Mathematical Models of Cancer are More Reminiscent of *Glasperlenspiel*

Manfred Sneps-Sneppe

***Abstract*— The article has two goals: firstly, to attract the interest of mathematicians to immunology and, secondly, to make some efforts (it must be admitted that quite limited) to promote the key achievements of Latvian virologists in the invention of cancer research, namely, the medicines Rigvir and Larifan for that going back in history to the 1960s.**

The immune system (innate and adaptive immunity) as well as lymphocyte sources are discussed, namely, T cells, B cells, dendritic cells, cytokines, chemokines, and interferons. The dual role of macrophages and dendritic cells is studied in many mathematical models.

Generally, cancers do not have danger signals and, therefore, cannot elicit strong immune reactions. Immunomodulators turn cancer from a cold to a hot state, to make cancer visible to the immune system. Immunomodulators Poly (I:C) and Larifan are compared.

Oncolytic virus therapy is a novel approach in the field of cancer treatment. The first oncolytic virus in the world was the genetically unmodified ECHO-7 strain enterovirus Rigvir, which was approved in Latvia in 2004 for skin melanoma treatment, but withdrawn in 2019 because did not reach the current standards for clinical use within the EU area.

***Keywords*—mathematical modeling, melanoma, immunomodulators, Larifan, virotherapy, Rigvir.**

I. Introduction

Cancer research ranges from epidemiology and molecular bioscience to the performance of clinical trials to evaluate and compare applications of various cancer treatments. These applications include surgery, radiation therapy, chemotherapy, hormone therapy, and immunotherapy. Starting in the mid-1990s, the emphasis in clinical cancer research shifted towards therapies derived from biotechnology research, such as cancer immunotherapy and gene therapy. Cancer research takes place in all countries. The National Cancer Institute is the major funding institution in the United States. In the 2016 fiscal year, the NCI funded $5,2 billion in cancer research and the funding is growing to $6,8 billion in 2022 [1].

Part of this rich funding also goes to mathematical research. Google Scholar gives 1,190,000 links to the “immune system mathematical models” question and even 17,500 links since 2020. Google Scholar gives 102,000 links to the “melanoma mathematical models” question and even 17,600 links since 2020. (It seems a little strange such a growth interest in melanoma studies.)

At the same time, we must admit that the mathematical research on cancer is not very successful because the immune system is too complicated and it must be noted that there are many unknowns and many black spots in immunity mechanisms.

The article has two goals: firstly, to attract the interest of mathematicians to immunology and, secondly, to make some efforts (it must be admitted that quite limited) to promote the key achievements of Latvian virologists in the invention of cancer research, namely, the medicines Rigvir and Larifan for that going back in history to the 1960s.

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II. An Insight into Mathematical Modeling with Some Medical Meaning

Let's consider just a few simplest models based on ordinary differential equations.

Lotka-Volterra Predator-Prey Model (1920). This is a popular model [2]. It assumes that 1) the population of prey (hares) grows exponentially in the absence of predators (wolves), 2) each wolf kills a certain fraction of the hare population per unit time, 3) the birth rate of wolves increases linearly with the rate of consumption of hares, and 4) wolves are constant death rate. This model has a neutrally stable equilibrium between prey and predator population sizes. This means that, at any initial starting point, the prey and predator population sizes are constantly fluctuating. The magnitude of these fluctuations increases with the distance of the initial conditions from the equilibrium point $x^* = c/d$, $y^* = a/b$.

The original model had four terms as follows:

$$x' = ax - bxy$$

$$y' = -cy + d(bxy)$$

where x is the population size of the hare and y is the population size of the volf, a is the per capita birth rate of the hare, b is the encounter probability between volf and hare (bx is the rate at which a volf individual kills hare), c is the per capita death rate of the volf, and d is the conversion efficiency of hare consumed by a volf into new volfs.

The numerical response (Fig. 1) describes the conversion of hare density into volf density, and the functional response captures the relationship between the consumption rate and food density. Type 1 shows the case that the rate of consumption of volfs is proportional to hare density. Type II relates to the case that the number of hares consumed increases rapidly with increased hare population density but plateaus at a carrying capacity. Type III is similar to Type II but assumes that at low hare density of hare consumption is slower than in Type II.

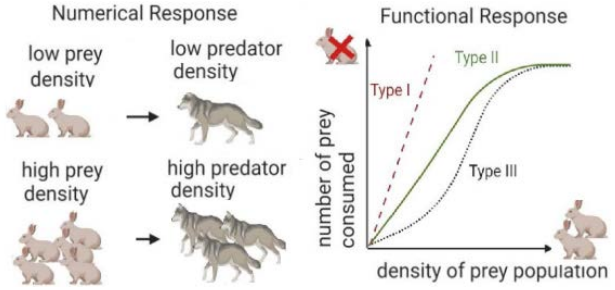

Fig. 1. Predator-Prey Model: numerical vs functional response [2]

Cancer model. The Lotka-Volterra model is useful for the description of the competition between two distinct cancer cell populations [3], between drug-sensitive (S) and drug-resistant (R) cells (Fig. 2), describing in the form of the following equations:

$$\frac{dS}{dt} = r_S\left(1 - \frac{S+R}{K}\right)S - \delta S$$

$$\frac{dR}{dt} = r_R\left(1 - \frac{C*S+R}{K}\right)R$$

where rS and rR indicate the intrinsic growth rates of S and R, respectively. The term $\delta > 0$ imposes a death rate on S due to therapy.

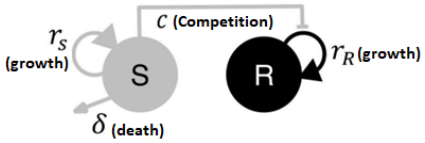

Fig. 2. The mathematical model of tumor growth and treatment [3]

Oncolytic virotherapy model [4]. Viruses are infectious agents that depend on a living host cell for replication. Oncolytic agents primarily move in tumor cells, leading to tumor cell lysis and severe antitumor effects. The oncolytic virotherapy model is the following: host cells are divided into susceptible (uninfected, S) and infected (I) cells, where the total number of tumor cells is C = S + I and viral population V. Term βII is described as directly affecting the number of virus particles (δ). The term for viral infection is γVS, and the term uV(t) simulates the effect of oncolytic virotherapy, and virus particles administered at time t (Fig. 3).

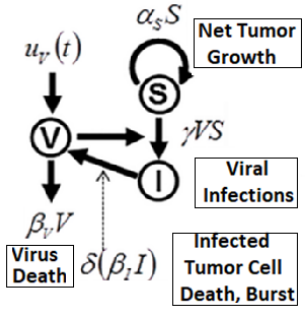

Fig. 3. Oncolytic virotherapy model [4]

Thus, we get the following equations:

$$\frac{dS}{dt} = \alpha_S S - \gamma VS$$

$$\frac{dI}{dt} = \gamma VS - \beta_I I$$

$$\frac{dV}{dt} = u_V(t) + \delta(\beta_I I) - \beta_V V$$

Birth–death process of phagocytosis. In [5], the well-known process of birth and death (a simple Markov model) is expanded in the modeling of phagocytosis. The model considers stochastic interactions between bacteria and immune cells and heterogeneity in the susceptibility of individual hosts to infection within a population. The aim is to study a dose-time response to intracellular bacterial infection dynamics after inhalation.

