NANOCELL THERAPEUTICS, INC.

Transforming cell & gene therapy ™

Spinning off for success: creating a comprehensive plan for NanoCell's orphan drug branch

NanoRare's Business Plan – 2023

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ABSTRACT

The following business plan presents an investment opportunity for potential investors in NanoCell's groundbreaking work on Ornithine Transcarbamylase deficiency (OTC), an ultra-rare metabolic disorder. Our focus is on creating an independent, separately funded and managed entity, called NanoRare, which leverages NanoCell's advanced therapy for the treatment of OTC. Our approach is based on a thorough market and competitive analysis to highlight the potential and profitability of this venture.

This review provides an understanding of the OTC landscape, assessing current incidence among patients and regions, reviewing available treatments, and identifying potential avenues for innovative interventions such as gene therapies. Simultaneously, the business plan deepens a comparative analysis, examining numerous biotechnology companies currently developing gene therapies for OTC treatment. The focus will be primarily on identifying the nature of these novel therapies and making a detailed comparison with the technology proposed by NanoRare. This assessment will not only highlight NanoRare's theoretical technical superiority, but also determine its prospective competitive position within the OTC gene therapy landscape.

The business plan includes a strategic evaluation of NanoRare, to capture future valuable partners and potential investors interested in this project. This analysis intertwines the business and technological aspects of the proposed therapy, aligning them with the initial steps that could translate into a clinical trial, if all proceeds as expected.

The feasibility of the current and any potential new technology with theoretical technical superiority is presupposed. The ambition with the following business assessment is to prepare a beneficial groundwork for NanoRare's current venture as well as for future projects. Through the subsequent sections of the plan, we aim to provide a detailed overview of OTC and the potential role of gene therapy offering a curative solution, emphasizing the possibilities for progress in the treatment of such diseases.

INTRODUCTION

Gene therapy, an emerging frontier in personalized medicine and healthcare, is paving the way for innovative treatment strategies for genetic disorders. It offers a transformative promise – a single therapeutic intervention that addresses the root cause of the disease, rather than managing its symptoms. Gene therapy is a novel technique defined as the treatment of disease by transfer of genetic material into cells. This might involve replacing a mutated gene with a therapeutic corrected version of itself, inactivating a mutated gene that is functioning improperly, or introducing a new gene to fight a disease. This revolution in healthcare presents an exciting and complex landscape of opportunities, challenges, and implications for both patients and the industry. The focus of the following thesis report is an in-depth exploration of this transformative field, specifically assessing the feasibility of spinning off a novel gene therapy approach for the treatment of OTC deficiency, from NanoCell Therapeutics B.V. into a separate, independently funded and managed company, named NanoRare.

NanoCell Therapeutics B.V. is a trans-Atlantic biotechnology company currently developing a novel targeted gene therapy platform, with the vision to make genetically engineered therapies truly affordable, scalable and accessible for patients. Upon successful development of their proof-of-concept, NanoCell is looking to expand its pipeline, by optimizing its technology platform for the treatment of genetic disorders. In this pursuit, the Urea cycle disorders have been identified as potential candidates to investigate, with the focus being the Ornithine Transcarbamylase deficiency.

OTC deficiency, an ultra-rare metabolic disorder could see a groundbreaking solution in gene therapy. In contrast to the prolonged symptom management seen in traditional pharmaceutical businesses, gene therapy aims for a single, impactful cure, potentially reducing and, in time, eradicating the disease and its associated burden from the population. This “one-shot” approach inherent to gene therapy reshapes the business model, requiring a comprehensive assessment of the market landscape and the identification of strategies for capturing and sustaining value. The premise relies on the potential of being the sole player in a specific arena (understanding by arena a specific genetic disorder, such as OTC) – the first company to successfully develop a gene therapy approach for a disease and secure its commercialization, will capture the entire target market, leaving no room for competitors.

The main goal with the following report was to undertake a market opportunity assessment that blends scientific understanding, market analysis and strategic planning to present a compelling case for investment in NanoRare’s groundbreaking venture. This evaluation entailed a scientific overview of OTC deficiency and available gene therapy technologies for its treatment; an analysis of the current market landscape with its dynamics, key players, opportunities, and challenges unique to this field; and a detailed examination of the distinct approach that NanoRare intends to develop and commercialize, leveraging a scientific differentiator strategy.

To address the central research question, the business plan focuses on the alignment between business strategy and the scientific development of the lead drug candidate (LDC), NR-001. This included, among others, an examination of third-party preclinical reference data, to validate our proposed approach and an overview of the initial research and development trajectory, from preclinical proof-of-concept to potential first-in-human clinical trials.

The following document unfolds in three block of contents. The first one includes the introduction to the business plan and a scientific review of OTC deficiency, aiming to provide a deeper understanding of the disease's pathophysiology, prognosis and current treatment options. The section concludes highlighting the disease's most important clinical unmet need.

The second block begins with an executive summary, presented as NanoRare's company profile. It then dives into the market analysis, assessing the competitive landscape, emerging opportunities and an overview of its proposed platform and product. It also provides a comparative analysis with key market players and potential disruptive advantages of NanoRare's proposed approach. Finally, a review of third-party preclinical data is presented to demonstrate that the therapy is based on proven methodologies, along with NanoRare's initial R&D trajectory and financial summary.

Finally, the last block assesses the competitive position that NanoRare could hold in the OTC gene therapy landscape, using market analysis tools. There, we examined current external market realities, as well as NanoRare's potential internal capabilities, that culminated in the identification of three long-term competitive advantages that NanoCell could transfer to the spin-off, in form of core competencies, to ensure the success of this project.

Overall, NanoRare's business plan presents a compelling narrative that connects the available scientific knowledge and recent technological advances in the gene therapy field, with the proposed business strategy to capture potential investors and partners.

ORNITHINE TRANSCARBAMYLASE DEFICIENCY: A comprehensive overview

Pathophysiology

Ornithine transcarbamylase deficiency (OTC) is an ultra-rare inherited disorder characterized by the accumulation of ammonia (nitrogen) in the blood, due to a deficiency of the OTC enzyme. The OTC enzyme is one of the five enzymes involved in the Urea cycle pathway that eliminates the excess of nitrogen, primarily from protein metabolism, by converting it into urea (in the liver) for excretion via the kidneys. OTC is responsible for catalyzing the synthesis of citrulline, in the second step of the Urea cycle, from carbamoyl phosphate and ornithine. Defects in these enzymes result in increased levels of ammonia, the so-called Urea cycle disorders (UCDs).

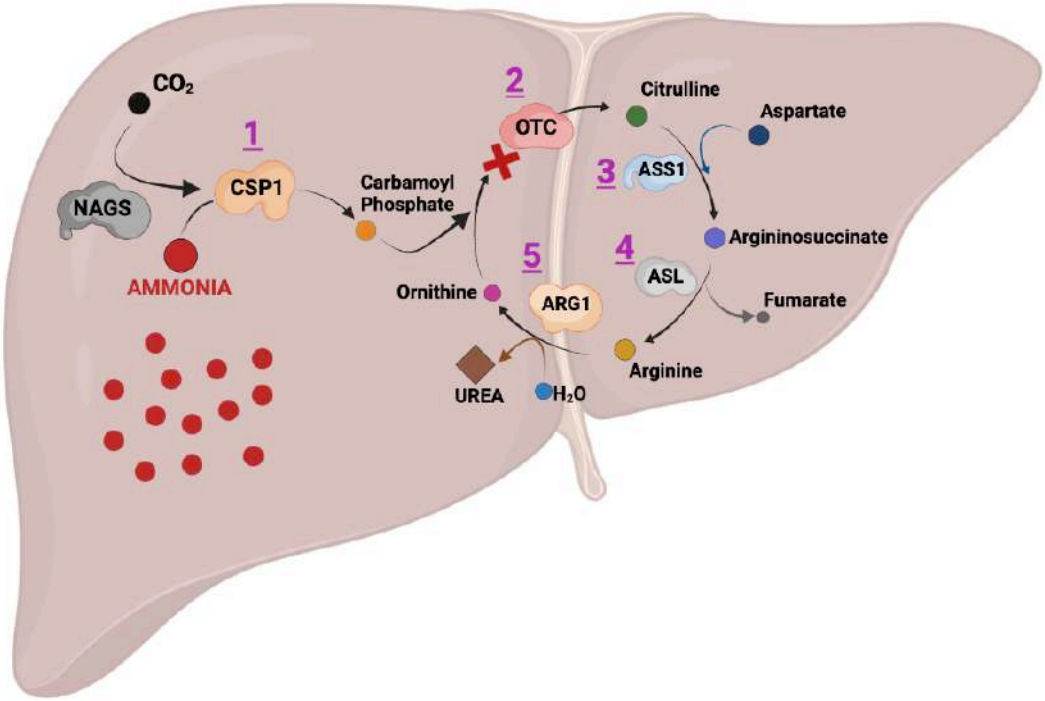


Figure 1: Urea cycle illustration: a series of five chemical reactions catalyzed by metabolic enzymes (CPS1, OTC, ASS1, ASL and ARG1). The first two steps of the cycle take place in the mitochondrial matrix, while the remaining last three occur in the cytosol. Ornithine transcarbamylase deficiency highlighted with a red cross in the second step, impending ureagenesis, leading to accumulation of toxic ammonia (red spots).

Ammonia is toxic when levels become too high, especially to the nervous system. OTC deficiency can occur at any age, with the most severe form affecting males more frequently in the first few days of life (neonatal-onset form). In some affected individuals, symptoms may be less severe and may not appear until later in life (late-onset form) (1). The range of symptoms affecting the patients are:

1. **Hyperammonemia:** the most significant symptom of OTC deficiency is the buildup of ammonia in the blood, which can cause a range of indications, including lethargy, vomiting, confusion, seizures, and coma. If left untreated, hyperammonemia can be deathly.

2. **Liver damage:** including cirrhosis and scarring, which can lead to liver failure and the need for a liver transplant.
3. **Irreversible neurological impairment:** it can also cause a range of neurological damage, including developmental delays, intellectual disability, seizures, and spasticity.
4. **Behavioral and psychiatric disorders:** patients can experience behavioral and psychiatric problems, such as aggression, depression, and anxiety.
5. **Growth and development issues:** children with OTC deficiency may experience growth and development issues, that includes delayed puberty, short stature, and poor weight gain.

Disease etiology and prognosis

OTC deficiency is caused by mutations in the OTC gene, which is X-linked recessive, and more than 500 mutations have been identified (see supplemental material Figure 1 for inheritance pattern) (2). Mutations that abolish OTC activity completely result in the severe, **neonatal-onset** form (less than 20% of residual enzyme activity), while mutations leading to decreased OTC activity result in the **late-onset** phenotypes (between 20% to 30% of residual enzyme activity) (1). Estimates of the prevalence of OTC deficiency range from 1 in 54,000 to 1 in 77,000 people.

The distribution of percentages between late-onset and neonatal-onset groups of OTC patients is not well-established in the scientific literature, primarily due to varying manifestations and diagnostic challenges associated with the disorder. The neonatal-onset form, which is more severe, tends to be diagnosed more readily due to the early appearance of life-threatening symptoms. In contrast, late-onset OTC deficiency presents with milder symptoms that can emerge later in life, resulting in delayed diagnosis and potential underestimation of its prevalence. Consequently, the available data may be biased towards the neonatal-onset form, and a clear understanding of the distribution between the two forms remains elusive.

Nonetheless, the paper titled “Three-Country Snapshot of Ornithine Transcarbamylase Deficiency” by Berna Seker (3), provides an updated detailed gender, age and clinical phenotype distribution of OTC deficiency patients in France, Turkey and England. Figure 2, below, depicts three graphs:

- **Panel A:** the gender distribution reveals that males are predominantly affected, because of the X-linked recessive nature of the disorder. *Blue: male; orange: female asymptomatic; grey: female symptomatic.*
- **Panel B:** shows the age groups affected by the disorder and its distribution. *Blue: 0-6 years old; orange: 6-12 years old; grey: 12-18 years old; yellow: >18 years old.*
- **Panel C:** offers a breakdown of the clinical phenotypes of OTC deficiency, what contributes to the heterogeneous presentation of the disease. *Blue; neo-natal presentation; orange: late-onset presentation; grey: asymptomatic.*

In addition, the article also describes (see Figure 2 in supplemental material):

- The heterogenicity of the clinical presentation of OTC deficiency: by measuring clinical symptoms, where altered neurological status (such as confusion, stupor, lethargy, or coma), and encephalopathy were the most common in all three countries. Gastrointestinal symptoms also ranked very high, with vomiting the most predominant.
- The variation in its treatment across countries: focusing on protein restriction diets, with higher rates in France (69%) and Turkey (79%) compared to the UK

(42%); and ammonia scavenger usage, that also varies, with dual therapy favored in Turkey and UK, while in France most patients were treated with only sodium benzoate.

The study advocates for increased awareness of the disease and the need for more uniform practices driven by recent guidelines, with an emphasis on **early detection**. It also underlines the importance of integrating data from similar studies into neonatal screening programs, to address potential underestimation of cases and ensure rapid intervention.

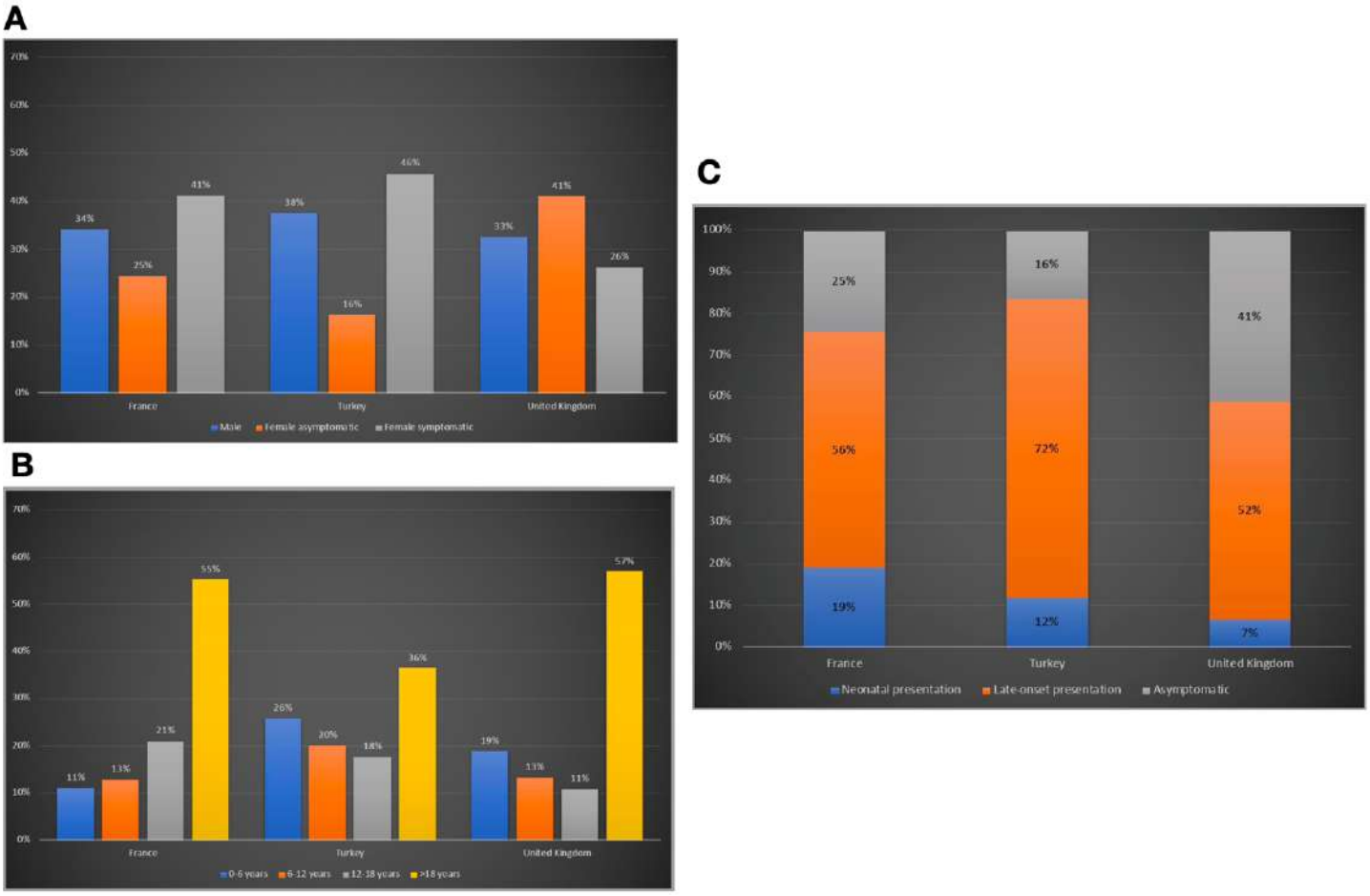


Figure 2: Graphs extracted from **Three-Country Snapshot of Ornithine Transcarbamylase Deficiency article.** Panel **A**: gender distribution; panel **B**: age groups of OTCD patients; panel **C**: clinical phenotypes.

Newborn screening programs (NBS) for OTC deficiency are crucial for early identification, significantly improving the prognosis and long-term outcomes for affected individuals. Tandem mass spectrometry (MS/MS) screening detects OTC deficiency by measuring the levels of citrulline and other amino acids in dried blood spots obtained from newborns. Low citrulline levels, combined with other biochemical markers (such as urine organic acids), can indicate OTC deficiency, although confirmatory testing, such as molecular genetic testing or enzyme activity analysis, is required to establish a definitive diagnosis. Prenatal diagnosis is possible in families with a known disease-causing mutation (1).

The implementation of newborn screening (NBS) for OTC deficiency varies across countries and regions. For instance, in the US, the screening programs are not uniform across states. Some of them have included the disease in these panels due to its severity

and available options, but others have not. In the European union, the implementation of OTC in NBS also varies, with some countries including it and others not. Figure 3, provided below, shows the disease inclusion in NBS panels across 30 European countries. The overall trend is towards the inclusion of OTC in these screenings programs, as the benefits of early detection and intervention become increasingly recognized.

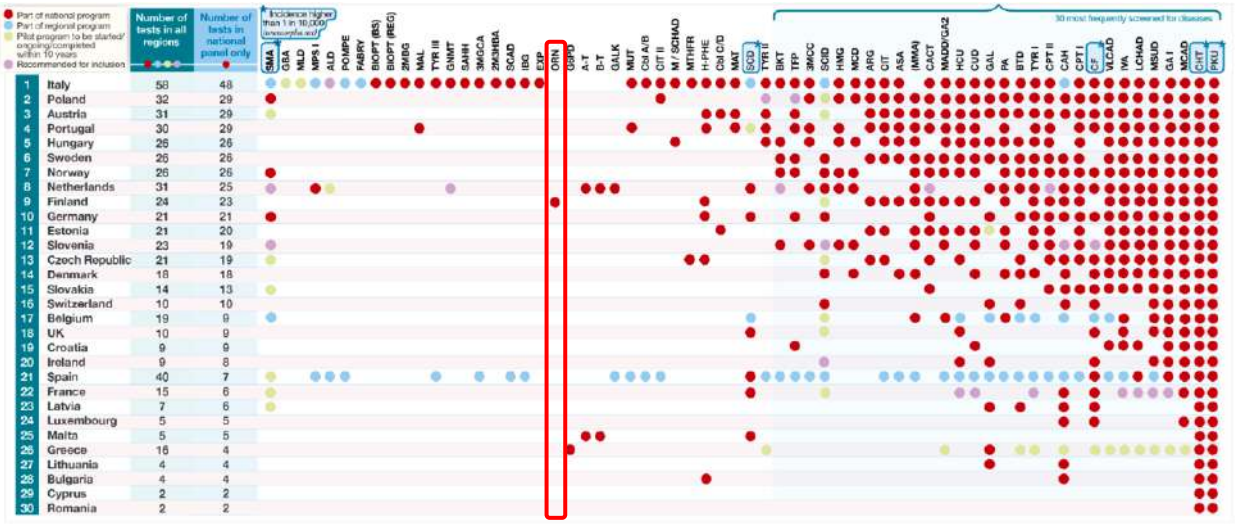


Figure 3: Mapping of disease inclusion in NBS panels in Europe. OTC, highlighted in red square, included only in Finland (date from 2021). Source CRA Insights **(4)**.

Current treatment options and clinical unmet need

OTC deficiency causes high mortality and morbidity, particularly in males who are hemizygous (only one copy of the gene) for the X-linked mutation. Fifty percent of infants with the disease die. Even if an infant survives a hyperammonemic coma, they will likely face intellectual disability if they were in the coma for over 24 hours. Patients diagnosed early and treated emergently have an improved prognosis, as do patients who adhere to low protein diets and receive liver transplants (5).

The **current golden standard treatment** for OTC deficiency includes a combination of pharmacological and nutritional interventions. These interventions are aimed at reducing ammonia levels in the body and preventing the hyperammonemic crises, which can cause irreversible neurological damage and/or death. Pharmacological interventions include the administration of nitrogen scavengers, such as sodium benzoate and sodium/glycerol phenylbutyrate, which help to eliminate excess nitrogen from the body. Nutritional interventions involve reducing protein intake and providing patients with specific amino acid blends, which help to reduce ammonia production. Existing treatments manage OTC deficiency symptoms but do not address the underlying genetic causes that prompt the disease.

The unmet clinical needs of OTC deficiency are significant, highlighting the necessity for new and more effective treatments. Current treatment options for OTC deficiency are not curative and liver transplantation is not a feasible option for all patients, as it critically depends on availability of matching donors and patient eligibility. This underscores the need for alternative treatments, **particularly for the neonatal-onset** group, which tackle the root cause of the deficiency, providing a life-lasting solution to patients from the day they are diagnosed.

Gene therapy requirements

Gene therapy offers a potential solution to the unmet clinical need of OTC deficiency. By introducing a functional copy of the OTC gene into affected cells (hepatocytes), the enzyme's activity could be restored, preventing the accumulation of toxic metabolites. Several preclinical studies have demonstrated the efficacy and potential safety of gene therapy in the treatment of other genetic disorders (6), (7), (8), providing a strong basis for further exploration of this approach for OTC deficiency.

To be effective, gene therapies must meet several key requirements:

- **Safety and tolerability:** must be well tolerated by the human body and have minimal adverse effects. Careful selection of delivery systems and vectors that target hepatocytes without triggering an acute immune response. Some of the main safety concerns are vector immunogenicity and risk of off-target effects.
- **Efficacy**: sustained and stable, life-long durable expression of OTC enzyme at physiological relevant concentrations needs to be ensured, for the therapy to be effective and curative.
- **Manufacturability:** they must have scalable production methods and cost-effective delivery systems to ensure the feasibility of the treatment.
- **Patient accessibility:** the therapies must be affordable to bring them to market and make available to patients in need.

Gene therapy has the potential to provide a transformative solution, enabling targeted treatment of the underlying genetic defect. The development of an efficient, safe, and accessible gene therapy for OTC deficiency represents a significant opportunity to improve patient's lives and create a valuable business opportunity.

Gene therapies for the treatment of OTC deficiency: comparatives

In recent years, the competitive landscape for the treatment of metabolic rare diseases has been rapidly evolving, with several companies investigating gene therapies for OTC deficiency, but also Methylmalonic acidemia, Propionic acidemia, Argininosuccinic aciduria or Phenylketonuria. NanoRare's innovative hybrid approach, which combines recombinant adeno-associated virus (AAV), transposase technology, and lipid nanoparticle (LNP) delivery vehicles, and robust preclinical data, may offer distinct advantages over alternative gene therapy methodologies.

The use of lipid nanoparticles include efficient delivery to target tissues, high transfection efficiency, and low immunogenicity (9), (10). LNP technology has been crucial in the development of several mRNA therapeutics, most notably: the COVID-19 vaccines, cancer therapies or genetic disorders, demonstrating its versatility in delivering different types of genetic material. On the other hand, AAVs have emerged as one of the most promising gene delivery vehicles for *in vivo* gene therapy applications due to their relatively high transduction efficiency of DNA. Furthermore, the therapeutic gene cassette can be packaged in different AAV capsid pseudotypes, with different transduction profiles and with enhanced tropism for the human liver (selectively transduces human hepatocytes *in vivo* (11)), what makes them potentially the best candidates to deliver the OTC corrected sequence. Combining these delivery vehicles might mitigate the limitations of using either AAVs or LNPs alone, such as vector immunogenicity and low delivery efficiency.

