**Company/laboratory/public institution:**

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**Supervision of trainee:**

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Internship period: 20 January - 18 July 2025

**Title of the project: Synthesis scale-up of EGFR targeted magnetic nanovectors**

**General context and positioning of the project:**

The research team "Nanomedicines and Nanoprobes" (NMNS) of the Center for Molecular Biophysics (UPR CNRS 4301 CBM) in Tours has recently developed magnetic siRNA nanovectors with the aim to deliver them via intravenous injection for targeted gene therapy in breast cancer.[1,2] These targeted, stealth magnetic siRNA nanovectors (NV-si) are composed of a core of functionalized superparamagnetic iron oxide nanoparticles (SPION) loaded with siRNA and cationic polymers (NV-si) via electrostatic interactions. The core of SPION is composed of magnetite ($Fe_3O_4$) and maghemite ($\gamma$-$Fe_2O_3$) labelled with a fluorophore (dylight™ 680), coated with $PEG_{5000}$ and functionalized with an epidermal growth factor receptor (EGFR) targeting moiety.[2,3] EGFR was chosen as target as it has been suggested to play an important role in TNBC.[4] Two types of targeting moieties are used, antibody fragments (scFv) for a PhD project and peptides for a collaborative project. The collaborative project entitled “Magnetic control for improved combination therapy in Triple Negative Breast Cancer (MAGCOT)”, include three French teams and one Italian team, is funded by the Institut National du Cancer (INCa, PLBIO22) and coordinated by S. DAVID. In this MAGCOT project, magnetic nanovectors, able to co-vectorize siRNA and conventional chemotherapy (NV-si-CT), are developed and will be evaluated on TNBC cell- and mice-models. However, one limiting step in this project, is the synthesis of the magnetic nanovectors and their CT drug loading. The synthesized quantities per batch are sufficient for the *in vitro* evaluation on cells but not for the development of the formulation and the *in vivo* experiments on mice.

**Objectives of the internship:**

The aim of this M2 Internship is to scale-up the synthesis of these magnetic nanovectors in order to reduce the number of syntheses that will be needed for the formulation experiments of NV-si-CT and their *in vivo* evaluation. In a first time, the existing protocols have to be mastered and literature research has to be done in order to understand the

different synthesis steps. In a second time, the protocols for the functionalization with the EGFR-targeting moiety and for the CT-loading have to be adapted to at least double the iron concentration of the obtained EGFR targeted nanovectors loaded with Doxorubicin as model drug.

**Summary of the scientific/technical program:**

In the 2 first month, the synthesis has to be mastered and literature research has to be done in order to understand all the synthesis steps and to identify the critical points for the scale up process. In the first step, the iron oxide nanoparticles are obtained using a simplified Massart's protocol and are then silanized with aminopropyl trimethoxysilane (APS). Then, the surface of SPIONs is functionalized with Dylight™ 680 fluorochrome and PEG (NHS-PEG-Maleimide). After purification by dialysis to eliminate free Dylight™ 680 and NHS-PEG-Maleimide, EGFR targeting moiety (antibody fragments (scFv) and peptides) are covalently grafted by maleimide-cysteine Michael addition reaction. The obtained targeted nanovectors are characterized by measuring the size, the zeta potential, the iron concentration and the fluorescence spectra of Dylight™ 680.[2,3] These functionalized SPION will then be electrostatically loaded with doxorubicin via a pH sensitive DOX-$Fe^{2+}$ complex (NV-DOX).[5] Currently, the obtained NV-DOX have an iron concentration of approximately 1 g/L. These can be used for biological evaluation on cells, but for formulation with siRNA in order to obtain NV-si-CT, the concentration must be at least 2 g/L.

In the second 2 months, the protocols, especially for the functionalization with the EGFR-targeting moiety, and for the DOX-loading have to be adapted. The obtained nanovectors have to be characterized and the experiments repeated in order to identify the parameters that can be changed without affecting the quality of the nanovectors. The objective is to optimize the EGFR-targeting peptide/SPION ratio and to obtain optimized NV-DOX with an iron concentration of at least 2g/L and a DOX loading ratio of at least 50mg DOX/g iron.

In the last 2 months, the identified parameters of the protocols can be further optimized either using the conventional way by changing one factor at a time or using design of experiments by changing several factors at a time.

The obtained results will be presented to the NMNS team and the partners of the MAGCOT project. A PhD and a post-doc student will use the obtained nanovectors for the *in vitro* (and *in vivo*) evaluation and can help with the NP synthesis and formulation experiments.

**References**:

1. Eljack, S. *et al.* Combination of Nanovectorized siRNA Directed against Survivin with Doxorubicin for Efficient Anti-Cancer Activity in HER2+ Breast Cancer Cells. *Pharmaceutics* **14**, 2537 (2022).
2. Nguyen, P. V. *et al.* Targeted nanomedicine with anti-EGFR scFv for siRNA delivery into triple negative breast cancer cells. *European Journal of Pharmaceutics and Biopharmaceutics* **157**, 74–84 (2020).
3. Hervé, K. *et al.* The development of stable aqueous suspensions of PEGylated SPIONs for biomedical applications. *Nanotechnology* **19**, 465608 (2008).
4. Nguyen, P. V., Allard-Vannier, E., Chourpa, I. & Hervé-Aubert, K. Nanomedicines functionalized with anti-EGFR ligands for active targeting in cancer therapy: Biological strategy, design and quality control. *Int J Pharm* **605**, 120795 (2021).
5. Gautier, J. *et al.* A pharmaceutical study of doxorubicin-loaded PEGylated nanoparticles for magnetic drug targeting. *Int J Pharm* **423**, 16–25 (2012).