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 virusmediated 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 first 18-months-period of the project, we established a joint collaboration framework including 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 installed an Intellectual Property Rights and Exploitation Board and an Advisory Committee, fulfilled all ethical requirements and had regular meetings of the whole consortium and the work package leaders, and separate meetings within selected working groups. In order to develop a scaffold as a carrier for all components, we first defined and produced initial prototypes of dendrimer-based, peptide-based and DNA-based scaffolds. Dendrimers were chemically modified to allow dye conjugation (for analytical purposes) as well as attachment of EEEs and targeting ligands. The same reactive groups that were introduced into the dendrimer scaffold were included in the peptide scaffold, and in the DNA scaffold by a specific new complementary strand technique. The EEEs and the targeting ligands were modified with click chemistry groups compatible to the modifications of the scaffolds. After

developing conditions to complex example effector genes, we finalized a first complete ENDOSCAPE prototype consisting of a scaffold equipped with EEEs, targeting ligand and the gene. We were able to define analytical procedures to ensure the quality of the equipped scaffolds by methods such as mass spectrometry, nuclear magnetic resonance, dynamic light scattering and fluorogenic detection.
Another objective is the functional characterization of the ENDOSCAPE prototypes in cell cultures and mouse models, and to investigate their molecular mode of action. Here we validated genes for hemophilia therapy and suicide genes for cancer treatment. In addition, we tested the expression of target receptors on different cell lines for several candidate ligands that have been recombinantly expressed and purified, or purchased. Currently, we are investigating the escape of nucleic acids from endosomes in living cells by confocal and light sheet microscopy using fluorescently labeled dendrimers modified with EEEs. We demonstrated efficient delivery of effector DNA in cell culture with low toxicity. 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 established various plant culture formats and evaluated different plant growth systems and culturing conditions.
We further conducted an expression analysis towards gene discovery in EEE producing plants with the intention to manipulate the biosynthesis of EEEs for higher yields. To date we are already able to extract and purify target EEEs from the biomass of greenhouse grown plants.
In addition, we started an early health economic evaluation by defining clinical example applications and planning analyses based on literature review. We are further collecting information on the expected cost of the ENDOSCAPE production process. 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

http://endoscape-2020.eu

ENDOSCAPE Consortium