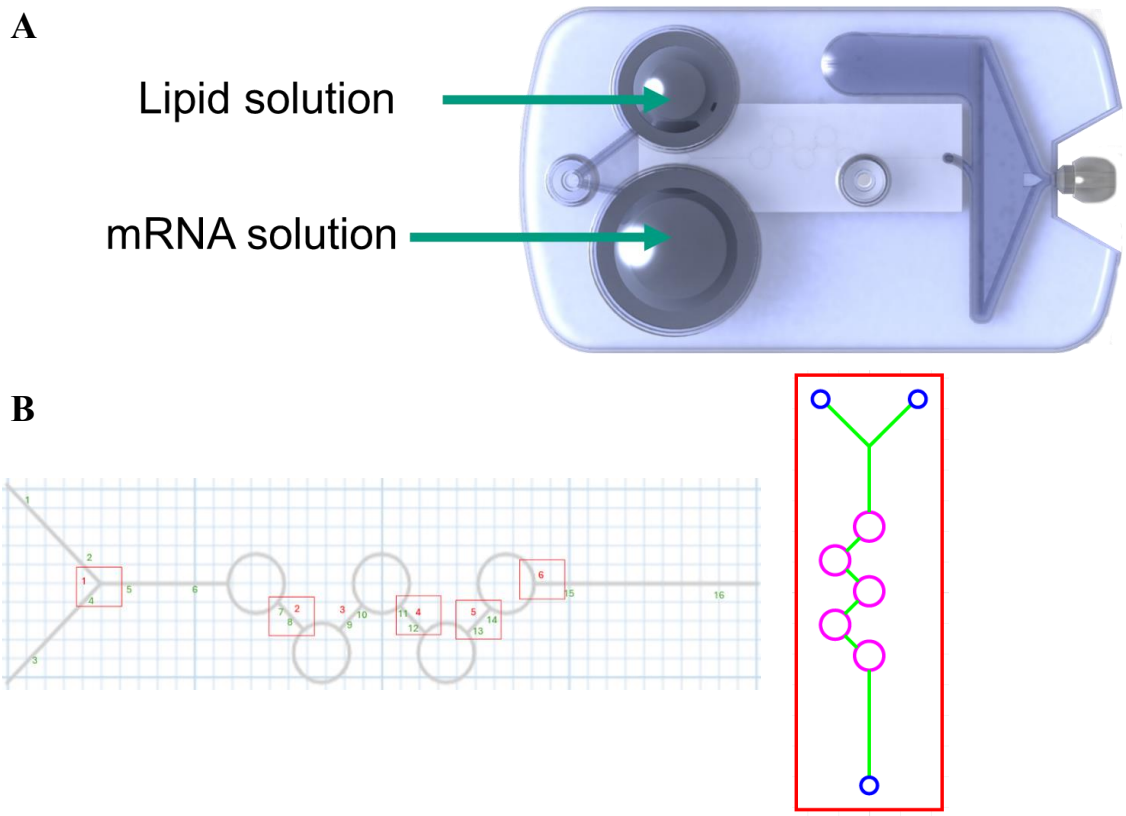
Supplementary material



Supplementary figure 1: Detailed schematic of the Nanospresso platform technology. A) Prototype of the cartridge design currently undergoing testing and optimization, featuring two blisters—one containing mRNA and the other a lipid mixture—that are mixed through a pressure-driven microfluidic system. B) Example of a microfluidic design under evaluation for the production of mRNA-encapsulating lipid nanoparticles.