When compared to mRNA technology, the hybrid approach offers improved stability and long-lasting gene expression, eliminating the need for frequent re-administration. While mRNA therapies have shown promise, they often require repeated dosing due to their transient expression, which can lead to increased healthcare costs and patient burden.

Retrovirus and lentivirus delivery vehicles suffer from safety concerns due to insertional mutagenesis, a significant drawback when transitioning to clinical trials (12), (13). Our hybrid approach bypasses this risk by delivering the well-characterized Sleeping Beauty transposase mRNA in a lipid nanoparticle, thus limiting the transient expression of the enzyme to a period of 24-48 hours. The transposon system, therefore, presents a safer integration profile as compared to retro/lentivirus.

In summary, NanoRare's distinctive hybrid approach addresses the current challenges in gene therapy, including vector immunogenicity, delivery efficiency, and safety concerns, while offering a potentially curative treatment for OTC deficiency. Preclinical reference data highlights the potential of this method to achieve long-lasting correction of underlying genetic defects. The use of mRNA as a transient delivery source for the Sleeping Beauty (SB) transposase further enhances the biosafety of our approach, as it eliminates the risk of chromosomal integration (transposase sequence into human's genome).

The proposed hybrid approach presents some notable anticipated advantages, as they (i) circumvent the requirement for repeated vector administration through our method's stable chromosomal transgene integration and expression, and (ii) may enable a reduction in the applied viral dose, thus mitigating vector-associated immune complications. NanoRare believes this approach has the potential to become the leading treatment option for OTC deficiency.

NANORARE'S COMPANY PROFILE: Executive summary

NanoRare is a new biotechnology company focused on the development of a novel gene therapy approach for the treatment of an inherited metabolic liver disease, with the lead indication being OTC deficiency. NanoRare's goal is to provide an innovative, safe, and effective treatment for patients suffering from OTC that currently have limited treatment options and high unmet clinical needs. By addressing the underlying genetic causes of this condition, patient's outcomes and quality of life can be improved significantly. The team consists of experienced scientists and business professionals with a track record of success in bringing new therapies to the market.

Technology's description

NanoRare's proprietary technology combines the use of adeno-associated viruses (AAVs) and lipid nanoparticle (LNPs) formulations to deliver and stably integrate a functional copy of the human OTC gene to hepatocytes. The approach has been designed to address a particular challenge of the OTC deficiency, which is to provide a one-shot, life-lasting solution. NanoRare's gene therapy utilizes an AAV virus to deliver a correct copy of the human OTC gene, while the LNP carries the transposase messenger RNA (mRNA), to integrate the gene into the cell's genome. This technology is being tested preclinically, and has the potential to provide the necessary long-term therapeutic benefits.

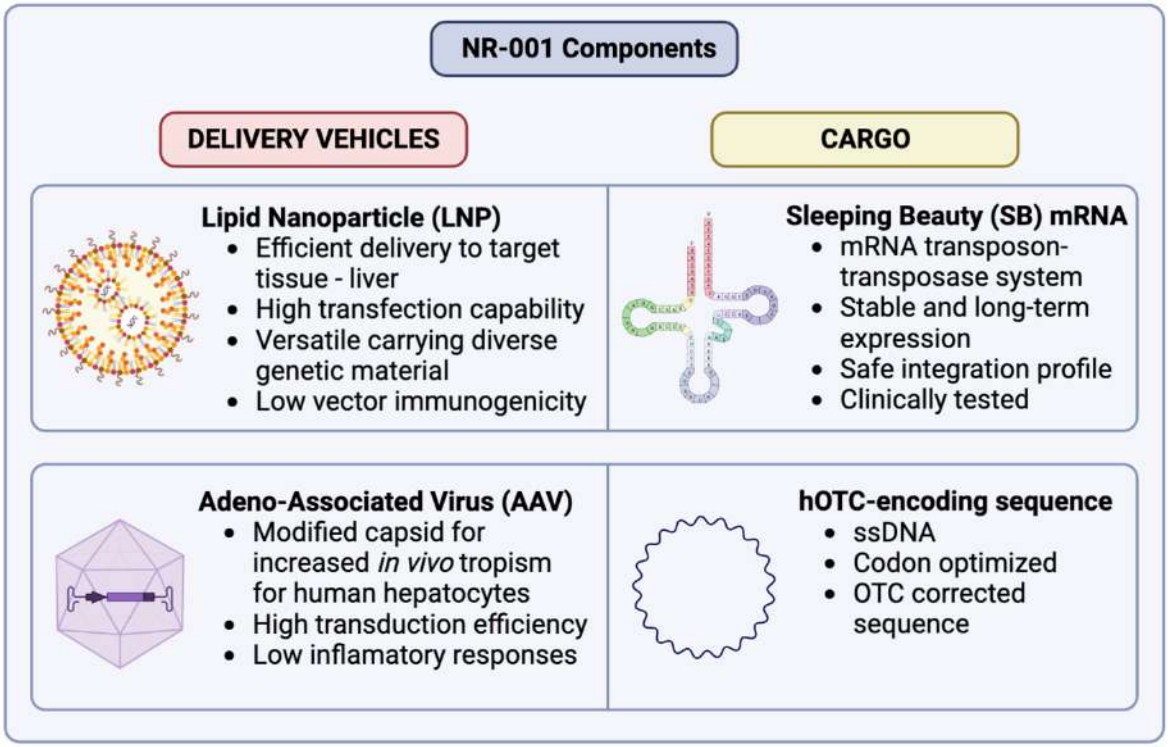


Figure 4: NanoRare's lead drug candidate components (NR-001). Schematic representation of both delivery vehicles: (i) LNP carrying the Sleeping Beauty transposase mRNA; and (ii) rAAV, transporting the corrected OTC sequence to be integrated into the hepatocytes' genome.

Proprietary technology

NanoRare is developing several proprietary technologies that make the gene therapy approach unique. The following technologies have shown promising results and are

poised to revolutionize the treatment of OTC deficiency. NanoRare technology's platform combines:

- **Proprietary lipid formulation:** a lipid nanoparticle formulation designed for optimal transportation in the blood circulation, enabling efficient cellular delivery of nucleic acid (transposase mRNA) to target cells (hepatocytes); and,
- **Proprietary rAAV capsid:** a recombinant adeno-associated virus (AAV) capsid (derived from the AAV-LK03 pseudotype) with improved *in vivo* tropism for human hepatocytes, carrying the hOTC-encoding DNA sequence; and,
- **Non-viral safe integration system:** a non-viral transposon/transposase-based genetic engineering technology that allows for precise and controlled gene editing, minimizing the risk of off-target effects and reducing the potential for adverse events. It provides stable integration of the hOTC sequence into the cell's genome.

Proof-of-concept and current studies

NanoRare's gene therapy for the treatment of OTC would provide patients with a single-shot, life-long curative treatment, through an intravenously administration of the NR-001 lead drug candidate, comprising the two drug components (AAV + hOTC sequence / LNP + transposase mRNA). This one-time, life-lasting solution has been designed to treat the neonatal group of patients. The proposed approach bypasses the underlying genetic disorder by providing a healthy, non-mutated copy of the OTC gene, potentially avoiding the costly factors associated to the treatment of hyperammonemic crisis and chronic medication.

Gene therapy for OTC has been shown to be safe and efficacious but only in late-onset patients. Proof-of-concept for the treatment of OTC deficiency with gene therapy was first demonstrated by Dr. James M. Wilson in 1996 (14). Since then, numerous studies have validated the potential of gene therapies to treat inherited metabolic liver disorders. From partial to total restoration of OTC enzymatic activity, achieving transient or stable expression (15).

As previously mentioned, these studies are being conducted on the late-onset group of patients (12 years or older), targeting non-dividing hepatocytes. Complications arise when treating the neonatal-onset group. The episomal expression achieved after transfecting dividing hepatocytes (such as those in the neonatal-onset) is diluted out as a result of cell division, decreasing the OTC enzymatic activity, thus necessitating the periodical repetition of this procedure. To overcome this issue, the gene sequence can be stably integrated in the hepatocytes' genome, thereby maintaining its expression in dividing cell populations, and providing life-long benefits.

Our proprietary approach incorporates novel technologies to optimize gene delivery and integration for improved clinical outcomes, aimed to show a prominent increase in OTC enzyme activity and reduction of toxic metabolites, such as orotic acid and plasma ammonia levels. NanoRare's team is devoted to advancing this technology towards clinical trials and, ultimately, its commercialization.

Exclusive rights to commercialize it

NanoRare holds a strong intellectual property position and has secured exclusive rights to develop and commercialize our gene therapy approach for the treatment of OTC deficiency and other genetic diseases. This provides a significant competitive advantage, with patent protection extending beyond 2040.

Overall strategy of business model

NanoRare's business model is focused on developing and commercializing gene therapies in rare genetic diseases. As a spin off from NanoCell Therapeutics, it plans to leverage the expertise from the CAR-T (Chimeric Antigen Receptor T-Cell therapy) and TCR (T Cell Receptor Engineering) adaptative immunotherapies to expand its pipeline and develop treatments for genetic disorders with high unmet clinical needs. In the case of OTC deficiency, the business strategy involves advancing the gene therapy approach towards clinical trials and securing collaborations with key stakeholders, such as regulatory bodies, like the **Medicine Evaluation Board** (Dutch regulatory authority for approval of medicines), patient advocacy groups, as the **VKS** (Dutch Association for children with metabolic diseases), or healthcare providers, as the **UMC/WKZ** (Utrecht Medical Center/Wilhelmina Kinderziekenhuis), that could help facilitating access to clinical trials, to, ultimately, bring this life-saving therapy to patients in need.

Because NanoRare's therapeutic approach is easy to expand, further advances derived from in-house research could potentially target other genetic liver diseases, by incorporating the corresponding genes of interest in each case. Thus, this technology platform is at the cutting edge for the treatment of metabolic rare diseases that require genetic reprogramming of hepatocytes in order to produce the enzymes affected by the corresponding mutations, such as Argininosuccinic aciduria (ASA deficiency) or Citrullinemia I (CTLN1 deficiency).

MARKET PERSPECTIVE

Market trends

The rare disease gene therapy space is rapidly expanding, with the United States Food and Drug Administration (USFDA) evaluating several new treatments in recent years. The "Rare Diseases Treatment Market Size, Share & Trends Analysis Report" by Grand View Research (16), reveals that the market is expected to expand at a compound annual Growth Rate of 12,8% from 2022 to 2030, reaching $335,84 billions of annual revenue forecast by the end of this period. The growth is attributed to the increasing prevalence of rare diseases, growing demand for personalized medicine, and advancements in gene therapy technologies that provide long-term therapeutic solutions.

To estimate the market size for OTC deficiency is challenging due to the limited data on the average cost per patient for diagnosis, treatment and management. The prevalence of OTC deficiency is approximately 1 in 70.000 newborns, based on the estimated prevalence of Urea cycle disorders in the US (1 in 35.000) and the proportion of OTC deficiency cases among them (~ 50%, (17), (18)). The cost of treating OTC deficiency can be substantial, but it varies significantly depending on factors such as healthcare systems, availability of treatment options, severity of the condition, and the region in question. Costs can include medications, dietary management, and hospitalizations, with ICU stays in the United States averaging between $1500 to $3200 per day (19). The length of care after a hyperammonemic crisis can range from several days to over a week, further contributing to the variability in treatment costs (20).

Nevertheless, the study “Barriers to drug adherence for the treatment of Urea Cycle disorders: assessment of patient, caregiver, and provider perspectives” by Shchelochkov O.A. **(21)**, reports that the annual direct cost of Urea cycle disorder treatments can range between $26.000 to $240.000 per patient, depending on the severity of the disorder and the type of therapy administered. While these figures are not specific to OTC deficiency, they give an idea of the potential cost associated with managing UCDs.

Given these complexities, a thorough estimation of the market size for OTC deficiency would require more accurate and region-specific cost data; however, some predictions can be made based on the findings above. In table 1, the populations of the respective regions were obtained from World Bank Data Org., and the number of OTC patients in each region was calculated. Assuming a constant average cost per patient of $100.000/year, the preliminary market size for OTC deficiency was estimated by multiplying the number of patients by the average cost per patient.

Regions	Total population	Birth rate / 1000 inhabitants	Estimated OTC total patient population	Number of OTC affected newborns per year	Average treatment cost per year ($ millions)
Europe	447.199.800	9,3	6.388,6	59,4	638,9
North America	370.203.720	11	5.288,6	58,2	528,9
Latin America & Caribbean	654.981.699	17,6	9.356,9	164,7	935,7
Africa	1.427.983.742	34,9	20.399,8	712,0	2.040,0
East Asia & Pacific	2370.204.350	11,4	33.860,1	386,0	3.386,0

Table 1: Preliminary market size estimation in five different regions.

Opportunities that arise: available technologies for OTC treatment

Urea cycle disorders represent a group of inborn errors of metabolism characterized by deficiencies in one of the five enzymes or transporters responsible for the conversion of toxic ammonia into non-toxic urea. These deficiencies result in the accumulation of ammonia and other toxic metabolites in the bloodstream, leading to hyperammonemia. Despite the availability of dietary and pharmacological management strategies, UCDs patients still experience recurrent hyperammonemia crises and suffer from the chronic long-term effects of them. Moreover, these treatments often fail to address the underlying genetic defects and may not be effective in all cases. Consequently, the development of gene therapy approaches has gained increasing attention in recent years.

Here, we have outlined several gene therapy technologies currently being explored for the treatment of OTC deficiency. Each of the following methods offer distinct advantages and limitations in terms of their expression, genomic integration, genetic correction or strength/duration of the effect. mRNA-based therapies, for instance, offer transient gene expression without genomic integration, while base/prime editing and viral vectors, such as retro and lentiviruses, provide stable expression with varying degrees of integration and genetic correction. These technologies aim to correct the genetic defect or provide a functional copy of the OTC gene to restore the normal metabolic pathway.

1. **mRNA:** protein production by mRNA template that produce the functional OTC enzyme. This approach does not integrate into the genome and provides a transient expression of the corrected gene sequence.

2. **Base/Prime-Editing:** precise genome editing technologies that can potentially correct the underlying genetic defect causing the disease, within its original locus, under an endogenous promoter. They provide stable expression and have the potential for life-long therapeutic effects. Base editing uses an engineered protein complex to make targeted single base pair changes, while prime editing uses a similar complex to make targeted insertions, deletions, or replacements in the DNA.

3. **AAVs (Adeno-associated viruses):** AAVs are small, non-pathogenic viruses engineered as gene delivery vectors. They can provide stable expression of the therapeutic gene if the targeted cells are not dividing. The therapeutic effect is expected to be life-long after 18 years of age, as the liver remains relatively stable in adults.

4. **Lentivirus**: lentiviral vectors are derived from retroviruses and can integrate into the host genome, providing stable expression of the corrected gene. This approach can correct the underlying genetic defect and provide lifelong therapeutic effects.

5. **Retroviral vectors:** Retroviral vectors are derived from retroviruses, like lentiviral vectors, but they have differences in their genome organization and integration preferences. Retroviral vectors can integrate into the host genome, providing stable expression of the corrected gene. This approach can correct the underlying genetic defect and provide lifelong therapeutic effects. However, due to their potential for insertional mutagenesis, they have been largely replaced by lentiviral vectors and AAVs in clinical applications.

The table provided below outlines the key characteristic of these technologies:

Technology	Expression	Integration	Genetic Correction	Strength of effect	Duration of effect
mRNA	Transient	No	No	High	Temporal
Base/Prime-Editing	Stable	Yes	Yes	Unknown, expected low	Anticipated Lifelong
AAVs	Transient (episomal)	No	No	High	Anticipated Lifelong after 18 years
Lentivirus	Stable	Yes	Yes	Expected high	Lifelong
Retroviral Vectors	Stable	Yes	Yes	Expected High	Lifelong

Table 2: Compilation of available gene therapy technologies to treat OTC deficiency.

While each gene therapy technology presents promising opportunities for the treatment of OTC deficiency, several clinical challenges remain. For instance, mRNA-based therapies require repeated administration due to their transient nature (22),which can be burdensome and costly for patients. AAVs, while generally safe and effective, may face issues related to vector dilution, as the episomal nature of the transgene expression could diminish over time, particularly in cases of cell division (23). Viral vectors, such as retroviral and lentiviral vectors, carry the risk of insertional mutagenesis, which could lead to oncogenic events (24).

Moreover, CRISPR/Cas9-based approaches, such as base and prime editing, may suffer from limited integration efficacy and off-target effects (25). Additionally, the heterogeneity of OTC deficiency, characterized by diverse mutations, presents challenges for single drug manufacturing for base and prime editing, where short templates might not be sufficient to address all possible mutations (26). The development of personalized gene therapies may be required to overcome this limitation, but this could be resource-intensive and pose manufacturing and regulatory challenges (27).

In summary, while each gene therapy technology offers unique features for the treatment of OTC deficiency, they also face various challenges that need to be considered and addressed to maximize their potential. Future research should focus on refining these technologies and developing personalized treatments to ensure safety and efficacy for the treatment of genetic disorders.

OTC gene therapy landscape: main competitors

The biggest challenge in gene therapy to treat inborn errors of metabolism is to provide a single shot, life-long therapy from the day they are born. This is the main reason why gene therapy approaches are poised to become the leader treatment option. In addition, the development of newer and more efficient delivery systems, such as LNPs; and the use of hybrid approaches utilizing AAV and LNP simultaneously, offer significant opportunities for gene therapy in OTC deficiency. From temporary solutions that can serve to mitigate the effects of hyperammonemic crises, by providing the mRNA coding sequence of the human OTC enzyme; to permanent solutions that will last a lifetime, when the approach is based on the integration of a non-mutated copy of the gene into

the hepatocyte's genome. These new technologies can potentially increase the efficacy of the gene therapy, enhancing its therapeutic effects, and overcoming some of the current limitations, such as the immune response or the limited transduction efficiency of liver cells.

As the potential of gene therapy for treating metabolic rare diseases becomes increasingly evident, new companies are entering this space. The most notable players in the gene therapy market for OTC, as well as the technologies that they are developing are listed in the following table:

Company	Delivery	Expression	Nucleic acid	Stage
ULTRAGENYX	AAV	Stable, non-integrated	DNA	Phase 3
Bloomsbury GT	AAV	Stable, non-integrated	DNA	Phase 1-2
Arcturus therapeutics	LNP	Temporary	mRNA	Phase 2
Moderna Therapeutics	LNP	Temporary	mRNA	Pre-clinical
Genevant	LNP	Temporary	mRNA	Pre-clinical
IECURE	Dual AAV delivery	Stable, integrated	DNA	Pre-clinical
Poseida	Dual delivery: AAV+AAV // LNP+AAV	Stable, integrated	DNA and mRNA	Pre-clinical

Table 3: List of biotechnological companies currently developing gene therapies for the treatment of OTC deficiency.

There are ongoing clinical trials for gene therapy in OTC deficiency, with some promising results. For example, Ultragenyx Pharmaceutical is developing DTX301, an investigational AAV-based gene therapy for OTC late-onset patients. A clinical-stage study currently in phase 3. Preclinical data have showed improved ureagenesis and ammonia control in NHP (non-human primates), with no serious adverse events reported.

Another example is Arcturus Therapeutics, developing an mRNA gene therapy that will enable OTC patients to make healthy functional enzyme in their liver cells transiently. Preclinical studies have shown that the technology platform used (LUNAR) effectively delivers OTC mRNA to hepatocytes through LNPs, resulting in a temporary restoration of the urea cycle function to normal levels and increased survival of the mouse model used (Spf-ash) (28), (29).

On the page below, a program, last updates and financial overview of the aforementioned companies in the OTC market is shown:

Company	Program Overview	Additional Notes	Latest updates	Partnerships		Financing rounds
ULTRAGENYX	**DTX301** to deliver stable expression and activity of OTC following a single intravenous infusion	Pre-clinical studies have demonstrated the approach's feasibility in NHP. **Orphan drug** designation (US, Europe and UK), **Fast Track** designation in US	Finalized Phase 3 study design with FDA for DTX301 OTC (**Enhance study**). Expected to begin Q1 2023	Collaborating with **Arcturus TX** to bring mRNA therapeutics for GSDIII, currently in phase 1/2. **Takeda asset investment** generates $65 millions in 2016	**Crysvita, Dojolvi** and **Mepsevii** medicines in the market. Crysvita revenue $325-340 millions and Dojolvi $65-75 millions in 2023	26% growth Q1 2023 vs Q1 2022. **Series A** raised $45 millions. **Series B,** $75 millions. **IPO** (in 2014) raised $121 millions, **valuation** at $436 millions
Bloomsbury GTX	**BGT-OTCD** liver-targeted AAV-LK03 gene therapy designed to provide a curative solution to OTC deficiency following a one-time intravenous injection	**BGT-OTCD** has completed preclinical efficacy, safety and biodistribution studies. Evaluated at **HORACE** clinical trial, with patients 16 years or younger	**BGT-OTCD** was granted **Orphan drug designation** by the EMA	Spin-out from **University College** London (UCL), in collaboration with CMRI (Children's Medical Research Institute)	Investigating as well **neurodegeneratives** diseases. **First-in-human** clinical trial for **BGT-OTCD** expected to initiate in **Q3 2023**	Seed funding raised 5 million pounds in October 2022
ARCturus therapeutics	**ARCT-810**. mRNA coding for hOTC formulated in an LNP to target periportal hepatocytes	Pre-clinical studies have shown the potential to restore OTC activitity, showing a clear dose response. Sustained OTC expression in mouse model for up to 28 days	Designated as **Orphan Medicinal Product** designation by European Commission	Lend proprietary technology to **Ultragenyx** to develop RNA medicines, since October 2015. Collaborating with **CureVac** (2018) and **Janssen** (2017). They acquired **Alcobra Pharmaceuticals** in 2017	**RNA Therapeutics**, focused on Influenza and COVID vaccines. Market capitalization of $1 billion. Arcturus is funded by **Biomedical Advanced Research and Development Authority (BARDA)**	Seed funding of ~ $2 millions. **Series A** raised $5 millions in 2013. **IPO** in 2020 raised $80,5 millions. They raised **$365,5** millions over 7 rounds
Moderna Therapeutics	**mRNA-3745**. Human OTC mRNA sequence delivered to hepatocytes through LNPs	Targeting late-onset patients, 12 years or older. **Transient expression** of OTC enzyme	**Not found**	**Not found**		**IPO** in 2018 raised $604 millions, and **valuation** was $7,6 billions. They have received 13 **funding rounds**, raising approaximately $3 billion dollars
Genevant	Built upon **LNP Onpattro formulation**, LNP mRNA-based therapy for OTC deficiency	Strategic mRNA-focused partnership in Rare Diseases with **BioNTech**	Optimized their LNP delivery platform, for the treatment of OTC deficiency, in NHP primates, in 2023	BioNTech and Genevant will develop **five products for rare diseases** with high unmet medical need **under the 50/50 co-development and co-commercialization** collaboration with the goal of having 5-10 programs at clinical stage by 2020		**Not found**
IECURE	**GPT-506** comprising two AAV vectors: **GTP-506A** (ARCUS nuclease to target PCSK9 site) and **GTP-506D** (hOTC sequence)	NHP data demonstrates stable insertion of therapeutic gene using ARCUS nuclease one year after dosing. **First-in-human study** on 2023	**Mutation-agnostic in vivo gene insertion approach**. Developing therapies for CTLN1 and PKU as well. **Orphan Drug** and **Rare pediatric disease designation** for GTP-506	Collaborating with Penn Vector Core and Dr. James Wilson **to develop LNPs,** at Gene therapy program of University of Pennsylvania	In association with **Precision Bioscience** for the development and use of the **ARCUS** nuclease	**Series A** financing round in September 2021 of $50 million. In November 2022, raised $65 million to advance gene editing programs
Poseida	**P-OTC-101:** liver-directed gene therapy combining piggyBac technology with AAV for *in vivo* treatment of OTC deficiency. 100-fold increase in OTC levels (neonatal-onset mouse model) with single injection	Dual delivery: **either AAV** (for transposon + transposase) or **AAV** (OTC transposon cassette) + **LNP** (SPB mRNA). 46% OTC positive hepatocytes with dual AAV and 56% positive hepatocytes if AAV+LNP approach	Ultimate **goal** transition to dual LNP approach, supplying SPB and GOI to hepatocytes. Also working on **PKU**, following same approach	**Roche:** strategic collaboration and license agreement to develop allogenic Car-T therapies. **Takeda:** collaboration and exclusive agreement to use Poseida's Piggybac, Cas-CLOVER, and DNA and RNA nanoparticle technology	$110 million upfront from **Roche,** could receive up to $110 millions in near term. With **Takeda**, received $45 million upfront, that could exceed $125 millions in the aggregate	**8 funding rounds,** generating $410,5 millions. **Series A** raised $23 millions. **IPO** in 2020 raised $224 millions. **Valuation** at $988,8 millions

Table 4: Overview of biotechnological companies developing gene therapies for the treatment of OTC deficiency. Financial data extracted from Crunchbase and Google Finance websites.