We have the following state definition {T, P} where T is the total number of extracellular bacteria, and P is bacteria-containing phagocytes. The birth, death, and survival rates are $\lambda > 0$ and $\mu > 0$ and $\alpha > 0$ respectively and the threshold for illness is M (Fig. 4).

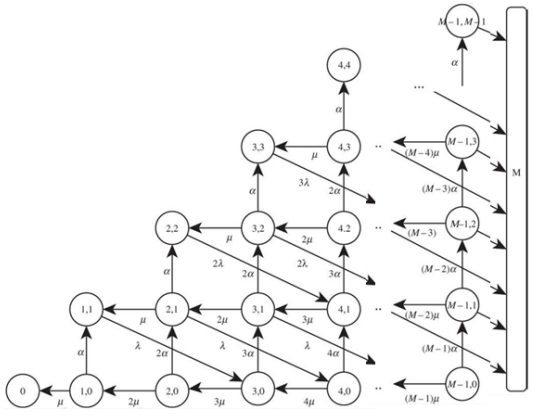

Fig. 4. The Markov chain of the birth-death-survival process [5]

The Kolmogorov equations (in the form of ordinary differential equations) are the primary means to solve a time-homogeneous Markov process.

These calculations are suitable for mathematicians – as an illustration of application in medicine and the use of some sophistical mathematical tools, but cannot give anything to doctors: if only because of the dimension of the model, for example, in a cancer of size as small as 1 mm3 there are 106 cells. We must note that the medical systems are too complicated. Let us give some preliminary insight into our organism.

III. The Sunrise of Immunology

Ilya Mechnikov (1845-1916) is best known for his pioneering research in immunology. He and Paul Ehrlich (1854-1915) – a German medical scientist – were jointly awarded the 1908 Nobel Prize in Physiology or Medicine "in recognition of their work on immunity" [6]. Working at the Bacteriological Institute, Odessa (1886-87), and at the Pasteur Institute, Paris (1888-1916), Mechnikov contributed to many important discoveries in the field of immune response. His most notable achievement was recognizing that phagocytes are the first line of defense against acute infection and that phagocytes are a type of white blood cell. In 1887, he noticed that white blood cells were attracted to certain bacteria. This work formed the basis of Metchnikov's cellular (phagocytic) theory of immunity (1892) and coined the term "pathogen". This hypothesis generated much resistance, especially from scientists who supported the so-called humoral theory of immunity and argued that only body fluids and blood solubles (antibodies). Although the humoral theory held sway for the next 50 years, in the 1940s scientists began to reconsider the role of cells in fighting

infection.

The hypothesis developed by Paul Ehrlich [7], to explain immunological phenomena, was a side chain theory that described how antibodies, protective proteins produced by the immune system, are formed and how they react with other substances. Ehrlich postulated that each cell has on its surface several side chains or receptors that function by attaching to specific food molecules. This theory about antibodies and cell surface receptors is working till nowadays.

Each side chain interacts with a specific nutrient (similar to how a key is inserted into a lock), it can also interact with other molecules, such as antigens (disease-causing toxins) produced by an infectious agent. Once the toxin binds to the side chain, the interaction is irreversible and blocks subsequent binding and nutrient uptake. The body then tries to overcome this obstacle by producing large numbers of replacement side chains – so many that they cannot fit on the cell surface and are secreted into the circulation. According to Ehrlich's theory, these circulating side chains are antibodies, they are tuned and capable of neutralizing a disease-causing toxin. Then they remain in circulation, thereby immunizing the individual against subsequent infestations of the infectious agent. Phagocytosis is primarily a protective reaction against infection and the penetration of foreign substances into the body (Fig. 5).

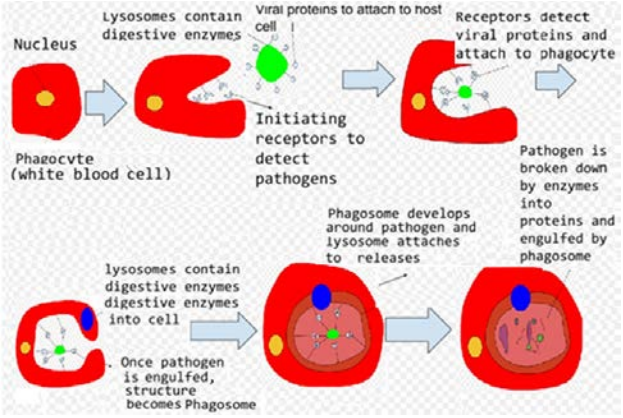

Fig. 5. Absorption of the pathogen by phagocyte [7]

IV. WHITE BLOOD CELLS - KEY FIGHTERS AGAINST INFECTIONS

White blood cells (leukocytes) are cells of the immune system that protect the body both from diseases and foreign invaders (Table 1). Leukocytes produce hydrogen cyanide during phagocytosis and can kill bacteria, fungi, and other pathogens, producing several other toxic chemicals.

T cells. The thymus is a specialized lymphoid organ of the immune system (Fig. 6). T cells born in the bone marrow migrate to the thymus to develop (or mature). After migrating to the thymus, progenitor cells mature into several types of T cells. T cells are critical to the adaptive immune system, where the body adapts to specific foreign invaders. T cells can be distinguished from other lymphocytes by the T cell receptor (TCR) on their cell surface.

B cells (B lymphocytes) function in the humoral component of immunity of the adaptive immune system. B cell activation occurs in secondary lymphoid organs such as the spleen and lymph nodes. After B cells mature in the bone marrow, they migrate through the blood to the spleen, which receives a constant supply of antigens through circulating lymph.

Table 1. White blood cells overview [8]

Type	%	Main targets
Neutrophil	62%	Bacteria
Eosinophil	2.3 %	Larger parasites and Modulate allergic inflammatory responses
Basophil	0.4 %	Release histamine for inflammatory responses
Lymphocyte	30%	B cells: releases antibodies and assists activation of T cells T cells: • CD4+ T helper cells: activate and regulate T and B cells • CD8+ cytotoxic T cells: virus-infected and tumor cells. • Gamma delta T cells: bridge between innate and adaptive immune responses; phagocytosis • Regulatory T cells: Returns the functioning of the immune system to normal operation after infection; prevents autoimmunity Natural killer cells: virus-infected and tumor cells.
Monocyte	5.3 %	Monocytes migrate from the bloodstream to other tissues and differentiate into tissue-resident **macrophages**.

