ISSN 1563-0218; eISSN 2617-7498

ӘЛ-ФАРАБИ атындағы ҚАЗАҚ ҰЛТТЫҚ УНИВЕРСИТЕТІ

ХАБАРШЫ

Биология сериясы

КАЗАХСКИЙ НАЦИОНАЛЬНЫЙ УНИВЕРСИТЕТ имени АЛЬ-ФАРАБИ

ВЕСТНИК

Серия биологическая

AL-FARABI KAZAKH NATIONAL UNIVERSITY

EXPERIMENTAL BIOLOGY

№3 (96)

Алматы
“Қазақ университеті”
2023

ISSN 1563-0218; eISSN 2617-7498

KazNU Science · ҚазҰУ Ғылымы · Наука КазНУ

ХАБАРШЫ

БИОЛОГИЯ СЕРИЯСЫ №3 (96) қыркүйек

04.05.2017 ж. Қазақстан Республикасының Ақпарат және коммуникация министрлігінде тіркелген

Куәлік № 16494-Ж

Журнал жылына 4 рет жарыққа шығады
(наурыз, маусым, қыркүйек, желтоқсан)

Журнал материалдарында ауқымды биологиялық мәселелері – ғылыми шолу, теориялық және эксперименталдық зерттеулердің нәтижелері қарастырылады.

Мақалалар биологияның келесі бөлімдері бойынша жарияланады: ботаника, биотехнология, биохимия, өсімдіктер физиологиясы, генетика және молекулалық биология, клеткалық биология, биофизика, адам және жануарлар физиологиясы, зоология және ихтиология, цитология және гистология, микробиология және вирусология.

Комитет по обеспечению качества в сфере образования и науки МОН РК

Crossref Content Registration

DIGITAL OBJECT IDENTIFIER

Национальный центр государственной научно-технической экспертизы

РОССИЙСКИЙ ИНДЕКС НАУЧНОГО ЦИТИРОВАНИЯ Science Index

ҚАЗАҚ УНИВЕРСИТЕТІ
БАСПА ҮЙІ

Жоба менеджері
Гульмира Шаккозова
Телефон: +7 701 724 2911
E-mail: Gulmira.Shakkozova@kaznu.kz

Компьютерде беттеген
Айгүл Алдашева

ИБ № 15125

Пішімі 60x84/16. Көлемі 21,0 б.т. Тапсырыс № 7045.
Әл-Фараби атындағы Қазақ ұлттық университетінің
"Қазақ университеті" баспа үйі.
050040, Алматы қаласы, әл-Фараби даңғылы, 71.
"Қазақ университеті" баспа үйінің баспаханасында басылды.

ШОЛУ МАҚАЛАСЫ

REVIEW ARTICLES

ОБЗОРНЫЕ СТАТЬИ

IRSTI 34.25.01 https://doi.org/10.26577/eb.2023.v96.i3.01

S.Zh. Khaydarov[1]*, E.D. Burashev[2]

[1]Al- Farabi Kazakh National University, Kazakhstan, Almaty
[2]«Research Institute for Biological Safety Problems»
of the Ministry of Health of the Republic of Kazakhstan , Kazakhstan, Almaty
*e-mail: logicalmind1984@mail.ru

THE SARS-COV2 EPOCH AND PROPER MANAGING STRATEGIES TO FACE THE CHALLENGES BOTH IN VIRAL RESEARCH AND TREATMENT

SARSCOV2 or COVID-19 caused 2020 the greatest pandemic since the end of WW1 and crippled the global healthcare and economy within a relatively short period of time. This article reveals a wide range of issues like vaccination issues by understanding the innate and adaptive immune responses and how tightly they are interconnected with each other. There are also to grasp the cellular and humoral parts of immunity and receptor binding recognition mechanisms, antibody neutralization, and antibody mediation both in adaptive and innate immunity to be discussed. The defense strategies of immunity are the objects along with clinical cases to discuss and how effective or not effective vaccination could be in a view of antibody role in creating immunity against this virus The problems of the viral genome are studied in an extent of functionality. There is also the thesis on antiviral treatment, strategies, and side-effects that could appear This review article will be interesting to those who are willing to design either antiviral research or develop the strategies for COVID-19 treatment and even vaccination as a drug design.

