MiNA Therapeutics

Investigating the biodistribution of MTL-CEBPA reveals delivery of small activating RNA into CD34+ cells and different types of immune cells *in vivo*

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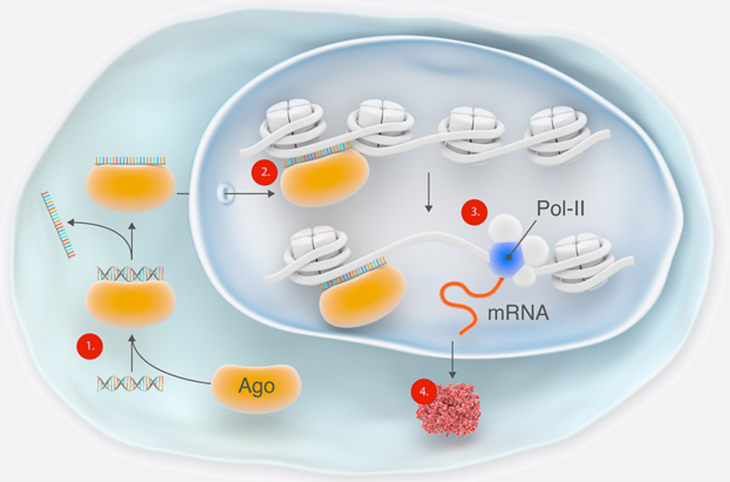
Background

- MTL-CEBPA is currently in Phase Ib clinical trials in patients with advanced hepatocellular carcinoma (HCC) (Clinical trial information: NCT02716012).
- MTL-CEBPA comprises a small activating RNA (saRNA) encapsulated inside a SMARTICLES® liposomal nanoparticle to activate CEBPA expression in cells.
- Our preclinical studies have demonstrated that MTL-CEBPA modulates the properties of immune cells in the tumor microenvironment in syngeneic mouse tumour models, improves liver function and suppresses tumor growth in tumour models in immunocompetent mice and rats.
- Administration of MTL-CEBPA to patients increases the CEBPA mRNA expression in circulating white blood cells.
- However, the cell types targeted by MTL-CEBPA in the immune system are not well defined.

Aim

- The aim of the study is to extend our knowledge on the biodistribution of MTL-CEBPA to cells of the immune system.

RNA activation mechanism



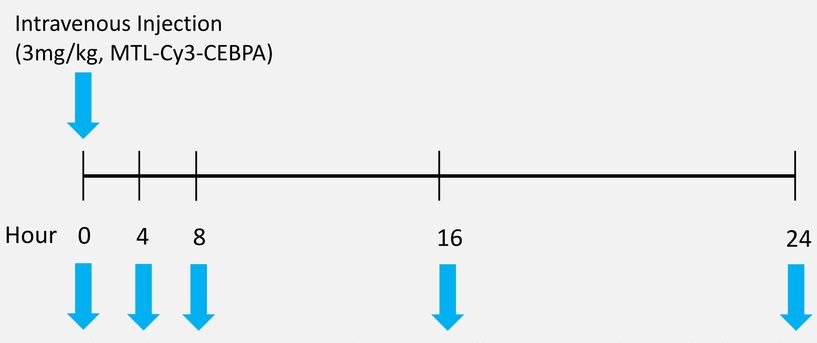
1. Loading of saRNAs into Ago2 protein
2. saRNA-Ago complex enters nucleus and associates with promoter region of target gene
3. Generation of a transcriptional activation complex to generate new mRNA
4. Synthesis of new protein

MTL-CEBPA Drug Product

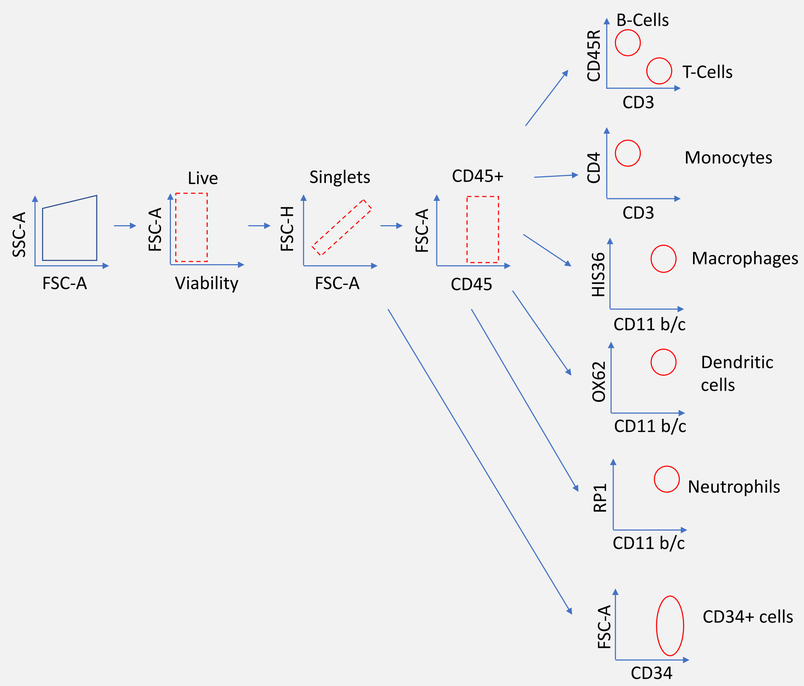
CEBPA-51 saRNA to upregulate CEBPA expression	NOV340 SMARTICLES® liposome	MTL-CEBPA
API	Formulation	Drug Product