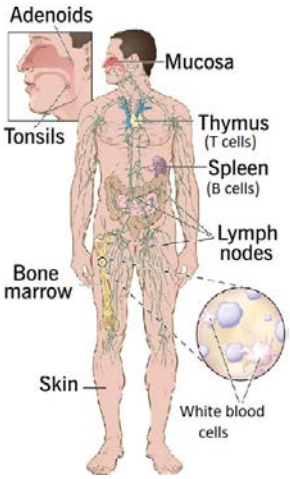

Fig. 6. Immune system and lymphocyte sources

B cells produce antibody molecules that can either be secreted or inserted into the plasma membrane, where they serve as part of the B cell receptors (BCRs). When a naïve

(or memory B) cell is activated by an antigen, it proliferates and differentiates into an antibody-secreting effector cell known as a plasma cell. In addition, B cells present antigens (these B cells are called *antigen-presenting cells*, APCs) (Fig. 7).

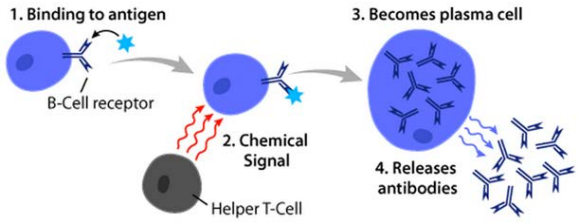

Fig. 7. The main functions of B cells are (1) to bind to antigens, (2) to receive help from a related T helper cell, (3) to differentiate into a plasma cell that (4) secretes antibodies

Regulatory T cells are a special population of T cells that provide a critical tolerance mechanism by which immune cells can distinguish invading cells from self. This prevents immune cells from reacting inappropriately against their own cells, known as an “autoimmune” response. Unfortunately, these same regulatory T cells can also be used by cancer cells to prevent tumor cells from recognizing and mounting an immune response against them. This is one difficult and unclear question.

Dendritic cells (DCs) may be called a nature's miracle carrying critical danger detection function. They are white blood cells that can carry on the task of antigen-presenting cells (APS) by monitoring human tissue. They receive signals from pathogens (inflammatory cytokines) and migrate to the lymph nodes. Migration of DCs from peripheral tissues to lymphoid organs is key to their antigen transport functions. Then they interact with T cells initiating and shaping the adaptive immune response [9]. Thus, they act as intermediaries between the innate and adaptive immune systems.

Different pathogens trigger different maturation profiles of dendritic cells, resulting in a polarization of different T cell subsets. An antigen-presenting cell (APC) displays antigen bound by *major histocompatibility complex* (MHC) proteins on its surface; this process is known as antigen presentation. T cells can recognize MHC complexes using their T cell receptors, TCRs (Fig. 8).

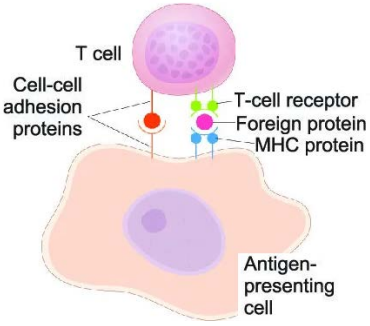

Fig. 8. How APCs work [10]

The exact genesis of dendritic cells and their interrelationship is only marginally understood now. It is worth noting that they can act in pro-cancer and anticancer roles.

Besides, it is worth mentioning *cytokines*, *chemokines*, and *interferons*. Cytokines are small cell-signaling protein molecules secreted by numerous cells and are a category of signaling molecules used extensively in intercellular communication. Hundreds of cytokines have been identified. Cytokines are the general category of messenger molecules, while chemokines are a special type of cytokine that directs the migration of white blood cells to infected or damaged tissues.

One popular cytokine signaling mechanism used by cytokines such as IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15 and the interferons. Interferons (IFNs) are key cytokines in both innate and adaptive antiviral immune responses. This family of cytokines comprises (1) the type I or viral IFNs (mainly α, β, ω, and τ) and (2) the type II or immune IFN (γ).

Interferon was discovered in the late 1950s. Interferon is an antiviral protein of mammals with prosperous immunomodulatory and antitumor properties (Fig. 9). Interferons are named for their ability to "interfere" with viral replication by protecting cells from virus infections. However, virus-encoded genetic elements can antagonize the IFN response, contributing to viral pathogenesis and viral diseases.

For the sake of historical justice, it should be said that the achievements of Latvian virologists - the medicines Rigvir and Larifan – were initiated after the invention of interferon.

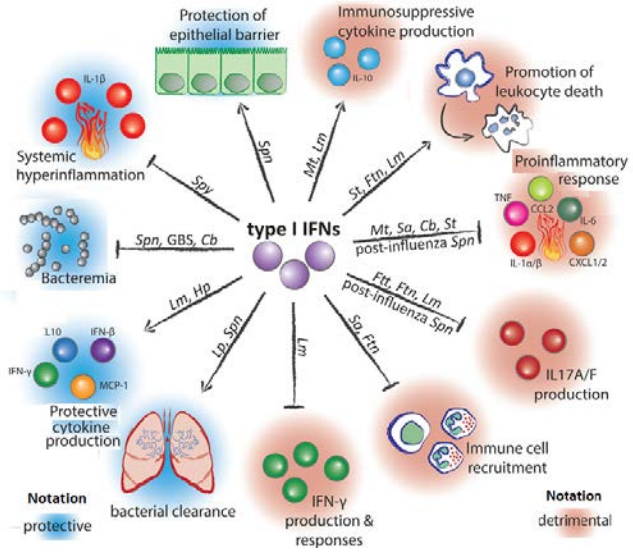

Fig. 9. Mechanisms of action and effects of type I interferons during infection with bacterial pathogens [11]. Arrow-headed lines represent stimulation and bar-headed lines represent inhibition by type I IFNs. Pathogen abbreviations: *Spn, Streptococcus pneumoniae*; *Spy, Streptococcus pyogenes*; GBS, Group B Streptococcus; *Cb, Coxiella burnetii*; *Lm, Listeria monocytogenes*; *Hp, Helicobacter pylori*; *Lp, Legionella pneumophilia*; *Sa, Staphylococcus aureus*; *Ftn, Francisella tularensis*; *Ftt, Francisella tularensis*; *Mt, Mycobacterium tuberculosis*; *St, Salmonella enterica.*

The *cluster of differentiation* (abbreviated as CD) is a protocol used for the identification and investigation of cell surface molecules. CD molecules can act in numerous ways,

often as receptors or ligands important to the cell. CD for humans is numbered up to 371 (as of 21 April 2016).

V. Immunity and Melanoma

Tumors are one unclear problem of immunology. They are objects that should not stimulate immunity, either because they are not associated with microbial stimulators, or because they are healthy growing cells that do not send alarms. Thus, to eradicate a tumor, we should:

• infect it,

• or create repeated lesions to alert local APCs,

• or we should revaccinate with an immune-stimulating tumor vaccine.

