NANEMIAR

Nanomedicine Approach to Normalize Erythrocyte Maturation in Congenital Anemia by Messenger RNA

D4.2 NANEMIAR REPORT 1

SHORT DESCRIPTION

This document briefly describes the progress of the project during its first year of execution (October 1, 2023 - September 30, 2024).

DISSEMINATION STATUS

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NANEMIAR

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LIST OF ABBREVIATIONS

Abbreviation	Description
DEP	Dissemination and Exploitation Plan
DMP	Data Management Plan
DoA	Description of Action
eGFP	Enhanced green fluorescent protein
GA	Grant Agreement
HSPC	Hematopoietic stem and progenitor cell
KPIs	Key performance indicators
LNP	Lipid nanoparticle
WP	Work Package

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Executive summary

NANEMIAR's project ambition is to develop a targeted mRNA-based therapy for congenital anemias involving a proof-of-concept study for β-thalassemia. This annual report provides a project summary, followed by an outline of the project management and implementation in the first year. The collaborative nature of the consortium supports the effective organization of the work, with all deliverables of action reached in time. A detailed description of the scientific progress can be found in the technical report. Here, the objectives of individual work packages (WP1-3) are listed as well as the status towards the achievement of each of these project objectives. Finally, the progress towards delivering impact is described in line with the communication and dissemination strategy of the action. The project has received significant attention through various communication channels managed by the project partners, and preliminary results have been shared at several conferences. The next steps for the coming year are to expand the data package following the description of the action, increase the visibility of NANEMIAR, and establish a pathway for exploitation.

On 1 July 2024, the NANEMIAR project monitoring meeting was held, with the presence of the project coordinator and partners, who are also the work package (WP) leaders. This meeting focused on the critical review of the status of project execution to date (M1 – M9), as well as planning for the next 3 months (M1-M12). Specifically, it was divided into two parts:

1. critical review of scientific progress (WP1-WP2), as well as management (WP4)
2. critical review of the implementation of the Dissemination and Exploitation Plan (WP5)

Critical review of scientific progress (WP1-WP2) focused on:

- Revision of operation development at WP level to ensure that the WP objectives are met.
- Identification of risks or deviations from plan (if applicable) and determining corrective actions (if necessary).
- Validation of the consortium targets and agreement on commitments for the coming months.
- Preparatory work on WP3 is also mentioned, although this WP has not yet started.

Critical review of management and central coordination tasks (WP4) focused on:

- Revision of upcoming deliverable reports and next deliverables to be prepared
- Revision of compliance with milestones and next milestones to be achieved
- Revision of risks and possible corrective actions
- Revision of the status of continuous reporting through the Funding and Tenders Portal

Based on the conclusions of this meeting, report M7 of the critical review of scientific progress (WP1-WP2), as well as the status of management tasks (WP4) has been prepared for internal use (August 28, 2024). The M7 report served as the basis for preparing this deliverable document.

A critical review of the implementation of the Dissemination and Exploitation Plan (WP5) is available in the M9 report prepared for internal use (August 28, 2024).

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1. Project summary

1.1. Context and overall objectives

The NANEMIAR project emerges amidst a transformative era in therapeutics, where conventional drug strategies have historically relied on small molecules. However, a paradigm shift is underway, with antibody-based drugs and genetic-based therapies for rare diseases gaining traction. This shift is driven by a growing recognition of the limitations of traditional approaches and the immense potential of innovative modalities to address previously unmet medical needs. In this context, NANEMIAR stands at the forefront of a therapeutic revolution, poised to harness the full potential of therapeutic mRNA.

Traditionally, mRNA has been primarily associated with its role in protein synthesis within cells. However, recent advances in mRNA technology have unlocked its therapeutic potential, paving the way for a new era of precision medicine. Unlike conventional approaches, which often lack specificity and precision, mRNA therapy offers the potential for highly targeted interventions, tailored to the individual needs of patients. As such, mRNA therapy offers a promising strategy, with the flexibility to address a wide range of diseases and conditions.

By fine-tuning mRNA expression and optimizing delivery mechanisms, NANEMIAR's project ambition is to develop a therapy for congenital anemias such as β-thalassemia and Sickle Cell Disease. These conditions, characterized by disruptions in normal blood cell production, pose significant health burdens to affected individuals and their families. Current treatment options, while providing some relief, often fall short in efficacy and are associated with undesirable side effects. By leveraging mRNA as a therapeutic agent, NANEMIAR aims to overcome the challenges and effectively restore normal blood cell production while minimizing off-target effects. Within the 3-year project, we aim to deliver proof of concept of a bone marrow-targeted nanomedicine in human and animal thalassemia models. To this end, we defined the following key objectives (Figure 1):

1. Development of a novel therapeutic mRNA with bone marrow specific delivery through targeted lipid nanoparticles (LNP). Activity will be analyzed in hematopoietic stem and progenitor cells (HSPCs) from healthy human donors and through (biodistribution) studies in mice.
2. Pharmaceutical testing in a thalassemic mouse model to determine dosing and toxicity of the therapeutic formulation.
3. Pre-clinical analysis of the therapeutic formulation in bone marrow cells from beta-thalassemic patients as proof of concept for efficacy.

