RNA ACTIVATION OF *CEBPA* REDUCES PROLIFERATIVE CAPACITY OF ACUTE MYELOID LEUKEMIC CELLS IN PRECLINICAL MODELS

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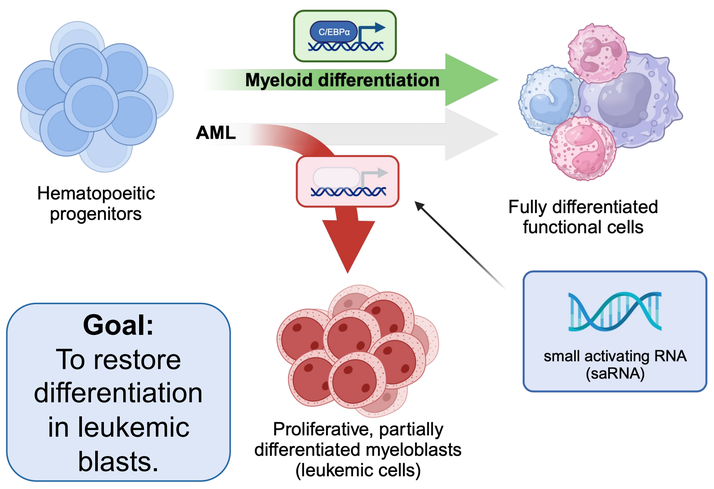
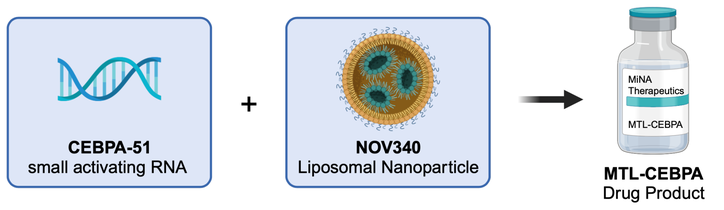
BACKGROUND

C/EBPα in Acute Myeloid Leukemia

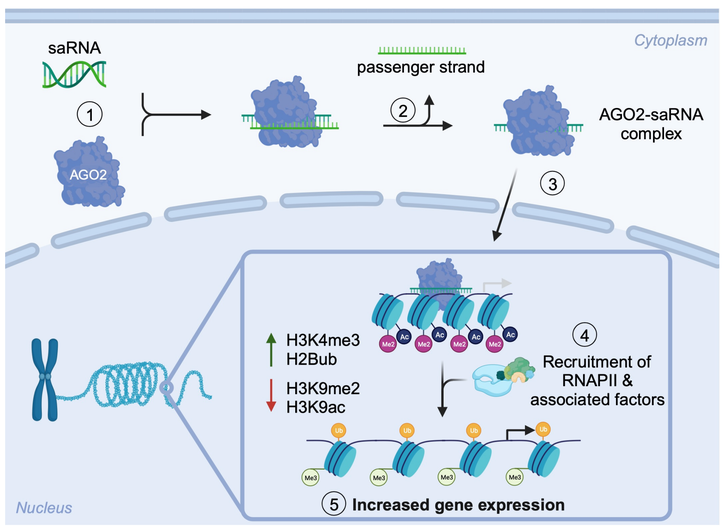


Goal: To restore differentiation in leukemic blasts.

MTL-CEBPA formulation



Mechanism of saRNAs



- Small activating RNAs (saRNAs) increase mRNA expression above endogenous levels by binding to the gene's promoter region.
- They recruit transcription factors and induce epigenetic changes, resulting in sustained gene upregulation.

