Peptide-decorated ferrocifen lipid nanocapsules for the targeting of multidrug resistant ovarian adenocarcinoma

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Andrea Bottasso[1] , Pierre Idlas[1], Milad Baroud[1], Pascal Pigeon[2], Gérard Jaouen[2], Elise Lepeltier[1], Catherine Passirani[1]

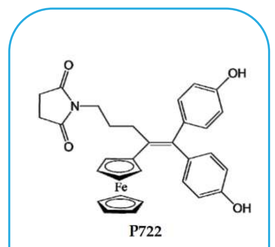
[1]Translational Micro and Nanomedecines (MINT, INSERM 1066 / CNRS 6021), Université d'Angers, Angers, France

[2]Chimie Paris Tech, PSL University, 75005 Paris, France

*Contact: elise.lepeltier@univ-angers.fr

Introduction and objectives

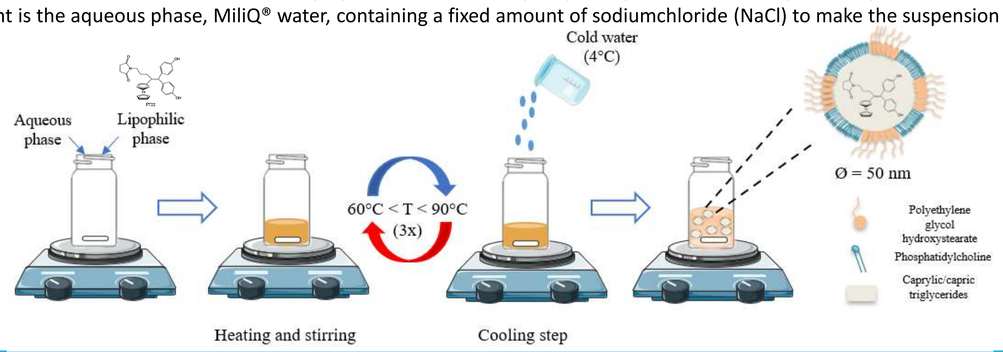
Ovarian adenocarcinoma reigns as one of the deadliest epithelial carcinomas impacting women, owing to its resistance to current treatments [1]. Thus, a novel approach to this condition is direly needed. Ferrocifen, an innovative organometallic complex, has the potential to be the answer for this hurdle. This molecule has shown very interesting antiproliferative and cytotoxic effects in both *in vitro* and *in vivo* studies [2]. However, this molecule is hindered by its lipophilicity: lipid nanocapsules (LNCs) are a much-needed vehicle to allow the delivery of this agent [3]. The aim of this project is thus to formulate the corresponding ferrocifen-loaded LNCs to target the ovarian adenocarcinoma. Furthermore, to improve the specificity of this formulation, the surface of LNCs has been decorated using different cell penetrating peptides such as TLS [4] or WSG [5], known from literature to be specific to the SKOV3 ovarian cancer cell line.