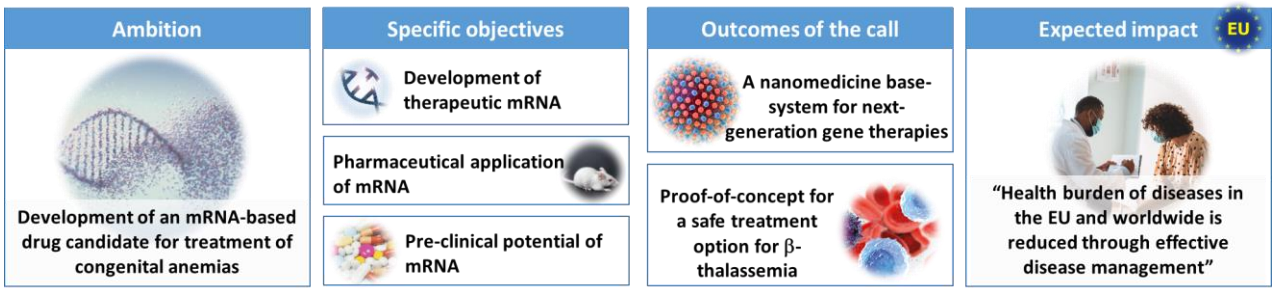

Figure 1. NANEMIAR's ambition.

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Upon success, NANEMIAR's impact is not limited to the realm of medicine. The societal and economic implications of the project's success are profound. By offering a safer, more cost-effective alternative to current treatment modalities, NANEMIAR's therapy has the potential to alleviate the burdens placed on healthcare systems and improve the quality of life for patients and their families. Additionally, by advancing our understanding of mRNA-based therapies and targeted delivery mechanisms, NANEMIAR contributes to the broader scientific and technological landscape, opening new avenues for innovation and discovery.

1.2. Work performed and main achievements

The first year of the project is mainly focused on the first objective of establishing the groundwork for a novel mRNA therapeutic that can be effectively delivered to specific cells in the bone marrow. We followed a parallel strategy on designing and testing mRNAs and LNPs in various models, including an immortalized cell model and primary HSPCs from human donors. Protocols for mRNA delivery have been validated across consortium partners and we demonstrated relevant levels of therapeutic protein expressed in HSPCs. Of note, LNP-mediated delivery appeared not straightforward in the HSPCs, so we are testing various LNP formulations for their activity. In addition, the physicochemical properties and shelf life of these new formulations are analyzed to ultimately generate stable nanoparticles that can be used for targeted delivery. Preliminary data on targeted delivery to the bone marrow suggests that we identified a robust conjugation technology to couple targeting moieties (e.g., peptides) to the shielding lipids of the LNP. Next steps include the investigation of biodistribution and activity of these formulations in mice.

1.3. Results beyond the state of the art

The project aims to deliver significant scientific and technological advances by *i)* creating new knowledge on targeted non-viral delivery (via LNPs) of mRNA that may be used for other oligonucleotide strategies, such as siRNA or gene editing (e.g., CRISPR) therapies, and *ii)* demonstrating bone marrow targeting may be useful for therapy development for other rare hematological diseases. Finally, mRNA-based supplementation of gene defects can be used to de-risk future gene therapy approaches. Hence, outcomes in this project provide insights that are broadly applicable at three levels:

1. At the highest level, our study will indirectly affect up to 6% of the world population suffering from a genetic disease that is potentially curable by cell and gene therapy. The benefits range from quality-of-life improvements to many additional years of survival, depending on the disease.
2. At the mid-level, it offers a model therapeutic that can be exploited for other rare inherited anemias ineffective erythropoiesis.
3. And at the most immediate level, approximately 1.5% of the global population is β-thalassemia carrier, with an estimated incidence of symptomatic cases in the EU of 1 in 10.000 individuals.

Based on the above numbers, the NANEMIAR project has a clear scale-up potential and we are exploring potential pathways to commercialization by involving end-users and stakeholders in the project. When the therapeutic formulation shows promising results, the consortium can establish the tools and network to prioritize and perform the activities that are required to bring the formulation to the next phase: pharmacology, pharmacokinetics, and toxicology assessments, GMP setup, safety and efficacy studies in

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humans (Clinical Phase I/II). Ultimately, we aim for co-development with large pharma as they have the experience for Phase III studies and market approval.

1.4. Policy relevance of NANEMIAR project

From a strategic standpoint, NANEMIAR is well-positioned to capitalize on emerging opportunities in the biopharmaceutical industry. With a multidisciplinary consortium comprising leading experts in molecular biology, biotechnology, and clinical research, the project benefits from diverse perspectives and complementary expertise. Through strategic partnerships and collaborations, NANEMIAR aims to translate its research findings into tangible therapeutic solutions, with the potential to transform the lives of patients worldwide. In this way, our approach and vision are completely aligned with the priorities of the Horizon Europe Strategic Plan with expected impacts in *Cluster 1 Health*, by delivering a technological breakthrough with future potential for destination 3 *tackling diseases and reducing disease burden*. Through the development of innovative therapeutic approaches, the project will specifically contribute to more effective, cost-efficient and affordable treatment for patients with a rare disease.

Additionally, it contributes to the Goals of the IRDiRC Orphan Drug Development on: *Goal 2 – 1000 new therapies for rare diseases will be approved, the majority of which will focus on diseases without approved options*, and *Goal 3 – Methodologies will be developed to assess the impact of diagnoses and therapies on rare disease patients*.

In summary, NANEMIAR represents a beacon of hope in the quest for effective treatments for hematological disorders such as congenital anemias. By harnessing the power of mRNA therapy and targeted delivery mechanisms, the project offers a promising pathway to improved outcomes for patients and a brighter future for healthcare innovation.

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2. Project management and implementation

2.1 List of participants and researchers involved in the project

Partner nº	Role	Short Name	Legal name	Country	Leader of WP
1	Coordinator	FFIS	Fundación para la Formación e Investigación Sanitarias de la Región de Murcia	Spain	WP3, WP4 and WP5
1.1	Affiliated entity	SMS	Servicio Murciano de Salud	Spain	
2	Beneficiary	MERCURNA BV	Mercurna BV	Netherlands	WP1
3	Beneficiary	CNRS	Centre National de la Recherche Scientifique CNRS	France	WP2

During the first year of the project, there have been no changes in the initial composition of the consortium.