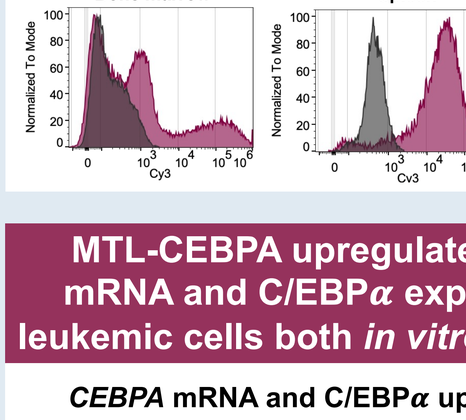
OBJECTIVES

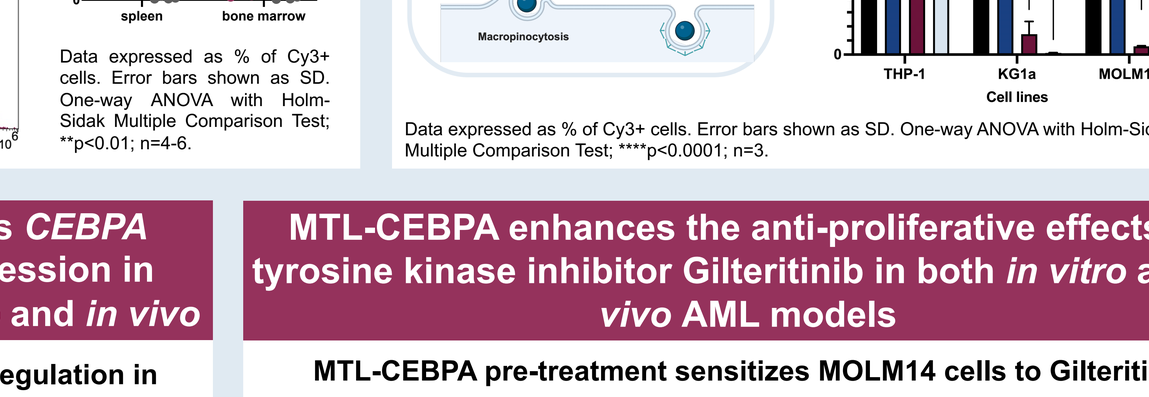
1. Characterize the uptake of MTL-CEBPA in AML cells
2. Validate MTL-CEBPA-induced *CEBPA* upregulation in AML cells
3. Evaluate the therapeutic potential of MTL-CEBPA for AML

RESULTS

NOV340 liposomal nanoparticle formulation preferentially delivers fluorescently labelled RNA to myeloid cells via macropinocytosis

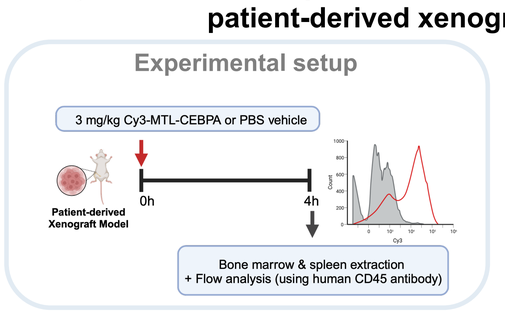
Cy3-MTL-CEBPA displays preferential uptake into myeloid leukemia vs lymphoid leukemia cell lines

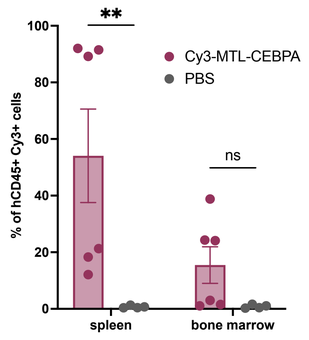




Data expressed as % of Cy3+ cells. Error bars shown as SD; n=3.