Main hurdles limiting the therapies

One of the significant hurdles in developing gene therapies for OTC deficiency is the limited understanding of the disease's pathophysiology and the factors affecting OTC gene expression, which complicates the identification of effective therapeutic targets. Other challenges include the high costs associated with developing and manufacturing new treatments, the small patient population, and the necessity for personalized therapies tailored to individual patients' genetic mutations and disease severity.

1. **Manufacturing complexities**

Gene therapies, although promising, are also associated with high costs due to the complexities of manufacturing, particularly for rare diseases with limited patient populations. Their development and production require specialized facilities and personnel, which can result in high treatment costs and limited accessibility for patients. Given the burden of hospital admissions, reduced life expectancy associated with the disease, and the high costs of existing treatments, there is a pressing need for effective, long-term, and cost-effective therapeutic solutions.

2. **Clinical challenges for gene therapies**

The current treatments for OTC deficiency primarily focus on mitigating the symptoms of the disease and do not offer a cure. Despite treatment, patients with severe OTC deficiency often require frequent hospitalizations and may experience neurological and developmental problems **(5)**. Although some symptoms can be managed, the underlying genetic defect remains unaddressed, and complete reversal of the disease is difficult to achieve.

Developing gene therapies faces various clinical challenges. One such challenge is the immune response triggered by viral vectors administered, which can potentially limit the therapeutic effect or even lead to adverse outcomes (30). However, AAVs have been shown to have several advantages for liver-targeted gene therapies, including relatively high transduction efficiency, ability to evade pre-existing immunity, and achieve long-lasting gene expression when cells are not dividing (31), (32). These features make AAVs an attractive option for delivering the therapeutic gene construct in the context of OTC deficiency.

Another challenge is the therapy’s limited efficacy. This issue can be addressed through the use of a hybrid approach that integrates the OTC corrected gene into the chromosome. Nonetheless, the potential for off-target effects must be considered and minimized to ensure its safety (33).

3. **Pricing and reimbursement**

The pricing and reimbursement strategies also present a complex challenge. These therapies often have high development and manufacturing expenses, which can translate into extraordinary treatment costs. Because of that, health insurance companies and governments may be hesitant to cover the costs due to their high price, potentially creating access barriers for patients. As an example:

- **Luxturna**, a gene therapy for a rare form of inherited blindness developed by Spark Therapeutics, is priced at $850,000 per patient.

- CAR-T therapies, which are used to treat certain types of blood cancer, also carry high price tags, such as **Kymriah** by Novartis, which is priced at $475,000 per patient for a one-time treatment.
- **Hemgenix,** a gene therapy for the treatment of Hemophilia B, is priced at $3,5 million, reflecting the significant investment required for their development and production.

To address these challenges, innovative pricing and reimbursement models are being proposed and explored, such as **performance-based agreements** (34), and value-based contracts. While performance-based agreements involve tying the payment for a therapy to specific clinical outcomes or performance measures; **value-based contracts** (35), on the other hand, link the price of a therapy to the value it provides to patients, healthcare systems, and society. Under this model, the cost of a therapy is determined based on its ability to improve patient outcomes, reduce healthcare costs, and deliver other measurable benefits, such as decreased treatment burden, enhanced patient adherence, prevention of complications or faster recovery times.

These models aim to align the price of gene therapies with the long-term value they provide, ensuring that patients have access to these life-changing treatments while maintaining the financial sustainability of healthcare systems. By exploring and implementing such innovative pricing strategies, NanoRare seeks to ensure that the therapy remains accessible and affordable, while still allowing to recoup the costs associated with its development and production.

Identifying OTC burdens

Patients with rare diseases, including OTC deficiency, often experience significant delays in obtaining an accurate diagnosis and appropriate treatment. These extended wait times can result in severe consequences for patients' health and well-being, as the progression of the disease can lead to irreversible damage. NanoRare is committed to addressing these burdens by minimizing wait times for diagnosis and treatment. To achieve this goal, several initiatives can be undertaken to expedite the diagnostic and treatment process for patients. These efforts could include:

1. **Enhancing disease awareness:** by collaborating with healthcare providers, patient advocacy groups, and other stakeholders, such as hospitals, research centers, etc.; NanoRare will work to increase awareness and understanding of OTC deficiency among medical professionals and the general public. This will aid in earlier recognition of symptoms and faster referrals to specialists.
2. **Improving diagnostic tools:** investing in the development and dissemination of advanced diagnostic tools and techniques, such as genetic testing and metabolic profiling, could enable more accurate and timely diagnoses of rare metabolic diseases.
3. **Expanding newborn screening programs:** advocating for the inclusion of OTC deficiency in newborn screening programs will allow for early identification and intervention, reducing the need for invasive treatments later in life.

NANORARE PLATFORM AND PRODUCT OVERVIEW

NanoRare's therapy for OTC deficiency employs a **hybrid approach**, using AAVs and LNPs delivery vehicles, to introduce a healthy, non-mutated copy of the OTC sequence into hepatocyte's genome, thereby restoring the Urea cycle, offering a curative treatment to patients. The recent advances in the development of gene delivery vehicles have made possible to design gene therapies without requiring the complex and expensive *ex vivo* cell manufacturing processes. NanoRare's technology platform allows for *in vivo* (e.g., within the patient's body) genetic reprogramming of hepatocytes. The manufacturing process of NanoRare's drug product is highly scalable and expected to cost significantly less than current life-long treatments and therapies for the management of OTC deficiency.

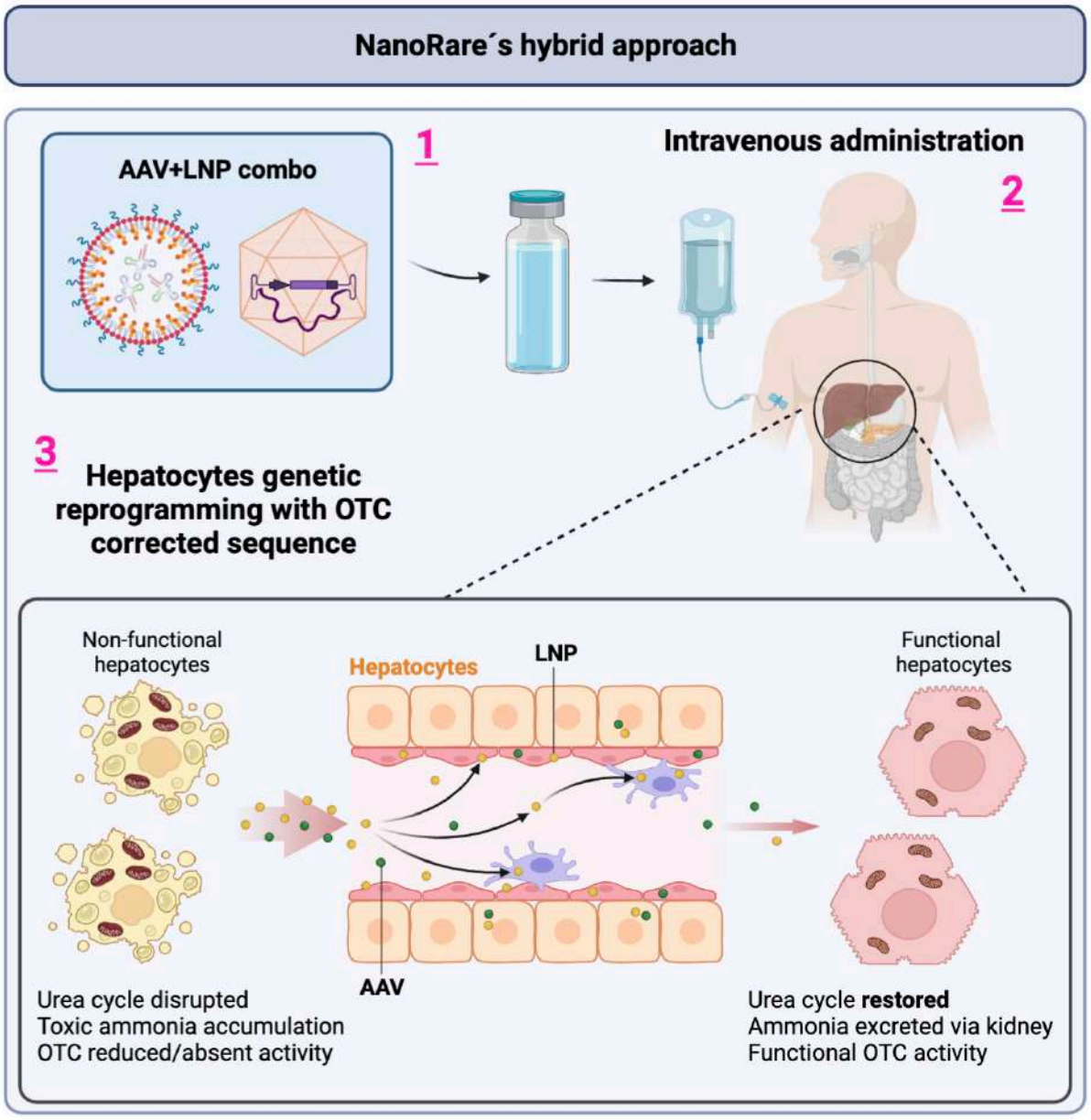


Figure 5: Illustrates NanoRare's proposed gene therapy OTC approach. **1)** Manufacturing process of delivery vehicles with respective cargos. **2)** Intravenous administration to OTC patient. **3)** Targeted liver delivery and hepatocytes' genetic reprogramming, Urea cycle restored.

Technical features of NanoRare platform

NanoRare's targeted gene therapy approach for OTC deficiency consists of different technology components that include:

- **A proprietary lipid formulation:** an LNP formulation for the delivery of therapeutic oligonucleotides (transposase mRNA) into cells; and,
- **Proprietary rAAV capsid:** a proprietary rAAV-capsid with improved *in vivo* tropism for human hepatocytes; and,
- **Non-viral safe integration system:** a genomic integration platform, the so-called Sleeping Beaty transposase system.

1) Lipid Nanoparticle formulation:

NanoRare's gene therapy platform is built on advanced technology, utilizing lipid nanoparticles (LNPs) that ensure optimal delivery of nucleic acid to liver cells. The LNPs in NanoRare's therapy aim to achieve a similar function as demonstrated in Onpattro's LNP formulation, developed by Alnylam Pharmaceuticals.

The FDA approved in 2018 the Onpattro formulation (medicament known as Patisiran, for the treatment of polyneuropathies induced by hereditary transthyretin amyloidosis), which has showcased the utility of lipid nanoparticle technology in nucleic acid therapy, especially for liver-targeted approaches. Onpattro's LNPs contain four main components: ionizable cationic lipid (DLin-MC3-DMA), which interacts with RNA and forms a core nanoparticle; phospholipid (DSPC) that lends stability to the lipid nanoparticle structure; cholesterol, added to further improve nanoparticle stability; and Polyethylene glycol (PEG)-lipid that increases the systemic circulation time of the nanoparticle and reduces interactions with serum proteins.

However, since the ionizable cationic lipid in Onpattro is proprietary technology, NanoRare is focusing on optimizing our LNP for intellectual property purposes. We are exploring alternative ionizable cationic lipids that could fulfill a similar role. Additionally, our differentiation strategy could be further expanded by incorporating targeting ligands, such as GalNAc, for specific uptake in liver cells. GalNAc is known to bind the asialoglycoprotein receptor (ASGPR), abundant in hepatocytes, making it an efficient targeting mechanism for NanoRare's approach (36).

Based on the insights extracted from "The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs" review, by Akin Akinc (37), successful clinical translation of LNP systems for the delivery of small nucleic acids to the liver, such as Onpattro, relies on criteria that includes: a size range of 100 nm or less, highly efficient encapsulation techniques, a low surface charge, and robust, scalable manufacturing processes. Importantly, the LNP formulations must also facilitate the intracellular delivery of these macromolecules into target cells.

These systems can efficiently encapsulate RNA, maintaining low surface charge, and mitigate toxicity associated with immune activation. In terms of size, using polyethylene glycol (PEG)-lipids that associate with the surface of the LNP enables precise size regulation, within the ideal range of 20-100 nm. This feature is crucial, considering that the diameter of human liver fenestrations is around 107 ± 1,5 nm (38).

In addition, recent studies such as "Optimizing lipid nanoparticles for delivery in primates" by Kieu Lam, suggest that while LNPs show promise as nucleic acid delivery vehicles,

their effectiveness varies greatly between rodent and non-human primates (NHP) models. By tailoring LNPs -specifically, reducing particle size to about 50 to 60 nm and increasing PEG concentration to 2,2-2,8% - researchers achieved an 8-fold increase in gene expression in NHP models in the context of LNP-mRNA treatment for OTC deficiency. These advances, though not predicted for rodent data, represent significant steps to test LNP technology in clinically relevant animal models. NanoRare will include similar optimizations to enhance the mRNA transposase delivery (9).

Relying on the successful trajectory of the FDA-approved Onpattro formulation, coupled with NanoRare's differentiation strategy, reinforces our approach to effectively deliver the messenger RNA that encodes the Sleeping Beauty transposase. The description of the endocytosis-mediated process by Onpattro LNPs can be found in the supplemental material Figure 3.

2) rAAV-capsid with enhanced *in vivo* tropism for the human liver:

NanoRare also brings its innovative approach to the realm of adeno-associated (AAV) viral vectors. Its primary objective revolves around using an AAV vector to deliver the corrected sequence of the OTC protein on a single strand DNA molecule (ssDNA). AAV vectors were chosen based on their proven efficacy in transfecting liver cells, as well as their safety profile. In addition, through the optimization of hOTC sequence, the coding sequence can be condensed to approximately 1065 base pairs, effectively fitting within the payload limits of these delivery vehicles.

To ensure optimal liver targeting, NanoRare draws inspiration from the pioneering work of Dr. Paul Gissen's group (discussed in detail in Preclinical reference data section). The AAV-LK03 variant identified in their research offers a strong foundation upon which we plan to develop our unique strategy. Notably, the AAV-LK03 vector displays a remarkable liver tropism, making it an excellent reference point for the development of the proprietary AAV delivery vehicle.

Building on the characteristics of the AAV-LK03 vector, NanoRare's efforts will focus on leveraging capsid engineering methods to differentiate its technology, by developing a proprietary AAV capsid. While maintaining the liver tropism that makes the AAV-LK03 promising, NanoRare aims to refine the AAV variant through strategies such as rational design, to find the best candidate with the capability to target the liver and evade pre-existing immunity (39). These processes would allow NanoRare to fine-tune the vector for optimal safety, efficacy, and manufacturability in the context of OTC treatment, as well as to strengthen its intellectual property assets.

Crucially, NanoRare's IP protection would come from the combined use of proprietary technology: LNP formulation and AAV capsid; and the use of the Sleeping Beauty transposon system. This combination of different, yet complementary technologies, would reinforce its unique approach to addressing OTC deficiency.

3) Genetic Engineering System – The Sleeping Beauty

To transfer the OTC-encoding DNA sequence from the cellular cytosol to the nucleus of the cell, as well as to mediate the chromosomal integration process, NanoRare's approach utilizes Sleeping Beauty (SB) transposon vectors. Transposons are non-viral, natural genetic elements with the ability to change positions within the genome. Since the transposon machinery can introduce the sequence of the gene of interest in the

genome, through a cut-and-paste mechanism, they provide the basis for long-term, permanent transgene expression.

Upon transfection (endocytosis of LNP carrying transposase mRNA) and transduction (endocytosis of rAAV loaded with OTC sequence) of targeted cells (periportal hepatocytes), the SB transposase (transcribed from its mRNA form to a protein complex) will recognize the inverted-terminal repeats (ITRs) that flank the OTC ssDNA and bind to them. Once completed, the SB system (ssDNA + transposase) will traverse the nuclear membrane through the nuclear pore complex via the NLS (nuclear localization sequence) of the transposase, where it will catalyze the transposition of the OTC sequence into the cell's genome.

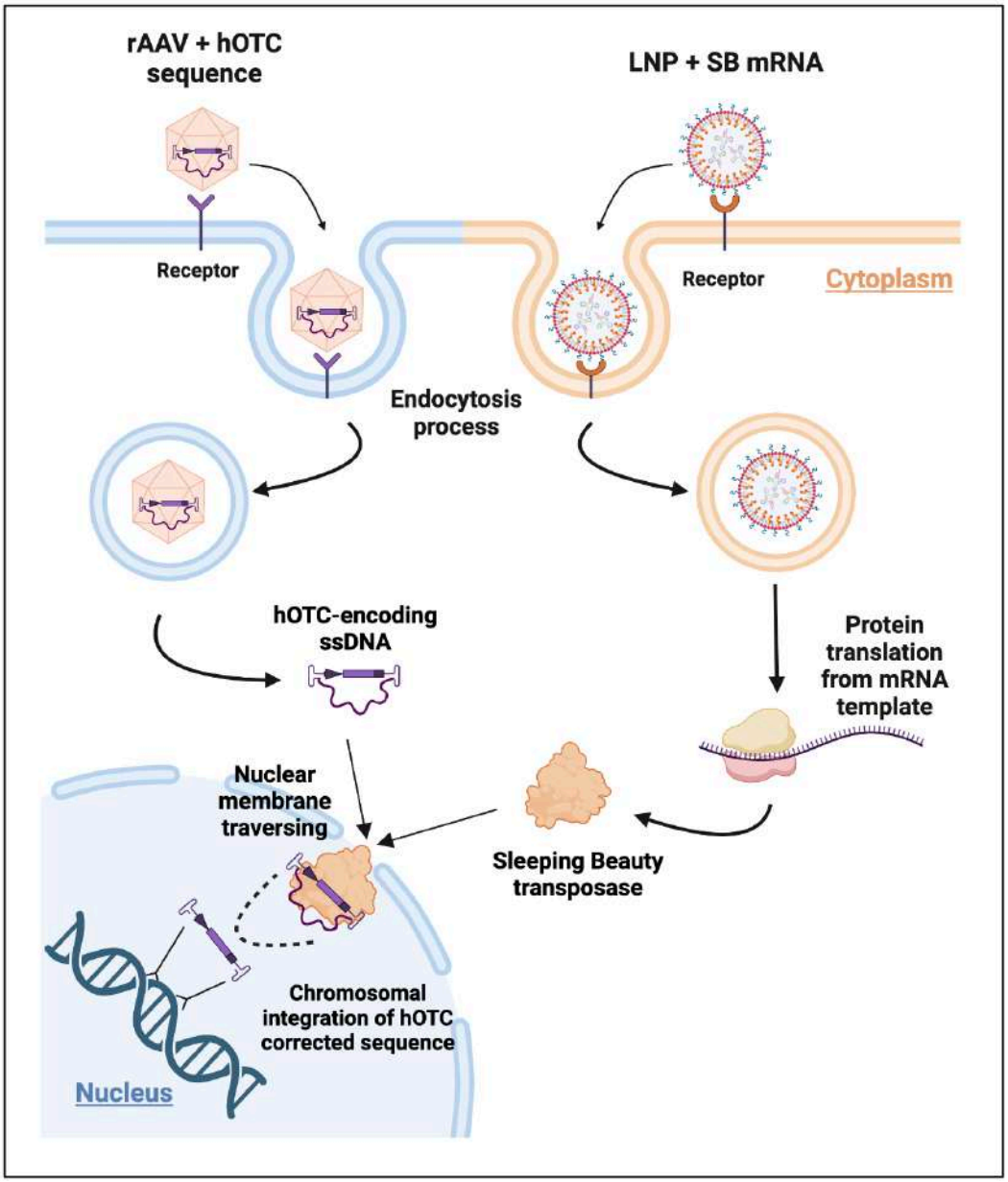


Figure 6: Process description of hybrid approach. The endocytosis of both delivery vehicles will result in the safe integration of the hOTC sequence into the hepatocytes' genome, through a transposition process catalyzed by Sleeping Beauty transposon system.

NanoRare's technology platform comprising the four components: NR-001

NanoRare's NR-001 represents a potential treatment not only for OTC patients, but also for those suffering from other liver metabolic diseases. Capitalizing on the proprietary Sleeping Beauty DNA modification system, NanoRare designs NR-001 to stably integrate the corrective human OTC (hOTC) gene into the patient's liver cells to restore the Urea cycle functionality. Compared to traditional liver transplantation or enzyme replacement therapies, NR-001 reduces the risk of immunogenicity, the requirement for re-administration, and the need for lifelong medication, marking a potential transformative leap in severe/neonatal OTC management.

In the broader context, the technology's adaptability sets it apart from existing therapies. The Sleeping Beauty system can theoretically be tailored to integrate other genes of interest, making it a potential therapeutic approach for a range of liver metabolic diseases beyond OTC. This would involve replacing the hOTC gene with the corrective gene associated with a specific condition, offering an efficient solution to other diseases with high unmet clinical needs.

Implementing NR-001 could lead to significant social and economic transformations. This therapy holds the potential to enhance patients' quality of life by freeing them from the burdensome routine of chronic medication and the fear of severe metabolic crises. Economically, it could ease long-term financial burdens on patients and healthcare systems by potentially eliminating the need for lifelong treatments and frequent hospitalizations. In essence, NR-001 targets not just symptoms, but the disease's root cause, presenting substantial economic advantages and serving as a valuable asset to patients, healthcare systems, and society as a whole.

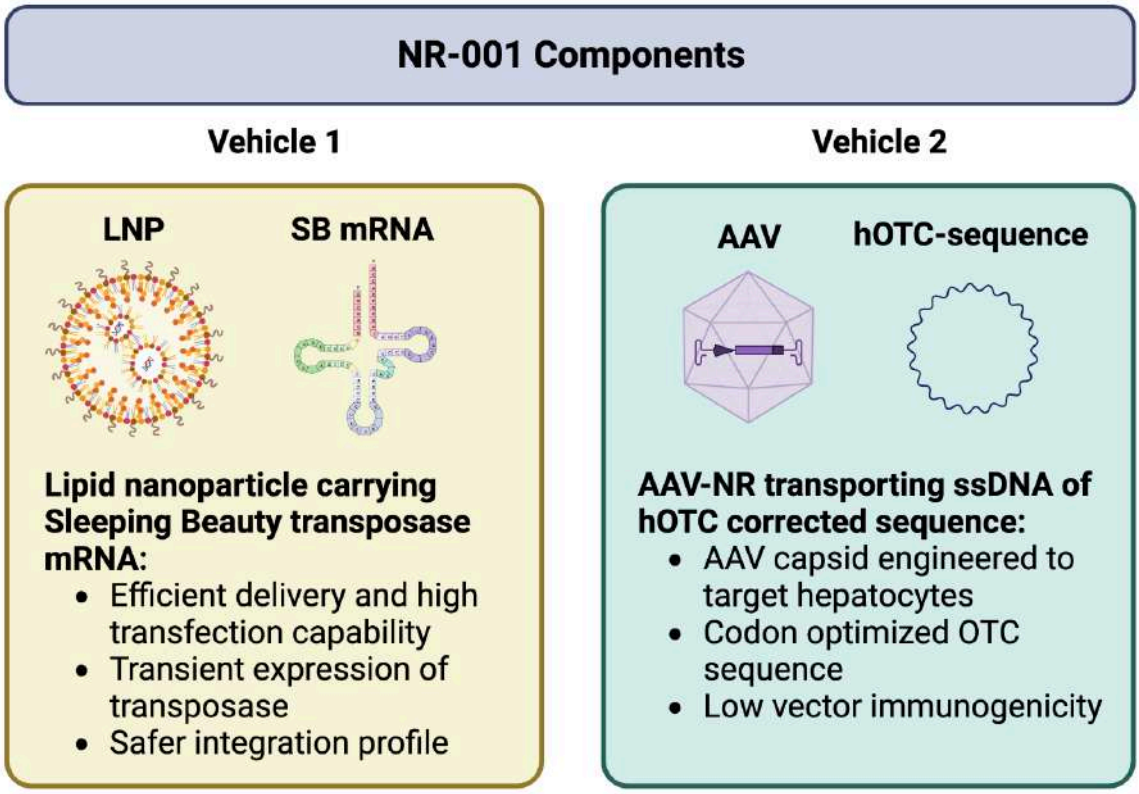


Figure 7: Schematic representation of NanoRare's lead drug candidate, the NR-001, and its two drug components, LNP + SB mRNA and AAV + hOTC.