Key words: SARS-COV2, mRNA-genome, ORFs (open reading frames), structural proteins, non-structural proteins (np), virus classification, immunity, antiviral drugs, antibodies, IgGs, vaccination, and lethal mutagenesis. steroids.

С.Ж. Хайдаров[1]*, Е.Д. Бурашев[2]

[1]Әл-Фараби атындағы Қазақ ұлттық университеті, Қазақстан, Алматы қ.
[2]Қазақстан Республикасы Денсаулық сақтау министрлігі «Биологиялық қауіпсіздік проблемаларының ғылыми зерттеу институты», Қазақстан, Алматы қ.
*e-mail: logicalmind1984@mail.ru

SARS-CoV2 дәуірі және вирустық зерттеулер мен емдеудегі қиындықтарға қарсы тұру үшін дұрыс басқару стратегиялары

SARSCOV2 немесе COVID-19 2020 жылы 1-ші дүниежүзілік соғыс аяқталғаннан бергі ең үлкен індетті тудырды және салыстырмалы түрде қысқа уақыт ішінде жаһандық денсаулық сақтау мен экономиканы құлдыратты. Бұл мақала туа біткен және бейімделген иммундық жауаптарды және олардың бір-бірімен қаншалықты тығыз байланысты екенін түсіну арқылы вакцинация мәселелері сияқты мәселелердің кең ауқымын ашады. Сондай-ақ иммунитеттің жасушалық және гуморальды бөліктерін және рецепторларды байланыстыруды тану механизмдерін, антиденелерді бейтараптандыруды және адаптивті және туа біткен иммунитетте антидене делдалдықтарын түсіну керек. Иммунитеттің қорғаныс стратегиялары – бұл вирусқа қарсы иммунитетті құрудағы антиденелердің рөлі тұрғысынан вакцинацияның қаншалықты тиімді немесе тиімсіз болуы мүмкін екендігі және клиникалық жағдайлармен бірге талқыланатын объектілер Вирустық геномның мәселелері функционалдық дәрежеде зерттеледі. Сондай-ақ пайда болуы мүмкін вирусқа қарсы емдеу, стратегиялар және жанама әсерлер туралы тезис бар Бұл шолу мақаласы вирусқа қарсы зерттеулерді әзірлеуге немесе COVID-19 емдеу және тіпті дәрілік дизайн ретінде вакцинация стратегияларын әзірлеуге дайын адамдарға қызықты болады.

Түйін сөздер: SARS-COV2, мРНҚ-геномы, ORF (ашық оқу шеңберлері), құрылымдық белоктар, құрылымдық емес ақуыздар (np), вирус классификациясы, иммунитет, вирусқа қарсы препараттар, антиденелер, IgGs, вакцинация және өлімге әкелетін мутагенез. стероидтар.

С.Ж. Хайдаров[1*], Е.Д. Бурашев[2]
[1]Казахский национальный университет имени аль-Фараби, Казахстан, г. Алматы
[2]«Научно-исследовательский институт проблем биологической безопасности»
Министерство Здравоохранения Республики Казахстан, Казахстан, г. Алматы
*e-mail: logicalmind1984@mail.ru

Эпоха SARS-COV2 и правильные стратегии управления для решения проблем как в вирусных исследованиях, так и в лечении

SARS-COV2 или COVID-19 вызвали в 2020 году самую крупную пандемию со времен окончания Первой мировой войны и за относительно короткий период времени нанесли серьезный ущерб мировому здравоохранению и экономике. Эта статья раскрывает широкий спектр вопросов, таких как вопросы вакцинации, путем понимания врожденных и адаптивных иммунных реакций и того, насколько тесно они взаимосвязаны друг с другом. Также необходимо обсудить клеточную и гуморальную части иммунитета и механизмы распознавания связывания рецепторов, нейтрализацию антител и опосредование антителами как в адаптивном, так и врожденном иммунитете. Стратегии защиты иммунитета являются предметом обсуждения наряду с клиническими случаями и того, насколько эффективной или неэффективной может быть вакцинация с точки зрения роли антител в создании иммунитета против этого вируса. Проблемы вирусного генома изучаются в степени функциональности. Существует также тезис о противовирусном лечении, стратегиях и побочных эффектах, которые могут возникнуть. Эта обзорная статья будет интересна тем, кто готов спроектировать противовирусные исследования или разработать стратегии лечения COVID-19 и даже вакцинацию как дизайн лекарства.

