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Self-assemblies of azacitidine prodrug: an innovative therapy against myelodysplastic syndromes

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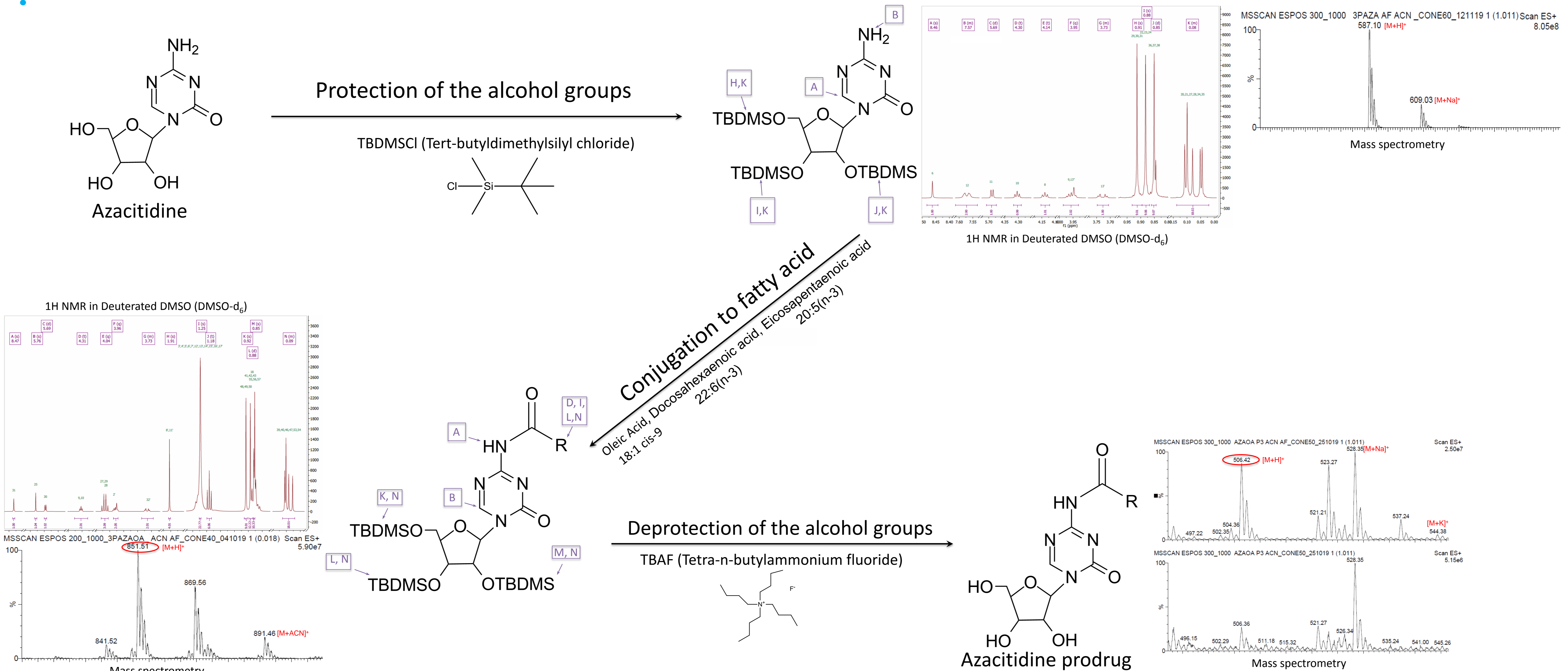
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Introduction

5-azacitidine, a cytidine analogue and a hypomethylating agent, is one of the main drugs being used for the treatment of myelodysplastic syndromes [1]. However, after administration, it exhibits several limitations including restricted diffusion and cellular internalization due to its hydrophilicity, and rapid enzymatic degradation. **Our objective is to improve the drug diffusion and to protect it from metabolic degradation via the formulation of an amphiphilic prodrug and its self-assembly into a nanoparticle.** Following the conjugation of the fatty acid to the azacitidine, the obtained prodrug was solubilized in acetone and mixed with water at different ratios to obtain self-assemblies by nanoprecipitation, thus protecting the active molecule from enzymatic degradation. This prodrug should be cleaved by cathepsin B [2], an enzyme overexpressed in cancerous cells [3], thus increasing the specificity of the drug.

1. Prodrug synthesis



The multi-step reaction for prodrug synthesis: starting with the protection of the alcohol groups with TBDMSO to avoid secondary conjugations during the next step of conjugation to the chosen fatty acid. Finally, the obtained product is deprotected using TBAF to give the prodrug (azacitidine-fatty acid conjugate). All of the products were identified and verified with either ¹H NMR spectroscopy, mass spectrometry or both.

