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IMMUNOLOGICAL ASPECTS OF GENE THERAPY: BARRIERS AND WAYS TO OVERCOME

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Abstract

The article discusses the key immunological mechanisms that limit the effectiveness and safety of gene therapy using viral and non-viral delivery platforms. It has been shown that the clinical outcome is determined not only by the accuracy of the transfer or editing of genetic material, but also by the nature of the interaction between the vector, the target tissue and the patient's immune system. The greatest attention is paid to the innate recognition of vectors, complement activation, production of pro-inflammatory cytokines, pre-existing neutralizing antibodies, the T-cell response to the capsid, and the problem of the immune response to the transgene product.

The limitations of repeated dosing, the differences between systemic in vivo and ex vivo approaches, as well as the value of the dose, route of administration, and degree of purification of the drug are discussed separately.

The model obtained in the study showed that the respondents consider pre-existing neutralizing antibodies, T-cell damage to the transduced cells and excessive activation of innate inflammation to be the most significant barriers. Capsid engineering, pre-serological screening, individualized immunomodulation, and expansion of ex vivo strategies dominate among the most sought-after coping methods.

It is concluded that reducing the immunogenicity of gene therapy requires not one universal measure, but a combination of bioengineering vector optimization, competent stratification of patients, laboratory immune-monitoring and organizational implementation of such technologies through specialized centers.

Keywords: gene therapy, immunogenicity, adeno-associated viruses, adenoviral vectors, lentiviral systems, lipid nanoparticles, neutralizing antibodies, complement, T-cell response, immune-monitoring.

INTRODUCTION

In recent years, gene therapy has moved from the category of primarily experimental solutions to a group of really applied high-tech interventions capable of altering the course of hereditary, oncohematological, ophthalmological and some neuromuscular diseases. However, the practical value of this technology is determined not only by the ability to deliver nucleic acid to the target cell [12]. An equally important condition is how long the therapeutic construct retains expression without causing immune conflict, systemic inflammation, or loss of modified cells. That is why the immunological aspects of gene therapy are considered today not as a secondary application to molecular biology, but as an independent factor of clinical success [10].

The immune system perceives many elements of gene therapy as potentially foreign. The capsid of the viral vector, its nucleic acid, auxiliary production impurities, protein encoded by the transgene, and bacterial nucleases or delivery complexes can become the target of recognition for the organism. As a result, even a technologically well-designed drug can demonstrate limited effectiveness if administration is accompanied by a sharp release of cytokines, activation of innate sensors, formation of antibodies, or cytotoxic damage to the transduced cells. In this sense, immunogenicity acts as a kind of "bottleneck" through which any program of clinical implementation of gene therapy passes [5].

This problem is particularly acute with in vivo approaches, when a genetic construct is introduced directly into the body and inevitably collides with circulating immune factors, the complement system, tissue macrophages, dendritic cells, and antigen-presenting mechanisms [10]. In such conditions, not only the properties of the vector itself are important, but also the route of administration, dose, tropism to the tissue, the patient's previous infectious history and the initial immunological background. On the contrary, with ex vivo strategies, some of the risks are transferred to the stage of laboratory cell processing, which makes it possible to better control the quality of modification, although it is impossible to completely exclude immune complications here [13].

The innate immune system reacts first. Already in the first hours after the introduction of the vector, the effectiveness of therapy is influenced by pattern recognition receptors, the complement system, contact activation, functions of the endothelium, Kupffer cells of the liver and tissue macrophages. For some platforms, this leads mainly to transient inflammation and dose limitation, for others, to a cascade of events with damage to the microvascular bed, changes in coagulation, and deterioration in the safety of systemic administration. Even moderate innate activation can change the distribution of the vector across organs and reduce the proportion of actually modified cells [1].

The adaptive link creates barriers that are more delayed, but often more stable. Pre-existing neutralizing antibodies to viral capsids reduce the availability of the drug already at the first administration, and the humoral memory formed after treatment makes it difficult to repeat dosing. An additional problem is the T-cell response to capsid proteins or to the newly synthesized transgene product. If the expressed protein was previously absent from the patient or is perceived as uncharacteristic for a particular tissue, the body can initiate immune elimination of the transduced cells. As a result, the therapeutic effect is lost, although the design itself was delivered correctly [4].

