CG-AD211: A New Therapeutic Paradigm for Alzheimer's Disease

Project Concept for the Development of a Genetic Pharmaceutical for Alzheimer's Disease (AD) Therapy.

The overall concept of the project is based on a comprehensive integration of traditional elements, which have stood the test of time, and innovative components that leverage the latest advancements in the field, all meticulously combined to create a supra-additive effect that delivers a unique, cutting-edge solution. Within this concept, the following components are included:

1. Focus on Pathology: Priority attention is given to the disposal of pathological intracellular protein aggregates and to the processes of neuroregeneration.
2. Multiplicity of Targets: Therapeutic versatility is achieved through the use of a composition of genes responsible for a range of biological processes — including the disposal of pathological protein aggregates, neuron restoration, and the regeneration of synaptic connections.
3. Unique DNA Vectors: Our therapeutic DNA vectors of VTvaf17 and GDTT1.8NAS series developed and patented as a platform solution, elegantly combine efficacy, safety, and the flexibility to vary with universal and tissue-specific promoters and coding sequences of target genes.
4. Precision Expression: The use of combinations of tissue-specific and inflammation-activated promoters within DNA vectors guarantees precise and efficient expression of target genes in specific cells, enhancing overall therapeutic efficacy and minimizing ectopic effects. The application of functionalized microspheres "DNA + Liposome + PEG" ensures affinity to target cells and contributes to achieving maximum efficacy while reducing aberrant effects.
5. Advanced Delivery Systems: A complex of cationic liposomes plus PEG functionalized with specialized reagent has been selected as the delivery system, which ensures the achievement of therapeutically significant concentrations of target proteins, thereby maximizing the drug's therapeutic effect.
6. Use of Native Genes: The incorporation of only natural human genes into the medicinal product ensures harmonious integration of the drug's action with natural biological processes, reducing the risk of adverse reactions and enhancing the drug's biocompatibility.
7. Regulatory Compliance: The composition of structural elements of the vectors, developed and patented as part of our platform solution, fully complies with FDA and EMA requirements, guaranteeing adherence to strict safety and efficacy standards.
8. Technological Efficiency. The use of proven technologies allows the drug to be manufactured at various standard biotechnological facilities, achieving both competitive pricing and high profitability.

Such an integrated combination of traditional and innovative approaches enables the development of a unique drug for AD therapy with a supra-additive effect that exceeds the sum of individual components. This solution ensures high efficacy and safety of treatment, meeting modern medical needs.

Components and Stages of Developing a Pharmaceutical for Alzheimer's disease Therapy:

I. Strategy Development
II. Therapeutic Implementation
III. Description of Tools
IV. Gene Selection
V. Delivery Methods
VI. Treatment Protocols
VII. Economic Feasibility

I. Strategy Development

To develop a reliable strategy it is proposed to use only proven approaches, facts, arguments in the project, i.e. to confirm experimentally the relationship between dynamic changes in biological indicators with changes in the expression level of target genes.

Alzheimer's disease is a multifactorial condition, and the development of a successful therapy requires not only considering the full spectrum of biological disturbances (factors) associated with the disease but also ranking them by significance with a mandatory assessment of the scientific and experimental evidence pertaining to each.

Understanding of the pathogenesis of Alzheimer's disease has significantly evolved in recent years. Studies indicate that critical cognitive and other physiological impairments are not caused by the accumulation of amyloid plaques (cerebral amyloidosis) but are predominantly associated with an excess of pathological tau aggregates. This leads to neurodegeneration, neuronal death, and ultimately atrophy of brain tissue. As a result, there is a slowing or complete disruption of signal transmission along neural pathways, which causes a wide range of neurological symptoms.

Based on this, the formation of amyloid plaques in the intercellular space is considered an insignificant manifestation within this project and does not entail targeted therapeutic intervention by the developed pharmaceutical on this process. However, the extent of cerebral damage by amyloid conglomerates can significantly influence the efficiency of drug delivery to target cells in various brain regions. When determining the final therapy regimen with the developed pharmaceutical for patients with Alzheimer's disease, it is advisable to consider the degree of cerebral amyloidosis. In cases where it exceeds the level of "Braak B (Stage II)—Moderate level of amyloid deposits," preventive administration of drugs to minimize amyloid formations, such as Aducanumab, Solanezumab, and others, should be employed.

Aspects related to the direct minimization of inflammation are also not considered priorities in composing the pharmaceutical formulation for AD therapy in this project. It is implied that this manifestation of AD will be subjected to indirect therapeutic effects during the implementation of the main objectives of the project, as well as medicinal correction using modern, highly effective pharmaceuticals available on the market (basic immunotherapy [immunosuppressants], glucocorticoids, monoclonal antibodies to block immune cell infiltration into the central nervous system, sphingosine-1-phosphate receptor modulators, etc.).

Similarly, aspects associated with the disruption of calcium homeostasis are not prioritized in the composition of the pharmaceutical for AD therapy in this project. It is assumed that these manifestations of AD will be addressed therapeutically using modern, highly effective drugs available on the market (Nimodipine and other L-type calcium channel blockers).

Tasks related to the direct prevention of hyperphosphorylation and aggregation of tau proteins are not considered within this project. Nonetheless, there may be an indirect positive therapeutic effect on this biological process during the achievement of the project's primary objectives. The development of a preventive pharmaceutical to inhibit hyperphosphorylation and aggregation of tau proteins is the subject of a separate project.

The project's strategy involves the creation of a pharmaceutical agent for the therapeutic implementation of the following biological processes directly related to the disease, for which there are currently no registered medications: the disposal of formed pathological (aggregated) intracellular protein formations; and the regeneration of damaged target regions of the brain.

A detailed rationale underpinning the biological processes and the therapeutic priorities is provided in the following table:

Disease Manifestations (Biological Processes), Pathogenesis	Therapeutic Task	Inclusion of the Therapeutic Task in the Project Strategy	Comment
Formation of Tau Aggregates	Disposal of Pathological Intracellular Tau Aggregates	Yes	The issue of disposing of pathological intracellular tau aggregates is a key aspect of Alzheimer's disease therapy and currently lacks widely accepted therapeutic solutions
Formation of Intracellular Amyloid Aggregates	Disposal of Pathological Intracellular Amyloid Aggregates	Yes	Disposal of intracellular amyloid aggregates is also an important aspect of therapy
Formation of Extracellular Amyloid Formations	Disposal of Pathological Extracellular Amyloid Formations	No	This aspect is not considered a priority, as it can be addressed with the existing market-available medications
Oxidative Stress	Minimization of Oxidative Stress	No	This aspect is not considered a priority, as it can be addressed with the existing market-available medications. There may be an indirect positive

