STATEMENT

by Prof. Dr. Maria Hristiyanova Nikolova, MD

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regarding a thesis for the award of the educational and scientific degree 'doctor'

Field of higher education: 4. Natural Sciences, Mathematics and Informatics, Professional field: 4.3 Biological Sciences; Doctoral program: Immunology

Doctoral student: Vancho Donev
Form of doctoral studies:regular
Scientific unit: Department of Immunology, National Center for Infectious and Parasitic Diseases
Topic:"Characterization of the potential immunoprophylactic and immunotherapeutic properties of human blood plasma"
Scientific supervisor: Assoc. Prof. Dr. Georgi Nikolov, MD

The set of materials presented to me includes the necessary documents, in accordance with the requirements of the Law on the Protection of the Rights of Persons with Disabilities, the Regulations for its implementation and the Regulations of the National Center for the Protection of Personal Data. I declare that I have no conflict of interest. within the meaning of the additional provisions of the Law on the Protection of the Rights of Persons with Disabilities.

Vancho Donev's dissertation is a scientific and practical development of undoubted interest in the field of immunoprophylaxis and immunotherapy. Despite being used for more than a century, human blood plasma is a unique biological product, the application of which has not stopped developing and expanding. The topic sounds especially relevant in the context of the Covid pandemic, when hyperimmune plasma from recovered patients was used by presumption, due to the lack of other specific means, as well as the increasingly strict requirements of international institutions for the characterization of medicinal products. All this is reinforced by the fact that Bulgaria is the first producer of blood plasma for medical purposes and the Bulgarian product Immunovenin Intact is the basis of the dissertation. For me, the meaning of the development is indisputable - to generate precise and comprehensive information about a biological product, as a prerequisite for its effective application in medical practice.

The dissertation is written sparingly, but sufficiently comprehensively on 130 pages, observing the generally accepted sections in the usual proportions (Introduction – 2 pages. Literature review with summary – 30 pages, Aim and objectives – 1 page, Materials and methods – 15 pages, Results – 26 pages, Discussion - 12 pages; Conclusions and contributions – 4 pages. The dissertation is illustrated with 7 tables and 23 figures, which are useful and completely sufficient to illustrate the

study. The development is based on a literature review, including 317 titles, with a worthy place given to Bulgarian research in this field, which is in many ways fundamental.

The overview covers the problem from all points of view: detailed characteristics of blood plasma components, historical review of purification and processing technologies, as well as its application as a replacement, preventive and immunomodulatory product. The doctoral student's knowledge of the mechanisms of immune response regulation, which are the basis for understanding and developing immunotherapeutic applications of the product, makes a very good impression. The necessary space is devoted to the problem of "pros and cons" of Covid-convalescent plasma, as well as the need to standardize intravenous immunoglobulin products. The review ends with very well summarized and clearly formulated "gaps" in our knowledge regarding the preventive and therapeutic properties of the product, convincingly justifying the purpose of the dissertation.

Six tasks have been formulated that adequately meet the set goal: comprehensive information regarding general immunological characteristics (content and ratio of total immunoglobulins and their isotype characteristics, multi-cytokine profile), pathogen-specific potential (with a focus on S.aureus; C.Albicans and SARS-CoV-2) and the presence of autoantibodies associated with a wide range of pathological conditions (antinuclear antibodies, ANA)

It should be emphasized the excellent **methodological approach**, the listed indicators were studied in parallel on: convalescent plasma and Immunovenin intact produced from it, on the one hand, plasma pools from the pandemic period, as well as plasma pools collected before 2019 and standard series of Immunovenin intact. In this way, the features of convalescent plasma and plasma pools from the pandemic period are distinguished and important conclusions are drawn regarding the possibilities for their application.

The methods used are very comprehensively described and include both classical and cutting-edge technologies, which are fully adequate for the tasks set. The inclusion of a home-made ELISA test developed in the laboratory makes a very good impression, which is a convincing check of the practical preparation of the dissertation candidate and convinces us of the possibility of universalizing the proposed algorithms. A comprehensive set of statistical methods has also been used, which convinces us of the reliability of the results.