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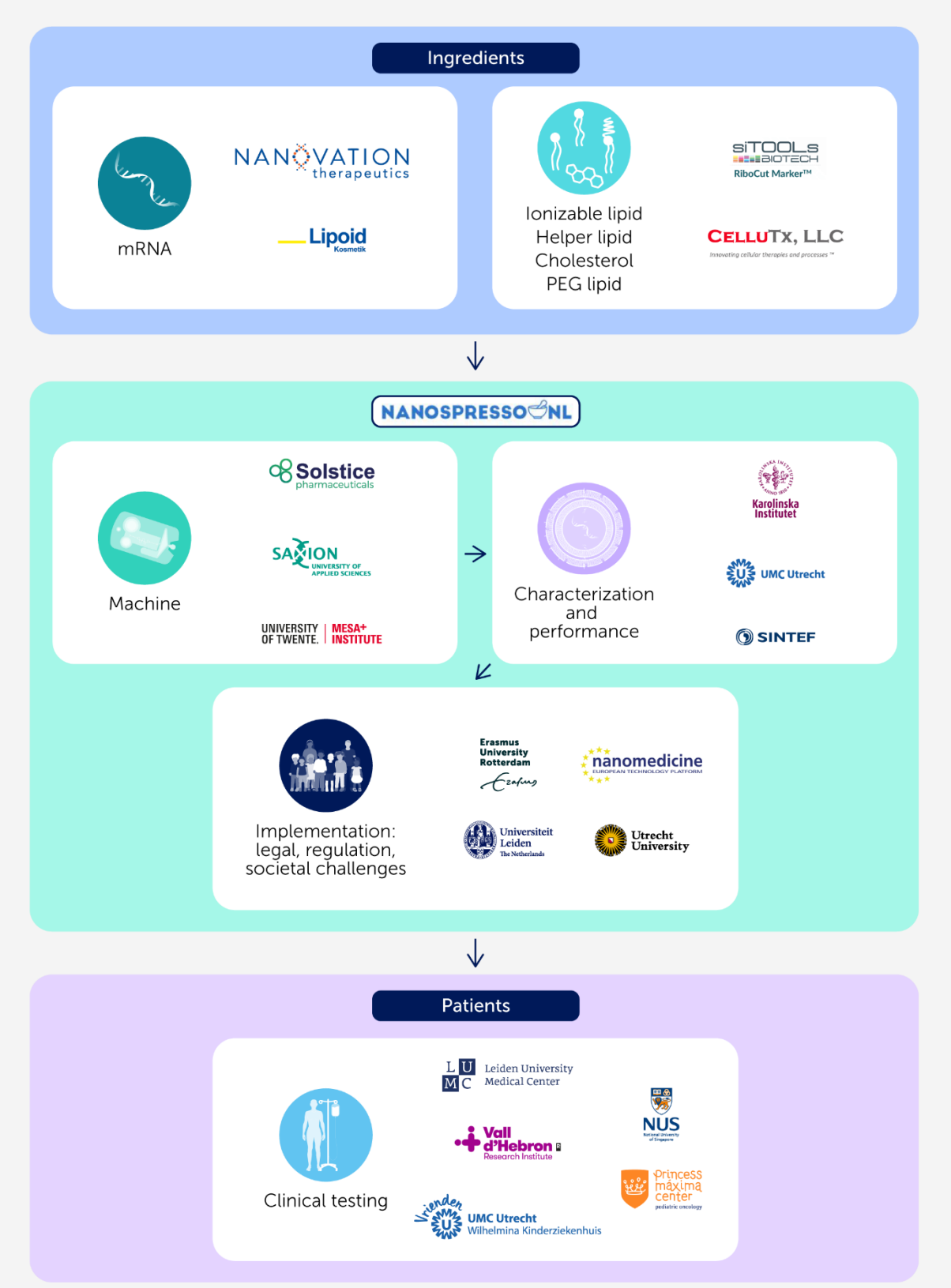

Supplementary figure 2: This figure provides a detailed overview of the project's organizational structure. It highlights the suppliers of active ingredients, including lipids and

mRNA, as well as the research partners responsible for developing the NANOSPRESSO machine. The machine incorporates a microfluidic system designed to enable consistent and reproducible mixing of lipids and mRNA, producing lipid nanoparticles encapsulating therapeutic nucleic acids. The figure also identifies the partners involved in characterizing the therapeutics produced by the NANOSPRESSO machine, conducting preclinical testing and addressing societal, legal, and regulatory challenges essential for implementing the NANOSPRESSO concept. Finally, it outlines the contributions of cooperation partners in developing a plan for clinical testing. Logos reproduced with permission from the featured institutions.

Supplementary section 1: Other drug delivery platforms

Antisense oligonucleotides and splice-switching oligonucleotides

Antisense oligonucleotides (ASOs) target specific gene expressions by binding to complementary mRNA sequences, which inhibits protein translation and the production of disease-causing proteins (**Figure 2B**) (1, 2). This precise modulation of genetic pathways makes ASOs effective for diseases with known genetic markers, notably orphan diseases.