Researchers involved in the project are listed below:

Beneficiary	Gender	Nationality	Career Stage	Role of researcher in the project
1-FFIS	Woman	Spain	Category B - Senior Researcher (Senior Researcher/ Associate professor)	Leading
1-FFIS	Woman	Spain	Category C2 - Recognised Researcher (Post-Doctoral Researcher)	Team member
1-FFIS	Woman	Spain	Category C2 - Recognised Researcher (Post-Doctoral Researcher)	Team member
1.1-SMS	Man	Spain	Category B - Senior Researcher (Senior Researcher/ Associate professor)	Team member
1.1-SMS	Man	Spain	Category B - Senior Researcher (Senior Researcher/ Associate professor)	Team member
1.1-SMS	Man	Spain	Category B – Senior Researcher (Senior Researcher /Associate professor)	Team member
1.1-SMS	Women	Spain	Category D2 – Other First Stage researcher	Team member
2-MERCURNA BV	Woman	Netherlands	Category A - Top Grade Researcher (Full professor/Director of research)	Leading
2-MERCURNA BV	Man	Netherlands	Category A - Top Grade Researcher (Full professor/Director of research)	Team member
2-MERCURNA BV	Man	France	Category C2 - Recognised Researcher (Post Doctoral Researcher)	Team member
2-MERCURNA BV	Man	Netherlands	Category D1 – First Stage Researcher (PhD student)	Team member
3-CNRS	Man	France	Category A - Top Grade Researcher (Full professor/Director of research)	Leading
3-CNRS	Woman	France	Category D2 – Other First Stage researcher	Team member

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Social impact Indicator: **Nº of researchers hired* under the project during the period (M1-M12) = 5**

Total Nº of researchers	Nº of researchers hired under the project during the period (M1-M12)
13	5
100%	38%

* Researchers hired under the project marked in bold in the previous table

Complementing our gender balanced NANEMIAR team, all hiring processes complied with transparency and inclusion, giving opportunities to both women and men. The FFIS-IMIB contracting process included the publication of the public open call through the EURAXESS portal.
The spirit of collaborative multidisciplinary approaches has been engrained in the appointed team members of NANEMIAR to form the new generation of early-career researchers.

2.2 List of Deliverables

WP Nº	Deliv. Nº	Deliverable Name	Lead Beneficiary	Type	Dissem. Level	Due Date	Delivery Date	Status
WP1	**D1.1**	**THERAPEUTIC mRNA**	**MERCURNA BV**	**DATA**	**SEN**	**31-mar-24**	**28-mar-24**	**Submitted**
WP1	**D1.2**	**Targeting moiety**	**MERCURNA BV**	**DATA**	**SEN**	**30-sep-24**	**Expected 28-sep-24**	
WP1	D1.3	Optimal biodistribution	MERCURNA BV	DATA	SEN	31-mar-25		Pending
WP2	D2.1	Mouse ex vivo efficacy	CNRS	DATA	SEN	30-sep-25		Pending
WP2	D2.2	Dosing protocol	CNRS	DATA	SEN	31-mar-26		Pending
WP2	D2.3	Proof Of Concept in mice	CNRS	DATA	SEN	30-sep-26		Pending
WP3	D3.1	Human ex vivo efficacy	FFIS	DATA	SEN	30-sep-26		Pending
WP4	**D4.1**	**Data Management Plan**	**FFIS**	**DMP**	**SEN**	**31-mar-24**	**26-mar-24**	**Submitted**
WP4	**D4.2**	**NANEMIAR report 1**	**FFIS**	**R**	**PU**	**30-sep-24**	**26-sep-24**	**Submitted**
WP4	D4.3	NANEMIAR report 2	FFIS	R	PU	30-sep-25		Pending
WP4	D4.4	Data Management Plan 2	FFIS	DMP	SEN	31-may-26		Pending
WP5	**D5.1**	**NANEMIAR Website**	**FFIS**	**DEC**	**PU**	**31-dec-23**	**27-dec-23**	**Submitted**
WP5	**D5.2**	**Dissemination and Exploitation Plan**	**MERCURNA BV**	**R**	**PU**	**31-mar-24**	**26-mar-24**	**Submitted**
WP5	**D5.3**	**Dissemination and Exploitation report 1**	**FFIS**	**R**	**PU**	**30-sep-24**	**Expected 27-sep-24**	
WP5	D5.4	Dissemination and Exploitation report 2	FFIS	R	PU	30-sep-25		Pending
WP4	D4.4	Data Management Plan 2	FFIS	DMP	SEN	31-may-26		Pending
WP5	D5.5	Dissemination and Exploitation report 3	FFIS	R	PU	30-sep-26		Pending

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Process Indicator: **Nº of deliverable submitted on time* / total Nº of deliverables to be submitted during the period (M1-M12) = 7 / 7 = 100%**

Total Nº of deliverables	Total Nº of deliverables to be submitted during the period (M1-M12)	Nº of deliverable submitted on time
16	7	7
100%	43%	100%

*On time means <= Due date / Submitted deliverable marked in bold in the previous table