			therapeutic effect during the implementation of the project's strategic objectives
Mitochondrial Dysfunction	Restoration of Mitochondrial Functions	No	This aspect is not considered a priority, as it can be addressed with the existing market-available medications. There may be an indirect positive therapeutic effect during the implementation of the project's strategic objectives
Disruption of Calcium Homeostasis	Normalization of Calcium Homeostasis	No	This aspect is not considered a priority, as it can be addressed with the existing market-available medications
Inflammation	Minimization of Inflammation	No	This aspect is not considered a priority, as it can be addressed with the existing market-available medications. The anti-inflammatory therapy regimen should be harmonized with the dosing schedule of the drug under development. There may be an indirect positive therapeutic effect during the implementation of the project's strategic objectives
Damage and Death of Neurons	Neuroregeneration of Damaged Neurons; Neurogenesis of New Neurons	Yes	Neurons damaged due to the accumulation of pathological intracellular protein aggregates undoubtedly require regeneration to restore bodily functions. The process of neurogenesis is also important in the context of this task, but the possibilities for its activation are limited due to the

			reduction in the population of neuronal stem cells.
Disruption of Protein Phosphorylation Balance, Especially Tau	Prevention of Hyperphosphorylation and Aggregation	No	This is a preventive aspect and is the subject of developing a separate pharmaceutical. There may be an indirect positive therapeutic effect during the implementation of the project's strategic objectives

II. Therapeutic Implementation

The therapeutic implementation (Integral Task) applied in this project consists of formulating therapeutic stages, incorporating into each of these stages a list of biological processes subject to therapeutic intervention, assigning weight coefficients to these biological processes, determining optimal native therapeutic agents (genes/proteins), appropriately selecting/designing therapeutic tools—non-viral DNA vectors with cloned target genes—and composing the optimal combination of DNA vectors for each therapeutic stage.

Therapeutic Stage No. 1: Disposal of Pathological Intracellular Protein Aggregates Formed in AD.

This stage is primarily focused on the elimination of tau aggregates. It is also implied that activating the degradation system will ensure the clearance of other pathological protein conglomerates, including intracellular Aβ aggregates (it is assumed that extracellular amyloid plaques constitute 70% of the total pathological aggregates of amyloid beta-protein, while intracellular Aβ aggregates make up about 30%), TDP-43 protein, intracellular polyamides, and so on.

Therapeutic Stage No. 1 encompasses the following Local Tasks:

1. Activation of ubiquitination of pathological protein aggregates for subsequent degradation in proteasomes.
2. Activation of binding and transport of labeled pathological protein aggregates into proteasomes.
3. Activation of proteasomal disposal.
4. Activation of ubiquitination of pathological protein aggregates for subsequent degradation by autophagolysosomes.
5. Activation of binding (adapter) and transport of labeled pathological protein aggregates into the lysosomal system.
6. Activation of autophagosome formation.
7. Activation of the fusion of autophagosomes with lysosomes and the formation of autophagolysosomes.
8. Activation of degradation and exocytosis of autophagolysosomal contents.

The weight coefficients and the rationale are listed in the table below:

Local Task	Weight Coefficient	Comment
Activation of ubiquitination of pathological protein aggregates for subsequent degradation in proteasomes	~0	The proteasomal system for the disposal of protein aggregates is less significant in this project since large protein aggregates like tau and Aβ are degraded through the lysosomal system
Activation of binding and transport of labeled pathological protein aggregates to proteasomes	~0	The proteasomal system for the disposal of protein aggregates is less significant in this project since large protein aggregates like tau and Aβ are degraded through the lysosomal system
Activation of proteasomal degradation	~0	The proteasomal system for the disposal of protein aggregates is less significant in this project since large protein aggregates like tau and Aβ are degraded through the lysosomal system
Activation of ubiquitination of pathological protein aggregates for subsequent degradation by autophagolysosomes	~0.2	Activation of ubiquitination of pathological protein formations plays an important role in the lysosomal system, as ubiquitin serves as a tag for the recognition and targeting of these proteins for lysosomal degradation
Activation of binding (adapter) and transport of labeled pathological protein aggregates into the lysosomal system	~0.2	Activation of binding by adapter proteins and transport of protein aggregates in the lysosomal system plays a significant role in cellular cleansing. Adapter proteins recognize and bind pathological protein aggregates, directing them to autophagolysosomes for degradation
Activation of autophagosome formation	~0.2	Activation of autophagosome formation in the lysosomal system is highly significant for the disposal of large protein aggregates and damaged organelles from the cell. Enhancing autophagy through autophagosome formation promotes effective degradation of pathological protein accumulations

Activation of fusion of autophagosomes with lysosomes and formation of autophagolysosomes	~0.2	Activation of the fusion of autophagosomes with lysosomes and the formation of autophagolysosomes plays a significant role in the lysosomal system, as it ensures the final degradation of pathological protein aggregates and damaged organelles
Activation of degradation and exocytosis of autophagolysosomal content	~0.2	Activation of degradation and exocytosis of autophagolysosomal content in the lysosomal system plays a crucial role in the effective disposal of pathological protein aggregates and toxic substances from the cell. This contributes to maintaining cellular homeostasis and prevents neuronal damage

Therapeutic Stage No. 2: Regeneration of Damaged Target Regions of the Brain.

Therapeutic Stage No. 2 includes the following Local Tasks:

1. Activation of neural stem cells (NSCs) proliferation.
2. Differentiation of NSCs.
3. Reprogramming of astrocytes into neurons in brain regions susceptible to inflammation and degradation.
4. Neuronal polarization.
5. Neuronal migration.
6. Synthesis of tubulin, assembly and stabilization of microtubules, and regulation of their dynamics.
7. Axonal and dendritic navigation.
8. Formation of synaptic connections.
9. Differentiation of glial progenitor cells into oligodendrocyte precursor cells (OPCs).
10. Differentiation of OPCs into oligodendrocytes; formation of oligodendrocytes.
11. Enhanced synthesis of myelin by oligodendrocytes.
12. Neuroprotection.