Ключевые слова: SARS-COV2, мРНК-геном, ORF (открытые рамки считывания), структурные белки, неструктурные белки (np), классификация вирусов, иммунитет, противовирусные препараты, антитела, IgG, вакцинация и летальный мутагенез. стероиды.

Introduction

Viruses are quasi-organisms that strongly depend on the cell host and on their replication – transcription – translation machinery. Viruses can infect both domains: the eucaryotic as well as procaryotic organisms (phages). It is really difficult to classify them as true parasites or pseudo parasites. Viruses are the smallest organisms with compact genomes enveloped mostly by capsid proteins that can operate not only DNA as a genetic footprint but easily mediate RNAs thanks to various types of transcriptase. named for their corona-shaped appearance in the electron microscope.

Severe acute respiratory syndrome-associated coronavirus 2 (SARS-COV2) is an acute respiratory infection caused by the SARS-CoV-2 virus, which belongs to the coronavirus family and genus Betacoronavirus. Single stranded positive-sense RNA virus (ssRNA$^+$). Variants of the SARS-CoV-2 coronavirus are continually emerging due to the ongoing transmission and evolution of this virus around the world. Since the pandemic was first declared by the World Health Organization (WHO) in March 2020 (1), B.1.17 (alpha), B.1.351 (beta), P.1 (gamma), B.1.617.2 (delta), and B.1.1.529 (omicron) [2–5]. **Kazakhstan had** the first case of human infection with coronavirus COVID-19 **Registered** in March 2020 [6]. According to the Johns Hopkins University database, as of January. In October 2022, the Republic of Kazakhstan had **1,484,400** registered confirmed cases**,** of which **19,052** died [7].

The coronavirus or SARS-COVID2 or COVID-19 belongs to the Coronaviridae family that are enveloped, positive-sense single-stranded RNA viruses [8]. The SARS-COVID2 consists of viral genome: fourteen open reading frames (ORFs), two-thirds of which encode sixteen nonstructural proteins (nsp 1–16) that make up the replicase complex [9,10]. The rest encodes the nine accessory proteins (ORF) and four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N), of which Spike enables the SARS-CoV entry into cytosol of target cell [11]. As any virus of these type, the Spike protein is the most variable and due to this capacity, the SARS-CoVs are capable to penetrate the various cell membrane types of mammals [9].

There are approximately 30,000 nucleotides in RNA; encoding 11 proteins. Retroviruses have caused a lot of harm over the years, and they're now a major threat to human well-being. These are the viruses that belong to the family retroviridae and typically carry their genetic material in the form of RNA. The genetic material of their hosts is in the form of DNA. Retroviruses are named after an enzyme called reverse transcriptase (RT). RT is responsible

for copying genetic information from one virus particle to another. The most known viruses of that family are Lentivirus (human immunodeficiency virus (HIV) and SARS-COV2 (COVID-19) [12]. It is important to mention that virus infections serve the aim to magnify the viral genome and assemble new viral units to be able to invade other surrounding cells and tissues, mostly such processes are carried out lethally to a cell or even to whole tissue systems like lungs. Thus, it is very difficult to classify any viral 'organism' as a parasite whose middle-term and long-term survival correlates with the host's wellbeing. 'The side effect' of viral infections as inflammatory or less obvious clinically distinguishable signs is the integration into the cell genome due to enzymatic activity of viral RNA of various tissue types; this in term causes various types of critical mutations in renewable tissues having a sometimes the devastating disease like pneumonia, renal failure or other chronic, irreversible diseases. The negative effect on the global healthcare state on the population which was exposed to the COVID-19 pandemic is only about to experience in near future. So, the harm potential of this type of viruses are never to neutralize completely and underestimation of its pandemic capacities is the highest priority to avoid of any authority [13-16].