Experimental setup

- The CEBPA saRNA was conjugated with a Cy3 fluorophore and encapsulated inside the SMARTICLES® liposome to form MTL-Cy3-CEBPA.
- MTL-Cy3-CEBPA was intravenously injected in Wister rats.
- Blood, bone marrow and spleen were harvested post-intravenous injection of MTL-Cy3-CEBPA and cells were isolated for flow cytometry analysis.



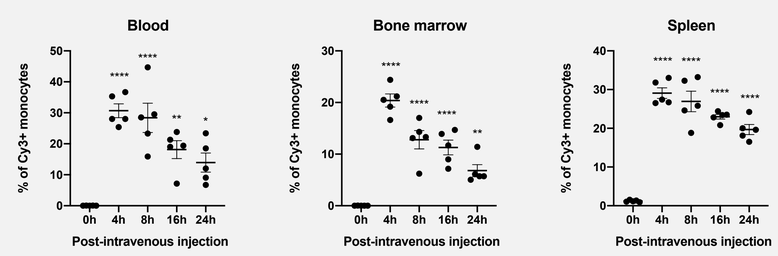
Flow cytometry gating strategy



MTL-Cy3-CEBPA uptake in myeloid cells

Monocytes

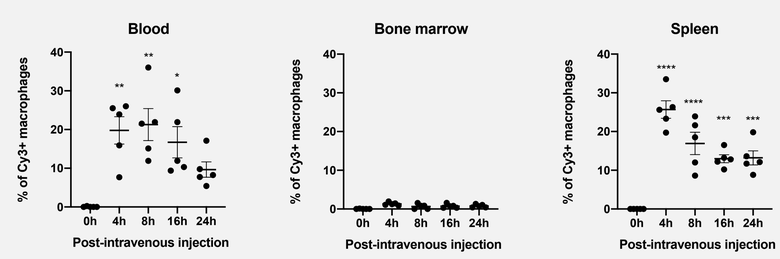
- The monocyte populations in blood, bone marrow and spleen mediate a significant uptake of MTL-Cy3-CEPBA.



Data are expressed as % of Cy3+ monocytes (CD45+ CD3- CD4+ cells). 1 way ANOVA was performed. *= p<0.05;**=p<0.01; ***=p<0.001; ****=p<0.0001 when compared to 0h.

Macrophages

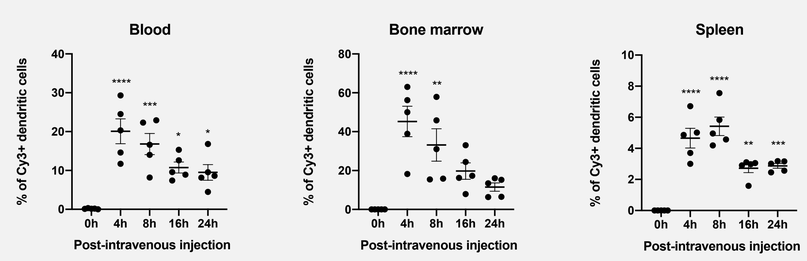
- The macrophage populations in blood and spleen, but not in bone marrow mediate a significant uptake of MTL-Cy3-CEPBA.



Data are expressed as % of Cy3+ macrophages (CD45+ CD11b/c+ HIS36+ cells). 1 way ANOVA was performed. *= p<0.05;**=p<0.01; ***=p<0.001; ****=p<0.0001 when compared to 0h.

Dendritic cells

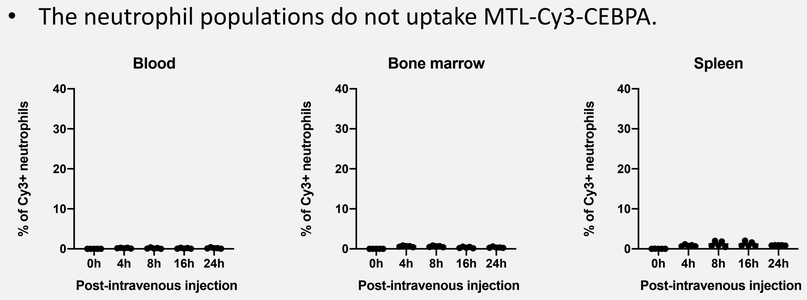
- The dendritic cell populations in blood and bone marrow mediated a significant uptake of MTL-Cy3-CEPBA.
- A small population of dendritic cells in spleen also takes up MTL-Cy3-CEPBA.