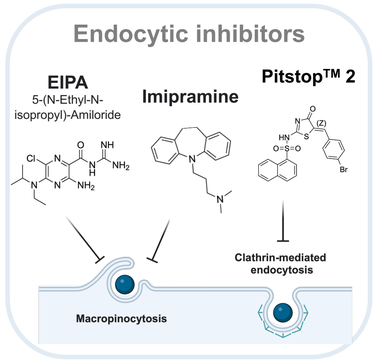
Cy3-MTL-CEBPA uptake in leukemic cells using *in vivo* patient-derived xenograft model

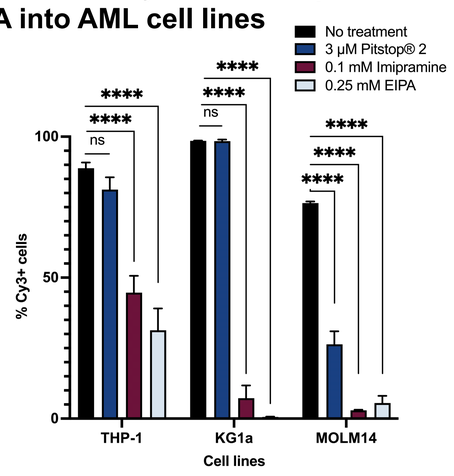




Data expressed as % of Cy3+ cells. Error bars shown as SD. One-way ANOVA with Holm-Sidak Multiple Comparison Test; **p<0.01; n=4-6.

Macropinocytosis inhibitors selectively block the uptake of Cy3-MTL-CEBPA into AML cell lines

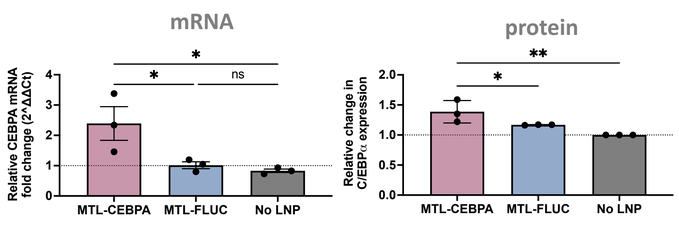




Data expressed as % of Cy3+ cells. Error bars shown as SD. One-way ANOVA with Holm-Sidak Multiple Comparison Test; ****p<0.0001; n=3.

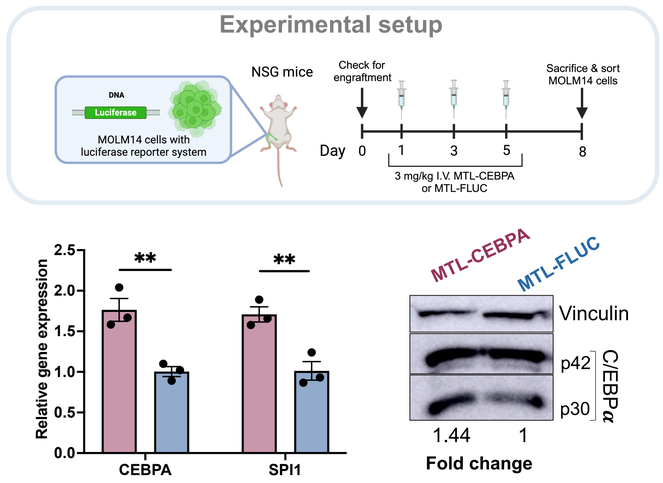
MTL-CEBPA upregulates *CEBPA* mRNA and C/EBPα expression in leukemic cells both *in vitro* and *in vivo*

CEBPA mRNA and C/EBPα upregulation in AML cell line THP-1



One-way ANOVA with Holm-Sidak Multiple Comparison Test; *p<0.05;**p<0.01; n=3. Error bars shown as SEM.

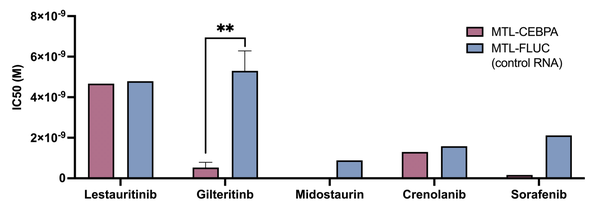
MTL-CEBPA upregulates *CEBPA* in MOLM14-xenograft mice



Two-tailed student t-test; **p<0.01; n=3. Error bars shown as SEM.