SARS – stays for severe acute respiratory syndrome. The majority of those diagnosed with SARS were healthy adults between the ages of 25 and 70. Children under the age of 15 have been the victims of a few alleged SARS cases. SARS typically has an incubation period of 2 to 7 days, but it can last as long as 10 days. People who have an illness that meets the current WHO case definition for probable and suspected cases of SARS have a case fatality rate of around 3%. Since COVID-19 infection became the subject of pandemic in 2020, United Nations considers this virus and related diseases to it as a global problem for health care systems worldwide. The consequence of SARS-COVID 2 infection could lead to chronic, long term health issue and sometimes to some extent of medical and mental impairment. In many countries the hospitalization rates reached the critical levels so many infected ones were forced to stay at home and get treated far from inpatient wards. Pneumonia is one of the most widely spread health condition among COVID-19 patients and needed to be separated according to the severity of the illness progress and lung damage surface. The more damage occurred the less oxygenation gained via lung breath, so many patients with acute lung damage were heavily dependent on artificial lung ventilation apparatus in intensive care. It was crucial to monitor whether the pneumonia patients with COVID-19 infection were regularly assessed for bacterial infection and try to detect bacterial co-infection and if a need occurred also the antibiotic treatment strategy would have been implemented to avoid pulmonary collapse [17].

The SARS-CoV-2/human/KAZ/B1.1/2021 strain

Strain SARS-CoV-2/ human/KAZ/Britain/2021 consists of 29,815 nucleotides and belongs to lineage B.1.1.7, according to the Pangolin COVID-19 database [18]. The SARS-CoV-2/ human/KAZ/B1.1/2021 strain was obtained from the Scientific and Practical Center for Sanitary and Epidemiological Expertise and Monitoring branch of the Republican state enterprise on the right of economic use, National Center for Public Health, Ministry of Health, Republic of Kazakhstan. Nucleic acids were extracted from the test sample using a *QIAamp* viral RNA minikit (Qiagen, Germany) according to the manufacturer's protocol. Reverse transcription was performed using the *SuperScript* VILO cDNA synthesis kit (Invitrogen, USA). For amplification to cover the entire genome of the virus, 65 primer pairs were designed using the online Primer-BLAST program (http://www.ncbi.nlm.nih.gov/tools/primer-blast) in order to generate amplicons ranging in size from 600 to 750 bp and tiled to overlap by about 100 bp. These amplicons were generated by PCR and visualized by 1.2% agarose gel electrophoresis (Sigma, USA). PCR amplicons were purified using the Pure Link PCR purification kit (Thermo Fisher Scientific, USA). Purified amplicons were sequenced using the Sanger dideoxy method using an AB3130xl (Hitachi Applied Biosystems) 16-capillary genetic analyzer autosequencer with the Big Dye Terminator 3.1 cycle sequencing kit (ABI, Foster City, CA, USA). Raw chromatograms were collected using *Sequencher* version 5 (Gene Codes Corp.) [18].

SARS-COV2 and its molecular feature

The viral Spike has an S1/S2 polybasic cleavage site that is proteolytically cleaved by cellular cathepsin L and the transmembrane protease serine 2 (**TMPRSS2**), and a receptor-binding domain (RBD) that mediates direct contact with a cellular receptor, angiotensin-converting enzyme 2 (ACE2) [8,19,20]. **ORF1a** and **ORF1b** are translated into viral replicase proteins as soon as the viral genome is inserted into the cytoplasm of the host

and cleaved into individual nsps (via host and viral proteases: PL^{pro}); The RNA-dependent RNA polymerase (nsp12, which is derived from ORF1b) is formed by these [21]. The components of the replicase move the endoplasmic reticulum (ER) into double-membrane vesicles (DMVs) at this location, which makes it easier for the virus to replicate genomic and subgenomic RNAs (sgRNA). The latter is turned into accessory or auxiliary proteins as well as viral structural proteins, which make it easier for the virus to form particles [22,23]. In conclusion, the secondary part of genome encodes the nine accessory proteins (ORF) that ensures viral mRNA genome to be translated, it is worth to mention that the replicase for accessory protein production is significantly bigger than the primary one. In addition, the four structural proteins: spike (S), envelope (E), membrane (M), and nucleocapsid (N), of which Spike enables the SARS-CoV entry into cytosol of target cell [Perlman, S., et al, 2009]. ORF1a and ORF1b are translated into viral replicase proteins as soon as the viral genome is inserted into the cytoplasm of the host and cleaved into individual nsps (via the host and viral proteases: PL^{pro}); The RNA-dependent RNA polymerase (nsp12, which is derived from ORF1b) is formed by these [Perlman, S., et al., 2009]. The latter is turned into an accessory or auxiliary protein as well as viral structural proteins, which make it easier for the virus to form particles [Snijder, E.J. et al., 2006, Wu, H.-Y. et al., 2010]. Unlike the HIV virus, which has a complicated capsid structure with sophisticated (negatively charged $dNTPs^-$ permeable) pores that are permeable for negatively charged dNTPs which serves as building blocks for the formation of RNA-host-DNA-hybrid. The SARS-COVs do not possess such protection from hostile enzymes inside the host cells [24]. So, the secondary part of genome encodes the nine accessory proteins (ORF) that ensures viral mRNA genome to be translated, it is worth to mention that the replicase for accessory protein production is highly important to be integrated into host genome or to let virus to reproduce itself.