Data are expressed as % of Cy3+ dendritic cells (CD45+ CD11b/c+ OX62+ cells). 1 way ANOVA was performed. *= p<0.05;**=p<0.01; ***=p<0.001; ****=p<0.0001 when compared to 0h.

Neutrophils

- The neutrophil populations do not uptake MTL-Cy3-CEPBA.

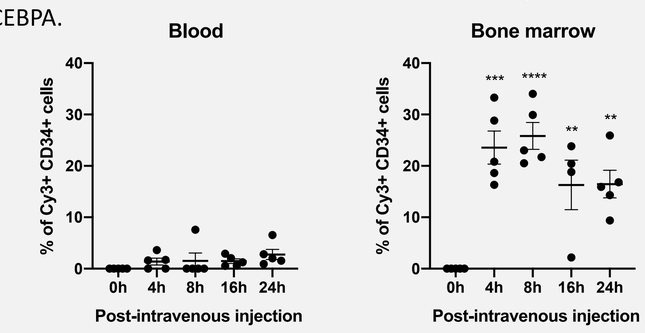


Data are expressed as % of Cy3+ neutrophils (CD45+ CD11b/c+ RP1+ cells). 1 way ANOVA was performed. No significant difference is observed between groups.

MTL-Cy3-CEBPA uptake in CD34+ cells

CD34+ cells

- The CD34+ cells in bone marrow but not in blood uptake MTL-Cy3-CEBPA.

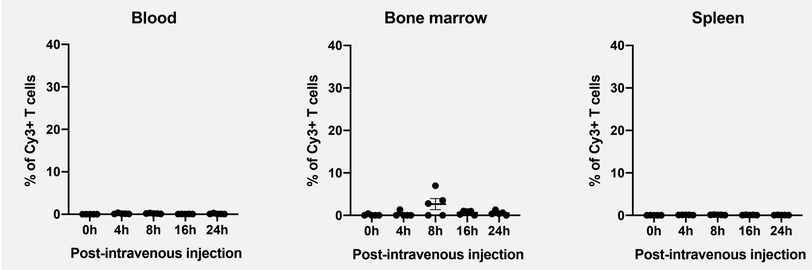


Data are expressed as % of Cy3+ CD34+ cells. 1 way ANOVA was performed.
*= p<0.05;**=p<0.01; ***=p<0.001; ****=p<0.0001 when compared to 0h.

MTL-Cy3-CEBPA uptake in lymphoid cells

T cells

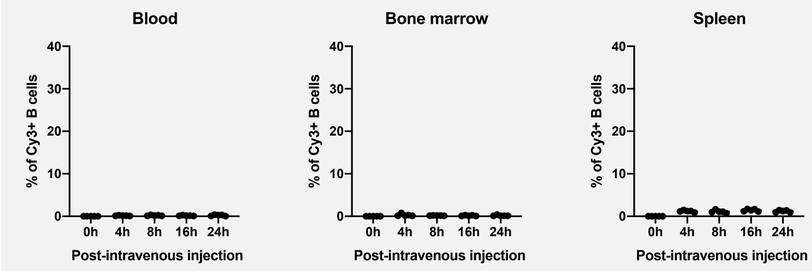
- The T cells in blood, bone marrow and spleen do not uptake MTL-Cy3-CEBPA.



Data are expressed as % of Cy3+ T cells (CD45+ CD3+ cells). 1 way ANOVA was performed.
No significant difference is observed between groups.

B cells

- The B cells in blood, bone marrow and spleen do not uptake MTL-Cy3-CEBPA.



Data are expressed as % of Cy3+ B cells (CD45+ CD45R+ cells). 1 way ANOVA was performed.
No significant difference is observed between groups.

Conclusion and future work

Conclusion

- MTL-Cy3-CEBPA can be taken up by some myeloid cell and CD34+ cell populations.

	Blood	Bone Marrow	Spleen
Monocytes	+++	++	+++
Macrophages	++	-	+++
Dendritic cells	++	++++	+
Neutrophils	-	-	-
CD34+ cells	-	++	ND
T cells	-	-	-
B cells	-	-	-

Summary of MTL-Cy3-CEBPA uptake in the cell populations 4 hour post intravenous injection. ++++ denotes ~40% of Cy3 uptake; +++ denotes ~30% of Cy3 uptake; ++ denotes ~20% of Cy3 uptake; + denotes between 5-10% of Cy3 uptake; - denotes no Cy3 uptake; ND refers to not determined.

Future work

- To investigate the CEBPA mRNA gene expression level in the cells which uptake MTL-Cy3-CEBPA.
- To apply our technology to target other diseases which can be treated by modulating myeloid and CD34+ cells.

References

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