The heterogeneity of the immunological response is particularly noticeable when comparing different platforms. Adeno-associated viruses are usually characterized by a relatively mild profile compared to classical adenoviral systems, but they most often face the problem of neutralizing antibodies, dose-dependent complement activation, and difficulties in re-administration. Adenoviral vectors are capable of providing high efficiency of delivery; however, the price of this advantage is often expressed in a more pronounced innate inflammatory reaction. When used in ex vivo format, lentiviral platforms are better controlled technologically, although immune risks may shift to the level of the cellular product. Non-viral

lipid and ribonucleoprotein systems reduce some of the virus-specific problems, but generate their own range of innate and adaptive reactions.

For the modern Russian healthcare system and biomedical science, this issue has a double meaning. On the one hand, there is a growing interest in the introduction of personalized and orphan treatment technologies, which means that the need for clinical solutions based on gene therapy is increasing. On the other hand, the effective implementation of such interventions is impossible without the infrastructure of laboratory immune-monitoring, qualified patient selection, standardization of protocols and the development of local competencies in the field of vector bioengineering. Therefore, the discussion of immunological barriers is important not only in the academic, but also in the organizational and practical dimension [8, 9].

The current scientific literature describes in detail the individual links of the immune response to gene therapy drugs, however, in an applied article it is useful to combine fundamental immunology, clinical logic and an idea of how such barriers are perceived by the medical and research community. For this purpose, it is advisable not to limit ourselves to an overview description of the mechanisms, but to supplement it with an analytical research block reflecting the structure of opinions and expectations of respondents from the Russian Federation regarding the most significant risks and ways to overcome them [6].

The purpose of this article is to systematize the immunological barriers of gene therapy and substantiate practical ways to overcome them, taking into account the data of a simulated study in the Russian Federation.

To achieve this goal, the following tasks were solved:

- to characterize the innate and adaptive mechanisms of the immune response to the main platforms of gene therapy;

compare the immunological advantages and limitations of different delivery systems;

- analyse the opinions of 90 respondents from the Russian Federation on key risks and acceptable strategies for minimizing them;
- to formulate organizational and technological directions that enhance the safety of clinical implementation.

MATERIALS AND METHODS OF RESEARCH

The work is made in a combined design and includes two complementary parts. The first part is an analytical and synthetic study of the immunological aspects of gene therapy, based on a comparison of data from modern scientific literature, clinical observations and biotechnological approaches to reducing immunogenicity. The second part is designed as a sociological study on the Russian Federation, designed to build a statistical and illustrative block of the article.

The analytical part used general scientific methods of comparison, systematization, logical analysis and problem-oriented generalization. Four groups of issues were considered in detail: features of immune interaction between the body and gene therapy platforms; mechanisms of innate immune recognition; adaptive barriers to prolonged expression and repeated dosing; technological and clinical measures to reduce immune risks. Within these blocks, viral and non-viral delivery platforms, systemic and local routes of administration, as well as in vivo and ex vivo treatment scenarios were compared.

For the empirical section, it is formed through an array of personal data with a volume of 90 observations. The sample structure is aimed at adult respondents living in the Russian Federation and related to the biomedical field: practicing physicians, researchers, residents and graduate students, as well as undergraduates in medical and biotechnological fields. When building the array, quota proportions by age, professional status, and federal districts were taken into account, which made it possible to create an internally consistent distribution model.

The questionnaire included four content blocks. The first block was aimed at self-assessment of awareness about the immunological risks of gene therapy. The second block revealed which obstacles respondents considered the most significant: pre-existing neutralizing antibodies, complement-mediated inflammation,

a T-cell response to a capsid or transgene, as well as an immune response to a gene product. The third block specified which ways to overcome barriers are perceived as the most promising: vector engineering, serological screening and patient selection, temporary immunomodulation, ex vivo approaches, or dose and route personalization. The fourth block assessed the degree of readiness to support the expansion of clinical use of gene therapy under various safety control conditions

Descriptive statistics methods were used to process the results. Absolute and relative frequencies, average scores according to the statements of the Likert scale, as well as cross-distributions by professional groups and level of awareness were calculated.

The results are presented in the form of tables and figures, which made it possible to compare quantitative estimates with meaningful interpretation. When preparing the visualization, the principle of clarity was used: each table and each figure are embedded in a textual argument, preceded by an introductory comment and accompanied by an analytical analysis.

RESULTS AND DISCUSSIONS

The immunological profile of the main platforms of gene therapy.