2. MTT and apoptosis assays:

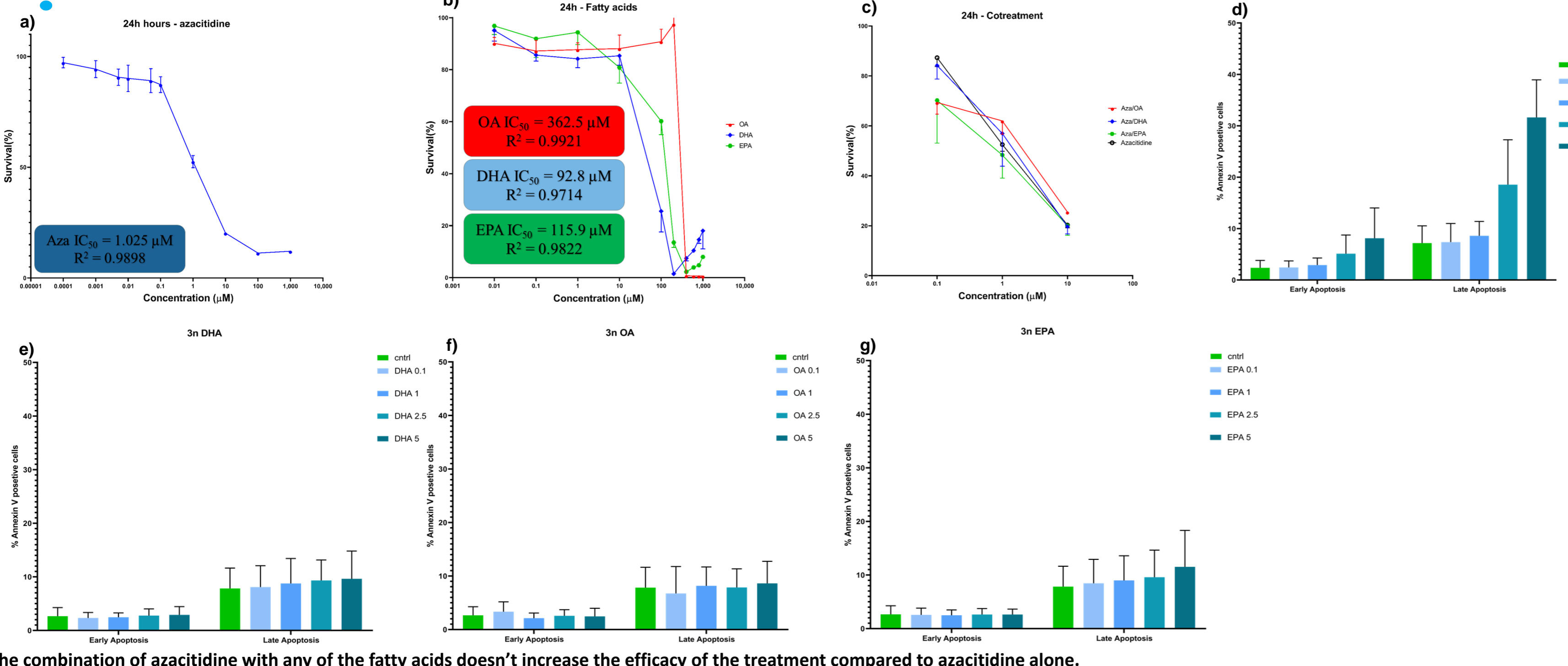


Figure a-c: MTT cell metabolic activity assay on HL-60 cell line (acute myeloid leukemia) to determine the IC₅₀ at 24 hours of azacitidine, the fatty acids and the co-treatments respectively (μM)
Figure d-g: Apoptosis assay on HL-60 cell line to determine the percentage of Annexin V-positive cells at 24 hours of azacitidine, docosahexaenoic acid, oleic acid and eicosapentaenoic acid respectively; post treatment with concentrations around the IC₅₀ of azacitidine (μM)

3. Upcoming studies :

a) Nanoparticle characterization

Determining size, zeta potential and shape :

- Critical Aggregation Concentration (CAC)
- Dynamic Light Scattering (DLS)
- Nanoparticle Tracking Analysis (NTA)
- Transmission electron cryo-microscopy (cryo-TEM)

b) In vitro studies:

The *in vitro* studies will be performed on several acute myeloid leukemia cell lines with varying sensitivity to azacitidine:

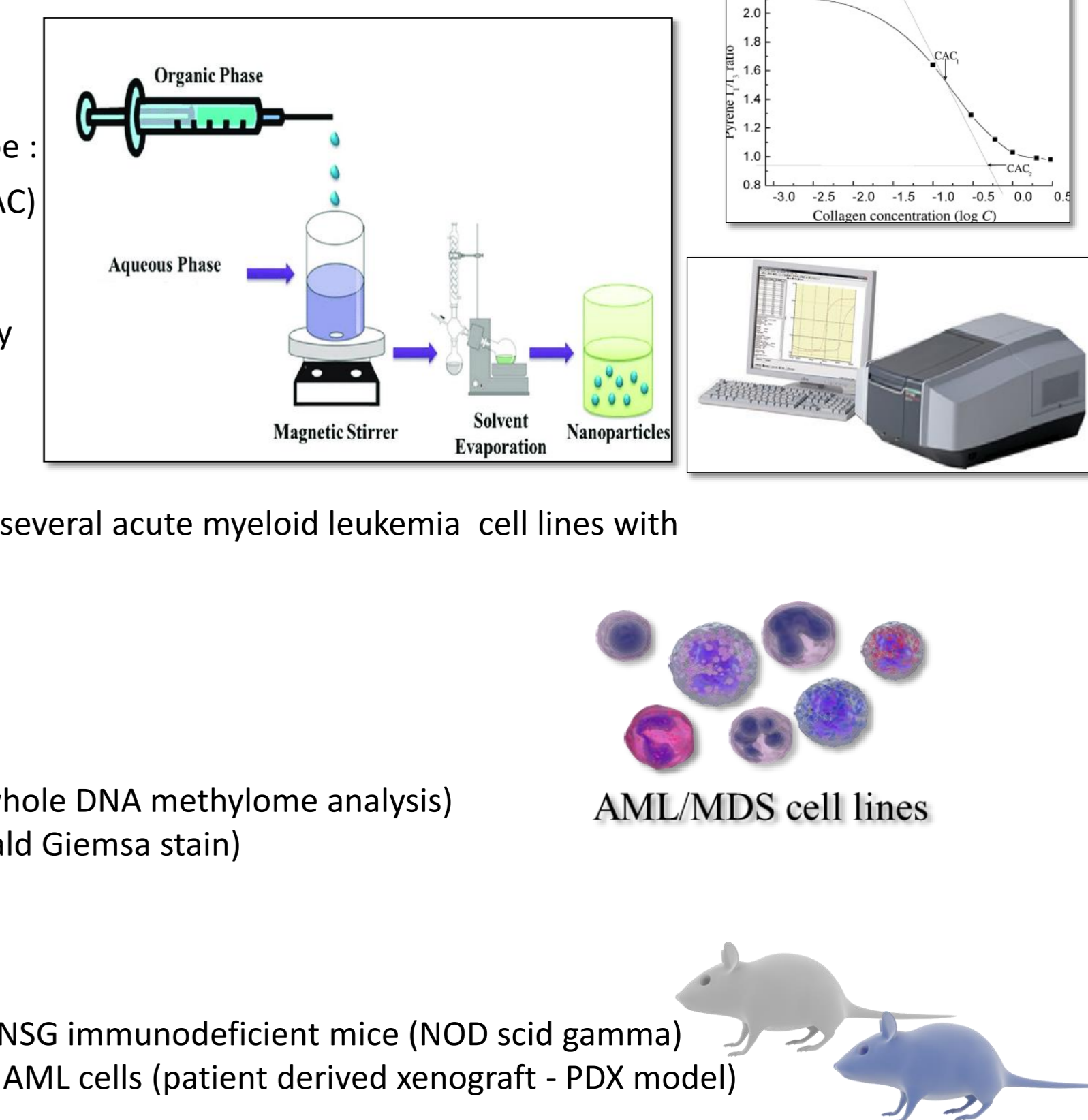
- HL-60
- SKM1
- K562

In vitro studies:

- Cellular uptake studies
- Cell cycle study (FACS)
- Methylation quantification (LINE -1, whole DNA methylome analysis)
- Differentiation studies (May - Grunwald Giemsa stain)
- Western blots

c) In vivo studies:

The *in vivo* studies will be performed on NSG immunodeficient mice (NOD scid gamma) that will be injected by a patient derived AML cells (patient derived xenograft - PDX model)



Conclusion

An azacitidine prodrug was achieved following a multi-step reaction to conjugate azacitidine to a fatty acid. The obtained prodrug will undergo a process of self-assembly to produce nanoparticles, that will be fully characterized. Finally, *in vitro* studies will be performed to determine the biological efficacy of these nanoparticles. The initial *in vitro* tests showed that the combination of azacitidine with any of the fatty acid doesn't increase the treatments efficacy, therefore any synergistic effect obtained later between will be attributed to the prodrug/nanoparticle approach.

References:

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[3] Alatrash, G. *et al.* Cathepsin G is broadly expressed in acute myeloid leukemia and is an effective immunotherapeutic target. *Leukemia* 31, 234 (2016)