The weight coefficients and the rationale are listed in the table below:

Local Task	Weight Coefficient	Comment
Activation of Neural Stem Cells Proliferation (NSCs)	~0.05	Neural stem cells are constitutively present primarily only in the dentate gyrus of the hippocampus and the subventricular zone, but as the disease progresses, even in these zones, a decrease in the number of NSCs is observed. In this regard, the

		process of activating NSC proliferation will be indirectly addressed through the influence of so-called hub proteins or master regulators
Differentiation of NSCs	~0.05	Stimulation of NSC differentiation is a process in which neural stem cells acquire the morphological and functional characteristics of mature cells necessary for performing specific tasks in the nervous system. This process will be indirectly addressed through the influence of so-called hub proteins or master regulators
Reprogramming of Astrocytes into Neurons in Brain Regions Prone to Inflammation and Degradation	~0	The process of replacing lost neurons through the transdifferentiation of astrocytes into new functional neurons is a promising approach that is not sufficiently studied at the moment but holds potential in the field of neuroregeneration. It is the subject of developing a separate pharmaceutical agent
Neuronal Polarization	~0.05	Proper polarization is necessary for the integration of new neurons into existing neural networks and the restoration of normal signal transmission. This process will be indirectly addressed through the influence of so-called hub proteins or master regulators
Neuronal Migration	~0.05	The process of neuronal movement is activated by various molecules and cellular interactions that guide neurons along specific paths to their target areas, where they differentiate and establish synaptic connections. This process will be indirectly addressed through the influence of so-called hub proteins or master regulators
Synthesis of Tubulin, Assembly and Stabilization of Microtubules, and Regulation of Their Dynamics	~0.2	Regulation of microtubule dynamics is important for the growth and directed projection of axons during neuron regeneration. Stable microtubules allow new neurons to properly integrate into the existing neural networks and restore synaptic connections
Axonal and Dendritic Navigation	~0.2	Following the repair of the damaged neurons, these nerve cells are supposed to establish proper connections with other neurons to form functional neural networks. Processes of axonal and dendritic navigation ensure directed growth of axons and dendrites to specific target cells, which is necessary for the restoration of synaptic connections and normal transmission of nerve impulses
Formation of Synaptic Connections	~0.2	Newly formed or restored neurons after damage must establish functional synapses with other neurons to ensure effective transmission of nerve

		impulses. If synaptic connections are not formed properly, the new neurons cannot integrate into the existing neural networks, thus restricting the recovery of cognitive functions
Differentiation of Progenitor Glial Cells into Oligodendrocyte Precursor Cells (OPCs)	~0	Aspects related to the activation of myelination processes are not considered priorities, as it is assumed that the body retains sufficient ability to myelinate new and damaged neurons
Differentiation of OPCs into Oligodendrocytes, Formation of Oligodendrocytes	~0	Aspects related to the activation of myelination processes are not considered priorities, as it is assumed that the body retains sufficient ability to myelinate new and damaged neurons
Enhanced Synthesis of Myelin by Oligodendrocytes	~0	Aspects related to the activation of myelination processes are not considered priorities, as it is assumed that the body retains sufficient ability to myelinate new and damaged neurons
Neuroprotection	~0.2	Activation of neuroprotection mechanisms helps prevent neuronal apoptosis and slows the progression of neurodegenerative changes in the brain. This allows preserving the function of existing and newly formed segments of neural networks

III. Description of Tools

At the stage of selecting tools for delivering genetic material, the possibility of using the following platforms was considered: viral vectors, DNA vectors, and mRNA platforms.

Justification for Vector Selection:

Which delivery system is better? The "battle" between proponents of viral and non-viral vectors is less a confrontation and more an evolution of two approaches that address their unique challenges. Viral vectors are effective instruments that deliver genetic material at high speed but come with potential risks, side effects, and the need for meticulous control.

Non-viral vectors, on the other hand, are flexible and safe: they can carry genetic material with minimal risks, delivering it precisely and specifically where controlled protein expression duration is required. When massive speed or systemic delivery of genetic material is not necessary, non-viral systems are optimal. Today, non-viral vectors have become significantly more effective thanks to technological "enhancements" such as liposomal transport systems, inspired by the technological leap that occurred during the COVID-19 pandemic.

The mRNA platform has also rapidly come to the forefront in drug development in recent years. However, despite all its advantages, this platform lacks effective mechanisms for ensuring tissue specificity and protection against ectopic effects.

The answer to the question "Which carrier is better?" is clear: each tool is suitable for its purpose. The flexibility and safety of non-viral vectors are ideal where targeted delivery, prolonged but limited expression period of the target protein, and minimal risks are essential. In this project, non-viral DNA vectors of the VTvaf17 and GDTT1.8NAS series fully realize their potential.

As a pharmaceutical agent meeting the criteria for solving Local Tasks and Therapeutic Implementation, a complex of non-viral DNA vectors carrying coding regions of **native human genes** was selected. Thus, the therapeutic effect is achieved by enhancing the natural functionality of the human body.

The delivery of genetic material to target cells within the medicinal product is facilitated by unique non-viral DNA vectors of the VTvaf17 and GDTT1.8NAS series, in combination with therapeutic "coding inserts." This system represents a **biomimetic mechanism** for therapeutic intervention, promoting organism regeneration and the restoration of normal functioning of its organs and systems.

Non-viral DNA vectors of the VTvaf17 and GDTT1.8NAS series comply with stringent regulatory requirements for genetic medicinal products, as outlined in the "Draft Guideline on the quality, non-clinical and clinical aspects of gene therapy medicinal products" (EMA/CAT/80183/2014, Committee for Advanced Therapies) and the "Reflection paper on design modifications of gene therapy medicinal products during development" (14 December 2011, EMA/CAT/GTWP/44236/2009, Committee for Advanced Therapies).

DNA vectors have been developed in alignment with the recommendations of FDA-2014-D-0663, ensuring no impact on cellular proliferative activity and morphology, reproductive health, or the potential transmission of antibiotic resistance genes to the environment.

Production and quality control—including safety indicators such as apyrogenicity, sterility, stability, purity, biological activity, and others—of the produced vectors are conducted in accordance with the requirements of the U.S. Pharmacopeia (USP 42 and NF 37, Chapter 1047: Gene Therapy Products), as well as the FDA guidelines FDA-2008-D-0205, *"Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs) Guidance for Industry,"* and FDA-2015-D-3399, *"Recommendations for Microbial Vectors Used for Gene Therapy."*

The non-viral DNA vectors of the VTvaf17 and GDTT1.8NAS series provide:

1. The highest level of safety due to:

- Absence of regulatory elements within the DNA vectors that represent nucleotide sequences from various viral genomes.
- No integration of DNA vectors into the genome of the target cell. Non-viral DNA vectors are used for delivering genetic material into cells but, unlike most viral vectors, remain in the cell as autonomous molecules and do not integrate into chromosomes. This avoids potential negative consequences associated with integration, such as activation of oncogenes or disruption of cellular gene function.
- Corresponding absence of risks of spontaneous oncotransformation.
- Absence of antibiotic resistance genes, thereby eliminating the risk of developing antibiotic-resistant strains.

- Targeted action: A specific promoter drives gene expression exclusively in the designated cells, thereby minimising ectopic effects, immune responses, and disruptions of cellular processes. Employing a dual-promoter architecture that operates on a logical “AND” principle—a tissue-specific segment combined with an inflammation-inducible segment—provides still greater precision, ensuring delivery not only to the target cell type but specifically to those target cells within the lesion that display inflammatory signatures.
- Encapsulation of the "DNA vector–cationic liposome" complex within an external PEG shell of low immunogenicity, which helps avoid a significant immune response.