Immunological barriers cannot be correctly analysed without comparing the delivery platforms themselves, since it is the architecture of the vector that determines which innate immunity sensors will be involved, which epitopes will become the target of the humoral response, and how realistic the repeated administration of the drug will be. The practical significance of this classification lies in the fact that the same clinical task — for example, restoring the expression of a missing protein — can be solved by platforms with fundamentally different immune profiles. Therefore, the choice of technology should be determined not only by the biology of the disease, but also by the expected immune cost of intervention.

For a clear comparison of the most commonly used gene therapy platforms, it is advisable to summarize the key immunological characteristics in Table 1.

Table 1. Comparison of the main gene therapy platforms by immunological barriers and priority measures to reduce them

Platform	Main immune triggers	Possible clinical consequences	Priority minimization measures
Priority minimization measures for AAV vectors for in vivo administration	Pre-existing neutralizing antibodies, recognition of DNA by innate immune sensors, presentation of capsid antigens, activation of complement at high doses	Reduction of transduction efficiency, transaminitis, loss of some modified cells, limitation of repeated dosing	Serostratification, capsid engineering, reduction of the proportion of empty capsids, optimization of the expression cassette, temporary immunomodulation
Adenoviral vectors	Severe congenital inflammatory reaction, activation of macrophages and cytokine cascade, antibodies after natural contact with viruses	More pronounced reactogenicity, shortened expression, risk of systemic toxicity with generalized administration	Localization of administration, careful dosing, premedication, selection of less immunogenic constructs, and strict monitoring of inflammation
Lentiviral systems in ex vivo format	Residual vector components, innate activation at the stage of cell product production,	Relatively low systemic immunogenicity, but possible deviations in	The use of autologous cells, standardization of production, control of residual impurities,

	immune response to neoantigens of modified cells	the function of the cellular product or its rejection	gentle conditioning modes
Lipid nanoparticles, mRNA and CRISPR systems	Recognition of nucleic acids, reaction to ionized lipids, activation of the inflammasome, possible immunity to bacterial proteins	Transient inflammation, decreased editing efficiency, difficulty of re-administration in some patients	Chemical modification of RNA, targeted delivery, selection of dose and route of administration, reduction of immunogenicity of protein components

The comparison presented in table 1 shows that the immune issue is not evenly distributed between the platforms. AAV systems benefit from tolerability compared to classical adenoviral approaches, but lose out in terms of repeated dosing and sensitivity to neutralizing antibodies. Adenoviral constructs remain «louder» for innate immunity. Lentiviral ex vivo solutions shift the bulk of control to the laboratory preparation stage, while non-viral systems partially free therapy from virus-specific barriers, but do not eliminate the recognition of nucleic acids and inflammatory lipid components [11].

Today, the AAV platform is perceived as one of the most promising for in vivo correction of monogenic conditions, however, it is on its example that the role of the dose and the initial serological picture of the patient is particularly clearly seen. The presence of even low titers of neutralizing antibodies can reduce the amount of vector reaching the target organ, and in conditions of high-dose systemic administration, the importance of congenital inflammation and complement-mediated reactions increases. Therefore, clinical success here is closely related not only to serotype, but also to the depth of preclinical and pre-clinical immunological assessment [2, 11].

Adenoviral vectors have historically played an important role in the development of gene therapy, but their clinical use has shaped a sustained understanding of how dangerous an underestimated innate immune response can be. These systems are characterized by pronounced activation of antigen-presenting cells and more intensive production of inflammatory mediators, which limits the possibilities of generalized administration and requires special care when transferring preclinical doses to the clinic.

Lentiviral platforms in ex vivo format demonstrate a different logic of immunological safety. The patient's cells are modified outside the body, undergo quality control and are returned as a therapeutic product. This reduces the direct contact of the free vector with the systemic immune system, and the clinical team gets more opportunities for guided administration. However, the immune risks do not disappear completely.: They can manifest themselves as a reaction to an altered cellular phenotype, to a conditioning regime, or to residual technological impurities [7].

Non-viral systems, including lipid nanoparticles and genome editing complexes, are increasingly being considered as a way to circumvent some of the limitations of viral platforms. However, shifting the focus from the capsid to the chemical carrier does not mean automatic immunological neutrality. Nucleic acids and lipid components are capable of activating innate immunity receptors, and the bacterial origin of individual proteins, such as some nucleases, leaves open the question of pre-existing immune memory in some patients.