2.3 List of Milestones

Milestone Nº	Milestone Name	WP Nº	Lead Beneficiary	Means of Verification	Delivery Date	Delivery Date (actual)	Achieved
1	**Partnership agreement**	**WP4**	**FFIS**	**Agreement signed**	**31-oct-23**	**20-oct-23**	**Yes**
2	**Project plan**	**WP4**	**FFIS**	**Existing SOPs exchanged**	**30-nov-23**	**20-nov-23**	**Yes**
3	**Recruitment completed**	**WP1, 2,3,4**	**FFIS**	**Employment contracts arranged**	**31-dec-23**	**31-dec-23**	**Yes**
4	**Therapeutic mRNA design**	**WP1**	**MERCURNA BV**	**Optimized expression in vitro**	**31-jan-24**	**29-feb-24**	**Yes**
5	**Reporter protein expression in HSPCs**	**WP1**	**FFIS**	**Transfection conditions selected**	**31-jan-24**	**29-jan-24**	**Yes**
6	**Screening target moiety completed**	**WP1**	**MERCURNA BV**	**Peptides with highest safety selected**	**31-may-24**	**28-may-24**	**Yes**
7	**Critical review of project execution and progress – 1**	**WP4**	**FFIS**	**Report discussed in quarterly project management meeting**	**31-aug-24**	**28-aug-24**	**Yes**
8	Critical review of project execution and progress – 2	WP4	FFIS	Report discussed in quarterly project management meeting	31-aug-25		Pending
9	**Critical review and updates of the Dissemination and Exploitation plans – 1**	**WP5**	**FFIS**	**Report discussed in quarterly project management meeting**	**31-aug-24**	**28-aug-24**	**Yes**
10	Critical review and updates of the Dissemination and Exploitation plans – 2	WP5	FFIS	Report discussed in quarterly project management meeting	31-aug-25		Pending
11	Targeted LNP formulation selected for in vivo application	WP1	MERCURNA BV	Coupling target moiety to LNP with high efficiency	31-oct-24		Pending

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12	Delivery and activity in mouse HSPCs confirmed	WP2	CNRS	Optimal expression THERAPEUTIC ex vivo	31-mar-25		Pending
13	Dosage for repeated dosing established	WP2	CNRS	Maximum tolerated dose established in single injection	30-sept-25		Pending
14	Treatment protocol established	WP2	CNRS	Pilot completed	31-mar-26		Pending
15	Transfection efficacy ex vivo patient samples	WP3	FFIS	Optimized expression ex vivo	30-jun-26		Pending

Process Indicator: **Nº of milestones achieved on time* / total Nº of milestones to be achieved during the period (M1-M12) = 8 / 8 =100%**

Total Nº of milestones	total Nº of milestones to be achieved during the period (M1-M12)	Nº of milestones achieved on time
15	8	7
100%	53%	46%

*On time means <= Due date / Milestones achieved marked in bold in the previous table

As outlined in the tables above, information on Milestones M1-M7 and M9 is uploaded to the Funding and Tenders Portal. The slight delay of submission of M4 by one month has no impact on the implementation (time and/or budget) of WP1 tasks. No deviation from the DoA is identified.

Next planned milestone for October 31, 2024, is:

WP Nº	Milestone Nº	Milestone name	Lead beneficiary	Delivery date	Status on September 30, 2024
1	M11	Targeted LNP formulation selected for in vivo application	MERCURNA BV	31-oct-24	Experimental work ongoing; description portal pending to be prepared

2.4 Project management – work package 4

Objective: Coordination of NANEMIAR

Main activity	As planned	Notes
Task 4.1: Central coordination (M1 – M36)		
Technical coordination	Yes	Project implementation follows the described action; milestones and deliverables have been uploaded to the EU portal in timely matter.
Partner Communication & dispute resolution	Yes	Kick-off meeting within 3 months after start of the project and follow-up progress meetings were held every 3-6 months. For ease of communication and collaborative work, a NANEMIAR dedicated Microsoft Sharepoint (including OneDrive and Teams) has been created.
Legal and Contractual issues	Yes	Grant Agreement and Consortium agreement have been signed; no issues to be reported
Financial & reporting	Yes	Prefinancing contributions have been distributed to partners upon receival by the coordinator. According to CA, to carry out the proper monitoring of the budget

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		implementation, the parties shall submit internal financial reports to the coordinator at M15. The first financial reporting is scheduled for M18.
Strategic coordination and connection with EC officers	Yes	Communication with EC officers is well established. The coordinator and WP leaders also met the project officer face-to-face during an EC-coordinated meeting in Brussels (March 14, 2024).
Task 4.2: WP, quality and risk management (M1 – M36)		
DMP	Yes	The DMP has been generated according to the HORIZON template data management plan and submitted as D4.1 (M6)
Management of WP operational development	Yes	According to milestone 2 (M3), existing protocols were exchanged between consortium partners to arrange a swift project start and manage operations.
Coordination of time and budget per WP	Yes	Work progress is discussed during regular meetings and no issues regarding time or budget have been reported so far.
Task 4.3: Strategic management (M1 – M36)		
Periodic validation of the consortium targets	Yes	Targets are discussed during bi-annual coordination meetings and relevant actions are taken to warrant project.
Communication to all project stakeholders	Yes	The coordinator is in close contact with all project partners and has dedicated personnel to deal with project management and communication.
Adjustments of the development trajectory in case of unforeseen events	Yes	New risks have been appropriately managed. For example, a new risk related to Task 2.1 was detected during the kick-off meeting, and effective mitigation measures were taken without major consequences to the planned scientific work.

During the 1st project implementation period, the coordinator has worked under **Task 4.1 central coordination** to ensure progress of the different WPs and manage the agreed workplan and framework with its deliverables and milestones. To facilitate collaborative work between the partners, a NANEMIAR dedicated Microsoft SharePoint (including OneDrive and Teams) has been created. Within Microsoft Teams, a general channel has been created for all consortium members to work on the scientific WP1-3, as well as two private channels for WP4 and WP5 with restricted access for management only. This warrants consortium communication and safe storage of documents. Mailing lists have been maintained and updated for the different WPs to ease information exchange. The consortium members work smoothly together and have facilitated necessary progress of the work.