Chemical structure of ferrocifen P722

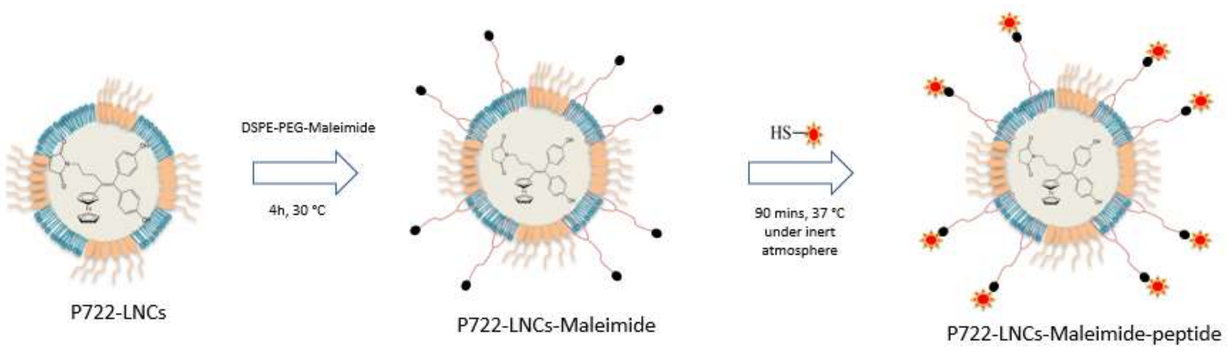
1. Formulation of LNCs

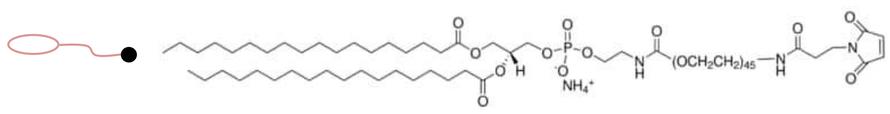
LNCs are composed of three main parts: an oily phase, an aqueous phase and nonionic surfactants. The oily phase is composed of caprylic/capric triglycerides (commercial name: Labrafac® WR 1349), nonionic hydrophilic polyethylene glycol (15)-hydroxystearate surfactant (Solutol® HS 15) and, as an amphiphilic cosurfactant, phosphatidylcholine from soybean (Lipoid®). The last principal component is the aqueous phase, MiliQ® water, containing a fixed amount of sodiumchloride (NaCl) to make the suspension injectable.



2. Surface decoration of LNCs

In order to perform a covalent grafting of the CTLS peptide at the surface of P722-LNCs, a PEGylated phospholipid DSPE-PEG_{2000}-Maleimide was post-inserted in LNC membrane in order to allow the Michael addition with the sulfur coming from the cysteine of the peptide. The peptides were grafted at concentrations of 1 mM and 3 mM.





DSPE-PEG_{2000}-Maleimide

Peptide C-TLS: (Cys-Thr-Leu-Ser-Gly-Ala-Phe-Glu-Leu-Ser-Arg-Asp-Lys)
or
Peptide C-WSG (Cys-Trp-Ser-Gly-Pro-Gly-Val-Trp-Gly-Ala-Ser-Val-Lys)

C-TLS and C-WSG peptides were synthesized by solid-phase peptide synthesis via the Fmoc strategy. The cysteine was added to the N-Ter position of the amino acid sequence to leave the C-Ter position accessible. They were purified and characterized by NMR and LC/MS chromatography. The final yields were 24 % for CTLS and 35% for CWSG with a purity higher than 95 % for both peptides.