2. Maximum efficacy due to:

- Inclusion of highly effective promoters within the vectors.
- Minimal size of the DNA vectors, allowing them to efficiently transfect target tissue cells without toxic effects.
- Encapsulation of DNA vectors in cationic liposomes and PEG, protecting nucleic acids from degradation by extracellular nucleases and increasing their stability in biological fluids. This transport system enables the most efficient delivery of genetic material into cells.
- Functionalization with specialized reagent, which is conjugated to the surface of the DNA–liposome–PEG microparticles. Functionalization with specialized reagent enhances the drug's penetration across the blood-brain barrier (BBB), and ensures maximum specificity, and affinity for cellular receptors.
- Additional efficacy—independent of the DNA-vector design or the DNA + liposome + PEG nanoparticle—is achieved through concomitant physiotherapeutic and pharmacological measures, such as focused-ultrasound (FUS), pre-emptive patient blood hydration, and other adjunctive approaches.

IV. Gene Selection

The medicinal product developer, in collaboration with engaged experts and leveraging advanced artificial intelligence-based intelligent systems and neural networks, has established an initial (baseline) list of native human candidate genes capable of executing the designated therapeutic functions.

The comprehensive task of evaluating and ranking candidate genes was addressed using the multi-round Analytic Hierarchy Process (AHP) with weighted criteria. This methodology involves decomposing the task into multiple hierarchical levels, where each subtask is individually analyzed. Therapeutic agents (candidate proteins) addressing each subtask are ranked independently, culminating in an integrated list (ranking) of candidate proteins based on these assessments.

For bioinformatics analysis, a scoring system was developed to evaluate "Benefit" ranging from +100 to 0 and "Harm" ranging from 0 to -100. This system is employed for ranking both local tasks and assigning an overall integral rating ("Weighted" final score) to each candidate gene. The "Benefit" metric accounts for the efficiency of the protein's function, as encoded by each gene, on biological

processes targeted at achieving the local task. The "Harm" metric considers the risks of oncogenic disease development, adverse events, hyperexpression, and ectopic expression.

The list of candidate genes/proteins, detailed analysis of selection stages, and results of the bioinformatics evaluation of candidate genes for implementing the therapeutic stages are presented in the tables included in the Appendix.

Optimal Promoters

To select optimal promoters by evaluating the efficiency/safety ratio for Alzheimer's disease (AD) therapy using DNA vectors that encode target genes, a separate bioinformatic analysis was conducted.

When choosing the best promoter for each gene, it is important to consider:

- Target and non-target cells/tissues
- Specific conditions in the target expression zone (inflammatory status, etc.)

Criteria for promoter selection—the ratio of effective expression level to the risks of ectopic expression and undesirable effects; duration of expression.

The use of cell-specific promoters can significantly reduce harm associated with ectopic expression of target proteins. Specific promoters ensure restricted gene expression only in certain cell types, minimizing the likelihood of undesirable effects in other tissues and cells. The use of a dual-promoter system based on the logical “AND” principle (a tissue-specific segment + an inflammation-inducible segment) enables even more targeted delivery—not just to the desired cell type, but specifically to those target cells located within the lesion area that exhibit signs of inflammation.

The most significant target cells for the chosen therapeutic strategy are neurons.

Considered options for promoters:

Universal Promoter EF1α:

The EF1α promoter is based on the gene encoding elongation factor 1 alpha, a protein involved in mRNA elongation during translation. This promoter is characterised by high basal activity and the ability to drive strong and sustained gene expression across various cell types.

Key features of the EF1α promoter:

- Constitutive activity: EF1α provides continuous gene expression regardless of external conditions, which is crucial for maintaining stable levels of a therapeutic protein.
- Broad expression profile: It is active in many cell types, including neuronal cells, making it suitable for neurotherapeutic applications such as Parkinson’s disease treatment.
- Low immunogenicity risk: EF1α elicits minimal immune responses, supporting long-term gene expression without significant inflammatory reactions.
- High expression efficiency: It ensures strong target gene expression, which is necessary to achieve a therapeutic effect.

Considered options for neuron-specific promoters:

1. CaMKIIα Promoter (Calcium/Calmodulin-Dependent Protein Kinase II Alpha):

- Specificity: Highly specific to mature postsynaptic neurons in the hippocampus and cortex, as well as in the amygdala and other limbic structures involved in emotional regulation. Also active in postmitotic, excitable neurons of the central nervous system—especially excitatory neurons.
- Advantages: Predominantly active in mature neurons, making it an excellent choice for targeting neuronal networks associated with memory and learning.

2. Thy1 Promoter:

- Specificity: Expressed in various central nervous system neurons, including motor and sensory neurons, many cortical neurons (e.g., pyramidal cells), and hippocampal neurons.
- Advantages: Widely used in neurotherapy due to its stability and neuronal specificity.

3. Synapsin I Promoter:

- Specificity: Primarily active in neurons of the central and peripheral nervous system, including cortical and hippocampal neurons, subcortical structures, brainstem centres, and spinal cord neurons.
- Advantages: Drives gene expression in synaptic cells, ensuring restriction to neural tissues.

4. Synapsin III Promoter:

- Specificity: More selective for certain neuronal subtypes compared to Synapsin I.
- Advantages: Enables precise expression in specific neuronal populations, which is useful for targeted therapies.

5. NeuroD6 Promoter:

- Specificity: Expressed in hippocampal and prefrontal cortex neurons involved in neurogenesis and the maintenance of neuronal plasticity.
- Advantages: Suitable for therapies aimed at neuronal regeneration and functional support.

Considered options for inflammation-activated promoters:

1. NF-κB Promoter:

- Specificity: Actively functions primarily in cells with activated transcription factor NF-κB. This includes cells involved in immune and inflammatory responses, such as activated immune cells (macrophages, lymphocytes), vascular endothelial cells, and cells of damaged or inflamed tissues. The NF-κB promoter is activated in response to various stimuli, including cytokines (e.g., TNF-α, IL-1β), stress factors, infections, and tissue damage.

- Advantages: Expression under the control of this promoter occurs predominantly in cells with activated NF-κB signaling, providing targeted gene expression in inflamed or damaged tissues. This allows the therapeutic genes to act only in pathological areas, reducing the risk of affecting healthy cells and minimizing side effects.

2. AP-1 (c-Fos/c-Jun) Promoter:

- Specificity: Actively functions mainly in cells with activated transcription factor AP-1, a heterodimer of proteins from the c-Fos and c-Jun families. The AP-1 promoter is activated in response to various external stimuli, including growth factors, cytokines, stress factors, ultraviolet radiation, and inflammatory processes. It operates in cells involved in processes such as proliferation, differentiation, apoptosis, and stress response.
- Advantages: Expression under the control of this promoter occurs in cells with active AP-1 signaling, providing selective gene expression in cells responding to specific stimuli or in certain physiological or pathological states. This allows targeting gene expression in cells involved in growth, development, regeneration, or responding to injury, reducing the likelihood of undesirable effects in other tissues.