NANORARE'S SCIENTIFIC DIFFERENTIATOR STRATEGY

NanoRare is pioneering an advanced gene therapy approach to address the OTC deficiency. This innovative dual-vector system, designated NR-001, is designed to offer a one-time, durable solution for OTC deficiency. The approach addresses the limitation of existing OTC treatments, which often inadequately target the root cause and may not be universally effective (20). The NR-001 lead drug candidate has the potential to offer a more precise and sustained treatment alternative.

Navigating the landscape: comparative analysis

The therapeutic market is poised for significant advancements, as there is an immense potential for novel treatment options that can outperform current standards of care and address the limitations of liver transplantation.

Three primary gene therapy approaches are being pursued by seven companies to tackle OTC deficiency. AAV-based therapies, in which a hOTC sequence is delivered for episomal expression, are the strategy of choice for Ultragenyx (DTX301, Phase 3) and Bloomsbury GT (BGT-OTCD, Phase 1-2).

Another group, composed of Arcturus Therapeutics (ARCT-810, Phase 2), Moderna Therapeutics (mRNA-3745, preclinical), and Genevant (preclinical) are leveraging LNP-mRNA based therapies, delivering the messenger RNA that encodes OTC to the cells, via lipid nanoparticles, for transient expression.

Lastly, Poseida (P-OTC-101, preclinical) and IECURE (GTP-506, preclinical) are utilizing integrative approaches for stable gene integration. IECURE employs a dual AAV delivery system to insert the hOTC sequence into the well-studied PCKS9 locus, using the ARCUS nuclease. In contrast, Poseida, combines AAVs and LNPs delivery systems to introduce the corrected sequence using the SuperPiggybac transposase system for chromosomal integration.

While each company aims to treat OTC deficiency with gene therapy, NanoRare's innovative dual-vector system may offer unique advantages. Here, we have outlined the superior technological and therapeutic aspects over its competitors.

NanoRare vs. AAV-based therapies: Ultragenyx and Bloomsbury GTX

NanoRare's hybrid gene therapy treatment presents significant advantages over the AAV-based therapies offered by Ultragenyx (DTX301) and Bloomsbury (BGT-OTCD). By utilizing a dual delivery system, the approach ensures efficient gene delivery and stable integration into the host genome.

This integration process, facilitated by a transposase, enables long-lasting therapeutic effects, offering patients a potential single-shot, life-lasting solution. On the other hand, Ultragenyx's and Bloomsbury's AAV-based therapies result in transient expression due to the absence of stable genome integration. This limits the duration of therapeutic benefits, especially for younger patients with rapidly dividing cells, such as those in the neonatal-onset group.

Stable chromosomal integration guarantees long-term OTC enzyme expression, improving therapeutic outcomes. While Ultragenyx and Bloomsbury's gene therapies

target late-onset patients (12-16 years or older, as they transfect non-dividing hepatocytes), they are less effective in addressing the needs of all patient groups. In contrast, NanoRare's approach has the potential to treat both neonatal-onset and late-onset patients, thereby expanding the target market and revenue opportunities.

Lastly, the potential for higher levels of OTC enzyme expression achieved by stable chromosomal integration might lead to an enhanced therapeutic effect. In summary, our hybrid delivery system outperforms Ultragenyx's DTX301 and Bloomsbury GTX BGT-OTCD gene therapies in several aspects:

- Stable expression of the hOTC sequence vs. transient expression of OTC sequence,
- Long-lasting vs. temporal therapeutic effects,
- Applicability to a broader patient population, including neonatal and late-onset patients vs. late-onset patients only,
- Enhanced OTC expression levels compared to the AAV approach.

NanoRare vs. LNP mRNA-based therapies: Arcturus, Genevant and Moderna Therapeutics

Moving on to the comparison between NanoRare's hybrid gene therapy approach and the LNP mRNA-based therapies developed by Arcturus, Genevant and Moderna, it is crucial to examine the key differences that arise from the distinct nature of their gene delivery vehicles.

The most significant advantage of NanoRare's gene therapy over Arcturus, Genevant and Moderna's therapies is achieving stable chromosomal integration, what results in long-term transgene expression. In contrast, the LNP mRNA-based therapies yield transient gene expression due to its nature, thus requiring of periodical re-administration.

Another distinction lies in the reduced risk of immune responses against the delivery vehicles used. NanoRare's hybrid system could enable the use of lower viral dosages, reducing the risk of potential side effects while maintaining efficient cellular transfection and endosomal escape (40). By combining AAV and LNP vehicles, the hybrid delivery system could effectively mitigate the immunogenicity commonly associated with either AAV or LNP therapies alone, as they typically require higher doses of delivery vehicles (41), (42). AAVs are known for inducing lower immune responses, while LNPs protect mRNA from degradation and facilitate cellular uptake without triggering significant immunogenicity. This combination allows NanoRare to capitalize on the strengths of both systems, minimizing the overall risk of immune responses (43).

In summary, NanoRare's hybrid delivery system for NR-001 demonstrates superiority over LNP mRNA-based therapies in several aspects:

- Stable integration and long-lasting therapeutic effects vs. temporary benefits,
- Reduced vector immunogenicity due to the combination of AAV and LNP elements, while maintaining efficiency,
- Improved cellular transfection rate and endosomal escape.

NanoRare vs. integrating approaches: IECURE and Poseida Therapeutics

Continuing the analysis, we next encounter two different technologies that involve stable integration into the host's genome, iECURE and Poseida Therapeutics.

While NanoRare uses a hybrid delivery system, iECURE's approach, in contrast, utilizes a dual AAV system to deliver the hOTC sequence and the ARCUS nuclease. This restriction enzyme (developed by Precision BioSciences) cuts the genome at the well-characterized PCSK9 site, serving as the insertion site for the healthy OTC gene.

One of the main differences between the two approaches lies in the gene-editing mechanism. While NanoRare's approach relies on the use of a transposase to integrate the therapeutic gene into the host's genome, IECURE's method employs a meganuclease to create a double-strand break, allowing the therapeutic gene to be inserted. While both methods aim for stable integration, the use of transposases, as in NanoRare's approach, could be considered a safer option.

The rationale behind this safer integration profile lies in the transient expression of the Sleeping Beauty transposase mRNA. The limited expression length reduces the risk associated with prolonged presence of gene-editing enzymes in the cellular cytosol. In contrast, iECURE's approach uses an AAV-delivered meganuclease for chromosomal integration, what results in prolonged expression of the restriction enzyme, increasing the risk of off-target effects and unwanted genomic alterations that could lead to mutagenesis.

Another advantage of NanoRare's hybrid approach is the reduced risk of immune responses. By combining AAVs and LNPs elements in their delivery system, NanoRare might diminish the overall risk of immune responses. iECURE relies solely on AAV vectors for gene delivery, which might present a higher risk of vector immunogenicity.

In summary, NanoRare's hybrid gene therapy approach offer certain advantages over iECURE's *in vivo* gene insertion method, including:

- A safer gene-editing mechanism using transposases with lower off-target effects compared to meganucleases,
- Reduced risk of immune responses due to the combination of AAV and LNP delivery vehicles vs. only AAVs.

Lastly, when comparing NanoRare's to Poseida's technologies, despite using a similar approach, some key differences can be highlighted.

Both technologies are based on a hybrid delivery of AAV and LNP delivery vehicles. The differences are found in the transposon/transposase-based genetic engineering system used. While NanoRare uses Sleeping Beauty, Poseida utilizes the SuperPiggybac, a hyperactive form of the Piggybac transposase.

As described in the article "Gene Therapy with the *Sleeping Beauty* Transposon System" by Partow Kebriaei (44), two fundamental properties can contribute to a transposon-based vector's genotoxicity: the genome-wide insertion profile of the vector, as well as the possible interaction between the transposase with endogenous human DNA sequences, or vice versa, interaction of human proteins with transposon vector sequences.

In line with this, Sleeping Beauty (SB) transposase appears safer in human cells concerning "off-target" cleavage. It has been reconstructed from fish genomes, and the mammalian lineage does not contain transposons similar enough to allow SB transposase cleavage, impeding remobilization of endogenous sequences. In addition, human cells do not express a protein with sufficient similarity to the SB transposase that

could remobilize an integrated SB vector. However, the PiggyBac present a cross-reaction between a catalytically active endogenous transposase (the human PGBD5 transposase-derived protein) and transposon vector sequences, which can mobilize insect piggyBac (PB) transposon vectors in human cells, potentially affecting the genomic stability of PB vector insertions in human applications.

Secondly, the SB transposon presents a safer integration profile, showing a close to random genome-wide distribution. This establishes a favorable integration mechanism, suggesting that SB might be safer for therapeutic gene delivery than the integrating viral vectors currently used in clinical trials, such as lentivirus, or its counterpart, the SuperPiggybac. Importantly, no SB-associated adverse effects have been observed in preclinical studies. In contrast, PB has a biased integration profile as it preferentially recognizes transcriptional start sites, which may lead to adverse effects due to changes in reading frames or insertional mutagenesis (see Figure 8). In fact, there have been clinically failed tests due to incidences of tumorigenesis in the CAR-T space using PB.

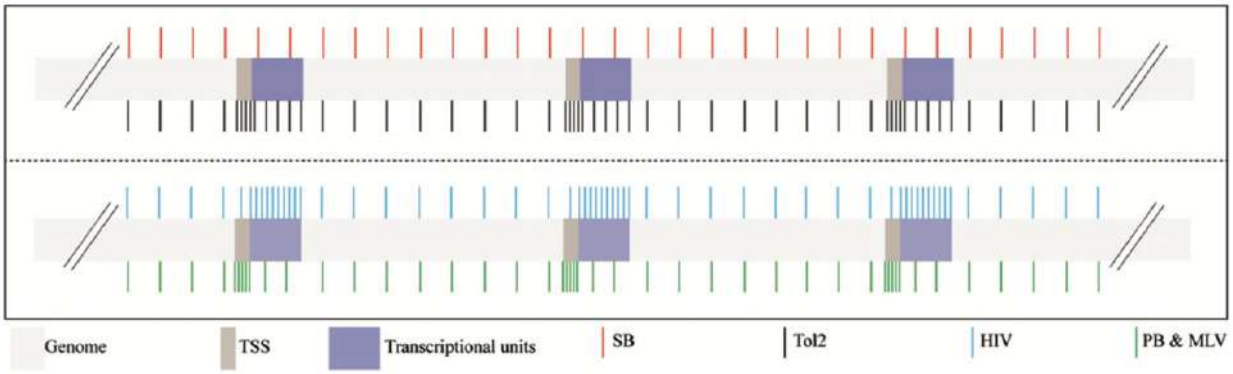


Figure 8: genome-wide integration profile of different integrating vectors. Picture extracted from research article: "*Sleeping Beauty* transposition: from biology to applications", by Suneel A. Narayanavari et. al., 2017. **TSS:** transcriptional start sites. **SB:** Sleeping Beauty. **Tol2:** fish transposon. **HIV:** Human immunodeficiency virus. **PB:** PiggyBac transposon. **MLV:** Moloney murine leukemia virus.

To conclude, the Sleeping Beauty transposase system offers a safer alternative for therapeutic gene delivery due to its reduced genotoxicity and a more favorable, close-to-random genome-wide integration profile. In contrast, the PiggyBac system presents increased risks associated with biased integration and potential genomic instability in human applications.

The next figure outlines the main areas of differentiation in comparison with the approaches just discussed:

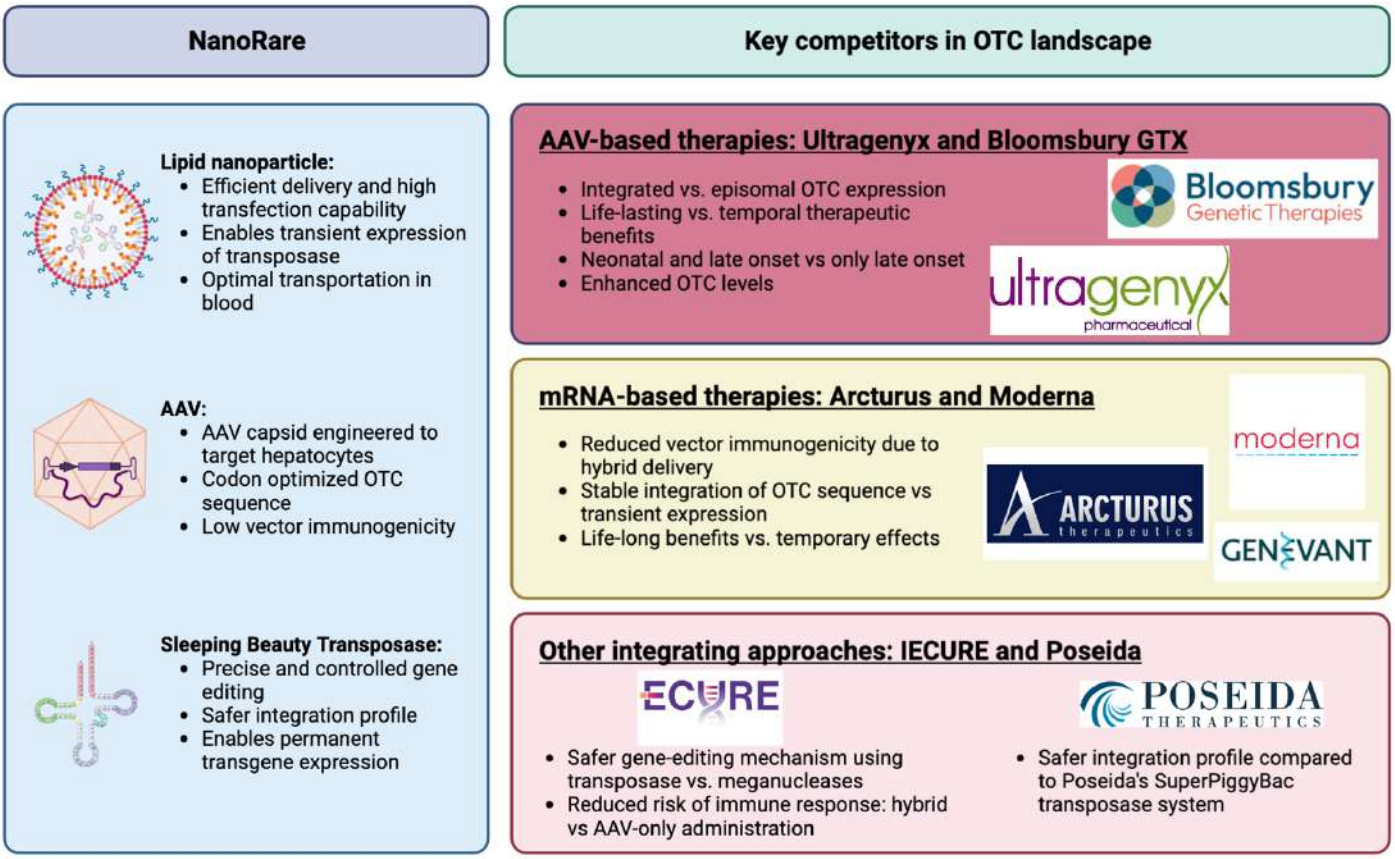


Figure 9: Main NanoRare's areas of differentiation in comparison to OTC relevant players.

Market disruptive potential and competitive advantages

In Figure 10 (shown below), the transformative potential of NR-001 is described. More than a therapeutic option, NR-001 could establish a new era in the treatment of OTC deficiency, not just because its capability to provide a permanent cure, but also due to the unique differentiators that set it apart from current therapies.

Consequently, a broader context highlights the importance of this development. Considering the rarity of OTC deficiency and the critical need for effective treatments, the market space eagerly awaits for a potential cure. Add to this we find the societal implications: it could radically change quality of life for patients and promote a substantial decrease in healthcare burdens. Elements that result in the incredible potential of NR-001, not only as a pioneering therapy but also positioning NanoRare as a key player in the landscape of gene therapy for metabolic disorders.

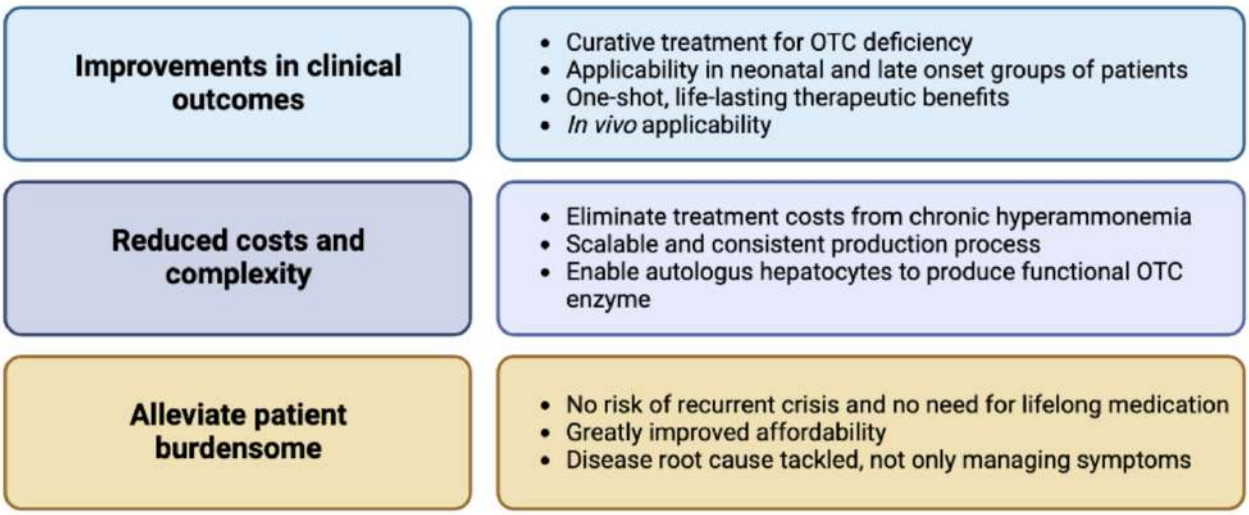


Figure 10: Summary of potential groundbreaking benefits of NR-001 approach.

As previously mentioned, the advantages associated to NanoRare's hybrid approach, that include reduced vector immunogenicity, efficient cellular delivery, safer biosafety profile, and integrated, stable expression of the correct OTC gene, collectively creates a strong case and business opportunity for NR-001 lead drug candidate. The aforementioned factors, in addition to the potential of treating the neonatal onset group of patients, reinforce our therapy to corner the market by offering a single-shot, life-lasting curative solution. A crucial fact, as the first entrant with a curative approach will capture the entire market, establishing itself as the most relevant player in the OTC landscape.

Because we bypass the root cause of the disease, not only manage its symptoms, we aim to create a game-changing solution that actually cures OTC deficiency. This therapeutic promise is supported by the favorable business fit, technical superiority and safety of NanoRare's proposed gene therapy. As NanoRare's in-house pre-clinical development progresses, we find ourselves in a promising position to translate the recent advancements towards a clinical trial.

In the following section, we present third-party preclinical data that serves as reference points for our intended study approaches. These are not our own findings, but valuable groundwork laid by other researchers in the field, which we aim to build upon.

PRECLINICAL REFERENCE DATA

The executive summary previously mentioned the groundbreaking study of Dr. James M. Wilson study in 1996, which first demonstrated the feasibility of treating Ornithine Transcarbamylase (OTC) deficiency with gene therapy, through metabolic correction in adult OTC mice using adenoviral vectors. However, an unfortunate incident in a clinical trial, involving a severe immune reaction to a high-dose of OTC-encoding adenoviral vector in 2002, resulted in the patient's dead, what slowed down the progress of gene therapy for metabolic diseases (14), (45).

Following the successful use of non-pathogenic adeno-associated viruses (AAVs) to treat other liver monogenic disorders like hemophilia A and B, gene therapy for OTC deficiency experienced significant advancement. One key development was the work of Paul Gissen's team, who conducted a breakthrough study on the safety and efficacy of a liver-targeting AAV-LK03 vector for OTC deficiency treatment (46), (47). The test subjects were juvenile cynomolgus monkeys, mimicking the growth of a pediatric liver, who were monitored over a 26-week period following the vector's administration. The results showed a favorable safety profile with no adverse clinical events, a strong hepatic biodistribution, and sustained OTC overexpression at levels higher than physiological.

Nonetheless, NanoRare's therapy offers notable benefits over the research conducted at the laboratory of Dr. Paul Gissen:

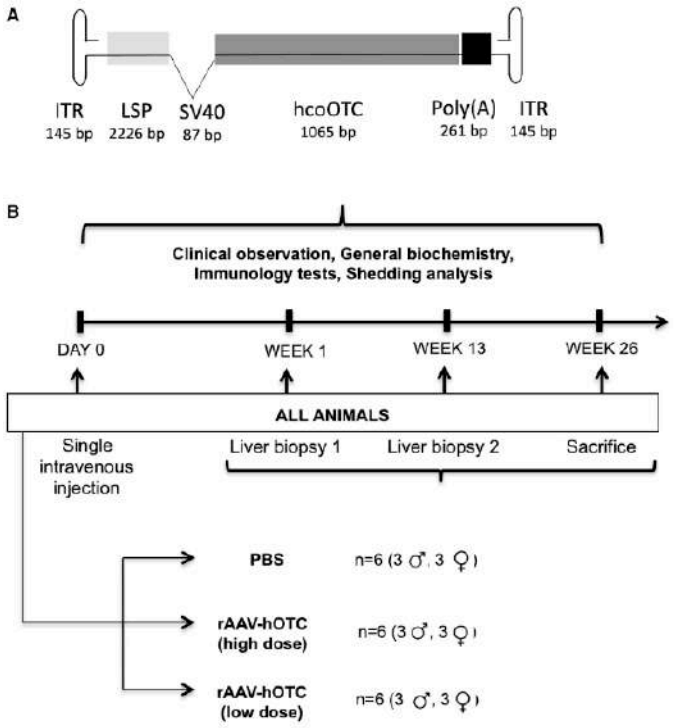
1. **Increased biosafety approach:** NanoRare's approach is based on a hybrid administration of AAVs and LNPs, what might enable the use of lower viral doses, reducing vector immunogenicity.

2. **Integrated and sustained OTC expression:** while the research article relies on the episomal expression achieved after transfecting hepatocytes with the AAV vector, it dilutes out after cell divisions, what hinders its applicability to treat the neonatal-onset group. NanoRare's approach uses the Sleeping Beauty transposase system, what allows for chromosomal integration of the hOTC sequence and sustained expression throughout the patient's life.

Despite these limitations, the extensive preclinical evidence produced by Paul Gissen's group is highly convincing and significantly applicable.