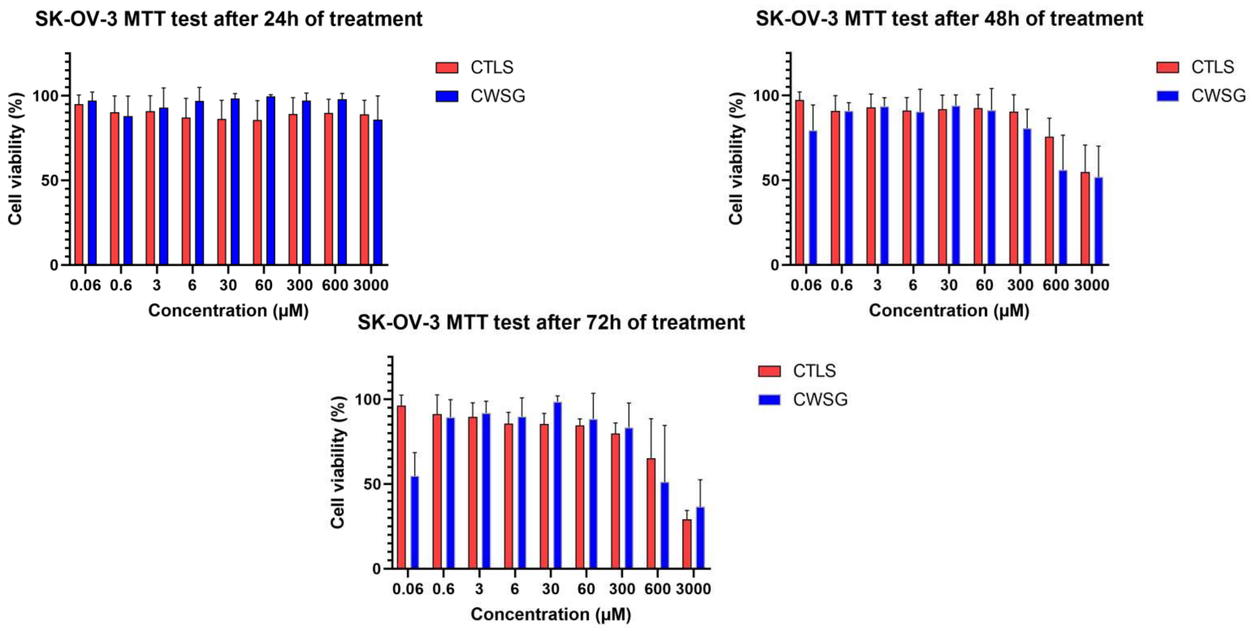
3. Characterization

Formulation	Diameter (nm)	PDI	Zeta potential (mV)	Loading P722 (% w/w)	Encapsulation efficacy (%)
P722-LNCs	49.3 ± 0.6	0.05 ± 0.01	-3.4 ± 0.6	0.56	> 95 %
P722-LNCs stealth	57.2 ± 1.0	0.06 ± 0.01	-18.1 ± 0.4	0.56	> 95 %
P722-LNCs-Maleimide	60.7 ± 0.6	0.12 ± 0.02	-17.0 ± 0.7	0.56	> 95 %
P722-LNCs-CTLS-1mM	66.3 ± 1.6	0.14 ± 0.01	-14.0 ± 0.9	0.56	> 95 %
P722-LNCs-CTLS-3mM	67.5 ± 3.2	0.15 ± 0.03	-7.2 ± 1.6*	0.56	> 95 %

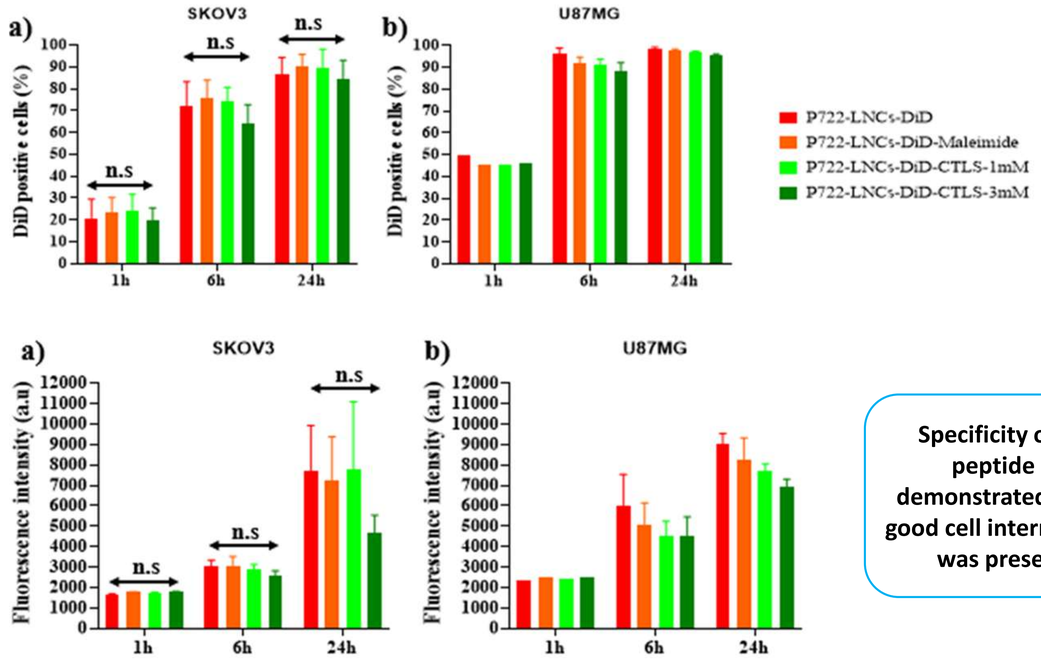
n = 3, Mean ± SD, *: $p < 0,05$; Kruskal-Wallis test

Tested concentrations:
1 mM and 3 mM
corresponding to 330 peptides and 990 peptides / LNC

4. *In vitro* studies



Kinetics of internalization of formulations (by flow cytometry)



Specificity of CTLS peptide not demonstrated but the good cell internalization was preserved

5. Perspectives

- Stability studies and characterization of P722-LNCs-peptide formulations
- Toxicity studies of the two peptides C-TLS and C-WSG when formulated with the P722-LNCs
- Internalization studies of P722-LNCs decorated with C-WSG
- *In vitro* confocal microscopy studies to determine the site of accumulation and action of these decorated ferrocifen loaded LNCs on ovarian adenocarcinoma
- *In vivo* experiments on Patient Derived Xenograft (PDX) murine models will commence shortly, to determine the biological efficiency of these formulations

Conclusion

P722-LNCs decorated with C-TLS cell penetrating peptide were successfully formulated as demonstrated by zeta potential values and 2D NMR.
Toxicity studies conducted on the C-TLS and C-WSG peptide demonstrated a promising anticancer synergic effect with the ferrocifen P722 at a concentration of 3 mM, which corresponds to the concentration of peptide covalently bound to our formulation of P722-LNCs.
Internalization kinetic on SKOV3 cells and U87MG cells did not show significant differences in cellular uptake using C-TLS modified P722-LNCs compared to non-modified formulations.

References:

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[3] Lainé AL. et al, Biomaterials 34, 6949-6956 (2013)
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[5] Ma C. et al, J. Pept. Sci. 19, 730-736 (2013)