The retroviruses (Retroviridae) family and their common features

There are six common characteristics that unite all retroviridae to one

1. Despite having DNA-dependent replication steps, retroviruses contain RNA as their genetic material.

2. Replicates via reverse transcription thanks to the presence of reverse transcriptase enzyme.

3. Due to the presence of the enzyme reverse transcriptase, reproduces via reverse transcription.

4. Integrase moves the viral DNA into the nucleus of the cell, where it is randomly and covalently integrated with the host genome.

5. Gene sequences like viral oncogenes and proto-oncogenes are found in retroviruses that are capable of rapidly transforming host cells into their needs.

6. Immune deficiencies, cancer, and neurological conditions can all be brought on by human retroviruses.

Structure, genome, and proteins

The typical retrovirus structure is **enveloped,** spherical to pleomorphic in shape, and they have diameter of 80–100 nm. The different genuses of retrovirus virions have diverse morphology, but they have their same **virion** component, which includes the outer envelope coat, two copies of the genetic material, and the viral proteins. **Envelope** consists of lipids that are obtained from the host plasma membrane during budding process and the **glycoprotein** such as **gp120** and **gp41** in case of HIV for example [25]. The outer lipid bilayer of the retroviral envelope protects it from the extracellular environment, aids in the entry and exit of host cells through endosomal membrane trafficking, and allows it to simply enter the host cells by fusing with their membranes are the **three distinct functions** of the retroviral envelope. A retrovirus has a **monopartite, linear, dimeric, ss RNA (+) genome** that is between 8 and 10 kilobases long and has a 5'-cap and a 3'-poly-A tail. Between the R regions are flanks for the group-specific gene (gag), pol, pro, and envelope (env) genes. The U3, R primer binding site (PBS), and U5 regions make up the 5'-long terminal repeats (LTRs). A polypurine tract (PPT), U3, and R regions make up the 3' end. During reverse transcription, a brief repeated sequence at each end of the genome is used to guarantee correct end-to-end transfer in the growing chain. On the other hand, U5 is a brief exceptional arrangement that sits between PBS and R [26]. The 18 bases in the PBS correspond to the tRNA primer's 3' end. The untranslated leader region known as the L region indicates how genome RNA is packaged. The gag, **protease**, pol, and env proteins make up the retroviral protein. The gag is the primary **retroviral** structural protein that orchestrates the majority of viral assembly processes. The interactions with the three gag subdomains—matrix (MA), capsid (CA), and nucleocapsid (NC)—effect the **majority of**

these assembly steps. While the gag subdomains are structurally distinct, their functions in the viral assembly process overlap [27,28].

Global COVID-19 spread and clinical consequences

According to the WHO dashboard, more than 6.4 million people worldwide died from COVID-19 by the August 18, 2022. The omicron strain has been diagnosed within over 590 000 000 people worldwide, a brand-new variant that appeared toward the end of November 2021, is now the most common strain worldwide and has contributed to the ongoing rise in several nations. In a number of high-income nations, vaccination is significantly reducing the number of cases and hospitalizations, but a lack of universal access to vaccines leaves many populations vulnerable. Even in people who have been vaccinated, there are still questions about how effective and for how long the current vaccines against Omicron and other new SARS-CoV-2 variants are. There is still a need for more efficient COVID-19 treatments as a whole. The COVID-19 pandemic, as well as the avalanche of research and false information, has shown how important it is to have reliable, easily accessible, and frequently updated living guidelines so that new findings can be understood and clear recommendations for clinical practice can be provided [29].

