibition than expected by an additive effect. An *in vivo* AML PDX model also revealed that combination therapy extended median group survival to 85 days in comparison to 51.5 days in the vehicle group, while STC-15 monotherapy outperformed Venetoclax (median survival 68 days *vs* 58 days, respectively). The survival results were supported by reduced numbers of circulating human CD45+ cells and lower spleen weight when compared therapy and control groups.

In conclusion, we demonstrated that METTL3 inhibition results in anti-tumour effects across different AML models. Moreover, we demonstrated a synergistic effect between the novel METTL3 inhibitor STC-15 and Venetoclax, both *in vitro* and *in vivo*. These studies provide evidence for the utility of METTL3 inhibitors as a new therapeutic agent to treat AML. Currently, STC-15 is in a phase I, First-in-Human multiple ascending dose study (NCT05584111).