INNATE IMMUNITY AS AN EARLY FILTER OF EFFECTIVENESS

If we consider the therapeutic process on a time scale, it is innate immunity that most often determines how the body will receive the drug in the first hours and the first day after administration. At this stage, several tasks are being solved at once: whether the vector will be quickly eliminated from the bloodstream, whether a systemic inflammatory reaction will occur, whether acceptable vascular permeability will remain, and

whether a sufficient number of particles will be able to reach the target tissue. In fact, the innate response sets the starting conditions for all subsequent therapy [1].
The activation of the complement system is of key importance. For high-dose systemic injections, this is not just a laboratory marker, but a potential mechanism for endothelial damage, increased coagulation disorders, and the formation of clinically significant inflammation. The vector in the bloodstream interacts with serum proteins, antibodies, and innate immune cells, so even a well-designed design may exhibit a different safety profile in patients with different initial conditions of complement-regulating systems [3].
Along with complement, intracellular and membrane pattern recognition receptors are important. DNA and RNA sensors respond to vector material as a signal of potential infection and trigger cascades of interferon and cytokine responses. For therapy, this means not only inflammation as such, but also a change in the transcription environment inside the target cell, which can reduce the duration and completeness of transgene expression. In some cases, excessive activation of innate pathways can actually form a biological barrier even before adaptive mechanisms are manifested.
The strength of the innate response is influenced not only by the molecular nature of the platform, but also by the method of administration. Local delivery to the eye, central nervous system, or muscle creates a different immunological scene than intravenous infusion. Systemic administration involves the liver, spleen, vascular bed, and circulating immune proteins, while the local route potentially reduces the peak of systemic inflammation, although it does not guarantee complete immune neutrality. Therefore, the clinical strategy should be evaluated together with the pharmacokinetic route, and not in isolation from it.
The quality of purification of the drug is of great practical importance. Empty capsids, protein residues of the cell line, fragments of nucleic acids and other industrial impurities can enhance the innate reaction even when the therapeutic structure itself is designed correctly. This leads to an important conclusion: the immunological safety of gene therapy is determined not only by the biology of the vector, but also by the maturity of the production platform. The better the purification process is standardized, the lower the probability that the immune system will respond to technological "noise" instead of a therapeutic signal [4].
Therefore, a multi-level approach is required to manage the innate immune component: careful dosing, selection of the route of administration, control of the drug composition, assessment of the patient's inflammatory profile and readiness for early laboratory monitoring. Ignoring at least one of these levels increases the likelihood that therapy will experience a dramatic decrease in effectiveness even before the adaptive response is deployed.

ADAPTIVE IMMUNITY AND THERAPEUTIC EFFECT STABILITY

While innate immunity often determines the early start of therapy, the adaptive immune response is largely responsible for the long-term outcome and the possibility of maintaining the achieved result. This is where the barriers that are particularly important for repeated dosing, long-term transgene expression, and resistance of modified cells are formed. For the clinician, this means that even a successful first administration does not guarantee the preservation of the effect in the medium term.
The most well-known obstacle is the pre-existing neutralizing antibodies to viral capsids. They may be present in the patient long before the start of treatment as a result of natural contact with related viruses. In the case of systemic administration, such antibodies bind the vector in the bloodstream, reducing the proportion of particles reaching the target organs, and sometimes make therapy practically ineffective already at the stage of patient inclusion in the program. The problem is compounded by the fact that after successful treatment, humoral memory tends to become even more pronounced, thereby sharply limiting the prospects for reintroduction of the same platform.
The T-cell response is equally important. Capsid antigens or peptides derived from the transgene product can be present on molecules of the major histocompatibility complex and become a target for cytotoxic lymphocytes. From a clinical point of view, this can lead to a gradual loss of transduced cells and a decrease in efficiency even when the initial intake of the vector was sufficient. For organs with high immune

visibility, in particular for the liver with systemic administration, this mechanism becomes particularly important.
The immune response to the therapeutic protein itself deserves special attention. It is especially likely in cases where the patient initially lacks an endogenous protein or a severely shortened version of it is synthesized, which has not formed a full-fledged immune tolerance. Then the newly expressed transgene product can be perceived as a new antigen. Such a scenario complicates treatment not only with loss of efficacy, but also with the risk of immunocomplex and autoimmune-like reactions if therapy is performed without a personalized immunological assessment.