Melanoma is a type of cancer that develops from pigment-producing cells (Fig. 10). Melanoma is known as the most dangerous and deadly type of skin cancer. It is a highly complex disease characterized by genetic mutations and an immune microenvironment that favors drug resistance and disease progression. Melanoma accounts for approximately 1% of skin cancer but it originates up to 60% of deaths from cutaneous malignancies.

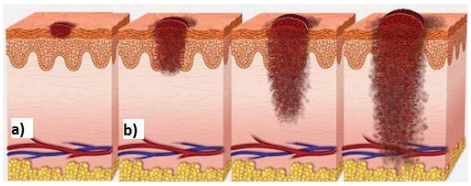

Fig. 10. a) Melanoma starts from pigment-producing cells, b) three stages of melanoma, in the third stage it produces metastases.

Let us demonstrate the complexity of the fight against melanoma [12]. We look at innate immunity and adaptive immunity (Fig. 11).

Innate immunity. Several therapeutic strategies to inhibit melanoma growth specifically target the activation of antitumor activities in innate subsets found in tumors:

- Natural killer (NK) cells bind tumor cells through receptor/ligand interactions and release cytolytic molecules, causing cell death.
- Phagocytes (such as polymorphonuclear neutrophils, PMNs), macrophages (Mφ), and dendritic cells (DCs) process dead tumor cells and present tumor-associated antigens (TAAs).
- DCs actively use cytokines released from activated NK cells.

Adaptive immunity. Long-term memory responses essential for melanoma remission include the activation and proliferation of adaptive immune cells, namely helper CD4+ T cells and cytotoxic CD8+ T cells. As mentioned above, DCs and to some extent macrophages are most capable of activating adaptive immunity to induce cytotoxicity of CD8+ effector T cells as well as promote the formation of memory immune populations involved in long-term remission. The recruitment of T- and B-lymphocytes by chemokines and the presentation of TAA molecules activate the adaptive part of immunity:

- Tumor-specific CD8+ T-cells bind tumor cells displaying TAAs on MHC molecules through the engagement of the T-cell receptor, which triggers the release of cytotoxic granules by the tumor cells.
- Tumor-specific CD4 + T-cells engage B-lymphocytes using TAAs presented by MHC molecules, leading to the release of TAA-specific antibodies. It causes tumor cell death through various mechanisms.
- Adaptive immune cells also reactivate innate immunity and kill tumor cells, this additionally releases TAA, which is processed by APCs.

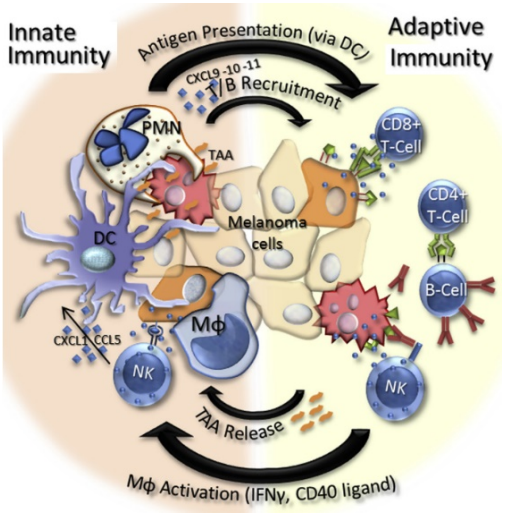

Fig. 11. Against Melanoma by Immune Cells [12]

The immune escape mechanism. The plasticity of melanoma cells leads to a phenomenon called “immune escape”, whereby cancer cells acquire a less immunogenic phenotype and suppress anti-tumor immune cells within the tumor microenvironment.

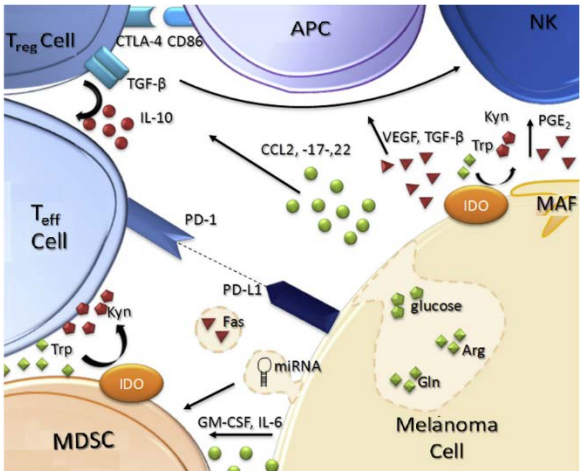

Fig. 12. Mechanisms of Immune Escape in Melanoma [12]

There are a lot of immune escape mechanisms in melanoma (Fig. 12), which contain many complex medical terms:

- Melanoma cells express IDO (Indoleamine 2, 3-dioxygenase), which inhibits the natural cytotoxicity of NK cells against tumor cells through the conversion of available tryptophan (Trp) to suppressive kynurenine (Kyn).
- Melanoma-associated fibroblasts (MAFs) further

inhibit NK cells through the secretion of Prostaglandin E2 (PGE2).

- Melanoma cells also secrete factors such as VEGF and TGF-β to inhibit the recruitment and function of APCs such as DCs.
- Immunosuppressive regulatory T-cells (Treg) are recruited by melanoma cells through chemokine secretion and these cells further inhibit APCs through engagement of CD86 by CTLA-4 expressed on the Treg surface.
- Treg also releases inhibitory cytokines to activated effector T-cells (Teff), which prohibits their melanoma-directed cytotoxicity, and express membrane-bound TGF-β, which inhibits NK cell action.
- Melanoma cells directly inhibit Teff action through the PD-L1 ligand and induce energy when binding the PD-1 receptor on T-cells.
- Secretion of apoptosis (inducing factors such as Fas ligand within exosomes) leads to apoptosis of Teff.
- Melanoma cells also recruit and convert myeloid-derived suppressor cells (MDSC) through the secretion of GM-CSF or IL-6 and deliver exosomes loaded with micro RNAs (miRNA).
- MDSC inhibits Teff through multiple mechanisms such as the expression of IDO.

Finally, melanoma cells deplete glucose and amino acids such as glutamine and arginine from the tumor microenvironment leading to immune cell starvation.

How to overcome these many immune escape mechanisms in melanoma, – is a hard issue. Since each patient would be unique in the features of their melanoma, it will be necessary to determine those features before treatment to select a balanced combination that will maximize the treatment efficiency and minimize toxicity.

