1. PUBLISHABLE SUMMARY

Summary of the context and overall objectives of the project (For the final period, include the conclusions of the action)

Gene therapy is one of the most promising options for future advanced treatments in a broad range of diseases. Successful gene delivery requires the recognition of target cells as well as cytosolic and nucleosolic uptake of the gene. Currently, non-viral based gene delivery is only suitable for in vitro applications. Clinical delivery of gene therapeutics is accomplished via viral vectors, which still have major safety concerns and involve complex and costly manufacturing procedures, preventing future implementation for the treatment of diseases affecting large patient groups. In currently available technologies, more than 98% of the therapeutic gene is transported to the cell but then, it accumulates inside cellular compartments called endosomes where it is finally degraded. In recent years, we have discovered and studied certain secondary plant metabolites called glycosylated triterpenoids or, as we call them, Endosomal Escape Enhancers (EEEs), which plants have evolved as a self-defense mechanism against pathogens. We are now adapting these metabolites to improve the delivery outcome of targeted, non-viral gene therapy. EEEs specifically accumulate in endosomal membranes and destabilize them, preventing gene degradation by endosomal escape and providing sufficient amount of the therapeutic gene to localize in the cell nucleus. However, to date such EEEs and the gene therapeutic product must be applied as two independent components, which makes clinical applicability and marketing approval complicated or even impossible.
The ENDOSCAPE technology aims to create a non-viral gene delivery technology comprising a scaffold that carries all required components, the EEE, a targeting ligand, and the effector gene. Proof of concept of the ENDOSCAPE technology will have a major impact on the therapeutic opportunities for current and future biopharmaceuticals with intracellular sites of action. Thus, overall objectives of the ENDOSCAPE project are to solve the longstanding problem of cytosolic and nucleic delivery of gene therapeutics, to minimize treatment risks by circumventing virus-mediated gene transfer, to enhance the efficacy of targeted gene therapeutic treatment in patients, to reduce the costs of gene therapy and make it available for a broad patient base, and to be compatible with personalized gene therapies.

Work performed from the beginning of the project to the end of the period covered by the report and main results achieved so far (For the final period please include an overview of the results and their exploitation and dissemination)

In the second 18-months-period of the project, we have worked together cooperatively and in constant exchange within the joint collaboration framework established in the first period. To successfully achieve our common tasks this framework includes a joint data server and common policies for structured and joint actions such as a Data Management Plan, a Publication Policy, and a Dissemination and Exploitation Plan. In addition, we had regular discussions with our Intellectual Property Rights and Exploitation Board and an Advisory Committee. We further held regular (video) meetings of the whole consortium and the work package leaders, and separate meetings within selected working groups.
After developing conditions to complex example effector genes, we analyzed a large panel of complete ENDOSCAPE prototypes consisting of a scaffold, EEEs, targeting ligand and the gene. To ensure the quality, ENDOSCAPE prototypes were analyzed by methods such as mass spectrometry, nuclear magnetic resonance, dynamic light scattering and fluorogenic detection which provided feedback for

further optimization cycles. The first ENDOSCAPE prototypes have been produced and chemically optimized before testing in vitro. We then continued with the functional characterization of the ENDOSCAPE prototypes in cell cultures and investigated their molecular mode of action. Here we first worked with the gene of enhanced green fluorescent protein as a reporter and surrogate for the therapeutic genes applied in the project for hemophilia therapy and suicide genes for cancer treatment. We further refined recombinant expression and purification of several candidate ligands and were able to select the most performing scaffolds and ligands to target hepatoma and cancer cells. To test toxicity and stability of the prototypes in human blood, we developed new assays for ex vivo measurements and have created the conditions for mouse experiments that have just started. To understand the molecular mechanism of the escape of nucleic acids from endosomes in living cells we used fluorescently labeled ENDOSCAPE modules and confocal microscopy for a subcellular analysis of their trafficking in hepatoma cells and primary hepatocytes.
The use of plant natural products such as EEEs entails the risk of heterogeneous source material and lack of availability. Therefore, an independent production process is vitally important. We selected and optimized different plant growth systems and culturing conditions including a post-harvest treatment of roots to increase the EEE quantity. We further conducted an expression analysis in EEE producing plants and discovered genes involved in the biosynthesis of EEEs with the intention to manipulate the biosynthesis of EEEs for higher yields. An extraction and purification protocol for highly pure EEEs and related compounds was established.
In addition, we continued the early health economic evaluation by defining clinical example applications and conducting analyses based on literature review and expert inputs. This included the definition of a production model for a Cost of Goods analysis and of cost-effectiveness model structures, clarification of comparator treatments and relevant dimensions of ENDOSCAPE-delivered therapies and documentation for two exemplary indications, acute lymphocytic B-cell leukemia and hemophilia B. Our project is regularly communicated, e.g., by a public website, a project video, and a LinkedIn account.

Progress beyond the state of the art, expected results until the end of the project and potential impacts (including the socio-economic impact and the wider societal implications of the project so far)

With the final success of the project, the ENDOSCAPE technology aims to achieve a higher efficacy of non-viral targeted gene therapy using EEEs, and to allow for any macromolecule to be delivered to target cells in the body, in particular to treat monogenetic diseases and cancer. ENDOSCAPE aims to provide a good alternative to viral gene delivery technology, while incorporating already existing medical drug candidates. It is safe, it can be produced at relatively low cost, thereby significantly impacting and changing the future landscape of therapeutic gene delivery. This will induce new biotech-based businesses, new research projects and create new technology platforms for development of new macromolecule therapeutics for a broad range of diseases. The ENDOSCAPE technology platform will broaden research agendas, facilitate the cross application of the most novel and appropriate techniques, provide integration of approaches, and widen the context for future gene delivery research involving private and public collaborations. ENDOSCAPE has the potential to allow the resurrection of drugs that clinically failed due to insufficient intracellular drug delivery and will strengthen the EU's competitive landscape in the global search for new advanced technologies.

Address (URL) of the project's public website

https://endoscape-2020.eu

ENDOSCAPE Consortium