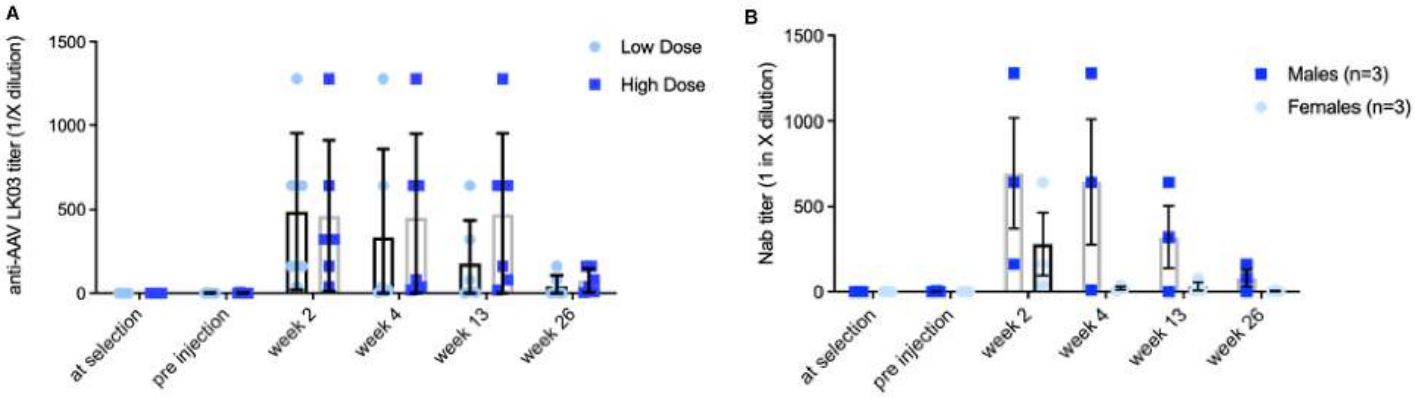
Below, from Figures A to D is a summary of the data that supports the clinical development of intravenous AAV-LK03 delivery to treat OTC deficiency:

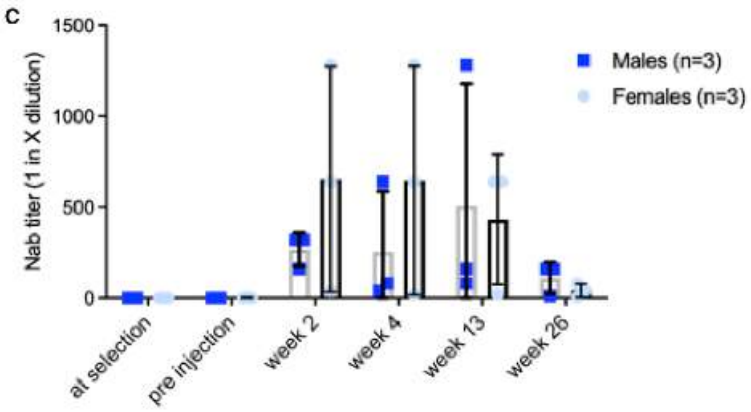
Figure A: Experimental design for preclinical assessment of AAVLK03.LSP.hOTC



Dr. Paul Gissen's team's conducted a good-laboratory practice-compliant investigational new drug-enabling study, where they assessed the safety of intravenous liver-tropic AAV-LK03 (an AAV3B-derived serotype) gene transfer of a human codon optimized OTC sequence. This gene delivery vehicle was administered to three gender-matched groups of six cynomolgus macaques, receiving either a high dose ($2*10^{13}$ vg/kg), a low dose ($2*10^{12}$ vg/kg), or a control group treatment (PBS, phosphate buffered saline). This dosage was selected based on a previous clinical trial targeting adult hemophilia B patients with an AAV8-derived vector. Because OTC enzymatic levels were restored in both treatment groups, the following study supports the clinical application of intravenous AAV-LK03 for neonatal-onset OTC patients.

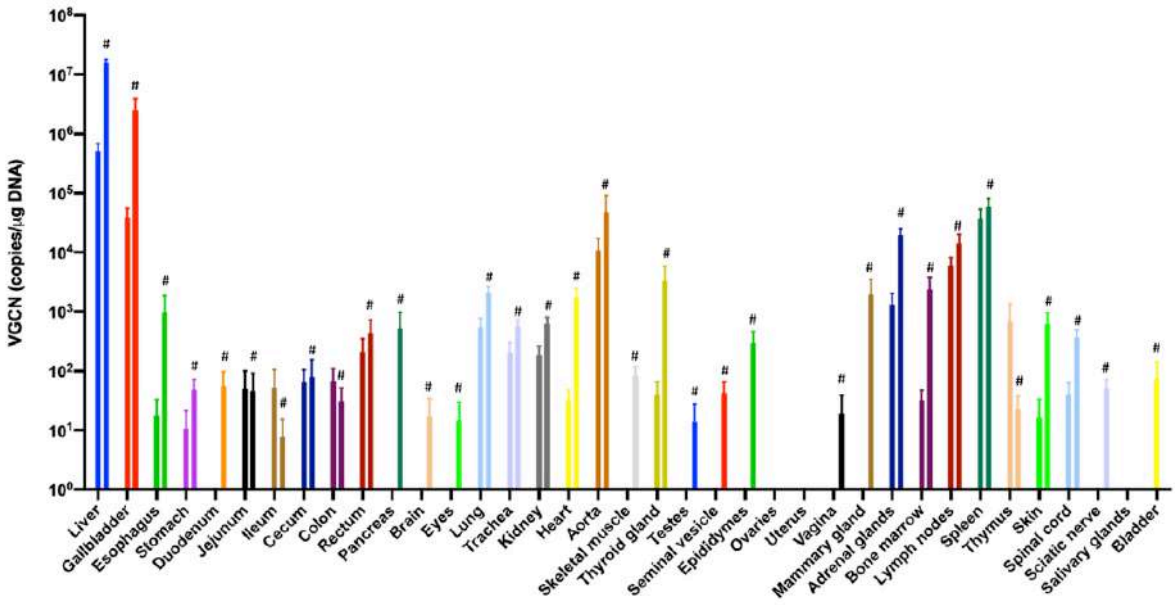
Figure B: Humoral immune response against AAV-LK03 capsid.





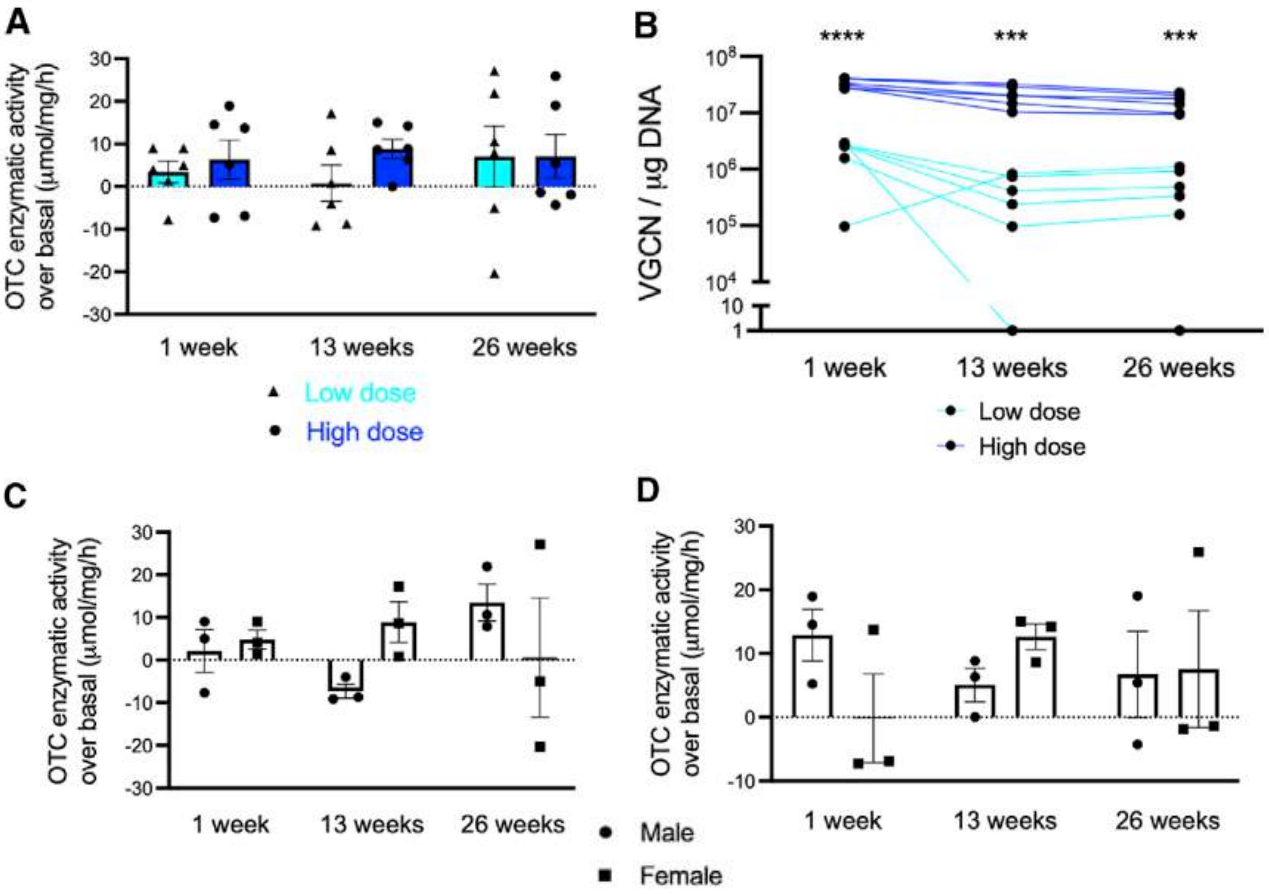
The researchers analyzed both humoral and cellular immune responses triggered by the administration of the AAV-LK03 vector. They monitored the humoral response over the 26-week period by checking for the production of antibodies that could neutralize the viral capsid in all test subjects. The results showed a transient increase in plasma neutralizing antibodies (Nab) titers, which decline by week 26, with no differences between genders. Conversely, there was no detectable cellular response against either the AAV-LK03 capsid viral protein 1 (VPN1) or the hOTC protein, implying that no capsid-specific T cells were activated following vector administration. The minimal humoral response and lack of cellular immunity, collectively reinforces the safety profile of this particular viral capsid pseudotype.

Figure C: Biodistribution of the AAVLK03.hOTC vector after 26-weeks following a single peripheral vein injection.



A biodistribution assay was conducted to determine the tropism of the administered vector, analyzing its ability to infect different cellular types. After euthanizing the animals at the 26-week mark, a qPCR analysis was performed in various tissues. The results revealed a significant liver tropism of the vector, with the liver displaying the highest number of genome copies. Interestingly, there was no evidence of transduction in the central nervous system and only minimal transduction was observed in genital organs.

Figure D: Supraphysiological liver OTC enzyme activity.



Lastly, increased OTC enzymatic activity was detected in the liver tissues from all treated animals at all time points. Differences in OTC activity between low- and high-dose groups suggest a trend for dose-response, and no gender differences were noted.

In summary, this study indicates the safety and efficacy of the hepatotropic AAV-LK03 vector for gene therapy in OTC deficiency, displaying remarkable liver tropism, limited off-target distribution, and absence of adverse events. It also demonstrated a transient humoral immune response and no observed cell-mediated immune responses against the viral capsid or the OTC protein, as well as a sustained increase in OTC activity throughout the 26-week period.

Following the successful preclinical evaluation of AAV-LK03 in addressing OTC deficiency, now we will describe another innovative gene therapy treatment that utilizes lipid nanoparticles (LNPs) as delivery vehicles. This approach has been recently explored in the work of Mary G. Prieve in the article “Targeted mRNA Therapy for Ornithine Transcarbamylase Deficiency” **(36)**. As NanoRare strives to design a hybrid therapeutic approach that combines the use of AAVs and LNPs vehicles, a deeper understanding of both platforms is crucial to leverage the strengths of each. This mRNA-based therapy represents a transient, yet powerful therapeutic approach. In this study, the experiments performed can be categorized in two sets:

1. **First set:** study of the properties and efficacy of their novel delivery system, designated HMT (hybrid mRNA Technology). To do so, they designed an mRNA encapsulating LNP (mRNA/LNP) to be employed simultaneously alongside a

liver-targeting polymer, named polymer micelle (see figure E below). The team used a luciferase-encoding mRNA to evaluate the system's specificity for liver cells. It was found that this HMT delivery system provided targeted and robust protein expression in the liver, while minimizing off-target effects.

2. **Second set:** here, the therapeutic potential of this method was tested by replacing the luciferase with a human OTC-encoding mRNA (hOTC mRNA). The efficacy of single and repeated doses of hOTC mRNA/HMT was analyzed. It was found that the treatment led to notable hOTC protein production and improved liver functionality in the mouse model of OTC deficiency.

Below, from Figures E to I is a summary of the data that demonstrates the safety profile and efficacy of a hybrid LNP delivery of mRNA-hOTC sequence in Spf^{ash} mouse model:

Figure E: Hybrid mRNA Technology delivery system.

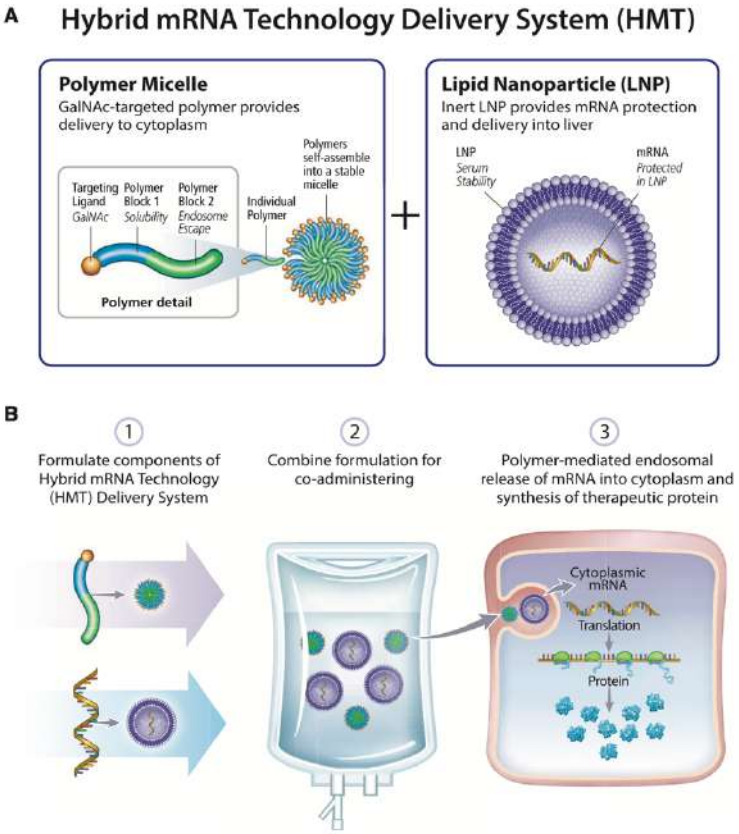
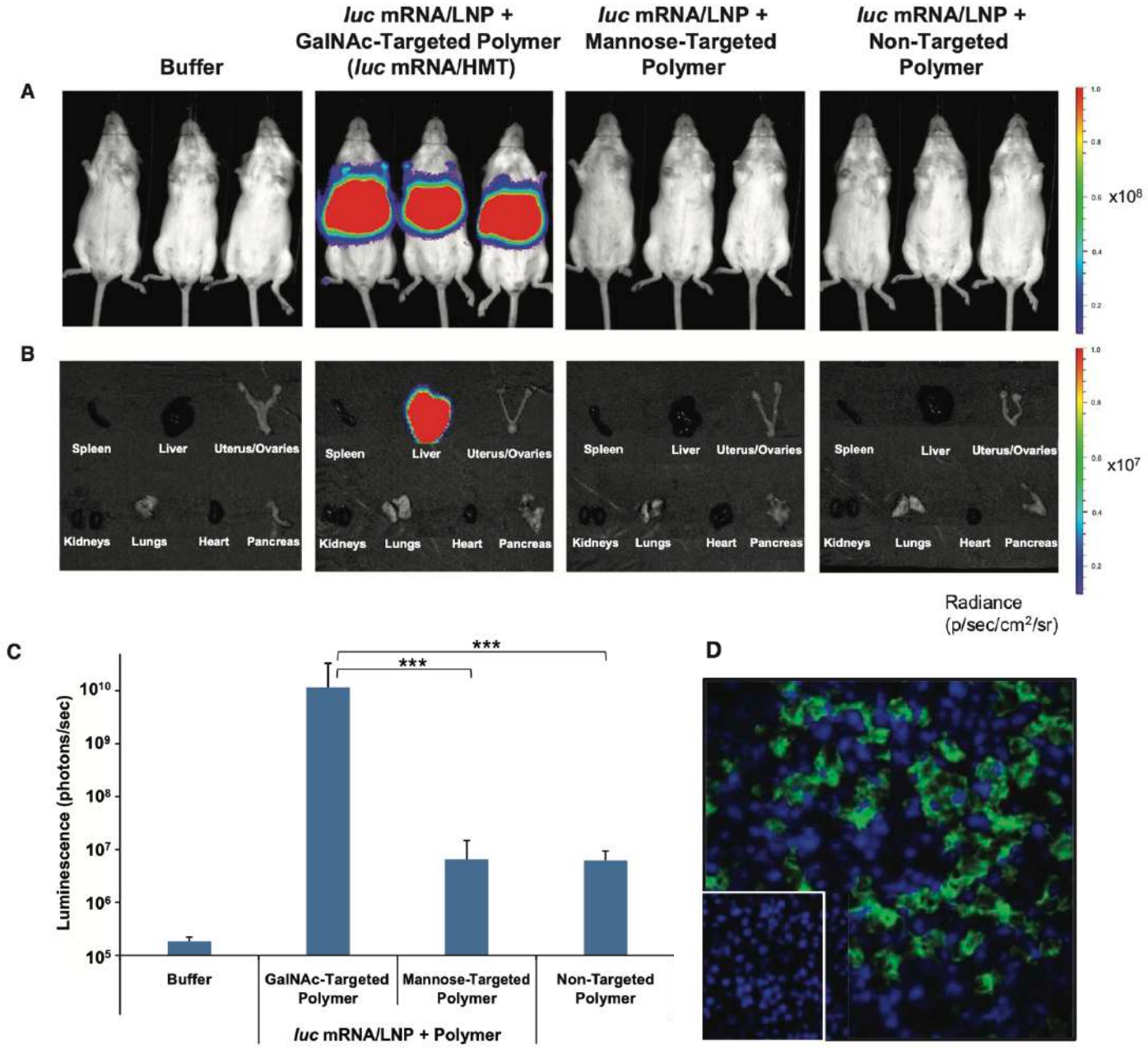


Figure E presents the Hybrid mRNA technology (HMT) delivery system, specifically design to target hepatocytes, for the release of mRNA encoding the human OTC protein. This study utilized an OTC deficiency mouse model, known as OTC Spf-ash. The liver-specific expression of the HMT formulation was confirmed through both, *in vivo* and *ex vivo* luminescence assays (Figure F), showing that the OTC mRNA expression was exclusive to hepatocytes. It is important to highlight that in this study the release of mRNA into the cytoplasm, which is carried by the LNP, and the subsequent synthesis of the therapeutic protein, is triggered by the polymer micelle, a key component that enhances endosomal release.

Figure F – 1st set: Liver specific expression of *luc* mRNA/HMT.



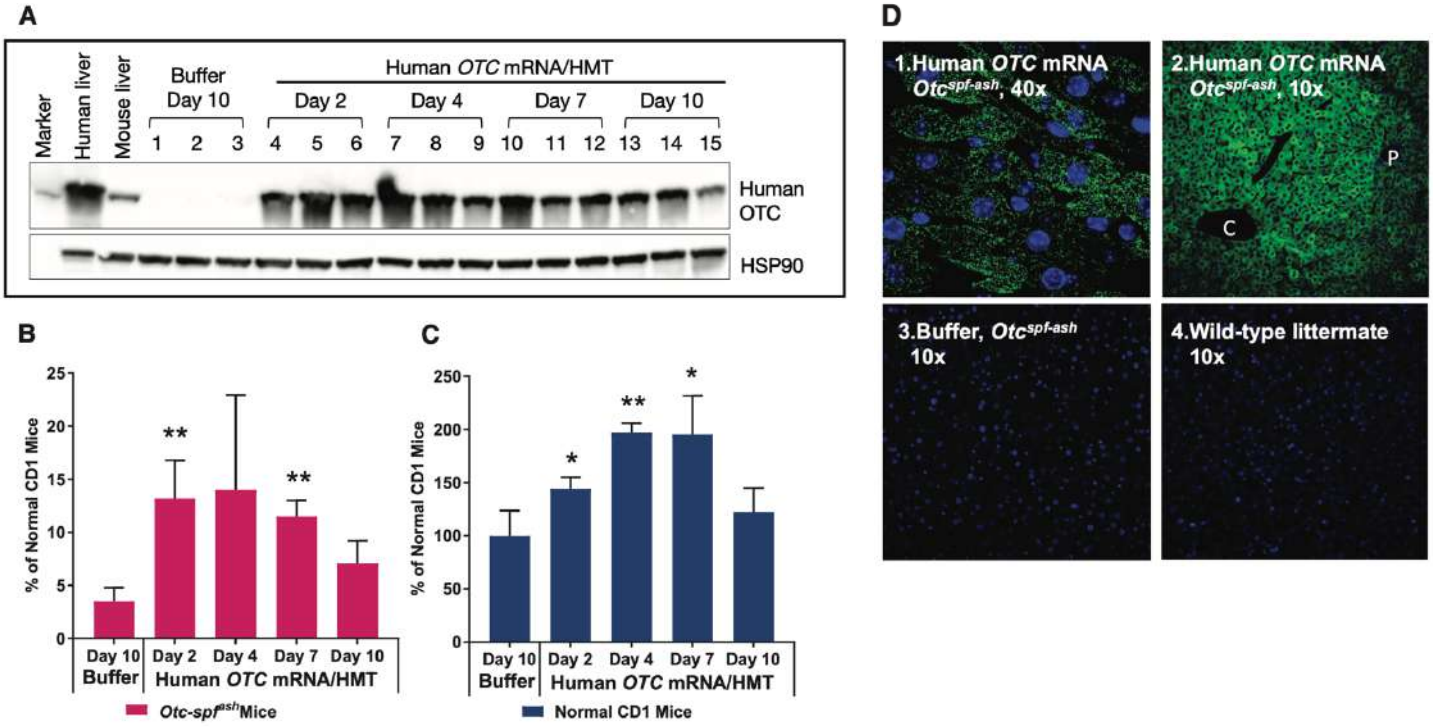
To evaluate liver-specific expression of HMT formulation, as well as the importance of the GalNAc-targeting component, they injected mice with an intravenous bolus of ***luc* mRNA/LNP** + GalNAc polymer micelle (i.e., ***luc* mRNA/HMT**) and the control polymers (a mannose-targeted polymer or a non-targeted polymer) to demonstrate targeting specificity.

In vivo and *ex vivo* luminescence showed that expression was 5 logs above the background level only when using the ***luc* mRNA/HMT** construct and limited solely to the liver. They did not observe expression of luciferase in spleen, heart, pancreas, kidney, uterus/ovary and lungs.

The luciferase expression observed in the HMT construct showed a ~2000-fold increase in comparison to that of the control polymers. No differences in luminescence expression was found between the control polymers either.

To confirm the liver-specificity of the *luc* mRNA/HMT construct, subsequent studies were performed where they observed that 95% of the GalNAc-targeted polymer was detected in the liver 2 hours post-administration. The immunofluorescence assays revealed that the administration of the HMT construct in the liver led to mRNA expression exclusively in cells that exhibit the characteristic structure of hepatocytes, which aligns with the anticipated hepatocyte-specific expression of the GalNAc receptor, ASGPR.

Figure G – 2nd set: OTC enzyme and activity levels after a single injection of hOTC mRNA/HMT construct.



Before conducting efficacy studies, the researchers assessed the expression and activity levels of human OTC protein following a single injection of hOTC mRNA/HMT construct in the mutant mice (Spf^{ash}), and compared them with the levels observed in normal/wild-type mice (CD1) treated and untreated with the same vector.

Western-blot analysis revealed detectable OTC protein in the mutant mice at various points up to 10 days after dosing. The OTC enzyme activity in these mice peaked 14% of normal mouse levels, four times higher than buffer-treated mice, and this increase was observed for up to 10 days after injection. Parallelly, normal mice treated with hOTC mRNA/HMT showed OTC enzyme activity reaching 200% of normal levels on day 4. The lower activity in mutant mice was expected, as control experiments had shown lower luciferase activity in these mice.

Immunofluorescence analysis confirmed the presence of hOTC protein in the liver cells of treated mutant mice, with no difference in protein expression between different liver regions. No hOTC-positive cells were found in the liver of buffer-treated mutant or wild-type mice. Additional qPCR experiments confirmed the liver specificity of hOTC

mRNA/HMT, showing a 40-fold increase uptake of mRNA by the liver compared to other organs.