In terms of meetings, the Kick-off meeting and 1st General Assembly meeting (hybrid session) was organized on November 20, 2023 (Murcia, Spain). This involved the introduction of the research teams, the review of the objectives of the WPs, as well as defining the next actions. Coordination Meetings (online) between the coordinator and WP leaders were scheduled every ~6 months (January 30 and July 1, 2024). These meetings involved monitoring the targets, overall project implementation, milestones achievements, deliverable production, risk and deviations as outlined in the DoA. In addition, the research team held conference calls for scientific discussion to manage scientific

Figure 2. NANEMIAR Kick-off meeting

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progress, organize their work and plan next steps (January 30, May 6, and July 1, 2024). The 2nd consortium meeting and General Assembly meeting is planned for October 17-19, 2024 (Marseille, France).
The Coordination Team (FFIS-IMIB) has been in close contact with the EU Project Officer. As mentioned above the coordinator and WP leaders also met the project officer face-to-face during an EC-coordinated meeting in Brussels (March 14, 2024).

Following **Task 4.2 (WP, quality and risk management)**, the DMP was drafted and submitted (D4.1, M6). To warrant smooth operational activities, general work protocols are being exchanged through SharePoint (Milestone 2: Existing SOP protocols). During the Kick-off meeting (November 20, 2023), a new risk related to Task 2.1 was detected, initially not foreseen in the GA. Immediately in the following month, appropriate measures were taken to mitigate it without major repercussions on the planned scientific work. The description of this risk will be available in the periodic report (M18), as well as in the continuous reporting section on Funding and Tenders Portal.

In relation to **Task 4.3 Strategic management**, the opportunity to include a new partner in the consortium has been reviewed, in response to the European Hop on Facility call. By consensus of all project partners, it was considered that the new potential partners did not fit or add extra value to the NANEMIAR project. Furthermore, by the time the Hop on Facility call is closed and awarded, the NANEMIAR project will be halfway its execution making it less attractive to add a new partner.
Further details on the continuous reporting are outlined below in sections 2.1 – 2.4.

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3. Technical report

3.1 Explanation of the work carried out and overview of the progress

3.1.1 Objectives

Core to our NANEMIAR project is the use of mRNA as a potential new treatment option for patients with congenital anemia, that is characterized by ineffective erythropoiesis (red blood cell production) and a deficiency in functional erythrocytes. Using mRNA is the active pharmaceutical ingredient, it needs to accomplish: i) proper engagement of the cellular ribosome to initiate protein translation and ii) avoid activation of the intracellular innate immune system. Additionally, it needs to be protected from degradation in the bloodstream and delivered to organ/cells of interest. Lipid nanoparticles (LNPs) are considered the most optimal vehicle for RNA delivery.

Within the first months of the project, we focused on a parallel strategy in terms of designing and testing selected mRNAs and LNPs in the various models available within the consortium. Next to the well-established immortalized K562 cells (an erythroleukemic model), an efficient protocol was established for the culture and differentiation of hematopoietic stem and progenitor cells (HSPCs) from mobilized bone marrow healthy human donors. Within WP1, we designed a strategy for therapeutic mRNA selection and tested several mRNAs in these relevant cell models. Through well-organized exchange of protocols and standard operating procedures, the delivery of mRNA to cells has been established across all consortium partners and mRNA-mediated therapeutic protein expression has been validated. For delivery of mRNA, we have also tested various LNP formulations, using different (proprietary and commercial) lipids in various molar ratios. Here, we analyze their physicochemical properties, shelf life, and activity. While most formulations show efficient mRNA delivery (expression of enhanced green fluorescent protein eGFP as reporter) in the K562 cell line, this needs further optimization for HSPCs (currently ongoing).

In line with the objective of the action, we are also focusing on establishing bone marrow targeted delivery. Of note, the field of tissue targeting through targeted LNPs is still in its infancy. While we have all necessary expertise and facilities present in our consortium to achieve active delivery of LNPs to the bone marrow, we have also increasingly realized the competition in the field as well as the inherent complexity of the avoiding the liver as main 'off-target organ'. As outlined in WP1, we started with a selection of candidate targeting moieties for bone marrow targeting. These include peptides known bind a well-known receptor present on erythroid progenitor cells that are involved in the pathogenesis of ineffective erythropoiesis. Preliminary analysis in K562 cells showed no toxicity or other adverse effects of the peptides, which will be verified in HSPCs (health volunteers) in the coming months. In addition, we are optimizing our conjugation technology for coupling of the peptide to LNPs through simultaneous testing of various strategies, with the goal to generate stable nanoparticles with good activity. Preliminary experiments in wildtype mice to study biodistribution of different LNP formulations are ongoing.

For WP2, the main objective is to assess the therapeutic formulation in thalassemic mice. A well-established model has been ordered and mice are backcrossed to expand the homozygous population. Also, a novel protocol is established for analysis of hematopoietic cell distribution using spectral flow cytometry, which will support future studies in this work package. And in relation to his, we are also optimizing the culture of HSPCs from mouse bone marrow for initial testing of the therapeutic mRNA.

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3.1.2 Explanation of the work carried out per WP

Work Package 1 - Development of therapeutic mRNA (M1-M18)

Objective: Optimize therapeutic mRNA and its efficient delivery to the bone marrow

Experimental work in this WP is running as described in the DoA, with good progress on the therapeutic mRNA. The design and testing of mRNA have been performed within the first six months of the project, and activity was confirmed in K562 cells and health donor HSPCs. Of note, we used the commercial transfection reagent Lipofectamin Messenger Max for the delivery of mRNA. Efficient delivery through LNPs, using eGFP reporter mRNA, is established in K562 cells (>90% showed eGFP expression) but needs further investigation for the HSPCs as they are likely harder to transfect cells (<5% with eGFP expression). An additional aspect could be the culturing and differentiation conditions of these cells. In the coming months, we will study various LNP formulations to *i)* validate mRNA delivery in HSPCs, *ii)* improve stability/shelf life (to at least 3 weeks), and *iii)* establish bone marrow targeting for the in vivo studies.