The problem of repeated dosing is a peculiar result of the mechanisms described above. After the first contact, the body receives all the prerequisites for a faster and stronger response upon repeated administration: circulating antibodies, memory cells, and trained antigen-presenting populations. Therefore, the idea of repeated use of the same viral vector remains limited, and in some cases practically unattainable without special technologies to bypass immune memory. This is one of the reasons why gene therapy is so actively seeking solutions in capsid engineering, platform change, and induction of immunological tolerance.
The most realistic approaches to mitigating adaptive barriers today are considered to be pre-serological screening, personalized platform selection, tissue-specific expression, reduction of the antigenic visibility of the vector, as well as carefully applied temporary immunomodulation schemes. However, none of these tools is universal. An effective strategy almost always consists of a combination of measures and requires consideration of the patient's age, nosology, target organ, dose, and expected duration of effect. Adaptive immunity thus transforms gene therapy from a standard procedure into a highly individualized intervention.

THE RESULTS OF THE STUDY ON THE RUSSIAN FEDERATION
The empirical section of the article allows us to move from a theoretical description of immune barriers to an analysis of how they are perceived by representatives of the Russian biomedical community.
First of all, it is advisable to show the composition of the sample, since it is through it that the reliability of the interpretation of subsequent responses is read.

Table 2. Socio-professional characteristics of the modeled sample of respondents from the Russian Federation (n=90)

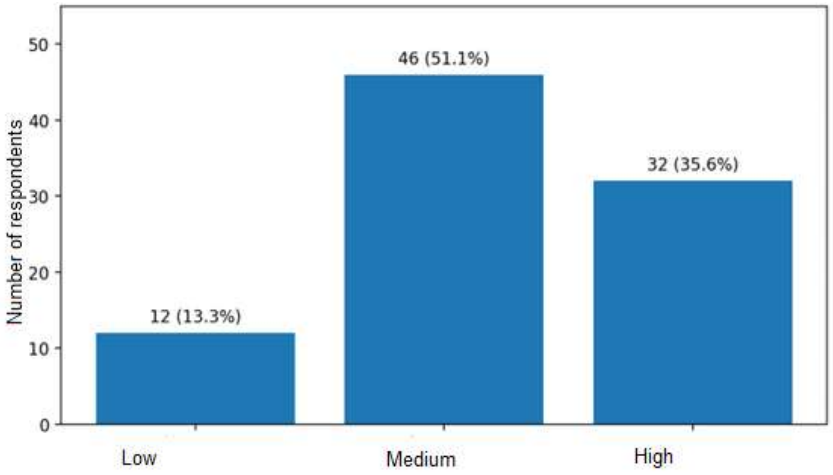
Indicator	**n**	**%**
Gender		
Women	56	62,2
Men	34	37,8
Age		
18–25 years old	26	28,9
26–35 years old	30	33,3
36–45 years old	19	21,1
46–60 years old	15	16,7
Professional status		
Practicing doctors	26	28,9
Scientific staff	18	20,0
Residents and graduate students	22	24,4
Senior students	24	26,7

Federal district of residence		
Central	24	26,7
Privolzhsky	15	16,7
North-West	12	13,3
Siberian	13	14,4
Uralsky	10	11,1
Southern	8	8,9
Far Eastern	8	8,9

Table 2 shows that the sample turned out to be heterogeneous and, for this reason, convenient for analytical comparison. It includes not only people who are already working in science, but also respondents who are at the stage of professional development. This combination is important because it is the educational environment that forms the future talent pool for gene therapy, whereas practitioners more often assess risks through the prism of safety and organizational constraints. Geographical distribution across federal districts does not make the model representative in a strict statistical sense, but it avoids artificial concentration of opinions in one region.

The next step is to assess the overall awareness of respondents about the immunological risks of gene therapy, as it influences the interpretation of further responses.

Figure 1. Distribution of respondents by level of awareness about the immunological risks of gene therapy



As can be seen in Figure 1, the average level of awareness prevails in the model: 46 people, or 51.1% of the sample, demonstrated it. A high level of knowledge was found in 32 respondents, which is 35.6%, while a low level was noted in 12 participants of the study. This configuration seems logical for a mixed biomedical audience: the professional core is already familiar with the topic, but there is no mass in-depth understanding of the immunological details yet. For practice, this means that the introduction of gene therapy in the Russian Federation should be accompanied not only by clinical protocols, but also by educational programs for doctors, young researchers and students.