The researchers then evaluated the efficacy of hOTC mRNA/HMT in a repeat-dosing study in the OTC deficiency mice model Spf-ash (Figure H). To mirror the clinical condition, they induced hyperammonemia by using an AAV vector that produces an OTC short hairpin (shRNA). This shRNA effectively reduces the levels of the residual mouse OTC mRNA and protein. Within a few days, hyperammonemia develops, and if left untreated, the mice succumb. This model closely mirrors the clinical condition observed in humans.

Figure H – 2nd set: Repeat dose efficacy study in the OTC Spfash mice.

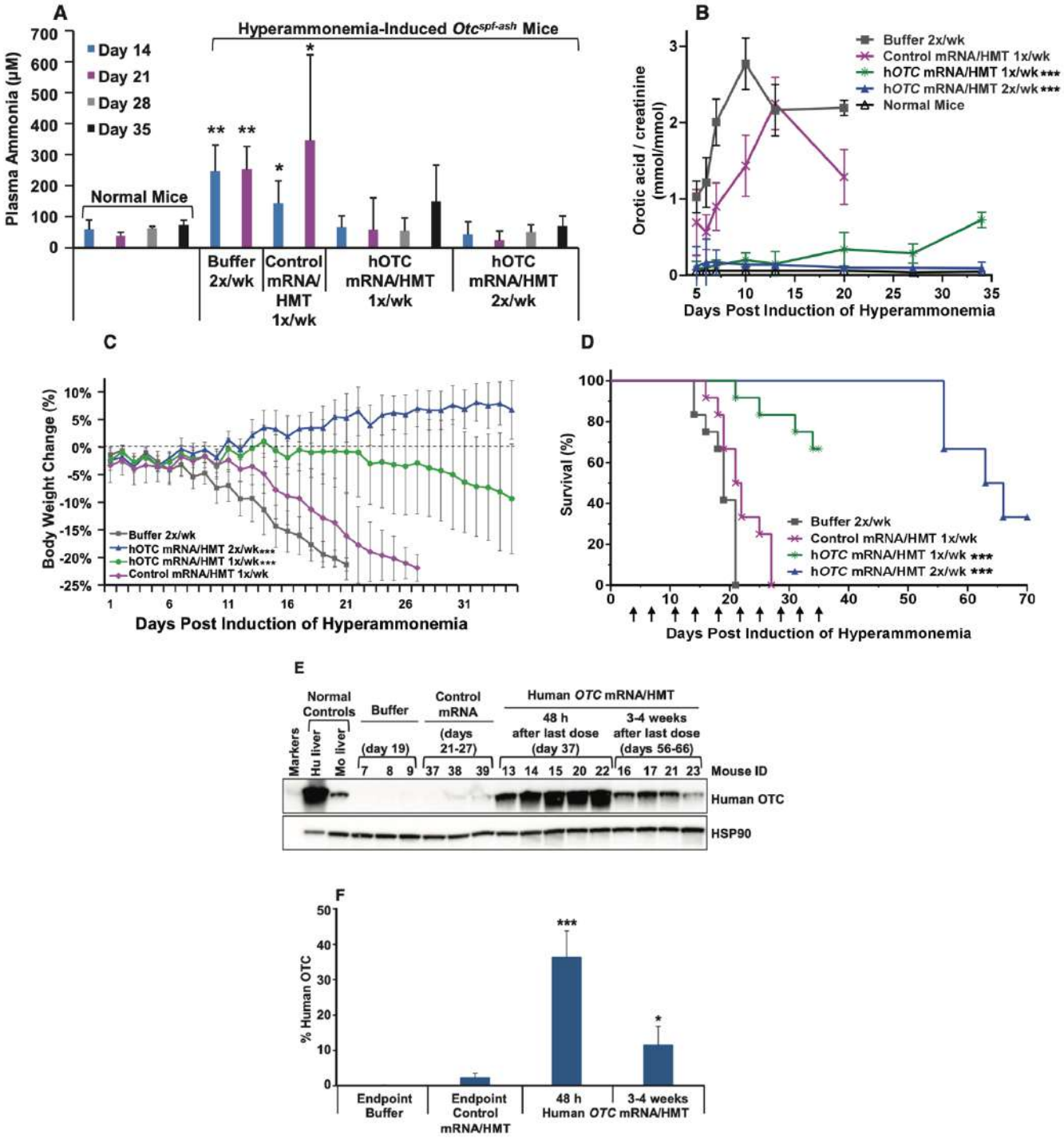


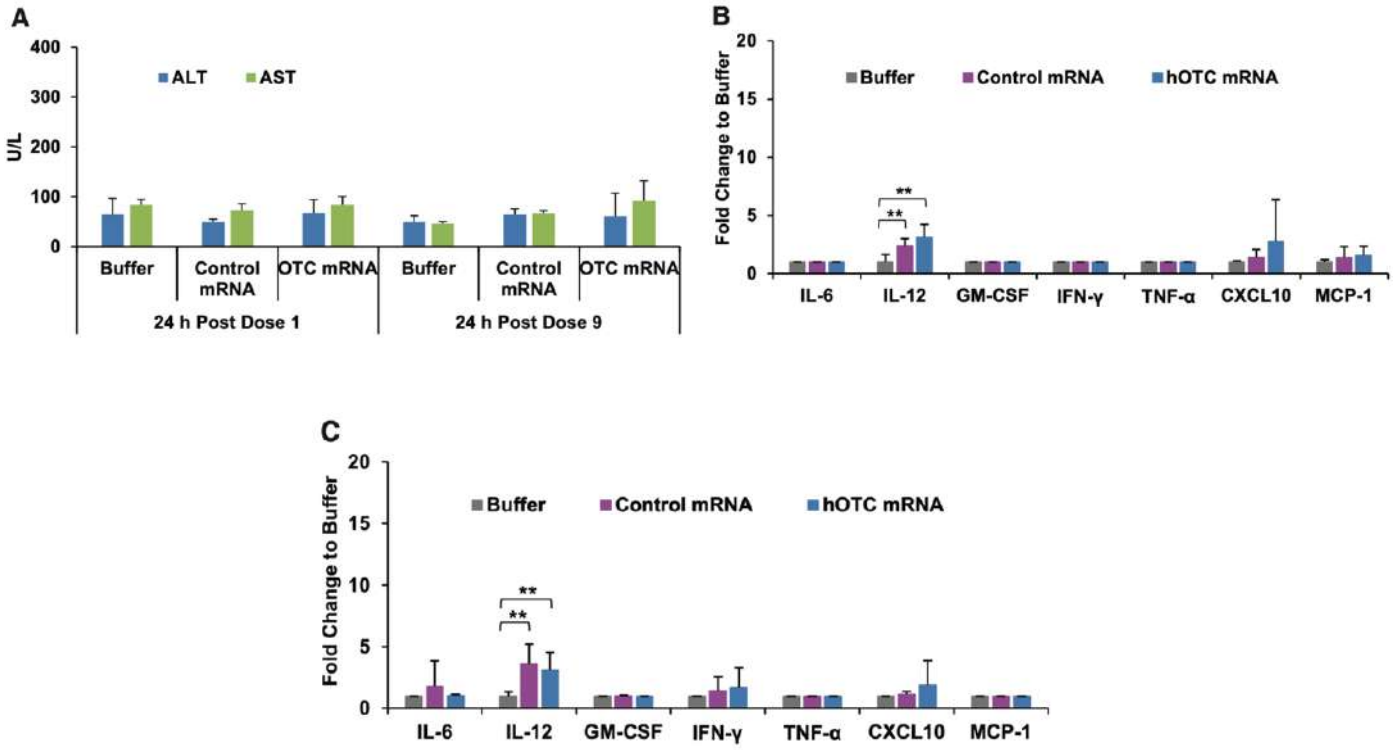
Figure H shows the experiments aimed to assess the survival and health of mice treated with different dosing regimens of hOTC mRNA/HMT. By day 35, no animals survived in the buffer and control groups, and the once-per-week treatment group also had to be sacrificed. However, the twice-per-week hOTC mRNA/HMT groups showed normalized plasma ammonia and reduced levels of urinary orotic acid 24 hours after the first dose and maintained throughout the study. This group also showed a significant increase in body weight, indicating both efficacy and good tolerability with the dosing regimen.

In contrast, the once-per-week hOTC mRNA/HMT group started showing elevated orotic acid levels and weight loss by day 20. All mice in the control mRNA formulation and buffer treatment groups died due to hyperammonemia or met the criteria for sacrifice before the end of the dosing period.

However, all mice in the twice-per-week treated group survived the 35-day dosing period, and those maintained after dosing termination survived for at least 3 additional weeks. The OTC protein levels in these mice were approximately 35% of normal human liver tissue control 48 hours after the final dose, which decreased to approximately 12% 3-4 weeks later. The researchers estimated the half-life of OTC protein to be around 12 days.

Lastly, they conducted a repeat-dosing study, using the same mutant male mice (Spf^{ash}) which had not received the AAV OTC shRNA, to evaluate the safety profile of the hOTC mRNA/HMT construct (Figure I).

Figure I – 2nd set: Repeat dose efficacy study in the OTC Spf^{ash} mice.



Here, the mice were administered the hOTC mRNA/HMT formulation, the control mRNA/HMT formulation or buffer twice-per-week for a total of four weeks, resulting in nine repeat doses. Normal levels of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) were observed 24 hours post-administration and 24 hours after the

final dose, indicating no adverse effects on liver function. Also, cytokines levels were monitored 3 and 24 hours after the final dose. No significant increases were observed in interleukin-6 (IL-6), granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon-gamma (IFN-g), tumor-necrosis factor alpha (TNF-a), or monocyte chemoattractant protein 1 (MCP-1) levels.

To conclude, a histopathological examination of internal organs was conducted 48 hours after the final dose. No significant pathological findings were observed in any of the tissues from the treated animals, indicating the absence of notable adverse effects, further supporting the safety of the hOTC mRNA/HMT construct.

The work done by Mary G. Prieve represents the first demonstration of a repeat, systemically administered mRNA therapy correcting OTC deficiency, also marking the successful use of non-viral delivery mechanism in this context. Notably, the administration of hOTC mRNA/HMT construct significantly reduced ammonia levels and extended survival time in the animals. Despite the differences with NanoRare's approach, the study conducted makes a compelling case for the hOTC mRNA/HMT system as an effective enzyme replacement therapy for OTC deficiency, yielding transient, but potent results.

The scientific investigations described throughout this section (Dr. Julien Baruteau – AAV-LK03 and Dr. Mary G. Prieve – LNP delivery) lay a solid foundation for the effectiveness and tolerability of the gene delivery systems that underpin NanoRare's innovative approach. Crucially, both studies have been conducted in clinically relevant animal models that imitate severe neonatal-onset OTC deficiency, what further contributes to support NanoRare's broader applicability.

The conclusions draw from both research projects provide key insights that validate the theoretical basis of our technology and also serve as invaluable guidelines for its development. In the upcoming section, we have outlined the step-by-step process of the initial R&D plan, building upon the theoretical proof of concept we have explored so far.

INITIAL R&D AND FINANCIAL PLAN

NanoRare's research and development (R&D) efforts will be directed towards the development of a novel liver-targeted gene therapy, the lead drug candidate (LDC) NR-001. This innovative approach will use an AAV-LK03-derived vector to safely and effectively deliver a corrected OTC sequence to hepatocytes. It will also leverage a proprietary LNP, designed to carry the transposase mRNA, for stable chromosomal integration.

Our R&D plan, outlined in the following section, is segmented into stages of increasing complexity – ranging from basic *in vitro* and *in vivo* studies, for the development of the proof-of-concept, to translational *in vivo* studies. NanoRare will then transition to pre-IND (investigational new drug) studies outsourced to external organizations; to the completion of the CMC (chemistry, manufacturing and control) package, ensuring the product is safe, effective and consistent between batches; and, lastly, conducting clinical studies. This process aims to lay the groundwork for the development and optimization of our LDC, followed by the evolution of the lead clinical candidate (LCC) program. The key activities, expected outcomes, and the interdependencies of these stages are shown on the tables below, and depicted in a Gantt chart in Figure 11, providing an estimated timeline.

The first set of objectives, included in the LDC program (aims 1 to 4), encompasses the design and synthesis of the therapeutic constructs, optimization of delivery vehicle components, and comprehensive preclinical assessment to achieve *in vitro* and *in vivo* PoC. This preliminary work is fundamental to understanding the distinct technology components (AAV + hOTC sequence / LNP + transposase mRNA) and their interplay within the context of the novel drug construct.

The second set of goals, integrated in the LCC program (aims 5 and 6), is set to begin after the successful design and optimization of NR-001, and will primarily focus on product and manufacturing process optimization. This steps aim to secure the application for IND-enabling studies, which is essential to obtain approval to conduct first-in-human clinical trials. During this stage, pharmacological, toxicological, and manufacturing parameters of NR-001 will be studied to develop the human clinical study protocols.

Given the severe nature and urgency of neonatal-onset OTC deficiency, our primary emphasis lies in formulating and validating the effectiveness of our therapy in this specific group. Each R&D goal is connected to a specific action plan. While the approach is based on existing knowledge and technologies, it allows for flexibility to incorporate new findings and insights as the research progresses. A financial overview and investor exist plan is described at the end of the section.

On the table below, a detailed description of NanoRare's aims 1 to 3 is shown:

Aims	Key objectives	Key activities	Description	Expected outcome
1	Development of **proprietary lipid nanoparticle** formulation	Confirm **optimal liver-targeting molecule**, if applicable*, and determine **optimal LNP composition**	Assess different targeting moieties that could potentially enhance liver-specific delivery of LNPs. Simultaneously, determine the best LNP formulation, focusing on efficiently packaging the transposase mRNA, stability in the bloodstream and uptake by hepatocytes.	**Optimized LNP that efficiently transfect hepatocytes**, as well as identification of proprietary ionizable cationic lipid for patenting purposes
		Evaluate **targeting capability of LNPs** in hepatocytes	Evaluate optimized LNP through *in vitro* assays using hepatocyte cell line. LNPs loaded with luminescence mRNA (such as luciferase) to visually track uptake and intracellular localization of LNPs. Observe differences in expression depending on cell type, as well as liver localization, periportal, perinuclear.	
2	Development of **proprietary adeno-associated (AAV)** vector	**Capsid engineering** for the LK03-derived vector to identify best candidate in terms of safety	Modify the AAV-capsid, through rational design techniques, to enhance safety profile of the candidate (ability to evade pre-existing immunity).	**Identified AAV vector** with superior safety profile and liver-targeting capability, to be used alongside the LNP formulation in the hybrid delivery. Also, potentially used as patentable asset by NanoRare
		Evaluate the **liver-transfecting efficacy** of the capsid candidate	Assess transduction efficiency of the engineered AAV capsid in *in vitro* (hepatocyte cell line) experiments.	
3	Development of **transposon DNA hOTC-encoding** DNA and the **transposase mRNA** to:	Determine **optimal ratio of AAV:pDNA** that maximizes cellular uptake	Perform *in vitro* transfection experiments to determine optimal ratio of AAV vectors to plasmid DNA (AAV:pDNA ratio) that enhances cellular uptake and transgene expression.	**Identified optimal loading** ratios between the vectors and their respective cargos, to maximize transfection and achieve higher chromosomal integration rates
		Determine **optimal ratio of LNP:mRNA transposase** that enhances chromosomal integration and long-term expression	*In vitro* transfection experiments to determine the best ratio of LNPs to mRNA (LNP:mRNA ratio) transposase that maximizes stable integration of hOTC gene into hepatocytes' genome and its long-term expression.	
		Confirmed **optimal AAV:LNP ratio** in NR-001 LDC	Identify optimal ratio of AAV-loaded and LNP-loaded that ensures maximal delivery and expression of the therapeutic constructs *in vitro* and *in vivo* (hepatocyte cell line and Spf-ash mice model) experiments.	

Table 5: NanoRare's aims 1 to 3 description. Optimization of NR-001 components for effective and liver-targeted delivery. The "if applicable" note in the LNP is subject to changes because of the current state of development of proprietary LNP technology by NanoCell therapeutics. If the lipid nanoparticles are observed to be directed to the liver, with significant hepatocyte uptake, the need for a ligand -targeting moiety- might not need to be required. However, if through the addition of a targeting ligand, an increase in transfection rates in hepatocytes is observed, it could be included in the development plan and used for IP purposes.

NanoRare's aims 1 to 3 articulate the objectives and their corresponding activities to optimize the different components of our LDC, NR-001. The rationale behind lies in establishing optimal configurations for our LNP and AAV delivery vehicles, and elucidating the most efficacious ratio of these vehicles to their respective cargo. A crucial

phase to consolidate the proprietary technologies that could be used as patentable assets. Once completed, NanoRare will then transition to the fourth aim: the optimization of the LDC, as a platform that combines the distinct technology components.

Aim	Key objective	Key activities	Description	Expected outcome
4	Development of Lead **Drug Candidate (LDC)** to achieve **Proof-of-concept** (PoC) for NanoRare's platform	Demonstrate **functional** and **specific targeting** of hepatocytes *in vitro* and *in vivo* Evaluate **efficacy *in vivo* by injection of NR-001** at various doses in Spf-ash mice and analysis of clinically relevant biomarkers Optimized encapsulation and **manufacturing processes of NR-001**	Conduct experiments in hepatocyte cell line *in vitro* and in a mouse model with OTC deficiency (Spf-ash mice), *in vivo*, to verify targeting specificity and functional effectiveness of NR-001. Establish information on *in vivo* pharmacokinetics, tissue distribution and stable integration. Administer different doses of NR-001 to the Spf-ash mice and monitor changes in clinically relevant biomarkers such as OTC enzymatic activity and plasma ammonia and orotic acid levels, to evaluate the effectiveness of the therapy. Later, assess the efficacy in non-human primate animal models. Refine formulation and production methods for efficient encapsulation of NR-001, ensuring streamlined, reproducible, and scalable good manufacturing practices (GMPs).	Lead Drug Candidate, **NR-001 efficiently transfects hepatocytes and demonstrates efficacy** in animal models

Table 6: NanoRare's aim 6 description. Optimization of LDC NR-001 to achieve Proof-of-concept.

As part of aim 4, NanoRare will test the NR-001 therapy to demonstrate the efficacy and specificity of our approach. Here, *in vitro* testing on hepatocytes cultures and *in vivo* studies using reporter genes in healthy animals will first take place. Later, clinically relevant animal models, such as the Spf-ash mice and juvenile NHP will help us to confirm NR-001's therapeutic potential in the neonatal-onset OTC context. The targeting capability will be measured with luminescence and immunofluorescence assays measured *in vivo* and *ex vivo*. The efficacy will be evaluated by injecting various doses of our therapy into the animal model and analyzing relevant biomarkers. Successfully passing these four aims will bring us to a significant milestone: achieving *in vitro* and *in vivo* proof-of-concept (PoC). This accomplishment paves the way for the beginning of the lead clinical candidate (LCC) program. Here, NR-001 will undergo comprehensive IND application-enabling studies and the filing process of an IND for our LDC, as shown in the table below:

Aims	Key objectives	Key activities	Description	Expected outcome
5	Complete **IND application-enabling studies** and file IND for NR-001	***In vivo* biodistribution analysis** and **evaluation of metabolic** and **pharmacokinetic profile** (PK) of NR-001	Conducting a range-dosing study in animal models. Histopathological and qPCR examination will be performed. Observation of clinical manifestations through the study to inform development of PK/PD relationships, and experimental design of non-clinical toxicology and safety pharmacological studies.	**Understand** the **biodistribution** and **pharmacokinetics** of **NR-001**. **Optimization** of **Good Manufacturing Practices (GMP)**. **Approval of IND application**, enabling start of clinical trails
		Secure an open **Investigational New Drug** (IND) **application** for NR-001	Engaging with regulatory bodies such as FDA and EMA for preliminary discussions upon satisfactory data from *in vitro* and *in vivo* Proof-of-concept. Production of NR-001 under GMP conditions. Complete IND-enabling studies including toxicology, metabolic degradation, safety pharmacology and stability parameters.	

Table 7: NanoRare's aim 5 description. Completion of Investigational New Drug application studies to clinically test the NR-001 LDC.

NanoRare's aim 5 is designed to make the progression from the laboratory to the clinic. Early efforts will be centered around extensive biodistribution and pharmacokinetic (PK) studies of NR-001, as well as to establish large scale production under GMP conditions to allow for scalability and reproducibility, ensuring consistent quality of the therapy. The subsequent achievement of securing the IND for NR-001, enabled by through engagements with regulatory bodies like the FDA and EMA, highlights NanoRare's commitment to transition towards clinical trials. Success in this phase signals a transformative shift, ultimately leading to the FDA/EMA-regulated clinical phase I/II study for the LCC (aim 6) program, described below:

Aims	Key objective	Key activities	Description	Expected outcome
6	Conduct **FDA/EMA - regulated clinical** phase I and II for NR-001	**Characterize** the **safety**, **tolerability**, **pharmacokinetic** and **pharmacodynamic** of intravenously administered NR-001 in OTC patients	Conduct multi-site, single-dose-escalating phase I/IIa study in OTC deficiency neonatal patients. Monitor for safety endpoints, such as spontaneous adverse events, physical exam, changes in vital signs, clinical laboratory measurements, and PK parameters. Therapeutic efficacy to be assess through relevant biomarkers.	**Completion of phase I/II clinical trials**, providing crucial safety and efficacy data in humans for NR-001

Table 8: NanoRare's aim 6 description. Completion of Lead Clinical Candidate program.

Finally, aim 6 signifies NanoRare's entrance into the clinical research landscape, through the initiation of FDA/EMA-regulated clinical Phase I/II. It encompasses a thorough multi-site, single-dose-escalating study to determine the safety and tolerability, alongside the pharmacokinetic and pharmacodynamic characteristics of the LDC NR-001 when tested in OTC deficiency patients. With stringent safety monitoring measures, ranging from spontaneous adverse events to detailed examinations and laboratory evaluations, patient safety will be prioritized at every step of the clinical trial. Meanwhile, the therapeutic efficacy of NR-001 will be concurrently assessed via relevant biomarkers measurements, thereby providing us with a detailed view of its performance in a clinical setting. The completion of this critical phase will mark the clinical validation of NR-001 and elevate NanoRare to the status of a clinical-stage biotechnology company.

It is vital to pinpoint key milestones that will drive NanoRare's journey from preclinical research to becoming a clinical-stage company. These significant checkpoints, embedded in the objectives, will help tracking the progress and validating the therapy. NanoRare's key value inflecting milestone covering the six aims are summarized below:

- **Milestone 1: Completion of *in vitro* and *in vivo* Proof-of-concept and Lead Drug Candidate optimization**. The first significant milestone will be achieved after successful development of PoC studies (aims 1 to 4), which is divided in three different phases (see chart below), and the finalization of the optimization of the LDC construct. It represents a critical validation point for the core science behind the project and is essential for confirming the therapeutic potential of our drug candidate, NR-001. This milestone is expected to be achieved with seed capital, but could also provide the momentum required to secure a second financing round, Serie A.
  - Estimated timeline: 18 – 24 months post-financing.
  - Estimated budget: €15 million.

- **Milestone 2: Securing FDA and EMA approval to initiate clinical trials**, the second significant milestone (aim 5) that marks the beginning of the lead clinical candidate program. As gene therapy is a relatively novel area, this regulatory milestone will be a

major accomplishment. It will require the successful establishment of a GMP manufacturing process and the satisfaction of all *in vivo* considerations, to corroborate our approach. Based on industry standards and the specific regulatory needs of gene therapy, it is projected that this milestone may be reached within 3 to 4 years from the start of the project. Achieving it could secure a third financing round, Serie B.

- Estimated timeline: 36 - 48 months post financing.
- Estimated budget: €30 million.

- **Milestone 3: Demonstration of safety and preliminary efficacy in OTC deficiency patients** (aim 6). This third and last milestone will also delineate the competitive profile of NanoRare's technology platform within the OTC gene therapy landscape, what could further enhance our potential for future partnerships and funding. It will provide the first indication that our LDC may ultimately be able to cure patients. Given the complexity of clinical trials and patient recruitment, this phase is projected to take place 4-6 years after project initiation. This milestone could help NanoRare to secure a Serie C funding, to develop new products or expand into new markets.
  - Estimated timeline: 48 – 54 months post financing.
  - Estimated budget: €30 million.