3. HIF-1α-Responsive Promoter (Hypoxia-Inducible Factor 1-alpha)

- Specificity: Activated under hypoxic conditions and tissue injury.
- Advantages: Drives gene expression only when oxygen is scarce or the tissue is damaged—situations commonly found in inflamed regions.

4. STAT3-Responsive Promoter (Signal Transducer and Activator of Transcription 3)

- Specificity: Triggered by immune and inflammatory signals.
- Advantages: Enables gene expression in direct response to inflammation, allowing precise targeting of neurons located within inflamed zones.

5. CREB-Responsive Promoter (cAMP Response Element-Binding Protein)

- Specificity: Activated by increased intracellular cAMP and other signalling pathways linked to neuronal activity and cellular stress.
- Advantages: Permits gene expression in neurons that are actively firing or under stress—conditions characteristic of damaged neural tissue.

To develop a gene therapy drug aimed at the clearance of pathological intracellular protein aggregates in neurons and neuroregeneration of damaged neurons in Alzheimer's disease, it is necessary to ensure specific expression of target genes precisely in the affected neurons. This can be achieved using a dual promoter that is activated only when two conditions are simultaneously met (logical "AND"): neuron specificity and activity in neurons with pathological changes.

As a result, the dual promoter will have the following construct:

1. Neuron-Specific Part: For example, the Synapsin I or CaMKIIα promoters.
2. Inflammation-Responsive Part: Options include the NF-κB response element (4–6 tandem repeats) or AP-1 (c-Fos/c-Jun).

Architecture: A "composite" promoter where regions for NF-κB and neuronal transcription factors are adjacent, controlling a single minimal promoter upstream of the gene.

Outcome: Expression occurs only in the simultaneous presence of neuron-specific transcription factors and inflammatory transcription factors (e.g., NF-κB). This ensures the logical "AND" function.

Thus, for gene therapy constructs intended for transfection of target cells exhibiting signs of inflammatory status in Alzheimer's disease, the following variants of a dual promoter have been selected:

Name	Target Cells	Description
*** + ***	Neurons	The dual promoter *** + *** is a powerful tool for ensuring selective gene expression in inflamed neurons. By combining the neuronal specificity with conditional activation, high precision in genetic intervention is achieved. This is particularly important in the development of gene therapy approaches for treating neurodegenerative diseases and other nervous system pathologies, where selective targeting of affected cells can enhance the efficacy and safety of the therapy.

For gene therapy constructs intended to transfect target cells lacking an inflammatory status, the *** promoter has been selected.

Selection of Reagent for Functionalization

Functionalizing gene therapy constructs with specific ligands or antibodies allows for targeted delivery of therapeutic genes directly into target cells. This enhances the effectiveness of AD therapy through:

- Enhanced Targeted Delivery: Specific binding to target cells.
- Reduced Side Effects: Minimization of impact on unaffected tissues.
- Improved Therapeutic Response: Increased intracellular DNA delivery and expression of therapeutic genes.

Reagent options considered for functionalization for target cells:

- Peptide Angiopep-2:

Description: Angiopep-2 is a 19-amino-acid peptide derived from the low-density lipoprotein receptor-related protein 1 (LRP1). It specifically binds to LRP1 receptors expressed on endothelial cells of the blood-brain barrier (BBB) as well as on cells of the central nervous system, including neurons and astrocytes.

Advantages: Angiopep-2 facilitates efficient crossing of the BBB and ensures targeted delivery of genetic material to neurons. This leads to increased expression levels of therapeutic genes in these cells, promoting disposal of pathological intracellular protein aggregates and neuroregeneration. The use of Angiopep-2 enhances the effectiveness of AD therapy by improving drug delivery to target cells and reducing side effects associated with non-selective distribution.

- Tetanus Toxin C Fragment (TTC):

Description: TTC has a high affinity for gangliosides on the surface of neurons.

Advantages: Used for specific delivery to neurons and transport along the axonal tract.

- Antibodies Against Neuronal Markers:

Description: Antibodies targeting neuronal cell adhesion molecule (NCAM) or other neuron-specific antigens.

Advantages: Provide high-specificity binding to neurons.

Based on the selection criteria, the following reagent was chosen for functionalization:

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Results of Bioinformatic Analysis of Target Genes/Proteins

Based on the processing results using a three-round Analytical Hierarchy Process methodology, and with due consideration of the weight coefficients of the Local Tasks, the following genes/proteins have been included in the final (key) list:

Therapeutic Stage No. 1:

Gene/ Protein	Local Task	Rationale for inclusion	Gene Coding Size (bp)	Protein Size (kDa)	Secreted	Localization
***	***	***	***	***	***	***
***	***	***	***	***	***	***
***	***	***	***	***	***	***
***	***	***	***	***	***	***
***	***	***	***	***	***	***
***	***	***	***	***	***	***
***	***	***	***	***	***	***

Therapeutic Stage № 2:

Gene/ Protein	Local Task	Rationale for inclusion	Size of Coding Region	Protein Size	Secre-ted	Localization

***	***	***	***	***	***	***
***	***	***	***	***	***	***
***	***	***	***	***	***	***
***	***	***	***	***	***	***
***	***	***	***	***	***	***
***	***	***	***	***	***	***
***	***	***	***	***	***	***

To ensure controlled interactions, the composition of the pharmaceutical formulation may include no more than four genes for each Therapeutic Stage. The final selection will be determined through in vivo experiments.

Achieving the therapeutic effect is ensured by the multiplicative impact resulting from the inclusion of genes that regulate biological processes across all Therapeutic Stages in the pharmaceutical composition: utilization of pathological intracellular aggregated proteins and regeneration of targeted brain regions. Additionally, the risks of ectopic gene expression included in the composition have been considered and minimized by selecting optimal promoters.

The technical implementation of this concept involves the creation of therapeutic non-viral DNA vectors based on the VTvaf17 and GDTT1.8NAS platform solution for each protein/gene included in the final (key) list. The list of therapeutic DNA vectors is provided in the table below:

DNA Vector	Therapeutic Gene	Promoter	Total size, bp
***	***	***	***
***	***	***	***
***	***	***	***
***	***	***	***
***	***	***	***
***	***	***	***
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Finished Dosage Form (FDF)

Criteria for Selecting FDF (in descending order of importance):

- Therapeutic Efficacy: Minimization of adverse effects.

- Ensuring Maximum Preservation of Drug Functionality.
- Ease of Application, Transportation, and Storage.
- Affordable Cost Characteristics.