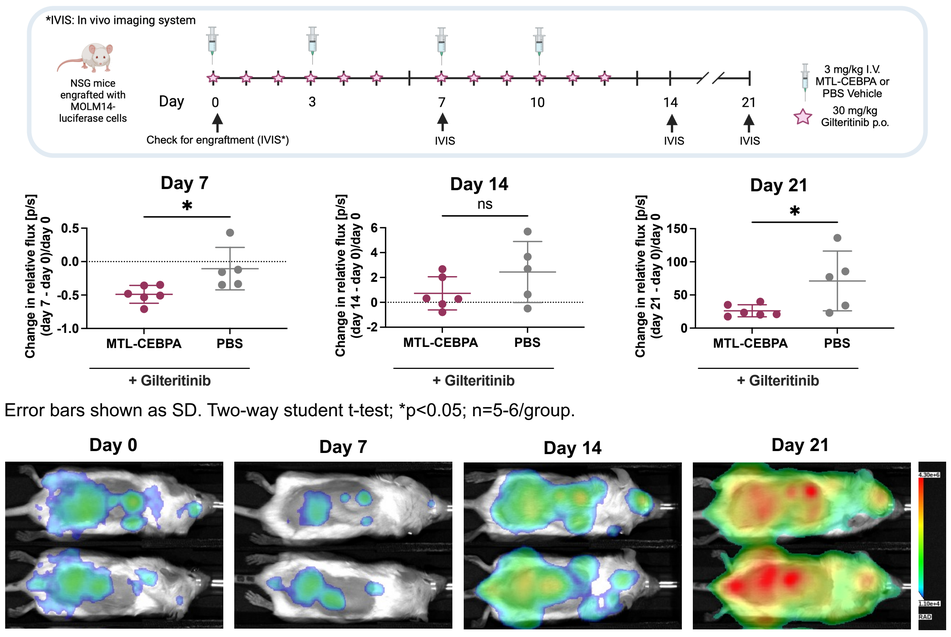
MTL-CEBPA enhances the anti-proliferative effects of tyrosine kinase inhibitor Gilteritinib in both *in vitro* and *in vivo* AML models

MTL-CEBPA pre-treatment sensitizes MOLM14 cells to Gilteritinib



Data shows 50% inhibitory concentration (IC50) of small molecule tyrosine kinase inhibitors following pre-treatment with MTL-CEBPA vs MTL-FLUC. Lower IC50 = more sensitive. Two-tailed student t-test; **p<0.01; n=3. Error bars shown as SEM.

Combination of MTL-CEBPA & Gilteritinib reduces leukemic burden in mice



Error bars shown as SD. Two-way student t-test; *p<0.05; n=5-6/group.

Bioluminescent images of mice harboring MOLM14-luciferase cells.
Higher bioluminescence = higher leukemic burden.

HIGHLIGHTS & FUTURE DIRECTIONS

- NOV340 liposomal nanoparticles **effectively deliver** fluorescently-labelled RNA to leukemic cells, both *in vitro* (AML cell lines) and *in vivo* (PDX mouse model).
- MTL-CEBPA **transcriptionally activates *CEBPA*** and increases C/EBPα expression in both AML cell lines and MOLM14-xenograft mouse model.
- MTL-CEBPA **improves Gilteritinib's anti-leukemic activity** and reduces leukemic cell growth in MOLM14-xenograft mouse model.

Next steps
- ❑Differentiation assays
- ❑MTL-CEBPA therapeutic effect in PDX model
- ❑Establish synergy between MTL-CEBPA & Gilteritinib

ACKNOWLEDGEMENTS

A special thank you to the various agencies funding my research, Profs. Maureen McKeague, Nathan Luedtke, and François Mercier for the mentorship, and MiNA Therapeutics for their ongoing support. Thank you to Christian Young and Cristobal Gonzalez-Losada for their assistance with the flow cytometry and cell sorting experiments, and Bahram Sharif-Askari and Véronique Michaud for their assistance with the *in vivo* experiments.

References

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