Regarding the targeting, we have also initiated the testing of several targeting moieties (peptide and antibody), which run simultaneously with optimizing the LNP formulation. This involves establishing the conjugation technology to the shielding PEG-lipids, assessing molar ratios of lipids in the LNP, analyzing its physicochemical parameters, validating activity in cells, and ultimately studying biodistribution in vivo. We expect to run several pilot biodistribution studies in the coming months, expecting the project to move along as described in the DoA. Further details are noted in the table below.

Main activity	Achieved	Outcome / Notes
Task 1.1: mRNA design and in vitro testing of activity (M1 – M6)		
mRNA design	Yes	As outlined in report D1.1 (M6), we will continue working with the wildtype sequence (using RIBOPRO's sequence optimization technology for de-immunization).
Transfection conditions	Partial	Lipofectamine-based transfection of HSPCs, isolated from mobilized healthy human donors led to good expression of reporter eGFP mRNA. The cells showed minimal expression when using LNPs, which will optimized by: 1. Testing new LNP formulations (different lipids and molar ratios) 2. Adjust dosing and timing of LNP addition to the cells 3. Use targeted LNPs (from Task 1.2)
Functional impact therapeutic mRNA	Yes	Therapeutic mRNA transfection (using Lipofectamine) did not significantly affect colony formation in healthy donor HSPCs.
Task 1.2: Identification and characterization of bone marrow targeting moiety (M4-M12)		
Selection of candidate targeting moieties	Partial	Following literature study, peptides have been selected based on binding to a well-known receptor on erythroid progenitor cells. Increasing peptide concentrations (up to 30M) were added to K562 cells and did not show adverse effects. Next step is to test the effect on healthy donor HSPCs. In addition, we plan to take along a CD117 antibody-based targeting approach as recently published (Breda et al., 2023). Of note, CD117 is expressed on short- and long-term HSCs and less specifically on the erythroid progenitor cells.
Targeted LNPs	Partial	The general strategy includes the conjugation of ligands (peptide or antibody) to functionalize PEG-lipids by click chemistry. These lipids are either incorporated into

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		the LNP prior to the conjugation or as a post-insertion method (after LNP generation). Preliminary data has been gathered on both approaches in K562 cells and needs to be further validated in healthy donor HSPCs and in mice. Afterwards, further optimization might be needed to enhance stability and/or activity (see risk 2).
Task 1:3 Biodistribution and safety of bone marrow targeted LNPs in mice (M1-M18)		
Biodistribution in wildtype mice	No	Training is ongoing and Pilot experiments with fluorescent mRNA are ongoing, and first data is expected in Q4 2024.

Work Package 2 – Pharmaceutical application of therapeutic mRNA (M1-M36)

Objective: Test the safety and efficacy of the therapeutic mRNA formulation in thalassemic mice

The B6.D2-Hbbd3th/BrkJ mice have been ordered. We received 5 heterozygous mice (1 male and 4 females) and started breeding 6 females for backcrossing before beginning to raise them. A total of 14 WT mice and 8 heterozygous mice were born. The team engineer is currently training for intravenous (IV), intraperitoneal (IP), and intrafemorally (IF) injections. mRNAs coding for fluorescent proteins will be injected for the initial experiments. Additionally, bone marrow will be collected from the first generation of homozygous mice. In parallel, we have been working on bone marrow extracts and blood from WT mice to develop a new protocol for analyzing hematopoietic cell distribution. This protocol is based on the combination of over 20 primary antibodies and spectral flow cytometry analysis. Thanks to precise computational analysis and gating of populations based on various markers, we can now identify the hematopoietic population in a single analysis. We have decided to write up this protocol and publish it.

Main activity	Achieved	Outcome / Notes
Ethical approval	Yes	Because of the change of mice (discontinued) we had to resubmit a project. Project is under the evaluation by the ethical committee, but small changes have been requested following the application of new European roles. Breeding of thalassemic mice is ongoing
Task 2.1 Evaluate the efficacy of therapeutic mRNA in restoring erythrocyte differentiation ex vivo using mouse bone marrow sample from thalassemic mice (M1-M24)		
Isolation and culture of HSPCs from bone marrow of thalassemic mice	No	Thalassemic mice have been ordered and received. We decided to backcross them once more before starting. This is now done. Isolation and culture of cells from bone marrow is expected to be up and running in the coming months.
Transfection conditions	No	Expected Q1 2025
Functional impact therapeutic mRNA	No	Expected Q2 2025

Work Package 3 – Pre-clinical potential of therapeutic mRNA (M19-M36)

Objective: Test the efficacy of the therapeutic mRNA formulation in HSPCs of beta-thalassemia patients.

WP3 is scheduled to start next year months 19, but the culture protocol of cells obtained from thalassemic patients has been optimized and established, as well as the CFU assay used for counting red blood cell colonies.

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Moreover, all patients were already informed about the project and informed consents were signed. So, one of the potential risks associated with WP3 is already solved.

1.1.2 Deviations from Annex 1 and Annex 2 (if applicable)

The testing of mRNA-LNP formulations is expected to take more time than initially scheduled in the DoA, since we are trying several different combinations of lipids to ultimately make a more informed decision on the formulation to take forward to WP2 and WP3. Additionally, there seems to be variation in the LNP shelf life, which would argue for using the LNPs as fresh as possible. As such, we propose CNRS obtain a microfluidic machine. This would allow for additional *ex vivo* and *in vivo* screening at CNRS. Mercurna can provide the necessary lipids and mRNA.