Proposed FDF Options:

1. Lyophilized DNA Vector Complex for Solution Preparation: DNA vectors without a coating are rapidly cleared in the cerebrospinal fluid and blood, which does not meet the criterion for preservation.
2. Lyophilized DNA Vector Complex Encapsulated in Liposomes and Polyethylene Glycol (PEG) for Solution Preparation: Double encapsulation requires a more thorough assessment of additional toxic effects. Additionally, this option may result in a slight increase in production costs.
3. Lyophilized DNA Vector Complex Encapsulated in Liposomes and Polyethylene Glycol (PEG) with Functionalization with specialized reagent for Solution Preparation: specialized reagent is conjugated to the surface of DNA vector + liposome + PEG microparticles. Functionalization with specialized reagent enhances drug penetration through the blood-brain barrier (BBB), specificity, and affinity to cellular receptors.
4. Lyophilized DNA Vector Complex with PEIpro Transfection Reagent (Polyethyleneimine) for Solution Preparation: Using polyethyleneimine as a transport system may cause side effects and undesirable phenomena.
5. Lyophilized DNA Vector Complex Encapsulated in Cationic Liposomes for Solution Preparation: Ensures maximum efficacy due to a high level of target cell transfection and preserves DNA vectors through encapsulation in cationic liposomes, with minimal adverse effects.

Final Selection:

The chosen final pharmaceutical form is a lyophilized DNA vector complex with double encapsulation—encapsulated in cationic liposomes and PEG with functionalization for infusion solution preparation. Encapsulation of DNA vectors with cationic liposomes in polyethylene glycol (PEG) can significantly enhance the efficiency of drug penetration into the target brain regions. PEG provides improved biocompatibility, prolongs the circulation time of liposomes in the cerebrospinal fluid and bloodstream, and reduces drug recognition by the immune system, thereby protecting it from degradation. Specialized reagent for functionalization enhances drug penetration through the BBB, specificity, and affinity to cellular receptors.

This type of FDF can substantially increase the efficiency of drug penetration into the target brain cells compared to the baseline level (DNA + cationic liposome).

V. Delivery Methods

To determine the preferential route of administration, the body systems/tissues are ranked according to their importance for AD therapy. Based on the results of the ranking, the predominant systems/tissues/divisions of the brain of a patient with AD are determined:

- Hippocampus - Very high significance
- Cortex (prefrontal and parietal) - Very high Significance
- Almond-shaped body (amygdala) - High significance
- Basal nuclei (striatum) - Moderately high significance
- Thalamus - Moderate significance
- Hypothalamus - Moderate significance
- Medulla oblongata - Low significance

Reaching the deep layers of the brain regions affected by pathological processes in AD with a drug is one of the most serious challenges and tasks of this project.

The method of administration should provide a significant therapeutic level of drug distribution in the target brain regions and create optimal conditions for effective transfection of target cells, with subsequent expression of therapeutic proteins.

The considered methods of drug administration are: intrathecal, intra-arterial. The choice of the method of drug administration should also take into account the criterion of drug preservation (DNA encapsulated in protective sheaths) for maximum prolonged presence/circulation in the medium in the area of administration and readiness for transfection of target cells.

Intrathecal Administration

When a pharmaceutical composition comprising DNA vectors encoding target proteins, combined with liposomes, is administered intrathecally (into the lumbar region of the spinal canal), it directly enters the cerebrospinal fluid (CSF). This method allows bypassing the blood-brain barrier (BBB) and provides direct access of the drug to the central nervous system (CNS).

1. Distribution of the Drug in the Cerebrospinal Fluid:
   - The cerebrospinal fluid is produced in the choroid plexuses of the brain's ventricles and circulates through the ventricles and the subarachnoid space of the brain and spinal cord.
   - Following intrathecal administration in the lumbar region, the drug ascends with the flow of CSF through the subarachnoid space of the spinal cord toward the brain.
2. Overcoming Barriers Between CSF and Brain Tissue:
   - The outer surface of the brain is covered by the pia mater and a thin layer of astrocytic processes (the glial limiting membrane).
   - The pharmaceutical penetrates this barrier via paracellular diffusion or through transport mechanisms such as transcytosis across endothelial cells.
   - The DNA, encapsulated in protective shells, utilizes perivascular spaces (Virchow-Robin spaces) surrounding blood vessels to infiltrate the deeper layers of brain tissue.

3. Interaction with Brain Cells:

- After penetrating the brain parenchyma, the liposomal DNA vectors are in close proximity to neurons. Cationic liposomes interact with the negatively charged cell membrane, stimulating uptake via endocytosis.

4. Release of DNA Vectors Inside Cells:

- Once inside endosomes, the liposomes disrupt the endosomal membrane, releasing the DNA vectors into the cytoplasm. The liposomal coating protects the DNA from intracellular nucleases until release.

5. Transport of DNA to the Nucleus and Gene Expression:

- A limited number of DNA vectors enter the nucleus of the transfected cell. Within the nucleus, mRNA synthesis occurs from the DNA vector, and target proteins are synthesized on ribosomes in the cytoplasm.
- The dual promoter ensures gene expression in target cells exhibiting an inflammatory status, minimizing ectopic expression in other cell types.

Arterial Infusion:

1. Preventive Administration of Mannitol and Intravenous Hydration

- Mannitol: As an osmotic diuretic, mannitol temporarily increases plasma blood osmolarity. Elevated osmolarity leads to dehydration of endothelial cells in cerebral capillaries, causing their volume to shrink. The reduction in endothelial cell volume creates temporary intercellular gaps, enhancing the permeability of the Blood-Brain Barrier (BBB).
- Intravenous Hydration: Nucleases are enzymes that degrade nucleic acids, including DNA. Increasing the fluid volume in the bloodstream through intravenous hydration dilutes nucleases. Reduced nuclease concentration decreases the likelihood of DNA complexes being degraded before reaching target cells. Additionally, hydration reduces blood viscosity, improving blood flow and promoting a more uniform distribution of DNA+liposome+PEG particles throughout the vascular system, thereby enhancing their access to areas with temporarily compromised BBB integrity.
- Maintaining Osmotic Balance: Hydration helps sustain osmotic equilibrium, preventing osmotic stress that could damage liposomal nanoparticles and the DNA vectors encapsulated within them.
- Compensation for Mannitol's Osmotic Effect: Mannitol, as an osmotic diuretic, may cause fluid loss from cells and tissues. Intravenous hydration compensates for this effect, maintaining overall fluid volume within the body.

2. Ultrasound Stimulation with Microbubbles

- Targeted Focused Ultrasound (FUS): Utilizing FUS enhances the efficiency of drug penetration into target brain regions and significantly improves the delivery of therapeutic agents. When combined with liposomes, FUS increases the permeability of brain tissues and temporarily opens the BBB.