VI. DUAL ROLE OF MACROPHAGES AND DENDRITIC CELLS AND THEIR MATHEMATICAL MODELING

Generally, cancers do not have danger signals and, therefore, cannot elicit strong immune reactions. To propose hypotheses regarding the biological mechanisms behind the observed discrepancies in experimental and clinical data, we need to have a better understanding of the interactions between the M1 and M2 macrophages and other cells in the microenvironment, such as the Th1 and Th2 cells with which the macrophages interact via type-I (e.g., IFN-γ, IL-12) and type-II (e.g., IL-4, IL-10) cytokines. It has been illustrated experimentally that the existence of the alternatively activated M2 cells promotes tumor growth (Fig. 13). Hence, it is necessary to consider the interplay between M1 and M2 macrophages during tumor evolution. Several mathematical models have been established to study the roles of M1 and M2 macrophages in tumor development and progression.

Cancer cells attract immature dendritic cells possibly through chemokines such as CCL20 or CXCL12. Dendritic cells can then be either blocked or skewed in their maturation—eg, by vascular endothelial growth factor—leading to induction of polarised CD4+ T cells that promote the expansion of cancer cells (pro-cancer) at the expense of CD8+ T cells that can cause tumor regression (anticancer). As mentioned above, the dendritic cells are a kind of nature's mirror, unfortunately far from knowing their role.

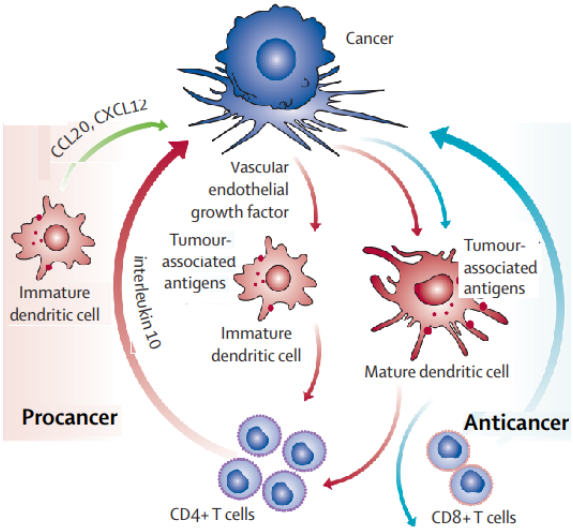

Fig. 13. Role of dendritic cells in the tumor microenvironment [13]

In 2016, den Breems and Eftimie [14] derived a new non-spatial mathematical model that describes the interactions between tumor cells, M1 and M2 macrophages, Th1 and Th2 cells, to investigate whether the variation in the M2/M1 ratio and the re-polarisation of macrophages accounts for the difference in tumor growth or tumor decay [14]. By numerical simulations and sensitivity analysis, they showed that the re-polarisation rates for M2 and M1 macrophages impact tumors. Type-II dominated response (i.e., more M2 and Th2 cells than M1 and Th1 cells) is associated with tumor growth and the M1 to M2 transition rate delay in tumor growth and size.

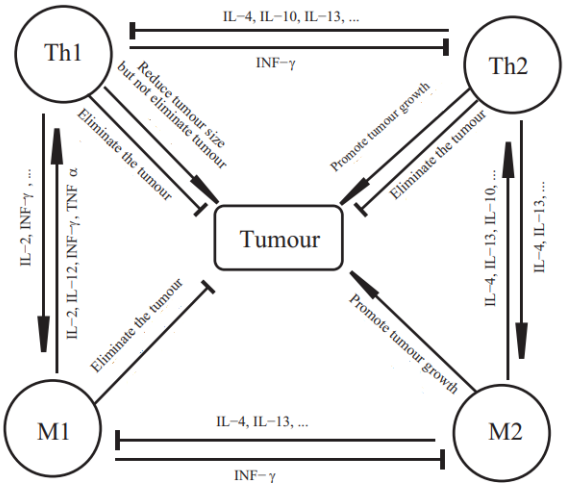

Fig. 14. Graphical description of the possible interactions between M1/M2 macrophages, Th1/Th2 cells, and tumor cells, via type-I cytokines (e.g. IFN −γ) and type-II cytokines (e.g. IL −4, IL −13) [15]

In 2017, Eftimie and Hamam [15] modified the previous model [14] to test hypotheses regarding the macrophage paradox in melanoma immunotherapies. They showed that tumor elimination both in the presence of a type-I dominated immune response (i.e., more M1 and Th1 cells) and in the

presence of a type-II dominated immune response. As observed experimentally, tumor growth occurs in the presence of a type-II dominated immune response. Moreover, tumor dormancy is the result of a delicate balance between the pro-tumor effects of M2 cells and the anti-tumor effects of Th1 and M1 cells (Fig. 14).

In 2018, Eftimie and Eftimie incorporated tumor therapy with an oncolytic virus into the M1 and M2 macrophages model [16]. They showed that the decay of tumor cells depends not only on the M2/M1 ratios that characterize tumor relapse but also on the number of tumor-infiltrating macrophages. Besides, they identified the parameters that can slow down tumor relapse.

VII. A VIVID EXAMPLE OF *GLASPERLENSPIEL*

Let us consider a generalized Lotka–Volterra model [17]. The purpose is to build the model of the Tumor-Immune Microenvironment (TIME). This model describes the relationships between tumor cell (T), pro-tumor immune cell (P), and anti-tumor immune cell (A) populations. The basic innovation here is parameter $\omega \geq 0$, which means a tumor-induced switching term from anti-tumor (ATI) to pro-tumor immune (PTI) cells. The authors [17] solve the following system of ordinary differential equations:

$$\frac{dT}{dt} = T\left(r_T - \frac{r_T}{K_T}T + \alpha_{TP}P - \alpha_{TA}A\right)$$
$$\frac{dP}{dt} = P\left(-d_P P + \alpha_{PT}T\right) + \omega AT$$
$$\frac{dA}{dt} = A\left(r_A - \frac{r_A}{K_A}A + \alpha_{AT}T - \alpha_{AP}P\right) - \omega AT$$

The parameters r_T and r_A describe the "intrinsic" growth rates of tumor and ATI cells each in the absence of other cell types, while K_T and K_A denote their carrying capacities (Fig. 15). The parameter α_{XY} is the effect of Y on the net growth rate of X. The key point here is a conversion parameter $\omega \geq 0$ controlling the rate at which tumor cells induce ATI cells to switch to PTI cells.

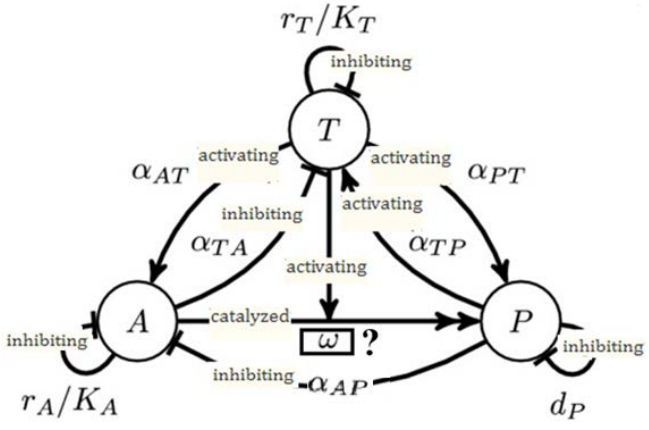

Fig. 15. Schematic diagram of interactions in the model [17]

The system includes terms reflecting the ability of tumor cells to induce some immune cells that inhibit tumor growth, such as M1 macrophages, to switch phenotypes into functionally pro-tumor states, such as M2 macrophages.