It is important to note that these are broad estimates and the exact timeline and budget could vary based on multiple factors such as the complexity of the R&D process, the regulatory environment, and the recruitment rate for clinical trials, among others. Regular project reviews will be needed to keep track of the progress towards this significant targets.

An estimated timeline of the lead drug candidate program (aims 1 to 4) during the first 18 months and milestone 1 can be found below:

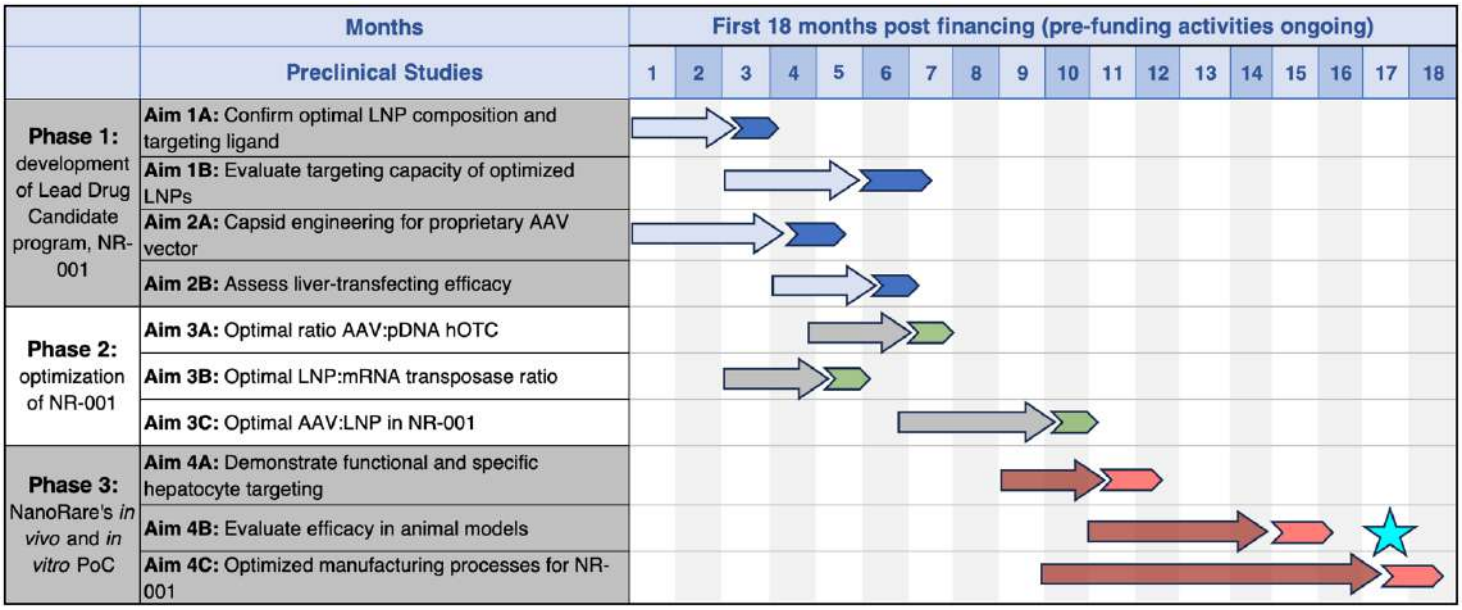


Figure 11: NanoRare's R&D timeline overview. **Milestone 1** represented with a bright-blue star.

Financing & Investor Exit

A detailed financial plan requires industry-specialized expertise and falls outside the scope of this project. However, a preliminary overview of the financial requirements and potential exit strategies for investors is described next:

1. **Financial overview:**

NanoRare's venture will begin with the closure of the first round of Seed Series Preferred I financing. An estimated €3 million will be necessary to establish the research team and maintain the infrastructure required to develop the *in vitro* PoC for the OTC gene therapy. The subsequent Seed Series Preferred II round aims to raise an additional €3 million to support the development of our lead clinical candidate program. These two rounds of financing could take NanoRare through a significant value inflection point, estimated to occur around 18-24 months from the closing of the financing. During this period, NanoRare anticipates achieving *in* vitro and *in vivo* proof-of-concept and making significant advances towards a first IND and CTA (clinical trial approval) filling in the US and Europe, respectively.

In terms of broader milestones, NanoRare would need to raise approximately €15 million for Milestone 1, a targeted €30 million for Milestone 2, and another €30 million for Milestone 3. The initiation of the IND-enabling lead clinical candidate program, which is NanoRare's aim 5, could start before the completion of aim 4, if supplemental capital is available.

2. **Partnering revenue:**

The strategic partnership for the development of NR-001 LDC could provide near-term, non-dilutive financing for the development of NanoRare's pipeline. In addition, NanoRare may also consider technology licensing agreements, upon successful development of the PoC, as a future source of revenue. By licensing the patented technology to other companies in the gene therapy space, the strategy aims to provide financial benefits as well as a validating-method for the platform in this niche market.

3. **NanoRare Investor exit plan:**

Potential investors in NanoRare could have three main avenues for return-on-investment exit:

- Early sale of the therapeutic application in areas outside NanoRare's focus.
- Merge or Acquisition (M&A) by a large pharmaceutical or biotechnology company.
- An Initial Public Offering (IPO) on a reputable stock exchange, such as NASDAQ.

Within the gene therapy industry, IPOs and M&As events have typically been routes for investor exit, taking place usually 3 to 4 years after initial rounds of financing. Similar to NanoCell Therapeutics, NanoRare aims consolidate a robust first financing rounds to achieve the milestones described above, with subsequent value inflection points. The most significant of these are the first IND approval and positive preliminary clinical safety and efficacy data, milestones 2 and 3. The timing of the IPO is market-driven, but is expected to occur upon IND approval.

As we conclude the initial R&D section and financial overview, it is clear that NanoRare's plan stands as a comprehensive strategy that seeks to leverage established methodologies in an innovative manner to develop an effective and safe gene therapy. Importantly, this plan is adaptable, capable of incorporating new findings and ideas as our project continues to unfold.

ASSESSING NANORARE'S POSITION IN THE OTC LANDSCAPE

In the last section, we will evaluate the position that NanoRare could hold within the OTC gene therapy landscape as part of our strategic planning. To do so, we will utilize various market analysis tools, such as Porter's five-force, a SWOT and VRIO analysis, to validate the divestment decision from NanoCell Therapeutics to NanoRare.

The scientific technical details that are discussed throughout the business plan, position NanoRare with a theoretical superiority over its competitors. As described, NanoRare's gene therapy presents the following key features:

- **Stable chromosomal integration:** this ensures OTC protein production (by autologous hepatocytes) throughout the patient's life, providing the necessary long-term benefits; and,
- **Increased biosafety profile: i)** the hybrid approach could enable the use of lower doses of delivery vehicles, potentially limiting vector immunogenicity, and **ii)** use of a transposase, Sleeping Beauty, with the safest integration profile of all genetic modifying mechanisms proposed; and,
- **Enhanced applicability:** offer a curative option to both, neonatal and late-onset OTC patients, potentially capturing the entire market.

Because of the aforementioned scientific advantages, NanoRare plans to leverage a differentiator strategy. These distinctive attributes shape the connection with the unmet clinical needs for OTC, constituting the business fit for NR-001, that potentially will offer:

- **Improvements in clinical outcomes:** a definite one-shot, life-lasting curative treatment for all OTC patients; and,
- **Reduced costs and complexity:** by eliminating treatment costs from chronic treatment and hyperammonemia crisis, as well as including scalable and consistent production processes; and,
- **Alleviate patient's burden:** by removing lifelong/immunosuppressive medications, improved affordability and tackling the disease's root cause providing a potential cure.

In line with our scientific differentiator strategy, these business advantages could later be developed as NanoRare's unique selling points for NR-001. Porter's five-force analysis allow us to investigate the competitive dynamics of the market, including entry barriers, bargaining power of suppliers and buyers/users, threats from substitute products, and the intensity of the competitive rivalry. By doing so, we can identify where the market power resides, which could help us leverage these forces to NanoRare's benefit.

Porter's Five-force analysis

1. **Threat of new entrants (moderate):** in the context of biotechnological research organizations, particularly the gene therapy field for rare diseases, a high capital investment is required to start operations, alongside substantial scientific and clinical expertise, as well as regulatory knowledge. These factors, that include material and human resources, make the entry barriers for new players high. In addition, NanoRare's platform technology, comprising the combined use of Sleeping Beauty transposase and a hybrid delivery system, will be protected by a robust patent portfolio, extending beyond 2040, what provides additional advantage over new entrants.

2. **Threat of substitutes products (high):** current treatments for OTC deficiency, such as dietary management, nitrogen scavengers and liver transplantation are not curative and come with their own set of challenges. This gives NanoRare's gene therapy a substantial advantage as a potential one-time, life-lasting solution.
   The most advanced gene therapy approaches for OTC deficiency, being Ultragenyx and Arcturus Therapeutics (AAV and mRNA-LNP based technologies, respectively), do not represent a significant risk, as their solutions are either temporary or do not cater the broad OTC patient population, limiting their potential as substitutes.
   Furthermore, other gene therapies in development, such as those pursued by IECURE and Poseida Therapeutics, could indeed be seen as potential substitutes, given they also propose integrative approaches. However, they are still in pre-clinical development, with the intrinsic uncertainties of its evolution. NanoRare's gene therapy approach holds potential in terms of an anticipated superior safety profile and broader applicability. These elements could significantly differentiate NanoRare from these potential substitutes, thereby reducing the degree to which this force could influence the strategy.

3. **Bargaining power of suppliers (low):** the suppliers in NanoRare's context would include the raw material providers for gene therapy production and conducting research activities, Contract Development and Manufacturing Organizations (CDMOs), and clinical research organizations for conducting trials. While there might be plenty of suppliers in the market – lowering their bargaining power, as the availability of options usually result in lower prices – specific requirements, such as strong adherence to Good Manufacturing Practices (GMPs), might reduce the number of qualified suppliers for some tasks, which could exert some pressure on their customers, increasing their power. Here, NanoRare's proprietary technology and control over the manufacturing process – optimized and clearly designed since the initial stages of development – should contribute to mitigate this risk.

4. **Bargaining power of customers (Moderate):** a distinction between users and customers is necessary to do in this context. While NanoRare's NR-001 potential users would include the broad OTC patient population; our customers could be considered healthcare systems and insurance companies who would pay for the therapy. As previously mentioned, gene therapies often come with high price tags, giving some bargaining power to these entities. However, because of the potential to transform patient's lives, the lifetime cost-saving benefits, and the lack of effective curative treatments currently, NanoRare's therapy could command higher control over the prize, offsetting some of this force. It's also important to note the shifting reimbursement climate, particularly in Europe, where cost-containment measures might impact how gene therapies are compensated. While this is not likely to be an issue for OTC treatment due to its urgent unmet medical need, those changes in reimbursement policies could influence the commercial landscape for gene therapies.

5. **Intensity of competitive rivalry (High):** the gene therapy space, while still a niche, presents a highly competitive atmosphere. Seven companies are developing gene therapies for OTC deficiency, and while each may have a different approach, the ultimate goal is the same: its commercialization. Because of the premise held by offering a curative option, the competitive rivalry is intense. To overcome this risk, NanoRare plans on leveraging the scientific differentiators that set it apart. In addition, the focus on early manufacturing optimization, rigorous IP protection, and defined development plan strategically position NanoRare to contend in this competitive space.

   The discrepancy between the moderate ratings of Porter's four external elements and the central conclusive one, the highly competitive rivalry, reflects the unique dynamics of

the gene therapy sector. The core of this competitive intensity is the race for a definitive cure for OTC deficiency, which if successful, would likely be the dominant treatment, providing a permanent solution via one-time application.

So, while current external elements suggest a moderate short-term impact on NanoRare, the highly competitive rivalry reveals a more dynamic, long-term view. This outlook anticipates increasing competitiveness within the broader gene therapy field, driven by the pursuit for a one-off cure for genetic disorders, which is crucial to understand the strategic landscape in which NanoRare will operate.

Porter's Five-force analysis reveals a market landscape that holds considerable promise for the spin-off. Within this competitive space, the unique approach and business strategy could place NanoRare in a favorable position. The proprietary technology, anticipated safety profile, and broader applicability gives distinctive edge over potential substitutes developed by competitors. The current absence of complete curative treatment restrict the bargaining leverage of customers, adding to our strategic advantage. The high barriers for new entrants, coupled with the control over manufacturing processes and access to a wide range of suppliers, reduce supplier power and further bolsters NanoRare's position.

This advantageous market structure suggests that NanoRare is well-positioned to success in developing a gene therapy for OTC deficiency. The translation of NanoRare's scientific advantages into a commercial product would likely lead to a strong market position and high return on investment, considering the unmet needs in OTC management. It's worth noting that the costs saved from eliminating lifelong treatments and frequent hospitalizations could result in substantial long-term cost savings for healthcare systems, thereby increasing its demand once developed. While our analysis doesn't guarantee, it offers a clear projection of NanoRare's potential to effectively navigate the market dynamics.

Having analyzed the competitive forces at play in the market, we transition into a detailed look at NanoRare's SWOT analysis. This provides a panoramic view of its strategic position, as it combines both, internal capabilities and external market realities. Here, we have identified intrinsic strengths that we can leverage, the weaknesses we need to address, the opportunities we aim to capture, and the threats we must mitigate to solidify NanoRare's position.

NanoRare's SWOT analysis

Strengths: Key selling points. The following NanoRare's assets make an attractive investment opportunity, showcasing the company's capabilities and unique features that differentiate it from others.

- **Unique gene therapy approach:** NanoRare's gene therapy for OTC includes stable integration, an increased biosafety approach, and broad applicability across both neonatal and late-onset patients. These attributes should differentiate significantly in a market where the threat of substitute products is moderate and the competitive rivalry fierce. Because of these unique scientific differentiators, NanoRare's position within the OTC landscape, in terms of safety and efficacy, is theoretically superior when compared to potential competitors.
- **Proprietary technology and IP protection:** NanoRare's intellectual property assets come from the combine use of a hybrid approach (proprietary AAV and LNP delivery vehicles) and a non-viral genetic engineering technology (Sleeping Beauty transposon system). This platform will be protected by a robust patent

portfolio that safeguards the combination of these different technologies, providing a strategic advantage when compared to new entrants.

- **Potential to diversify pipeline:** besides NR-001 for OTC deficiency, the Sleeping Beauty transposon system can be tailored to the treatment of other genetic disorders, as well as the delivery vehicles, by incorporating the genes of interest (GOI) in each case. Because NanoRare's proposed technology has potential applications to treat other metabolic disorders (such as Argininosuccinic aciduria or Citrullinemia I), it plans to advance its pipeline; after successful development of the proof-of-concept.

Weaknesses: Areas of strategic improvements. They reflect the challenges ahead and can be viewed as opportunities for growth and development.

- **Early development stage:** because NR-001 would be in pre-clinical development, uncertainties about safety, efficacy, and potential complications when transitioning towards human trials might be raised. By achieving *in vivo* PoC, the risk should be substantially reduced.
- **Financial risks:** like any early-stage biotechnological company, NanoRare might face financial challenges. To begin operations, NanoRare will require substantial funding to carry out research activities, as well as subsequent financing rounds (seed A, B, etc.), to advance our research to clinical trials, and eventual market launch.
- **Manufacturing challenges:** gene therapy manufacturing processes are often complex and costly. Challenges could arise when scaling up these processes, ultimately affecting timelines and financial resources moving forward. For that reason, NanoRare is committed to its optimization, alongside the adherence to GMPs, during the development of the lead drug candidate. A crucial step that we intend to leverage as well, when outsourcing this process to a CDMO, aiming at lowering their bargaining power.
- **Dependance of regulatory and market success of NR-001:** NanoRare's future heavily relies on the successful development and approval of its first product, NR-001. Therefore, any setbacks during this process could significantly impact its valuation, as well as future prospects.

Opportunities: Value expansion prospects. External market realities that NanoRare could capitalize to expand its value and potential return on investment.

- **High unmet medical need:** NanoRare's approach to OTC deficiency would satisfy its most critical unmet clinical need, which is to provide a one-shot, life-lasting curative solution. It also has the potential to capture the market, as the first company that develops a curative treatment will establish itself as the most relevant player.
- **Growing gene therapy market:** the expanding market suggests strong potential for growth, future partnerships, and technology licensing agreements, that could be used as different sources of revenue in the future. Consequently, this would offer significant advantages to further advance NanoRare's pipeline.
- **Potential for long-term economic impact:** NanoRare's therapy could result in substantial long-term cost savings for patients and healthcare systems, by potentially eliminating lifelong treatments and frequent hospitalizations.

Threats: Risk considerations. These areas can be seen as potential hurdles that the company might face.

- **Competitive landscape:** NanoRare faces competition from other companies developing gene therapies for OTC deficiency, such as Ultragenyx and Bloomsbury GT with their AAV-based therapies; Moderna, Genevant and

Arcturus with LNP mRNA-based therapies; or IECURE and Poseida that are currently developing integrative approaches.

- **Regulatory obstacles:** obtaining regulatory approval for gene therapies can be challenging due to stringent safety and efficacy requirements. This could lead to unpredicted delays or denial of approval. By proactively engaging with regulators to ensure our clinical development plans aligns with their expectations, NanoRare aims to minimize potential setbacks.
- **Pricing and reimbursement challenges:** the high costs of gene therapies can pose challenges when it comes to pricing and reimbursement. Because of that, NanoRare will explore different pricing strategies, such as performance-based agreements of value-based contracts, to avoid them and ensure that the therapy remains accessible and affordable, while still allowing to recoup costs associated with its development and commercialization.
- **Clinical challenges:** regarding safety, that would include: vector immunogenicity and risk of off-target effects; and efficacy, if the liver cells are not successfully targeted with the delivery vehicles and the hOTC sequence is not properly transposed to the genome. Other challenges related to OTC deficiency include the small patient population (ultra-rare disease), and the need for personalized therapies that are tailored to individual's necessities.

Upon a detailed analysis of NanoRare's strategic environment through both SWOT and Porter's Five-Force frameworks, several key insights and strategic connections emerge that present a compelling investment case for NanoRare's gene therapy.

A key strength that NanoRare plans to leverage is NR-001 anticipated superior safety profile and broader applicability, which are instrumental to establish it as a potential dominant player in the OTC gene therapy space. Coupled with the intellectual property protections, NanoRare expects to increase the entry barriers and reduce the risk of substitutes, specially from IECURE and Poseida Therapeutics.

To mitigate the hurdles, NanoRare's primary focus is on demonstrating the proof-of-concept in *in vitro* and *in vivo* conditions, what we think will substantially reduce them, as well as enhance its appeal to potential investors and future partners. In addition, NanoRare's emphasis on manufacturing optimization could assist managing the complexities when scaling-up, limiting potential pitfalls and their financial implications.

NanoRare envisions future partnerships and possible licensing agreements, what could provide significant revenue sources. The intention to broaden NanoRare's pipeline further strengthens the case for its long-term value. Since we aim to satisfy the most critical OTC unmet need in a continuously expanding market, we see an opportunity to establish NanoRare as a key player, creating a reputation among the field that could have significant financial implications in future milestones, such as an Initial Public Offering.

Our choice of proven methodologies with unparallel results will give NanoRare scientific advantage to mitigate the clinical challenges devised. After successful development and optimization of NR-001, the strategic plan includes early engagement with regulatory authorities such as the FDA and EMA to complete IND-enabling studies. This proactive commitment is meant to reduce potential regulatory risks and improve the market acceptance of NR-001, thereby minimizing the extent to which rivalry and substitutes could pose a threat (the strongest market forces that affect NanoRare's position).

NanoRare believes that offering a curative solution, combined with the potential long-term economic impact will help exert greater control over pricing, addressing the bargaining power of customers. By fostering relationships with regulatory agencies and developing innovative pricing strategies, NanoRare aims to overcome reimbursement and regulatory challenges ahead.

Consolidating insights from the SWOT and Porter's Five-force analyses, NanoRare emerges favorable positioned to initiate operations in the OTC gene therapy landscape. This approach is based on a solid scientific groundwork, early engagement with regulatory authorities, exploring potential partnerships and licensing technology agreements, and envisioning the expansion of NanoRare's pipeline to reinforce its market leadership potential. These insights lay the foundation for the VRIO analysis that follows, where we detail key resources and capabilities that could be transferred to ensure NR-001's development.

NanoRare, as a potential spin off, plans to leverage key resources and capabilities from NanoCell Therapeutics B.V. Exploiting them will allow to effectively engage with the challenges of gene therapy in the OTC context, as well as to establish a robust presence in this competitive landscape. Guided by our SWOT and Porter's analyses, we aim to fortify NanoRare's competitive positioning by utilizing the following NanoCell's key competences.

VRIO analysis

Resource or capability	V	R	I	O	What is the result?
	Is the available resource Valuable?	*How Rare is the resource?*	*Is it hard to Imitate?*	*Is the Organization capable to manage the resource?*	
Optimized Gene Editing Technology	High	High	Yes	Yes	**Long term competitive advantage**
Intellectual Property Assets	High	Medium	Yes	Yes	**Long term competitive advantage**
Scientific and Tecnical Resources	High	Medium	Medium	Yes	**Temporary competitive advantage**
Manufacturing Processes	Medium	Low	Low	Yes	**Competitive parity**
Strategic Partnerships	High	Low	Low	Yes	**Temporary competitive advantage**
Regulatory Expertise	High	High	Medium	Yes	**Long term competitive advantage**

Table 9: summary of NanoCell's key resources and capabilities that NanoRare could utilize for NR-001 development.

1. **Optimized gene editing technology:**
   In the core of NanoRare's strategy is the utilization of NanoCell Therapeutics' advance gene editing technology, the Sleeping Beauty transposon system. NanoCell has broad experience in the technological and functional aspects of Sleeping Beauty, its integration pattern, and safety features as a result of the CAR-T program. We plan to harness this knowledge for the development of NanoRare's curative approach for OTC.

2. **Intellectual property assets:**
   NanoCell's patent portfolio will be instrumental to efficiently manage and protect NanoRare's unique innovations derived from in-house research projects. These patented technologies and proprietary knowledge can provide NanoRare with a competitive advantage against competitors and new market entrants. NanoRare's novel technology platform, combining the use of the transposase with the hybrid delivery system, is predicted to lead to significant patenting activities during the R&D process. Consequently, this will lead to new composition of matter and method claims, strengthening its position in the market.

3. **Scientific and technical resources:**
   NanoRare will take advantage of NanoCell's proven expertise, fortifying its credibility and technical competence. NanoCell's team incorporates highly experienced business and scientific professionals with a track record of success in bringing new therapies to the market, what would be invaluable for advancing NR-001 from PoC stage to clinical trials, and eventual commercialization. The specific knowledge and skills required have been gathered over years of dedicated research, what makes them significantly difficult to replicate, providing NanoRare with unique advantages. This expertise will be crucial when addressing the challenges identified in the SWOT analysis.

4. **Manufacturing processes:**
   Benefiting from NanoCell's manufacturing capabilities and expertise, NanoRare aims to exercise significant control over the production process, ensuring product quality, regulatory compliance, and reducing third-party dependencies. Specialized suppliers and manufacturing companies already associated with NanoCell could also contribute to the production of NR-001. In addition, a preferred vendor suitable for GMP process development and a GMP compliant manufacturing company will be selected once the research-scale manufacturing optimization is completed.

5. **Strategic partnerships:**
   Similarly, NanoCell's existing strategic alliances offer NanoRare potential benefits in terms of funding, resources, and ultimately market reach. NanoCell has identified numerous GMP-compliant suppliers and academic partners that could provide access to critical resources – ranging from laboratory facilities to conduct the research project, raw materials for the production of the gene therapy, crucial scientific and business expertise to advance the therapies to clinical stage and market authorization, or access to healthcare providers, such as the Utrecht Medical Center, to secure patient's availability when transitioning to the clinical phase. These partnerships could help mitigating the weaknesses and threats that NanoRare might face, ensuring a resilient growth trajectory.

6. **Regulatory expertise:**
   With NanoCell's extensive experience navigating the regulatory landscape concerning gene therapies; after successful developing PoC and conducting clinical trials, NanoRare could expedite the approval of its novel therapy. This expertise will be of vital importance when engaging with regulatory agencies, such as the FDA and the EMA, or specifically the Medicine Evaluation Board in the Netherlands, for IND-enabling requirements and

eventual drug approval. Transferring this knowledge to NanoRare could significantly alleviate the identified clinical challenges and regulatory hurdles, providing a formidable competitive advantage.