- Ultrasound Impact: Exposure to ultrasound causes microcavitation of microbubbles near endothelial cells.
- Mechanical Disruption: The mechanical effects of ultrasound can temporarily disrupt the tight contacts between endothelial cells, increasing the permeability of the BBB.
- Precision Targeting: Focused ultrasound allows you to target specific areas of the brain.

3. Intraarterial Drug Administration

- Ensures rapid attainment of high drug concentrations within the arterial system supplying the brain. Administering the drug directly into an artery leading to the brain enhances its efficacy and reduces systemic exposure to other organs.

4. Overcoming the Blood-Brain Barrier (BBB)

- Mannitol and ultrasound stimulation jointly provide a temporary, reversible, and controlled reduction of the BBB's barrier function. The formation of intercellular gaps and the expansion of existing pores allow macromolecules and nanoparticles to penetrate the brain parenchyma. Additionally, functionalizing microspheres with specialized reagent further enhances drug penetration through the BBB.

5. Transport of DNA+Liposome+PEG Complexes to Brain Tissue

- DNA+liposome+PEG complexes have an average size of about 150 nm: The size allows them to pass through the enlarged pores in the BBB. Polyethylene glycol (PEG) provides increased circulation time in the bloodstream and decreased uptake by the reticuloendothelial system.
- Circulation in the Bloodstream:
  - The first circulation: Maximum concentration of "DNA-liposome-PEG" complexes reaches brain capillaries immediately after administration.
  - Passive Penetration: Complexes diffuse into the brain interstitial space through temporarily opened BBB gaps.

6. Interaction with Brain Cells

- Upon penetrating the brain parenchyma, liposomal DNA vectors are in close proximity to neurons. Cationic liposomes interact with the negatively charged cell membranes, promoting uptake via endocytosis. Functionalizing microspheres with specialized reagent further enhances specificity and affinity for cellular receptors.

7. Release of DNA Vectors Inside Cells

- Once inside endosomes, liposomes disrupt the endosomal membrane, releasing DNA vectors into the cytoplasm. The liposomal shell protects DNA from intracellular nucleases until release.

8. Transport of DNA to the Nucleus and Gene Expression

- A limited number of DNA vectors enter the nucleus of transfected cells. Within the nucleus, mRNA synthesis from the DNA vector occurs, and target proteins are synthesized on ribosomes in the cytoplasm.

- Dual Promoter System ensures gene expression in target cells exhibiting inflammatory status, minimizing ectopic expression in other cell types.

At the current project stage, a comprehensive analysis of the outlined administration methods was conducted based on the criterion of maximum drug delivery to target brain regions. The following methods were selected: intrathecal administration, arterial infusion with preventive mannitol administration, intravenous hydration, and focused ultrasound stimulation (FUS).

The selected administration methods ensure a substantial therapeutic level of drug distribution within the target brain regions and create optimal conditions for the effective transfection of target cells, with subsequent expression of therapeutic proteins.

Priority Administration Method: Intraarterial infusion with preventive administration of mannitol, intravenous hydration, and FUS has been identified as the priority method. The final decision regarding the most effective administration route will be made based on the results of animal experimentation.

VI. Treatment Protocols

The flexibility of therapeutic regimens is ensured by tools for adapting the schedule/scheme of drug administration with due regard to the effect and adverse events:

- Duration: The therapeutic agent exhibits a prolonged (~20 days) yet observable period of action (expression of therapeutic genes) in the target regions of the brain.
- Frequency: The therapeutic agent allows for multiple administrations in the absence of immunogenicity.
- Dosage: Personalized therapeutic scenarios can be developed based on actual efficacy and adverse events.

The administration of the therapeutic agent can be organized according to various protocols depending on the disease characteristics, therapeutic objectives, and the pharmacokinetics of the drug. The main administration schemes are outlined below:

Single Administration

- Description: The drug is administered once to achieve an immediate therapeutic effect. It is used when a quick intervention is needed to treat a disease or symptom.
- Advantages: Rapid action, simplicity.
- Disadvantages: Potential for a transient effect, high initial concentration may cause side effects.

Revolver Administration with Cumulative Effect

- Description: The drug is administered multiple times with gradual accumulation in the body until therapeutic concentration is achieved. The cumulative effect is ensured by the drug's prolonged half-life or its accumulation in tissues.
- Advantages: Achieves a stable therapeutic effect during prolonged treatment. Maintains an even concentration of the therapeutic agent in the body.

- Disadvantages: Risk of accumulation and toxicity, necessity for monitoring drug concentration.

Revolver Administration with Complete Reduction of Therapeutic Effect (Intermittent)

- Description: The drug is administered multiple times, but the therapeutic effect diminishes between administrations. This may be due to the drug's rapid metabolism and elimination. Restoration of the therapeutic effect requires subsequent administrations.
- Advantages: Controlled impact on the body with the ability to adjust dosages.
- Disadvantages: Risk of "peak" effects and side reactions during high concentration levels of the therapeutic agent.

Pulse Administration (Pulsed Therapy)

- Description: The drug is administered in large doses at intervals to achieve a significant effect over a short period while minimizing side effects.
- Advantages: Rapid symptom relief, reduced side effects between administrations.
- Disadvantages: Risk of adverse events at high doses.

Multi-Stage Administration

- Description: The drug is administered sequentially, with each stage targeting a specific objective (e.g., utilization of pathological intracellular protein aggregates; neuroregeneration).
- Advantages: Effective adaptation to changing treatment conditions.
- Disadvantages: Requires meticulous planning and monitoring.

Selected Treatment Scheme:

A treatment regimen was selected to maximize drug efficacy and minimize adverse events:

Multi-Stage Pulse Administration, Multi-Course (as Indicated):

Initially, each therapy course involves the pulse administration of the first stage drug, containing DNA vectors encoding genes responsible for implementing Therapeutic Stage No. 1: Disposal of Pathological Protein Aggregates formed in Alzheimer's disease. After a certain period (~14 days), the administration of the second stage drug occurs, containing DNA vectors encoding genes responsible for implementing Therapeutic Stage No. 2: Regeneration of Targeted Brain Regions damaged by AD.

Course Treatment Scheme:

1. First Pulse Administration of Stage One
2. Exposure Period: 6 days
3. Second Pulse Administration of Stage One
4. Exposure Period: 6 days
5. First Pulse Administration of Stage Two
6. Exposure Period: 6 days
7. Second Pulse Administration of Stage Two
8. Exposure Period: 60 days
9. Disease Remission Control

Therapy courses may be repeated as indicated until a significant therapeutic effect is achieved.

VII. Economic Feasibility

According to external experts involved, the economic indicators for the holder of the drug's registration certificate are expected to be quite impressive, provided that preclinical and clinical studies are successfully completed and the drug subsequently enters the market.