Apart the severe acute respiratory syndrome and acute respiratory destress syndrome (ARDS) causing serious health impairment, COVID 19 is also capable to cause post COVID 19 health conditions like cognitive impairment states Other neurological and non-neurological deficits, such as **fatigue** and **mental health** symptoms, may overlap or cluster with cognitive deficits. In conditions following COVID-19, fatigue or exhaustion manifests as severely depleted systemic energy levels that are unrelated to activities or exertion and unaffected by usual rest or sleep. The quality of one's life, physical and cognitive function, social participation, and employment are all negatively impacted by fatigue. The core symptoms of depression following COVID-19 include a persistent low mood and sadness for at least two weeks and a markedly diminished interest in enjoyable activities. Depression can also cause problems sleeping, changes in appetite, fatigue, thoughts of self-harm or suicide, and feelings of worthlessness. Anxiety symptoms can include restlessness, racing or uncontrollable thoughts, difficulty concentrating, a sense of dread, difficulty sleeping, a lack of appetite, and irritability [30].

Innate Immunity and SARS-COV2

The issues of the innate immune system are a very complicated matter and deserve detailed view in a distinct paper. However, within this article, it is important to include innate immunity to grasp the real pathogenic nature of COVID-19. Innate immunity in humans is the first defense line that helps our body to clear and distinguish the viral invasion and start to respond to it. Human cell immunity mostly heavily relies on two types of innate immunity: The macrophages that absorb the pathogen and tend to disintegrate viruses inside and neutrophils that are able to initiate the cell death to stop the virus from spreading, because one single by SARS-COV 2 infected cell is capable to produce up to 10.000 viral units until it experiences the cellular burst [31].

Limiting viral entrance, translation, replication, and assembly, assisting in the detection and extermination of infected cells, and coordinating and speeding up the development of adaptive immunity are all functions of innate immune responses. Pathogen-associated molecular patterns (PAMPs) are recognized by cell surface, endosomal, and cytosolic pattern recognition receptors (PRRs), which then cause inflammatory reactions and programmed cell death (neutrophils) to prevent viral infection and encourage clearance [32]. The COVs (coronaviruses) developed innate immune system suppressors thanks to ORFs (ORF3 and 3CL) that are responsible for accessory protein encoding that sustains the viral replication and translation types of machinery and mitigates the antiviral response as an evasion strategy [33]. The cyclic GMP-AMP synthase (cGAS) is a STING (Stimulator of interferon genes) signaling pathway that gets activated by cytoplasmic DNA. cGAS-STING is the protective cascade-driven reaction that significantly limits both DNA and RNA viruses during the active phase of infection [34-37]. The SARS-COV 2 disintegrates the organelles' unity and one of its victims is mitochondria. Mitochondria get seriously damaged and their DNA freely swims in the cytosol by which the cGAS gets activated to fight the invaders' genome [38]. Last but not least, it makes sense to mention the cellular components of innate immunity against viral infections. Macrophages, monocytes, dendritic cells, neutrophils, innate lymphoid cells (ILCs) such as natural killer (NK) cells, are capable to resists virus invasion with a help of PRRs (Pathogen resistance receptors) that recognize PAMPs or damage-associated molecular patterns (DAMPs) to induce inflammatory signaling

pathways and immune responses [39]. To sum up this chapter, SARS-COV-2 or COVID 19 like other COVs and retroviruses has an extremely high potential to invade a cell and replicate itself fast and effectively enough to overcome the innate immunity and sometimes to escape the B-cells antibody formation. Adaptive immunity faces also high challenges due to the high variability of spike – proteins that enable it to form new strains with novel defensive evasion strategies. The virus is capable to cause serious health and life-threatening conditions to almost all groups of people worldwide. It is able to make its survival effective in a host cell thanks to accessory proteins and thanks to rapid integration into replication and translation cell machinery and actively defending the positive RNA-original genome. The virion rate production is well synchronized by the assembly process allowing COVID-19 to produce its copies in an average amount of 10.000 units per single eukaryotic cell, allowing it to achieve high infection rates among human and animal populations. So, we can surely say that COVID 19 is still very dangerous pathogenic organism whose potential is not fully understood.