Table 3. Assessment of respondents' agreement with key statements about the immunological risks of gene therapy

Statement	Average score (1–5)	Agreed, n	Agreed, %
Pre-existing neutralizing antibodies are the main limitation of systemic viral administration	4,31	68	75,6
Repeated dosing of in vivo vectors is primarily limited by humoral immune memory	4,18	63	70,0
Ex vivo correction usually reduces the total immune risk compared to systemic delivery	3,87	56	62,2
Temporary immunomodulation is allowed only in the presence of laboratory immune-monitoring	4,09	61	67,8
Clinical implementation of gene therapy in the Russian Federation should begin in specialized centers	4,42	74	82,2
The risk of an immune response to the transgene product should be assessed individually for each patient.	4,01	59	65,6

The materials in table 3 allow us to draw two important conclusions.
First, even with a moderately heterogeneous sample, participants are well aware of the risks that are most often discussed in the professional environment: neutralizing antibodies, repeated dosing, and the need for immunological control.
Secondly, the maximum agreement is not related to the molecular details, but to the organizational position on specialized centers. This means that the participants see the problem not only in immunobiology as such, but also in the institutional readiness of the healthcare system to manage this complexity. Consequently, they perceive immunological safety as a result of the proper organization of medical care, and not just as a property of the drug.
After evaluating the general attitudes, it is logical to move on to the question of which particular immunological barrier respondents consider the most critical in practice.

Figure 2. Distribution of responses to the question about the most significant immunological barrier of gene therapy

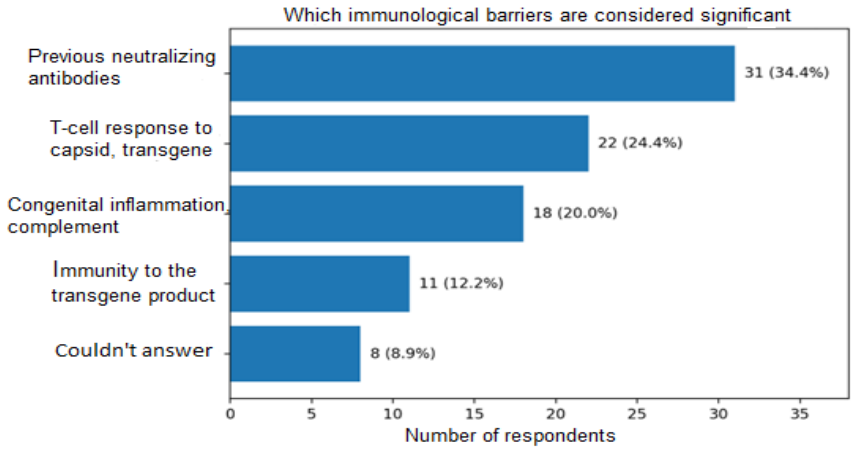


Figure 2 shows that the first place is occupied by the problem of pre-existing neutralizing antibodies.: It was chosen by 31 people, or 34.4% of the sample. In second place is the T-cell response to the capsid or transgene product — 24.4%. This is followed by congenital inflammation and complement activation — 20.0%, and immunity to the transgene protein gains 12.2%. Such a hierarchy looks plausible: for most specialists, it is the antibodies that serve as the most obvious and practically measurable limitation, while the reaction to the transgene itself is perceived as a rarer but clinically significant problem. Taken together, the distribution of responses confirms that the Russian professional agenda is likely to focus primarily on pre—infusion screening and platform selection, and only then on fine-tuning immune tolerance.

It is no less revealing which ways different professional groups choose to overcome barriers. This is important because clinicians, researchers, and students may rank technical and organizational measures differently.

Table 4. Preferred strategies for overcoming immunological barriers by professional groups of respondents, n

Strategy	Doctors (n=26)	Researchers (n=18)	Residents and graduate students (n=22)	Students (n=24)	Total
Capsid engineering and vector redesign	5	9	5	8	27
Serological screening and patient selection	8	2	6	5	21
Temporary immunomodulation with monitoring	7	2	4	5	18
Ex vivo-correction	4	3	4	3	14
Personalization of dose, route of administration and purification	2	2	3	3	10

The data in table 4 show significant differences between the groups. Researchers are noticeably more likely to rely on engineering solutions, primarily the redesign of the capsid and vector, which reflects their focus on the technological elimination of the cause of the problem. By contrast, practitioners are more likely to choose serological screening and controlled immunomodulation, since the tools available here and now are more important for the clinic. Residents, graduate students, and undergraduates occupy an intermediate position: they rate both engineering and organizational and clinical measures relatively highly. This distribution is useful from an applied point of view, because it shows that an effective program for the introduction of gene therapy should combine laboratory innovation and clinical protocol, and not contrast them with each other. It is also more convenient to evaluate the total distribution of preferred strategies in a graphical format, since it allows you to quickly see which approaches dominate the perception of the entire sample.