The analysis of the effects of tumor-induced immune cell conversion in a simple model of the TIME is carried on, finding that an immune cell conversion term allows for bistability between a cancer-free state and a state with a non-zero tumor cell density (Fig. 16). These results suggest an important role for immune cell conversion in the early stages of tumor growth before the TIME has been shaped into a pro-tumor state.

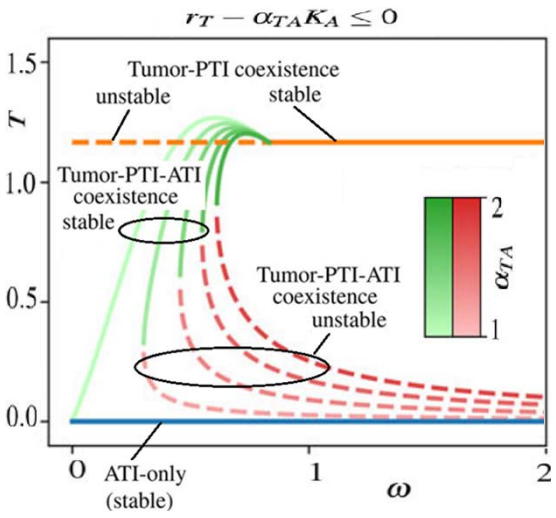

Fig. 16. Bifurcation diagrams over ω for different ATI tumor-killing rates α_{TA}.[17]

Unfortunately, this excellent paper [17] is nothing more than a purely mathematical exercise, it is a case of *Glasperlenspiel* since the key parameter ω is unknown controlling the rate at which tumor cells induce ATI cells to switch to PTI cells (How to overcome many immune escape mechanisms shown in Fig. 12?). The hard work is needed to uncover the role of tumor-induced immune cell conversion on cancer dynamics.

We hope – such intricate mathematical models will come in handy over time.

Table 2. dsRNA based drugs: modern trends of pharmaceutical developments (an excerpt from [18])

Name	Structure	Developer	Current status	Prescription
Natural dsRNAs				
Ridostin	Mixture of dsRNA and single stranded RNA from *Saccharomyces cerevisiae*	SRC VB "Vector" (Russia) 1994-2015	Approved for medical application	Influenza, herpes, chlamydia, tick-borne encephalitis
Ridostin Pro	Mixture of dsRNA and single stranded RNA from *Saccharomyces cerevisiae*, stabilized with polyvinylpyrrolidone	SRC VB "Vector" (Russia) 2001	Registration stage	Influenza, SARS
Larifan	DsRNA of phage f2	Larifan Ltd. (Latvia) 2018	Approved for medical application	Arbovirus and rhabdovirus infections, herpetic diseases
Rifastin	DsRNA of phage φ6	SRC VB "Vector" (Russia) 1990-2018	Laboratory studies	Viral infections (Omsk hemorrhagic fever, influenza)
Synthetic dsRNAs				
PolyA:PolyU				
Poludan	Double-stranded complex polyriboadenylic and polyribouridylic acids	LANS-Pharm Ltd. (Russia) 1982-2018	Approved for medical application	Viral eye diseases (adenovirus and herpetic infections), influenza and SARS
Polyadenur	Double-stranded complex polyriboadenylic and polyribouridylic acids	Beaufour Ipsen (France), Hemispherx Biopharma (USA)	Clinical trials 2003-2019	Hepatitis B and C (as a part of IFN-α based complex therapy), breast cancer
PolyI:PolyC				
PolyI:PolyC	Double-stranded complex of polyriboinosinic and polyribocytidylic acids	University of Alabama at Birmingham, Hemispherx Biopharma, Inc. (USA); Medical University of South Carolina, Gibbs Cancer Center and Research Institute, Eli Lilly and Company (USA), etc.	Clinical trials 2014-2019	Hepatocellular carcinoma, hepatitis B, influenza

VIII. IMMUNOMODULATORS TURN CANCER FROM A COLD TO A HOT STATE

There are a lot of attempts in search of immunomodulators to make cancer visible to the immune system, in other words, to turn it from a cold to a hot state. Let us refer to the summary [18]. Unfortunately, they all are to a great extent in laboratory studies.

Table 2 summarizes literature data on the development of drugs based on natural and synthetic high-polymeric double-stranded RNA (dsRNA), and their antiviral, immunoadjuvant, and antitumor properties. It has been shown that enhancing the innate immune response with dsRNA can effectively improve methods for treating and preventing infectious and cancer diseases. The further use of dsRNA to treat pathological processes of different origins is discussed.

Our goal is to compare immunomodulators Poly (I:C) and Larifan, both of which are double-stranded RNA (named in Table 2).

Immunomodulator POLY(I:C): state-of-the-art. Polyinosinic: polycytidylic acid, or Poly(I:C), is a *synthetic* double-stranded RNA (dsRNA). Poly(I:C) has been extensively investigated for decades for its immune-stimulatory properties and potential use as a vaccine adjuvant. Poly (I:C) has been known as a potent inducer of type I IFN ([19] published in 1967). During these 50-60 years, Poly (I:C) is investigated in detail, as depicted in Fig. 17.

Due to poly(I:C) capacity to activate many immune cell types, directly and indirectly, is distinctly known for its immunostimulatory activity:

- It is primarily renowned as a priming agent for activating antigen-presenting cells, particularly, dendritic cells (DC). Indeed, poly(I:C) activates DC to strongly upregulate signals required for antigen-specific T-cell priming, which include: co-stimulatory molecules CD80, CD86, and CD40; pro-inflammatory cytokines such as IL-12; and, chemokines that attract T cells, e.g. CXCL10.
- Furthermore, poly(I:C) treatment of DC in a booster phase stimulates secondary T-cell expansion to a much greater extent than other TLR agonists, through type I IFN and IL-15 signaling.
- In addition, poly(I:C)-treated DC activates natural killer (NK) cells through both IL-12 secretion and cell-cell contact.