Aspects Considered in Evaluating Economic Indicators:

- Multifactorial Nature of the Disease: The extraordinary complexity of Alzheimer's disease and the absence, as of the date of this material, of widely accepted therapeutic solutions with confirmed efficacy and safety have led to a significant unmet demand.
- Utilization of Established Technological Approaches: The project employs proven technological methods, enabling the drug to be manufactured at various standard (non-unique) biotechnological facilities. This approach aims to achieve acceptable pricing while ensuring high profit margins for the holder of the drug's registration certificate.
- Convenient Logistics and Storage Conditions: The developed pharmaceutical— a lyophilized complex of DNA–cationic liposomes–PEG—offers ease of logistics and storage, facilitating widespread use in various countries.
- Aging Population and Rising Prevalence: The aging population, increase in patient numbers, and earlier detection rates of AD create prerequisites for the formation of a continually growing, substantial market segment with currently unmet demand. As of 2021, the global prevalence of dementia, predominantly attributed to Alzheimer's disease, exceeds 55 million individuals. Projections indicate a significant upward trend, with the number of affected individuals expected to triple, reaching 152 million by 2050. This escalating prevalence underscores the imperative for enhanced public health strategies, early diagnostic interventions, and the development of effective therapeutic modalities to mitigate the impending global healthcare burden associated with Alzheimer's disease.
- Scope of Preclinical and Clinical Studies: Due to the extensive nature of the required preclinical and clinical studies, which necessitate the involvement of a global player, the implementation envisions the creation of an alliance or consortium for further advancement of the project.

Accordingly, project participants using the following approaches in the development of drugs namely:

- modern nature-like molecular genetic tools (unique non-viral DNA vectors);
- complex bioinformatics algorithms for realization of therapeutic stages (evaluation and ranking of candidate genes/proteins by methods of multi-round analytical hierarchy, delivery methods, finished dosage form composition, etc.);
- combinatorics (optimal combination of genes/proteins responsible for disposal of pathological intracellular protein aggregates, regeneration of target brain sections; the use of tools for

specific delivery of the medication to target cells (promoters, reagents for functionalization); optimization of the finished dosage form composition, determination of the method of administration; application of additional physiotherapeutic methods in therapy),

taking into account the indicated market factors have increased chances of successful launch of the drug into the market and its commercialization.

It should also be considered that the indications for the use of the developed pharmaceutical may, in the future, be expanded to other conditions associated with the accumulation of pathological protein aggregates in neurons (e.g., tauopathies, Parkinson's disease) and neurodegeneration (e.g., multiple sclerosis, depression).

Completed Steps

1. Bioinformational Elaboration: Identification of Base List of Candidate Genes/Proteins

Utilizing extensive bioinformatics research conducted by both engaged molecular biology experts and artificial intelligence, an initial (base) list of candidate genes/proteins was established. This list includes genes/proteins responsible for the utilization of pathological intracellular protein aggregates and the regeneration of targeted brain regions.

2. Bioinformational Elaboration: Ranking and Formation of Key List of Candidate DNA Vectors

The genes/proteins included in the initial (base) candidate list were ranked using the Analytic Hierarchy Process with Weighted Criteria (AHP). This method involves breaking down the task into multiple levels, where each local task is analyzed separately. Therapeutic tools (candidate proteins) for solving local tasks are ranked individually, and an integrated list (ranking) of candidate proteins is subsequently formed. As a result, a key (condensed) list of candidate genes/proteins was made up.

3. Development of coding cassettes for each vector candidate and local therapeutic task for subsequent incorporation into DNA vectors.

Identification of optimal cell type–specific promoter elements, inflammation-inducible regulatory promoter regions, and accordingly the optimal dual-promoter configuration. Construction of promoter segments for each vector candidate and local therapeutic task for subsequent incorporation into DNA vectors.

4. Creation of DNA Vectors

Platform DNA vectors of the VTvaf17 and GDTT1.8NAS series were developed.

5. Depositing Production Strains

Original producer strains for the industrial production of the specified vectors were deposited in international repositories such as NCIMB and others.

6. Intellectual Property Registration

All necessary patent procedures were completed to register intellectual property rights: patents were filed for inventions related to the VTvaf17 and GDTT1.8NAS platform DNA vectors and the

corresponding producer strains. List of patents: US11149279, SMT202400120, EP3673061, CN111065738, JP6918231, ES2975070, CA3073258, KR102276373, IL272823, US20210310011, US2022000855, EP3847252, CN113166771.

7. Production and Research

Preliminary in vitro preclinical studies of the platform DNA vectors were conducted. Production technology was developed, and laboratory and pilot-industrial manufacturing protocols were established. Preclinical studies and Phase I clinical trials of another drug (for a different indication) have been conducted using the same platform DNA vectors.

8. Development of the Finished Dosage Form (FDF)

The transport system selected was cationic liposomes. In vitro and in vivo experiments were conducted to evaluate the transfection/expression efficiency of the target gene by the DNA-liposome complex. The preferred variant of Finished Dosage Form in the form of DNA-cationic liposome-PEG with functionalization was developed. Ligands for nanoparticle functionalization have been identified. The optimal size of the DNA + liposome + PEG nanospheres has been determined. Production technology options for the Finished Dosage Form were developed.

9. Determination of Administration Method

A comparative evaluation of administration methods was performed: intra-arterial and intrathecal. The preferred administration method was identified as intra-arterial. Additional pharmacological (mannitol, hydration) and physiotherapeutic (Focused Ultrasound, FUS) methods were selected to ensure maximum penetration of the therapeutic agent into the patient’s target brain tissues.

10. Development of Preclinical Trials Plan

A comprehensive preclinical trials plan was developed to assess the efficacy and safety of each candidate gene/protein and to determine the final composition of DNA vectors within the drug formulation.

Upcoming Steps

- Creation of therapeutic DNA vectors - candidates carrying target genes and the corresponding producer strains. Preliminary work on the development (design) of DNA vectors is underway.

- Conducting a series of experiments on animals (to determine DNA vectors included in the final composition of the drug; verification of the method of drug administration),

- Following the results of animal experiments: determination of the final composition, DNA vectors ratio, DNA/liposome/PEG ratio in the drug product. Preparation of the final Finished Dosage Form,

- Transfer of DNA vector and the Finished Dosage Form manufacturing technology to an industrial pharmaceutical company,

- Conducting a set of regulated preclinical studies of the drug to assess efficacy / safety,

- Conducting a set of clinical trials of the drug to assess efficacy / safety, and in case of successful implementation of these stages

- Registration and placing the drug on the market

- Expansion of the indications for use and the number of patients covered, respectively, prescription of the medicinal product - treatment of tau-pathies, including Parkinson's disease, multiple sclerosis, etc.