CNRS has agreed to a trial period of 2 months for the machine with provider Cytiva, to test the abovementioned actions. Once agreed with all consortium partners that this strategy works well, we would like to propose the purchase of the machine by CNRS. As there is currently no equipment expense included in the budget in the GA, the coordinator will then contact the Project Officer to inform and review whether any amendments are necessary. According to the GA, the project has budgetary flexibility - this allows changes between the budget items granted. It is noted that the eligible costs for equipment are only depreciation costs, so a large part of the purchase amount would have to be charged to indirect costs or other financing sources.

There are no further deviations from the DoA detected; the implementation of the project continues as planned and described in the GA.

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4. Impact

As described in the DoA, outcomes of our NANEMIAR project will uniquely contribute to delivering proof of concept for an innovative targeted mRNA-based therapy treatment of beta-thalassemia that is aimed at having a societal and economic impact once brought to market. The project also has the potential to generate a wider impact by delivering significant scientific and technological advances on *i)* creating new knowledge on targeted non-viral delivery that may be used for other oligonucleotide strategies, and *ii)* demonstrating effective bone marrow targeted LNPs that may be useful to drug development programs for other rare hematological diseases. NANEMIAR´s key element of the impact (target groups / outcomes / impacts) is shown below:

TARGET GROUPS	OUTCOMES	IMPACTS
Who will use or further up-take the results of the project? Who will benefit from the results of the project?	*What change do you expect to see after successful dissemination and exploitation of project results to the target group(s)?*	*What are the expected wider scientific, economic and societal effects of the project contributing to the expected impacts outlined in the respective destination in the work programme?*
Scientific community: field of congenital anemia. **Pharma companies**: future licensees for bringing new drug to market **Clinicians**: potential new β-thalassemia treatment **End-users**: β-thalassemic patients	High use of the scientific discovery published (measured with the relative rate of citation index of project publications). A big pharma company shows interest in partnering for future drug development plans. mRNA therapy uptake: clinicians and patients express positive for participation in future clinical trials.	**Scientific**: New breakthrough scientific discovery on mRNA therapy opening the way for treatments of non-iron anemia beyond β-thalassemia. This innovative therapeutic approach will lead to the more effective disease management and the reduction of the health burden of disease. **Economic**: Future treatment that reduces seriousness of the disease or its co-morbidities (by means of improved efficacy and safety of the new drug), reduces costs related to transfusion and chelation drug costs, and secondary healthcare costs. Definitely, the health care systems benefit from innovation expertise. **Societal**: Outreach and communication make patients (and general public) more knowledgeable of new treatment alternatives and better adhere to new knowledge-based disease management strategies. Ultimately, treatment may increase patients' ability to work/participate in society.

4.1 Project pathway to impact

Based on the early stage of the project, the current levels of impact are minimal. Importantly, we have established a dissemination and exploitation plan (D5.2, M6) that outlines the communication and dissemination strategy towards reaching the identified target groups and initiated a path for exploitation. The latest contributions to communication and dissemination are described in the recent report (D5.3, M12) and summarized in the sections below.

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Since this report refers to the first year of project implementation, the results and publications will be produced in the following period.

4.2 Open Science

A data management plan has been prepared (D4.1, M6) where the consortium described their strategy towards the accessibility of data and the options for its re-use. Given the early project stage, the consortium has not yet published or openly shared their research. Of note, protocols and methods are being exchanged through a shared consortium folder to ensure swift data collection and reproducibility of results across consortium partners.

4.3 Dissemination and exploitation – work package 5

During the 1st year of project implementation, the focus was on creating the visual identity of the project, as well as materials, tools and channels to support NANEMIAR´s communication and dissemination strategy.

Main activity	As planned	Notes
Task 5.1: Creation and update of the Dissemination and Exploitation Plan, including Communication activities (M1-36)		
Creation DEP	Yes	Described in deliverable D5.2 (M6)
Periodic update DEP	Yes	Described in deliverable D5.3 (M12), and next one is scheduled for M24
Task 5.2: Open dissemination and collaboration (M1-36)		
Active dissemination through partner channels and networks	Yes	Various meetings were attended where the project (and preliminary results) was presented. Further details in deliverable D5.3 (M12)
Participation in events	Yes	Further details in deliverable D5.3 (M12)
Task 5.3: Creation and promotion of the NANEMIAR project (M1-36)		
Project identity package	Yes	The project website (D5.1; M3) and promotion material (inc. project flyer, roll-up) has been produced. Further details in deliverable D5.3 (M12)
Periodic communication	Yes	Social media accounts are open and actively managed, and various communication activities have been carried out for promotion of NANEMIAR. Further details in deliverable D5.3 (M12)

Looking at the tasks in more detail, the Dissemination and Exploitation Plan (DEP), including Communication activities was established under Task 5.1 and submitted (D5.1, M6). A detailed record of all dissemination and communication activities has been created and kept. It is available to the consortium in the dedicated shared folder in Teams to continuously monitor, assess and improve DEP effectiveness.

The project visual identity, website (www.nanemiar.eu) and the promotional materials produced under Task 5.3 (Creation and promotion of the NANEMIAR project) have been designed and developed (Figure 3, Figure 4).