Notations in Fig. 17:

- CCL/CXCL, C-C/C-X-C motif chemokine ligand;
- IL, interleukin;
- IL-1RN, IL-1 receptor antagonist;
- ISG15, IFN-stimulated gene 15;
- M0 – undifferentiated macrophages with the potential to polarize into specific macrophage subtypes;
- MGMT, O^6-methylguanine-DNA methyltransferase;
- MX1, MC dynamin-line GTPase 1;
- Noxa, phorbol-12-myristate-13-acetate-induced protein 1;
- PD-L, programmed death 1 ligand;
- TGF, transforming growth factor;
- TAM, tumor-associated macrophage;
- TAP1/2, transporter 1/2, ATP binding cassette subfamily B member;
- TNF, tumor necrosis factor

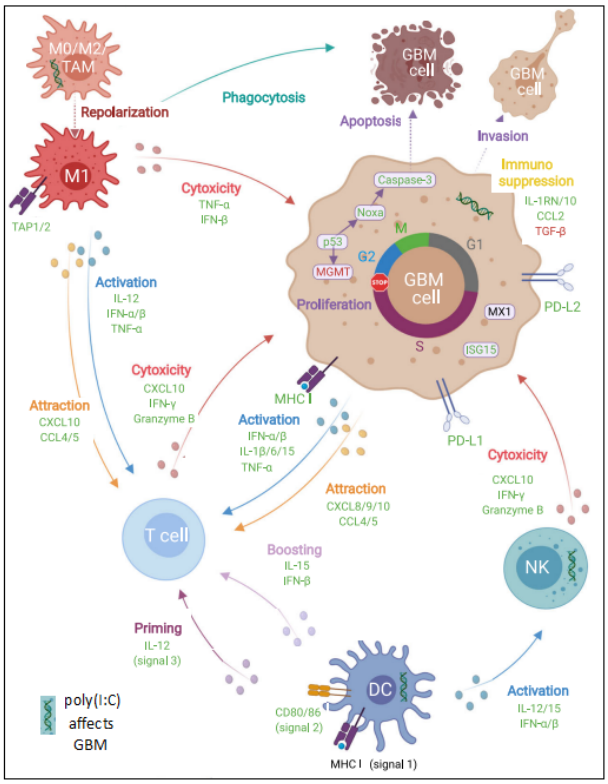

Fig. 17. An integrated overview of how poly(I:C) affects GBM and immune cells on molecular and functional levels. Poly(I:C) activates several immune cells directly and indirectly. Note that macrophages repolarize to M1 [20]

Unfortunately, although several Poly(I:C) modifications have been developed, none of these derivatives have passed any clinical trials, primarily due to the range of endotoxin-like side effects. Although the Poly(I:C) complexes did not alter phagocytosis, all of the complexes inhibited drug metabolism by liver microsomal enzymes. Poly(I:C) complexes are effective IFN inducers in humans, but their toxicity limits their use in cancer patients. Poly(I:C) agents induced pulmonary thrombosis and hepatic necrosis. Thus, there is a continued search for potential dsRNA analogs.

Immunomodulator Larifan: state-of-the-art. Bacteriophage-derived dsRNA (bf-dsRNA), also known as Larifan, is a heterogeneous population of dsRNA, which have been isolated from E. coli cells infected with a single-stranded RNA bacteriophage f2 mutant.

The original active substance Larifan was developed in 1976, commercial production has been performed since 1994. By the decision of the specialized expert commission on antiviral drugs at the Pharmacological Committee dated 03/06/1991, the test results were approved and the drug was sent for registration to the USSR Pharmaceutical Committee. Registration ended due to the collapse of the USSR. The Larifan registration process continued in Latvia, the injection form was registered in 2004.

Larifan is best known for its ability to induce type-I IFNs and is therefore used as an antiviral agent to treat herpes, papilloma, and influenza virus infections. However, the

effect of Larifan has not been analyzed systemically.

In the study [21], for the first time, in 2019, the immunomodulatory effects triggered by bf-dsRNA and poly(I:C) on freshly isolated human peripheral blood mononuclear cells (PBMCs) in ex vivo cultures are directly compared. The study was conducted at the Latvian Biomedical Research and Study Centre.

There are two data displays. All data are displayed as the means ± 95% confidence interval. The first one contains data from 12 molecules (IFN-α2, CCL17, TNF-α, CCL4, IL-12p70, GM-CSF, CCL1, CXCL10, IL-1β, CCL13, IL-23, IL-9) from 29 total changing power up to 5 days (isolated and stimulated by the Luminex 200).

The second data collection is flow cytometry-based. The effect of bf-dsRNA and poly(I:C) on the expression of lymphocyte molecular markers ex vivo (n = 9). The collected PBMC samples were labeled with 18 different markers (11 markers are displayed):

1) 14 fluorophore-conjugated monoclonal antibodies for the following surface markers: anti-CD45-V500, anti-CD8-V450, anti-CD25-V450, anti-HLA-DR-FITC, anti-CD38-FITC, anti-CD4-FITC, anti-CD56-PerCP-CyTM5.5, anti-CD3-APC-H7, anti-CD28-APC, anti-CD95-APC, anti-CD152-PE, anti-CD119-PE, anti-CD16- PerCP-CyTM5.5, and anti-CD19-PE.

2) 4 intracellular markers were also used: anti-Perforin-PE, anti-Granzyme B-FITC, anti-IFN-γ- Alexa Fluor 647, and anti-FoxP3-Alexa Fluor 647.

The conclusion from both experiments follows: the effect of bf-dsRNA on *ex vivo* cultivated PBMCs was similar to that induced by poly(I:C). Both exhibited the potential to promote the release of proinflammatory cytokines and chemokines *ex vivo*, which could translate to the *in vivo* activation of the innate immune response and subsequent T-cell activation.

The current obvious future work is for statisticians, namely, the numerical analysis of 29 different cytokines and chemokines (from the first data display) in 29-dimensional space by the support vector machine tools as two classes (immunomodulators POLY(I:C) and Larifan) as well as the change in time by regression analysis.

The same numerical analysis of 18 lymphocyte molecular markers (from the second data display) in 18-dimensional space by the support vector machine tools as two classes (immunomodulators POLY(I:C) and Larifan) as well as the change in time by regression analysis.

For immunologists, the hard work should be done to describe the Larifan features, following Poly(I:C) (as Fig. 17 shows).

IX. Oncolytic Virus Rigvir

Oncolytic virus therapy is a novel approach in the field of cancer treatment. Oncolytic viruses can occur naturally or they can be created using genetic engineering. The virotherapy method is based on the selective effect of viruses on tumor cells, causing their death or oncolysis, practically without damaging healthy cells and at the same time stimulating normal immunity and its resistance to the tumor (Fig. 18).