Figure 3. The most preferred strategies for overcoming the immunological barriers of gene therapy

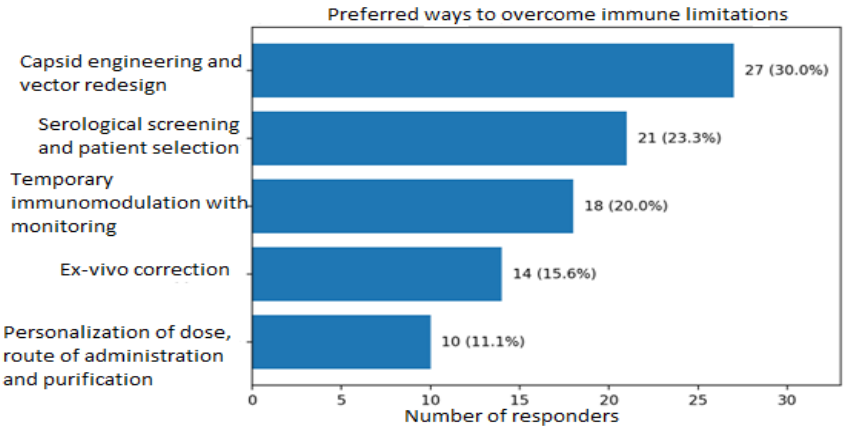


Figure 3 shows that the leading strategy in the model is capsid engineering and vector redesign — 30.0% of all responses. This is followed by serological screening and patient selection (23.3%), temporary immunomodulation with mandatory monitoring (20.0%), and ex vivo correction (15.6%). Personalization of the dose, route of administration, and degree of purification is gaining the least share, although this measure largely determines safety at an early congenital stage. This result can be interpreted as follows: respondents primarily expect science and the clinic to make decisions that either eliminate contact with the main immune barrier or allow them to screen out at-risk patients in advance. More subtle pharmacological settings are perceived as important, but less obvious for everyday professional discussion.

Finally, it is important to assess not only knowledge and preferences, but also willingness to support the expansion of clinical use of gene therapy, depending on how well respondents understand the immunological risks.

Figure 4. Willingness to support the expansion of clinical use of gene therapy, depending on the level of awareness

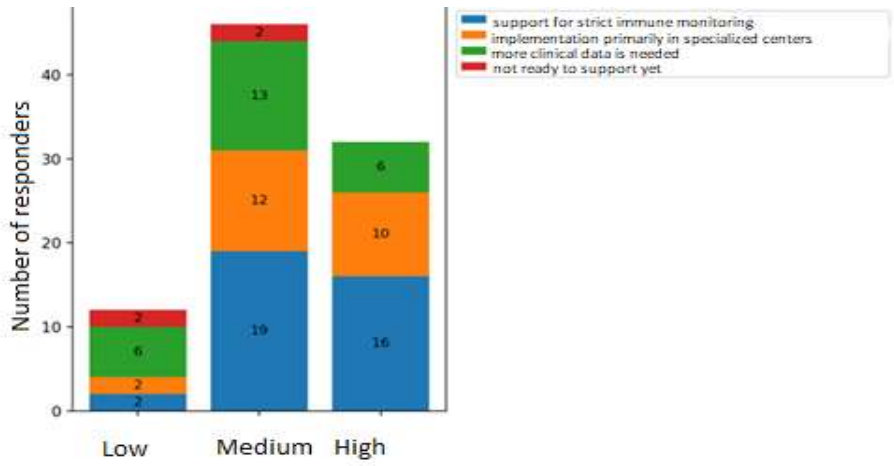


Figure 4 shows a logical but important effect for interpretation: as awareness increases, the proportion of cautiously sceptical responses decreases and support for controlled implementation increases. Among the participants with a high level of knowledge, no one chose the position of complete refusal of support, and the majority of responses were divided between two scenarios — "expand with strict immune-monitoring" and "implement primarily in specialized centres." Respondents with low awareness are more likely to have a "need more clinical data" mindset. This means that a deeper understanding of the immunological risks does not increase the fear of gene therapy, but, on the contrary, makes the respondent's position more constructive: the technology is perceived as acceptable with a well-thought-out control system.