The chemical synthesis of oligonucleotides allows for the introduction of non-natural nucleotides that enhance the molecule's stability against nuclease degradation and increase selectivity for target mRNA. Notable modifications include phosphorothioate linkages, where sulfur atoms replace non-bridging oxygen atoms on the phosphodiester backbone, enhancing stability and binding affinity (2–5). For instance, fomivirsen employs a fully thioated backbone to increase nuclease resistance (6, 7), while inclisiran incorporates six thioate bonds and multiple sugar modifications, such as 2’-O-methyl, 2’-fluoro, and 2’-O-methoxyethyl groups (8–10), substantially extending its half-life in the liver to several months after subcutaneous administration—contrast this with unmodified oligonucleotides, which generally persist for just hours (4, 11, 12).

These structural enhancements also improve protein-binding capacity and reduce water solubility, which aids in the oligonucleotide's passive diffusion across cell membranes (13–15). Consequently, certain ASOs, like the splice-switching oligonucleotides golodirsen, viltolarsen, and casimersen, have been approved for therapeutic use without the need for complex delivery systems (15, 16).

The creation of milasen, customized for a single patient with Batten’s disease, showcases the rapid development potential of ASOs. Targeting a specific mutation identified through

genomic screening, milasen progressed from concept to treatment in just a year, significantly reducing the patient’s symptoms with manageable side effects through biweekly intrathecal injections (17). This case exemplifies the potential for rapid, personalized ASO therapies, particularly for severe conditions, leveraging chemical modifications that confer stability and enhance cellular uptake (2, 14, 18).

N-acetylgalactosamine

Targeting ligands can be introduced to improve uptake by specific cell types, e.g., in inclisiran. Inclisiran achieves accumulation in hepatocytes owing to one additional modification, a tri-antennary N-acetyl galactosamine (GalNAc) residue that specifically binds the asialoglycoprotein receptor that is almost exclusively expressed on hepatocytes (19). Together, these modifications allow a treatment schedule based on twice-yearly subcutaneous injection (12, 20, 21).

This strategy is, to a certain extent, generic: provided that the target mRNA is in the hepatocytes, the enhanced stability provides the therapeutic nucleic acid ample time to arrive there (22). This has been successfully exploited to build a pipeline of N-acetylgalactosamine (GalNAc)-targeted siRNA molecules for various orphan diseases (23, 24). This approach can potentially be expanded with alternative ligands binding cell surface receptors in different organs and on other cell types.

Nanoparticles

The chemical modifications that enable therapeutic use of oligonucleotides cannot be applied to longer mRNA or DNA constructs. Because of their length, chemical synthesis is impractical and biological production of these agents is mandatory, necessitating alternative protection and delivery methods. Nano-sized delivery systems can accommodate these larger nucleic acid payloads while being small enough to enter cells. Historically, the field was

divided into viral and synthetic carriers (25). The synthetic carriers are detailed in Section 3, while the viral vectors will be briefly outlined below.

Viral vectors

Viral vectors benefit from millennia of co-evolution with humans, which has led to natural selection for highly efficient transducers of genetic material into human cells. Retroviruses, lentiviruses, adenoviruses, and adeno-associated viruses (AAV) are the most popular approaches (26). Several products are currently on the market (27, 28). As well as vaccines, viral vectors are used as *ex vivo* transducers of T cells to make CAR T cells (29), offering hope in the realm of cancer treatment. In the sphere of rare diseases, viral vectors have played a pivotal role (30); for instance, onasemnogene abeparvovec (Zolgensma) employs an AAV vector to deliver a functional copy of the SMN1 gene to patients with spinal muscular atrophy (SMA) (31, 32), illustrating the transformative potential of viral vector technologies in addressing genetic disorders previously deemed untreatable. The field has also witnessed some important retractions from the market, such as betibeglogene autotemcel (for treatment of transfusion-dependent β-thalassemia), elivaldogene autotemcel (for treatment of severe combined immunodeficiency due to adenosine deaminase deficiency), and alipogene tiparvovec (for treatment of lipoprotein lipase deficiency). The production of viral vectors remains challenging and costly, and the range of vectors suitable for therapeutic applications is limited (33–35). Also, viral vectors may elicit an immune response: following initial exposure, the viral vector becomes unsuitable for subsequent use in the same patient as the body will recognize and neutralize it. Should the vaccine or gene therapy prove unsuccessful in clinical studies, the vector is precluded from being repurposed for different treatments in that individual. Furthermore, the patient may already possess immunity against the chosen viral vector, thereby compromising the efficacy of the treatment (36–38). In addition,

unexpected interactions between viral infections may occur. For example, an AAV2 outbreak amidst the COVID-19 pandemic appeared to be associated with hepatitis in children (39–42).

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