NanoCell's key resources and capabilities highlighted in green on the table above – gene editing technology, intellectual property assets, and regulatory experience – have been identified as NanoRare's potential long-term competitive advantages. The strategic plan aims to transform these advantages into NanoRare's Core Competencies, harnessing their potential to overcome upcoming challenges and gain a distinctive edge in an increasingly competitive landscape.

The successful transference of these resources to NanoRare underlines the strategic proposition for the development of NR-001. On one hand, it strengthens the investment appeal for NanoRare, showcasing the unique scientific advantages that are intended to be leveraged in this arena. On the other hand, it reinforces the rationale behind NanoCell's strategic divestment to NanoRare, highlighting a clearly designed and focused strategy to exploit these core competencies, maximizing resource utility and market potential.

The strategic analysis began with Porter's Five-force, identifying external pressures that might influence NanoRare's venture. Then, we used a SWOT analysis to better comprehend NanoRare's internal capabilities and how to use them to confront external market realities. This exploration led us to a VRIO examination, where NanoCell's key resources were identified to discern the long-term competitive advantages that could be transferred to NanoRare as core competencies. The insights derived from this analysis allowed us to crystallize this competitive landscape into a perceptual map, as a clear visual representation for our positioning in the market.

The following perceptual map is a graphical tool that offers an overview of the gene therapies currently being developed and where they locate within the competitive landscape, by carefully analyzing two core dimensions: **therapeutic efficacy** and **technological innovation**.

The therapeutic efficacy encompasses measures such as the safety profile and the curative potential of a therapy, including the consideration of its duration of effect. Each player was scored on a scale of 1 to 3.

- **Safety profile:** is based on the curative potential offered by each therapy and the need for re-administration. In this way: 1 point for treatments with potential vector immunogenicity due to vehicle re-administration on a monthly basis; 2 points for treatments with moderate safety profiles, requiring re-administration every few months – yearly; and 3 points for therapies with a strong safety profile, that offers a one-shot cure with no re-administration.
- **Expression:** associated with the length of effect. Here, 1 point for therapies that yield transient expression (weeks to a month); 2 points for episomal expression (months to several years, if cells do not divide); 3 points for stable chromosomal integration (life-long benefits).

The technological innovation pertains to the novelty and sophistication of each approach, its potential for future adaptability, and the competitive advantages derived from the technology. Each competitor was scored on a scale of 1 to 3.

- **Sophistication of approach:** 1 points for technologies that represent minor improvements to existing methods; 2 points for technologies that introduce novel

mechanisms; 3 points for breakthrough technologies that significantly change the existing landscape.

- **Future adaptability:** 1 point for technologies with limited adaptability; 2 points for technologies with moderate versatility; and 3 points for technologies with high adaptability that can be applied in other therapeutic areas.
- **Derived competitiveness:** 1 points for technologies with a limited competitive edge; 2 points for technologies with a moderate advantage; and 3 points for unique technologies with strong intellectual property protections that renders a clear competitive superiority.

Through this classification, we intend to demonstrate NanoRare's unique position in this space, with a distinct combination of high therapeutic efficacy and cutting-edge technological innovation. Consequently, the following perceptual map aims to manifest the compelling investment opportunity that NanoRare represents within the realm of gene therapy for OTC deficiency.

Perceptual map

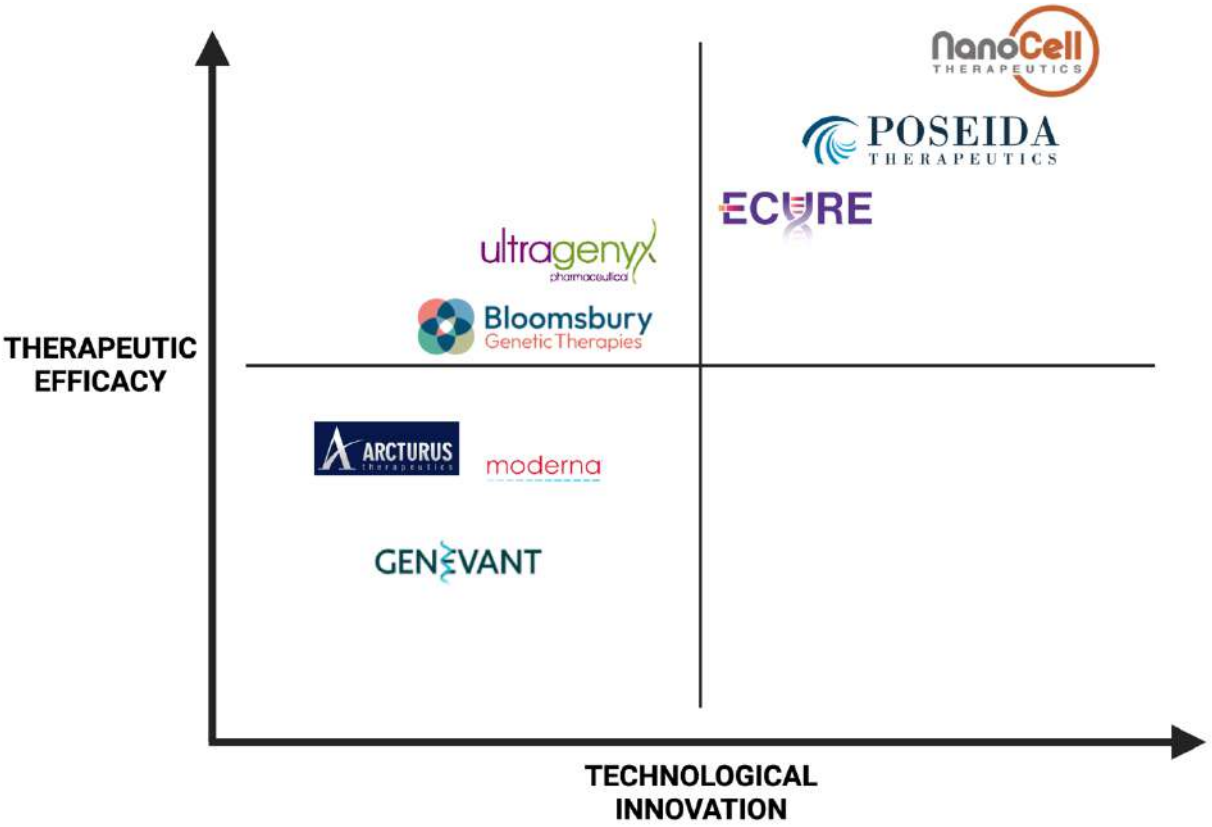


Figure 12: Perceptual map of relevant competitors within the OTC gene therapy landscape defined by therapeutic efficacy and technological innovation dimensions.

As it has been carefully described on our scientific differentiator section, NanoRare's therapy present itself as the most promising and innovative solution for the treatment of OTC deficiency with a gene therapy approach.

In the bottom-left quadrant of the perceptual map, we find the LNP mRNA-based therapies. These present a temporary therapeutic solution, as their functionality depends

on the repeated translation of the mRNA molecules to produce physiologically relevant OTC protein levels. Arcturus Therapeutics, for instance, is currently exploring a twice-a-month dosing regimen for OTC patients aged 12 and above. However, the recurring need for re-administration of this therapy inherently limits its curative potential and increases the risk for vector immunogenicity.

In the top-left quadrant, AAV-based therapies are positioned. These offer increased therapeutic efficacy when compared to LNP mRNA-based approaches due to the prolonged duration of effect. This is attributed to the nature of the approach, where a single-strand DNA molecule, delivered by an AAV, leads to continued mRNA production. However, the therapeutic effect of these therapies is temporary when administered to neonatal-onset patients due to high cell division rates, leading to dilution of the delivered hOTC-corrected sequence.

The top-right quadrant includes the integrative approaches, such as those proposed by IECURE and Poseida Therapeutics. Both therapies aim for permanent integration of the hOTC sequence, albeit using different mechanisms. IECURE integrates the hOTC sequence into the PCSK9 locus using a meganuclease, while Poseida Therapeutics utilizes the SuperPiggybac transposon system. In contrast, NanoRare leverages the optimized gene editing technology developed by NanoCell Therapeutics, offering a safer and more thoroughly characterized genome editing tool.

In contrast to Poseida Therapeutics' method, the distinguishing factor in NanoRare's approach lies in the choice of transposon system for the stable integration of the hOTC-encoding sequence. We utilize the Sleeping Beauty system, which has demonstrated lower genotoxicity and a more favorable integration profile, thus establishing itself as a safer alternative. Additionally, the concise window of our gene-editing machinery's expression, ranging from 2 to 4 days, affords a greater control and enhanced safety profile. This is particularly important when compared to IECURE's approach, where the meganuclease sequence is delivered via an AAV in a ssDNA molecule. This process usually entails protein production for a considerably extended period of time, potentially lasting for weeks or even months, depending on the cell division rate. As previously indicated, this factor hinders IECURE's safety features: the longer time gene-editing technologies remain in the cellular cytosol, the higher probability of unwanted genomic alterations because of off-target effects is originated.

The integrative approaches, nonetheless, represent the pinnacle of innovation, offering a potential safe, efficacious, and curative solution for OTC deficiency. Furthermore, these approaches could also present applicability that spans beyond OTC deficiency management for the treatment of other genetic disorders.

Taking into account our strategic positioning analysis, NanoRare's scientific differentiators, as well as key resources and capabilities that are available to us, a highly compelling argument for investment is presented. Our unique approach to gene therapy, bolstered by the insights derived from our Porter's Five-force and SWOT analyses, illustrates the potential value that could be obtained from funding NanoRare's venture.

Moreover, the strategic divestment from NanoCell Therapeutics would further enable NanoRare to exploit the envisioned core competencies, to successfully navigate the complexities of this competitive and challenging landscape. This approach not only aligns with NanoRare's strategic intent – to become the most relevant player in the OTC gene therapy landscape – but also amplifies the potential for substantial returns for investors.

CONCLUSION

The essence of the business plan presented was to assess the potential feasibility and market opportunities for a breakthrough gene therapy called NR-001, to be developed by NanoRare - a potential spin-off of NanoCell Therapeutics B.V - and intended to provide a curative solution for the treatment of OTC deficiency. This assessment is based on a detailed exploration of the scientific rationale behind the approach, an analysis of the current market landscape and the design of a strategic plan to ensure its development.

It is undeniable that gene therapies are set to revolutionize the treatment of genetic disorders. They have emerged as potential game-changing approaches, as their functionality is based on the correction/reversal of disease-causing genetic alterations. Thus, gene therapy could offer a definitive and curative solution, representing a transformative leap in the treatment of genetic disorders for which there is currently no cure. Given their disruptive potential, they have triggered fierce competition, with the first successful market entrant likely to secure a significant advantage, as it could capture the entire target market.

There is a pressing need for more effective and durable therapeutic alternatives, which represent the most important unmet clinical need for OTC deficiency. Current standard treatments are not curative, and liver transplantation remains inaccessible for many, due to lack of donor availability and patient eligibility issues. In response, NanoRare designs its lead drug candidate, NR-001, to provide a one-time, lifelong curative treatment at the time of diagnosis.

This novel drug construct leverages recent scientific and technological innovations with the proposed strategic business plan to deliver a technically superior approach that could differentiate it in the competitive landscape. Based on proven methodologies, the curative potential of NR-001 relies upon: stable chromosomal integration, enhanced safety through the hybrid approach and Sleeping Beauty transposase, and broader applicability to severe/neonatal and late-onset patient groups. These scientific advantages, while "theoretical," are grounded in rigorous scientific research and, importantly, have been tested in clinically relevant OTC models. Also, they will be crucial when addressing the clinical challenges devised to increase the long-term benefits of our solution. NanoRare will work to translate these recent achievements into a first-in-human clinical trial.

These scientific advantages lay a solid foundation for the business strategy, which translates into the business fit of NR-001. NanoRare’s approach is designed to deliver improvements in clinical outcomes, reduce healthcare costs and substantially mitigate patient burden. These distinctive attributes shape the connection to the unmet clinical needs of the OTC, and will manifest as unique selling points for the proposed treatment.

At the core of NanoRare’s strategy is the successful development of our LDC, reinforced by the scientific review of third-party preclinical reference data and detailed overview into the research and development plan. NanoRare anticipates significant patenting activity during the R&D trajectory, with several new composition of matter and method claims. Crucially, NanoRare's intellectual property protection will come from the use of proprietary technology (LNP and AAV delivery vehicles) combined with the Sleeping Beauty gene editing system. With an adaptive and comprehensive R&D strategy, NanoRare is confident that NR-001 will be optimized for clinical trials.

To ensure NanoRare's sustainability, we used analytical tools such as SWOT, VRIO and Porter's Five Forces to identify strategic advantages and current dynamics that could be leveraged to navigate the complex rare disease market and address anticipated threats. This allowed to highlight three long-term competitive advantages currently utilized by NanoCell's Therapeutics, which could be effectively transferred to NanoRare, in the form of core competencies, to overcome the challenges ahead.

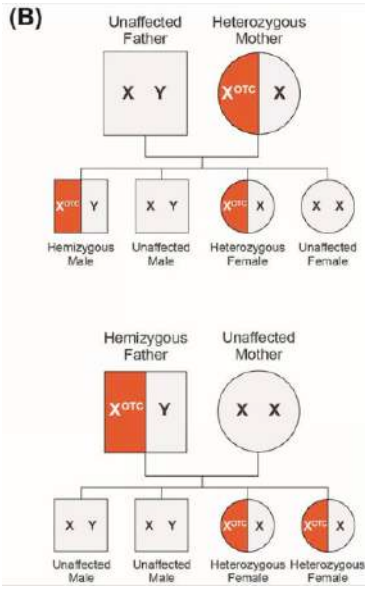
Because integrative approaches represent the pinnacle of innovation, offering safe and potentially curative solutions for genetic disorders, our analysis indicates a strong prospective position for NanoRare in this space. This venture is meant to be beneficial for all stakeholders, ensuring an investment in NanoRare extends beyond financial means, offering valuable assets in terms of skill-set, intellectual property and key resources as described. Importantly, NanoRare anticipates substantial growth and opportunities in this rapidly expanding industry, because of the potential for strategic partnerships and technology licensing agreements.

Investor exit strategies predict three possible avenues – early sale of the therapeutic application outside NanoRare’s focus, a merge and acquisition by a larger pharmaceutical or biotechnology firm, or an IPO on a reputable stock exchange. Notably, the timing of these exits is aligned with major milestones and value inflection points at NanoRare’s development pipeline, offering a clear path for an increased return on investment.

As NanoRare navigates this competitive landscape, the adaptability will allow to incorporate new findings and ideas, to stay at the forefront of these innovative field. By divesting from NanoCell Therapeutics, NanoRare seeks to exploit the identified long-term advantages to become a key player in the OTC gene therapy market.

SUPPLEMENTAL MATERIAL

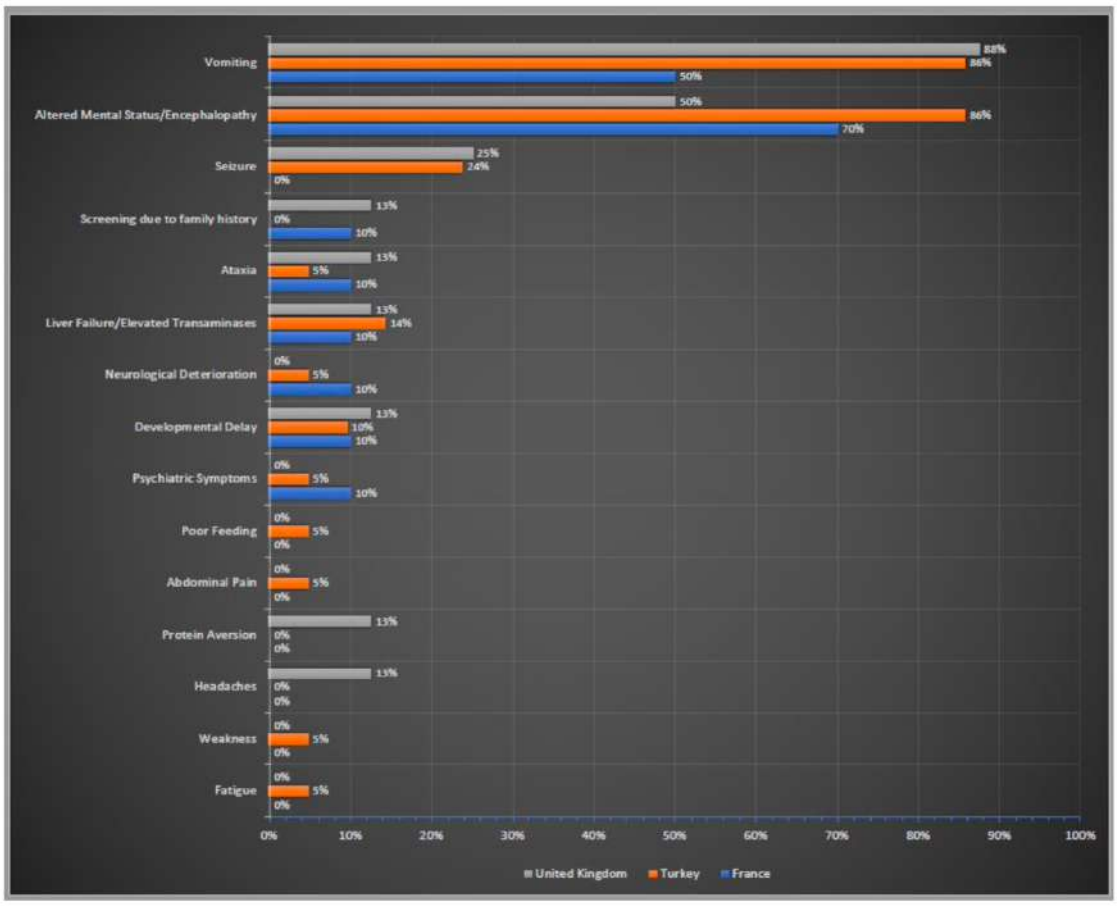
<u>Supplemental Figure 1:</u> OTC deficiency follows a pattern of X-linked inheritance. Picture extracted from the research article: "A complex case of delayed diagnosis of ornithine transcarbamylase deficiency in an adult patient with multiple comorbidities", by Jessica Abbott (48).



Woman who carry one copy of the OTC deficiency gene have a 50% chance of passing the pathogenic variant to their child during pregnancy. All daughters from men with OTC deficiency will inherit the mutated gene. However, sons of men with only one copy of the gene will not inherit it. Woman who carry one copy of the gene are commonly referred to as carriers; however, this term can be misleading because they may actually experience symptoms of OTC deficiency.

<u>Supplemental Figure 2:</u> Graphs extracted from **Three-Country Snapshot of Ornithine Transcarbamylase Deficiency article.** Panel **D**: initial clinical symptoms of OTC; panel **E**: protein restriction intervention; panel **F**: ammonia scavenger usage.

D



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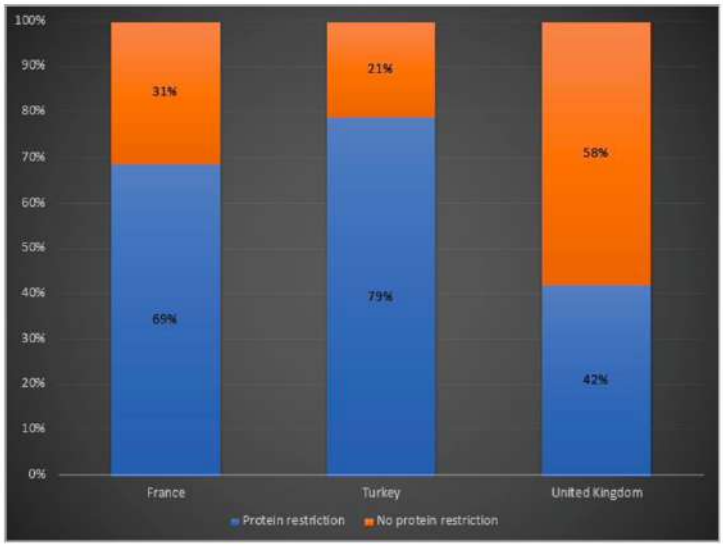

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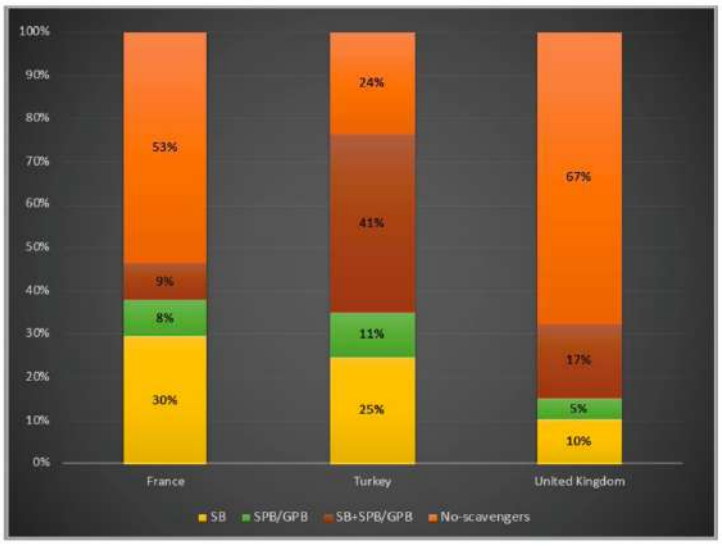
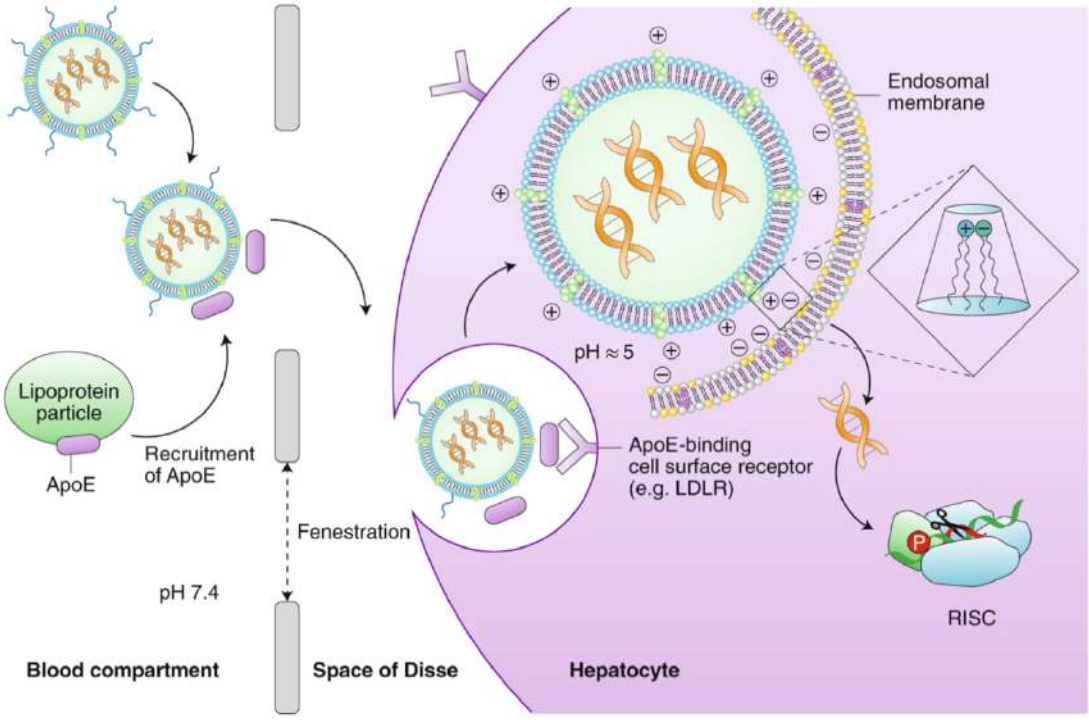


<u>Supplemental Figure 3:</u> Onpattro lipid nanoparticle (LNP)-mediated delivery of siRNA to hepatocytes *in vivo*. Picture extracted from the paper "The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs" review, by Akin Akinc et. al. 2019.



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