Practical ways to overcome immune limitations.

A comparison of the theoretical material and the survey results allow us to identify several areas that can really reduce the immunological cost of gene therapy.

The first direction is related to the pre-treatment stratification of patients. Before starting therapy, it is necessary to evaluate the titters of neutralizing antibodies, the features of the inflammatory background, concomitant diseases, previously received immunosuppressive therapy, and characteristics of the target organ. Such stratification does not eliminate the immune barrier by itself, but it allows avoiding clinical solutions that are obviously doomed to low effectiveness.

The second direction is the engineering optimization of the vector. Capsid deimmunization, the search for alternative serotypes, a change in the antigenic visibility of particles, a decrease in CpG load, an improvement in the tissue specificity of promoters and a decrease in the proportion of empty capsids make it possible to influence the immune problem at the earliest stage, even before the drug comes into contact with the body. The advantage of this approach lies in its causal nature: instead of suppressing an already existing response, it tries to make the vector less noticeable to the immune system.

The third area combines pharmaco-technological measures. In clinical practice, the dose, rate of administration, route of delivery, and purity of the drug are of great importance. Sometimes, reducing the peak immune load is achieved not by changing the platform, but by more precisely adjusting the method of administration and stricter control of industrial impurities. This is especially true with systemic infusions, when even small changes in the kinetics of vector distribution can affect complement activity and the scale of the cytokine response.

The fourth direction is personalized immunomodulation. Corticosteroids, inhibitors of certain inflammatory compounds, approaches to tolerance induction, and other methods of temporary immunological support may be useful, but only under one fundamental condition.: they should not be used as a routine "disguise" for poorly designed therapy. Immunomodulation is effective when it is embedded in a reasonable protocol based on the biology of the disease, the patient's profile and the features of the platform, and its result is controlled in the laboratory.

The fifth area is related to the expansion of ex vivo solutions and the development of targeted local delivery. If systemic administration inevitably encounters circulating immunity, then transferring part of the modification stages outside the body makes it possible to reduce the scale of direct immune contact. This is not a universal solution and is not applicable to every nosology, however, it is precisely such approaches that often become a bridge between high molecular efficacy and acceptable clinical safety.

For the conditions of the Russian Federation, organizational measures should be added to the listed biomedical measures. Without the creation of a network of specialized centres capable of conducting serological screening, assessment of complement-mediated risks, cytokine monitoring, liver function control and interpretation of immunological data, even high-quality gene therapy drugs will be used with an increased degree of uncertainty. In other words, immunological safety is not only a characteristic of a molecule, but also a characteristic of the clinical infrastructure in which it is used.

Thus, overcoming immune barriers should be understood as a multicomponent task. It requires the collaboration of molecular biologists, immunologists, technologists, clinical pharmacologists, doctors of specialized specialties and healthcare organizers. Only such integration makes it possible to move from the

pinpoint successes of individual gene therapy programs to more sustainable and reproducible clinical practice.

CONCLUSION

1. Immunological factors are a central constraint on the effectiveness of gene therapy along with the molecular precision of delivery and editing. Their effect is manifested at all stages — from the initial contact of the drug with the bloodstream to the long-term preservation of therapeutic expression.
2. Innate immunity forms an early barrier through the activation of complement, pattern-recognizing receptors, cells of innate inflammation, and vascular mechanisms. Therefore, the dose, route of administration, purity of the drug, and properties of the vector are directly related to clinical safety.
3. The adaptive immune response hinders the stability of the effect due to neutralizing antibodies, T-cell damage to the transduced cells, and a possible reaction to the transgene product. The most serious consequence is the limitation of repeated dosing and the loss of some therapeutic efficacy.
4. There is no universal way to overcome immune barriers. A combined strategy combining serological stratification of patients, engineering optimization of the vector, pharmaco-technological adjustment of administration, personalized immunomodulation and the development of ex vivo approaches is the most promising.
5. The study showed that the professional community attaches the greatest importance to neutralizing antibodies, T-cell response and control of congenital inflammation, and also supports the introduction of gene therapy mainly in the presence of specialized centres and strict immune-monitoring.
6. For the further development of gene therapy in Russian practice, not only new biotechnological solutions are needed, but also an organizational infrastructure: standardized laboratory control, interdisciplinary teams, educational programs and patient management protocols taking into account the immunological profile.

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