The results consistently provide answers to the six tasks related to the characterization of the general immunological and pathogen-specific properties of the studied objects. The discussion testifies to the good theoretical preparation of the doctoral student, who is familiar with current publications in the field and skillfully interprets and compares the results of his and related research, expressing logical hypotheses. For me, the following results and conclusions are most significant:

A comprehensive characterization of the content of immunoglobulin classes and subclasses, antinuclear antibodies, as well as the content of specific antibodies against common pathogens

(*S.aureus, C.albicans*) in standard batches of Immunovenin Intact has been performed for the first time. These data have an important bearing on the immunotherapeutic, immunoprophylactic and immunomodulatory properties of Immunovenin Intact and are a significant addition to the product characteristics.

Convalescent plasmas were comprehensively characterized in terms of the content of general and specific immunoglobulins, including those with neutralizing activity, as well as the concentration of 25 cytokines with pro-inflammatory and regulatory effects, and were compared with plasma pools from the period of mass spread of SARS-CoV-2. There are three main conclusions: a significant proportion of convalescent plasmas lack RBD-specific IgG and IgA, with the levels of SARS-CoV-2 specific immunoglobulins in Immunovenin intact, produced from pools collected during the pandemic, being significantly higher than the average levels in convalescent plasmas; both donor plasmas and pools collected during the pandemic lack high concentrations of pro-inflammatory cytokines; convalescent plasmas collected in 2021 have high neutralizing activity against 6 studied variants of SARS-CoV-2, including appeared after 2021

A methodological algorithm has been developed for characterizing the protective, therapeutic and immunomodulatory properties of immunoglobulins for intravenous administration, which could be introduced as a mandatory part of the production and therapeutic protocols, as well as expanded to include additional microorganisms and plasma components.

I generally agree with the contributions made, but I have significant reservations regarding the formulation of Theoretical Contribution 2, which, in my opinion, remains completely incomprehensible to those unfamiliar with the details of the study results. The established cross-protective effect towards subvariants other than the one circulating during the sampling period should have been emphasized much more categorically.

The autoreference meets the requirements by presenting the main results of the dissertation in a synthesized version.

The results of the research in the dissertation arepresented in 3 publications in scientific journals in English, two of which with an impact factor, and participation in two international and 4 scientific forums on the topic.

My critical remarks are mostly technical in nature. The text to the figures is not detailed enough or unclearly formulated, which makes them difficult to perceive. As examples, I note Fig.10 and Fig.13 (Multiple increase in the level of 25 tested cytokines?) Although Bulgarian is not the native language of the dissertation author, the work is too good to allow massive spelling and stylistic errors. I am also not satisfied with scheme 3, entitled Study Design. Again, for the unfamiliar reader, it will be difficult to navigate the thesis concept without a detailed explanatory text of what and why was studied.

My question to the dissertation candidate is: "If you had the opportunity to continue the study, what other immunological parameters and pathogen-specific antibodies in human plasma would you characterize?"

In conclusion, I believe that Vancho Donev's dissertation is a relevant scientific and applied work, developed in response to the growing need for well-characterized and standardized immunotherapeutic and immunomodulatory agents, under the natural pressure of increasing immune-mediated diseases and emerging pathogens with pandemic potential. The results obtained suggest ideas for the further improvement of a long-known Bulgarian biological product and for the development of therapeutic strategies based on immunomodulatory and immunocorrective therapy. Last but not least, V. Donev's doctoral studies are an example of successful science/business cooperation, which is one of the priorities of the National Center for Immunopathogens as a leading partner in the center of competence "Immunopathogen". The dissertation work fully meets the scientific criteria in the Law on the State Agency for the Development of Immunopathogens and Immunomodulatory Drugs of the Republic of Bulgaria and the Regulations of the National Center for the Development of Immunopathogens for the Acquisition of the Doctoral Degree. This gives me reason to give my categorically positive assessment and I propose to the esteemed scientific jury to award the educational and scientific degree of 'doctor' to Vancho Donev in the doctoral program "Immunology"

17.08.2025 **Author of the statement**: .....

Prof. Dr. Maria Nikolova, MD