# Enhanced drug delivery via antibody–drug conjugates and siRNA-linked nucleic-acid aptamers targeting EGFR in glioblastoma cells

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**Résumé**

Active targeting in drug delivery is based on the binding of ligands to receptors present on the surface of targeted cells in order to promote the internalization of ligand conjugated drugs. The most well-known conjugates are antibody-drug conjugates (ADCs), which combine the specificity of monoclonal antibodies with the cytotoxic of chemotherapeutic drugs. In addition to antibodies, nucleic acid aptamers, known as single stranded DNA or RNA oligonucleotides referring to chemical antibodies with high affinity and selectivity for their target (*Zhou et al, 2016*), are promising to deliver conjugated drugs or therapeutics nucleic acids such as siRNA by active targeting delivery (*Mercier et al, 2017*).
In our team, we are interested in developing an aptamer-siRNA chimera called AsiC targeting EGFR (epidermal growth factor receptor), a receptor, internalized by endocytosis (*Ivaska et al, 2011*) and often overexpressed in glioblastoma cells, the most aggressive tumour of the central nervous system. Our AsiC consists of a targeting part: a 2' fluoro modified RNA aptamer E07 targeting EGFR (Cruz da Silva, Foppolo et al, 2022) and a therapeutic part: a siRNA for gene silencing. The therapeutic efficacy of such conjugates not only depends on target specificity but also on efficient internalization into tumor cells. However, so far, no therapeutic approach to enhance endocytosis of conjugates is available.

In recent studies, we showed that gefitinib, a tyrosine kinase inhibitor directed against the EGFR, induces a massive, non-physiological endocytosis of EGFR, known as gefitinib-mediated endocytosis (GME), in different glioblastoma cell lines (*Blandin et al, 2021 ; Cruz Da Silva et al, 2021).* We thus hypothesized that besides promoting endocytosis of EGFR, gefitinib could also promote endocytosis of its ligands. In this study, we proved by quantitative fluorescence bioimaging, that gefitinib is indeed able to strengthen the endocytosis of

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fluorophore-conjugated EGFR-specific antibodies and aptamers. We also showed that the GME potentiates the toxicity of an ADC and the efficacy of an AsiC. Our results suggest the development of a new therapeutic combination with gefitinib, to potentiate the delivery of ADC, AsiC and likely other conjugates targeting EGFR in glioblastoma, while limiting side effects on non-targeted cells. Our results have been submitted for publication and are already available online as a preprint (https://www.biorxiv.org/content/10.1101/2024.10.22.617611v1).

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**Mots-Clés:** antibody, drug conjugates, aptamer, siRNA conjugates, bioimaging, epidermal growth factor receptor, gefitinib, glioblastoma, endocytosis, nucleic acid aptamers, RNAi