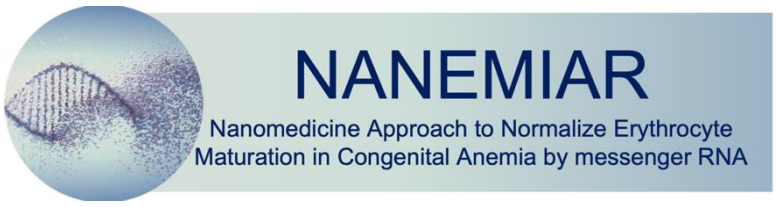

Figure 3. NANEMIAR logo

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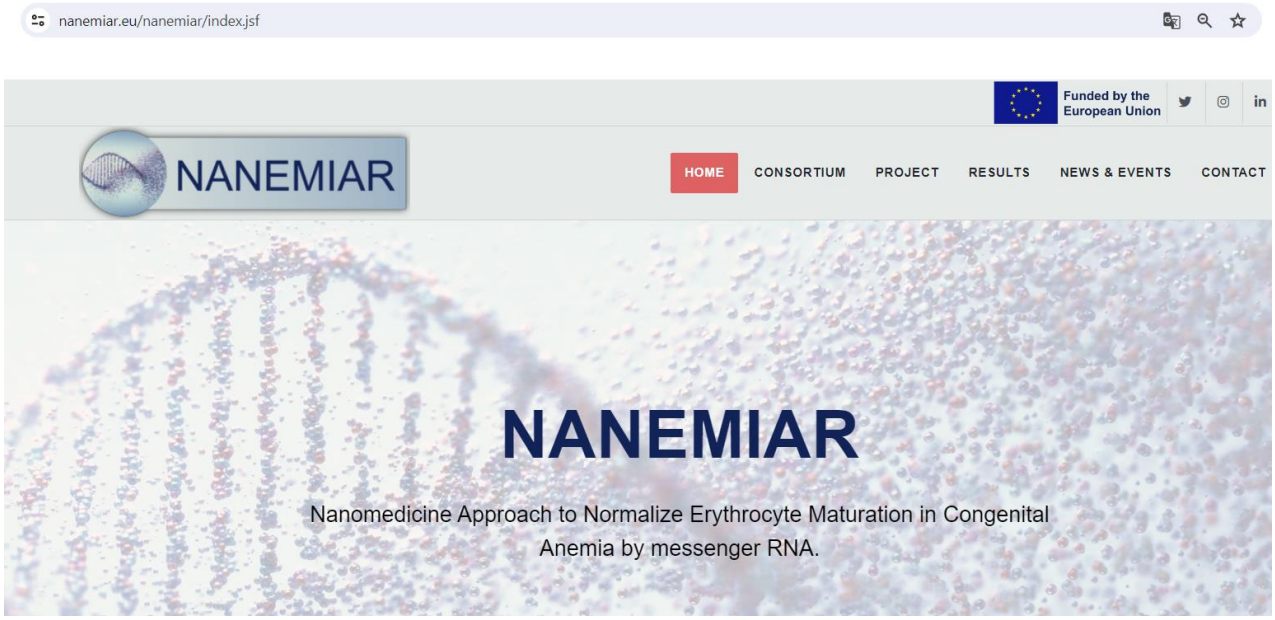

Figure 4. NANEMIAR website (homepage screenshot)

NANEMIAR social media accounts have been opened and are actively managed to Increase project awareness, engage stakeholders, and generate new leads for exploitation. Specifically, NANEMIAR has accounts at **X, Instagram, and LinkedIn:**

X – Profile: @NANEMIAR https://x.com/nanemiar

Instagram – Profile: @NANEMIAR https://www.instagram.com/nanemiar

LinkedIn – Profile: https://www.linkedin.com/showcase/nanemiar

#innovation #research #EUproject #HorizonEurope #EUfunded #congenitalanemia #mRNA

The project partners have also actively participated in different scientific events, and initiatives focused on the general public (e.g., radio interview), as part of Task 5.2 (Open dissemination and collaboration). More detailed information on these activities carried out during the 1st year of the project is available in the deliverable D5.3 Dissemination and Exploitation report 1 (M12).

4.4 Update of the plan for exploitation and dissemination of results

List of the communication and dissemination activities carried out in the context of the project during its 1st year of implementation (period M1-M12) is available in the deliverable D5.3 Dissemination and Exploitation report 1. These activities are adjusted to those planned in the deliverable D5.2 Dissemination and Exploitation Plan submitted in M6. No update to this plan is required currently.

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5. Conclusions

5.1 Scientific progress

Scientific progress of the project is going as expected and is executed along the lines of the DoA. We have shifted more focus to screening of LNPs as it has become increasingly clear how intricate the LNP formulation (lipid composition and percentage) is in order to achieve milestone 11 (selecting targeted LNP formulation selected for in vivo application). Nevertheless, there has been good progress on the characterization of the targeting moiety and conjugation technologies, as will be further outlined in Deliverable 1.2 Targeting Moiety. Depending on the outcome of the LNPs within the coming months, we may have to anticipate a slight delay in Deliverable 1.3 Optimal Biodistribution. But as indicated, we are testing various LNP formulations (different lipids, compositions) to resolve this. And, as outlined above, we have included a potential mitigation measure for the distribution of LNPs and increasing capacity by CNRS opting for a demo model of Cytiva. Moreover, the patients from WP3 are already enrolled, which provides us with extra time to develop the best LNP formulation.

5.2 Management tasks and continuous reporting status

As outlined above, the project management is executed well in close contact with the EU project officers and by meeting nearly all deadlines for submission due dates.

The summary for publication has been uploaded on Portal, together with the NANEMIAR project information sheet to promote the project. Further information in the subsection regarding project results and main achievements will be included alongside the project duration. Additional information regarding the continuous reporting is being completed on the Funding and Tenders Portal (e.g., foreseen and unforeseen risks that materialized in the first year of the project, communication activities and dissemination).

The 2nd consortium meeting and General Assembly meeting is planned for October 17-19, 2024 (Marseille, France). During this meeting, the progress of the project will be reviewed, as well as the strategy for exploiting results. Also, NANEMIAR consortium will review within the coming months the possible purchase of equipment by CNRS, initially not foreseen in the GA.

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NANEMIAR

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