Table 3. Approved oncolytic viruses up to 2021 [22]

Year	Oncolytic virus	Type of tumor
2004	ECHO-7 Rigvir (Latvia)	melanoma
2005	H101 (China)	late-stage refractory nasopharyngeal cancer
2015	T-VEC (USA)	advanced melanoma
2021	Teserpaturev (Japan)	malignant glioma

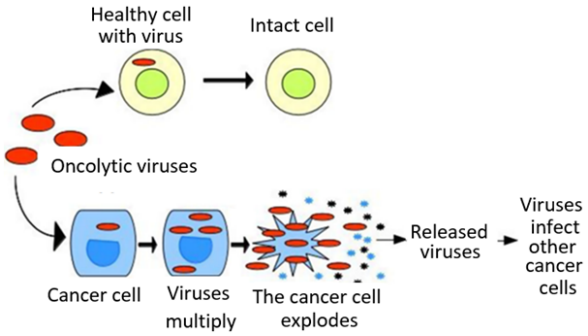

Fig. 18. Schematic representation of oncolytic virotherapy. Viruses can specifically infect cancer cells and then multiply until the cancer cells burst. The newborn viruses are then released to infect (and then burst!) other cancer cells

Only four oncolytic viruses are registered up to 2021 (Table 3). The first oncolytic virus in the world was the genetically unmodified ECHO-7 strain enterovirus Rigvir, which was approved in Latvia in 2004 for skin melanoma treatment. Three other viruses mentioned in Table I are genetically modified [22]. Therefore, RIGVIR is kind of a miracle of nature. An oncolytic adenovirus, a genetically modified adenovirus named H101, was approved in China for head and neck cancer treatment in 2005. In 2015, talimogene laherparepvec (T-VEC), an oncolytic herpes virus, which is a modified herpes simplex virus, became the first oncolytic virus to be approved for use in the U.S. and the European Union, for the treatment of advanced inoperable melanoma.

T-VEC is not the best option as a monotherapy but its administration combined with cancer immunotherapy could prove particularly effective. The fact that OVs are injected locally into the tumor to avoid pre-existing antiviral immunity is also considered a limitation because, in this case, the virus may not reach tumors in organs that are difficult to reach with an injection [23].

On December 16, 2022, the U.S. Food and Drug Administration approved Adstiladrin (nadofaragene firadenovec-vncg) for adult patients with unresponsive non-muscle invasive bladder cancer.

On the history of Rigvir. In 1960, a previously unknown phenomenon in the world was recorded, namely, that human intestinal viruses (enteroviruses of the ECHO group) obtained from young children can destroy certain types of human tumors (angiosarcomas) vaccinated in hamsters [24]. The research started in Latvia in 1959. About 60 types of viruses were isolated from the gastrointestinal tract of healthy children. One of these viruses turned out to be the most suitable for oncology. RIGVIR activates immune cells (lymphocytes) – T-cells and B-cells. RIGVIR administration was based on the changes in $CD4^+$, $CD8^+$, and $CD38^+$ lymphocytes. The first clinical trial was approved in April

1968. The RIGVIR pre-registration clinical studies had been performed from 1968 to 1991. Registration ended due to the collapse of the USSR. The first oncolytic genetically unmodified ECHO-7 strain enterovirus RIGVIR was approved in Latvia in 2004 for the treatment of skin melanoma. In March 2019, Rigvir's approval as a virotherapy agent was withdrawn in Latvia by Latvia's State Agency of Medicines due to discrepancies between the laboratory testing of Rigvir samples and previously reported results. A series of clinical trials should be performed according to the current standards for clinical use within the EU area.

Discussion on Rigvir. The future of Rigvir is currently unknown. Meanwhile, genetic research was carried out in Finland [25]. Here are a few quotes from [25]: "Rigvir is a melanoma cell-adapted (genetically unmodified) formulation of echovirus 7 (E7) isolate. Rigvir claims to have both oncolytic and oncotropic properties while being safe to use and free from adverse effects to the patient (…)

Rigvir contained nine unique mutations in the viral capsid proteins VP1-VP4 across the whole data set, with a structural analysis showing six of the mutations concerning residues with surface exposure on the cytoplasmic side of the viral capsid (…) Rigvir and five other isolates were also subjected to cell infectivity assays performed on eight different cell lines.

However, there have not been conventional clinical trials using Rigvir (…) We conclude that Rigvir's claim of being an effective treatment against multiple cancers is not warranted under the evidence presented here. Bioinformatic analyses do not reveal a clear mechanism that could elucidate Rigvir's function at a molecular level, and cell infectivity tests do not show a discernable difference in either the oncolytic or oncotropic effect between Rigvir and other clinical E7 isolates used in the study."

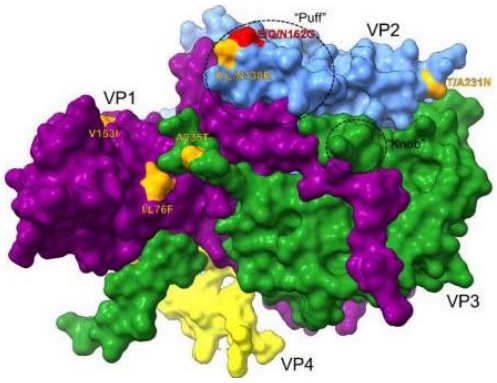

Fig. 19. Echovirus 7 capsid protein structure depicting unique amino acid mutations. Visible unique mutations of Rigvir (in orange and red) exhibit sufficient surface exposure [25]

There were objections from representatives of the Rigvir Group [26], to which the authors of the genetic studies responded [27]: "We call for further studies regarding Rigvir's efficacy, especially regarding what potentially makes it unique compared to clinical E7 isolates, which possess a seemingly similar infection profile across native and cancer cell lines. It is our understanding that clinical trials performed with Rigvir are small in terms of the number of trials and test subjects. Dr. Alberts refers to earlier clinical studies carried on in 1968–1991. However, as we wrote, these data are either difficult to obtain, written in Russian, or do not reach the current standards for clinical use within the EU area."

Some attempts to attract artificial intelligence tools to future work on Rigvir were made recently [28].

X. Conclusion

The article has two goals: firstly, to attract the interest of mathematicians to immunology and, secondly, to make some efforts (it must be admitted that quite limited) to promote the key achievements of Latvian virologists in the invention of cancer research, namely, the medicines Rigvir and Larifan for that going back in history to the 1960s.

The immune system (innate and adaptive immunity) is described in short. Lymphocyte sources are discussed: T cells, B cells, dendritic cells, cytokines, chemokines, and interferons. The dual role of macrophages and dendritic cells is studied in many mathematical models as well as a model of bifurcation curves called Glasperlenspiel.

Generally, cancers do not have danger signals and, therefore, cannot elicit strong immune reactions. Immunomodulators turn cancer from a cold to a hot state, to make cancer visible to the immune system. Immunomodulators Poly (I:C) and Larifan are compared.

Oncolytic virus therapy is a novel approach in the field of cancer treatment. The first oncolytic virus in the world was the genetically unmodified ECHO-7 strain enterovirus Rigvir, which was approved in Latvia in 2004 for skin melanoma treatment, but withdrawn in 2019 because did not reach the current standards for clinical use within the EU area.

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