Adaptive immunity and SARS-COV2

Viruses are in capsid coated relatively small genome carrying (mRNA or DNA) 'nano-living-being' with so called ORFs (open reading frames – the viral genome sequence) when they enter the host cytosol [40].

Many times, in medical history the viral infections showed the high potential of increasing their population in host cells. The virus load speed is extremely important in viral spread before the secondary immune response occurs that definitely will be able to cap any viral threat spreading. The corona viruses can reproduce their copies up to 10.000 viral particle per an infected cell [41].

The occurrence of repetitive, i.e., monotonically repeating, letter sequences is a characteristic that is extremely baffling in many genomes of higher organisms and some viruses, the latter to a very limited extent. The human genome of $3.2x10^9$ hereditary letters utilizes under 2% of those letters to store the data of working qualities, to the extent that we know up until this point. Sequences of letters that are repeated millions of times make up well over 40% to 50% of the genetic letters that are housed in our 46 chromosomes. The purpose and significance of these repetitions are unknown to us [42].

However there some theories that suggest that the human genome is so profound with a purpose, a defensive way to secure the vital genes or group of genes, to keep them relatively stable against mutations or exposures that cause various mutations that interferes or even blocks the essential expressions, and maybe also to withstand many viral genome integrations both in replication and translation processes as well.

The following options were utilized when vaccination options were highly specialized: vaccination with viruses that have been killed (inactivated) and are no longer able to reproduce or with parts of the viruses. Accidents also occurred during the early stages of vaccine development, primarily as a result of incomplete inactivation or contaminated vaccine derived from virus-infected cells. One well-known instance is the contamination of an early preparation for the polio vaccine with the rodent tumor virus SV40, a previously unknown simian virus. Fortunately, this accident did not result in any problems: there is no evidence that receiving the contaminated vaccine increased the risk of tumor development [43].

The safest vaccines available today are the recombinant virus vaccines produced by using genetic engineering technologies. Since the virus genes whose products are responsible for antibody production are isolated from the virus genome. The proteins of these viral genes are then expressed in bacteria or in yeast, the viral proteins synthesized in this way are then purified and used as a vaccine. These vaccines are virus free [44].

Innate immunity – the primary response and cell defense.

The immune system consists of specific as well as unspecific mechanisms that fight viral invasion with various efficiency as well as time reaction. There are unidentified antiviral compounds in the mucous membranes of the inner surfaces. While some defense cells can kill viruses by phagocytosis (eating), this method isn't very effective without supporting mechanisms. Some viruses, like HIV, have the capacity to grow in the macrophages that phagocytize them, allowing them to get past this antiviral defense The generation of interferons, or molecules that stop virus replication and spread, is a very intriguing and common defense mechanism. The formation of these proteins occurs far earlier than the development of antiviral antibodies following viral infection. The interferon α, β, γ are currently the most well-known [45].

Table1 – The interferon types and cells that generate them as unspecified auxiliary virus-fighting agents in humoral immunity.

Interferon	Functions and cells
interferon α	induced mainly in leukocytes by foreign cells, virus-infected cells, tumor cells, or virus envelopes.
interferon β	induced by viruses and foreign nucleic acids in many different cells of the body.
Interferon γ	formed by T lymphocytes when foreign proteins enter the body.

The cellular unspecified immunity consists of macrophages, basophils, and natural killer T-cells.

NKTs and NKs, also known as natural killer T-cells, are a class of leukocytes. White blood cells called leukocytes help the body to fight various infections. Less than 1% of the body's lymphocytes are these uncommon cells. Are T-cells specialized or generalized? The only T-cell subset regarded as non-specific is natural killer T-cells, which facilitate communication between the non-specific immune system and the specific immune system. These cells take direct aim at microbial intruders [43].

Leukocytes called basophils have previously been misinterpreted. Scientists found it difficult to study these cells because of their short lifespans of one or two days. According to recent studies, basophils are the only white blood cells that express histamine and congregate in connective tissues. The com-ponent histamine is what causes allergy symptoms to manifest in the body. Basophils have the ability to eliminate cancer cells in the early stages before they pose a threat to the body [42].

The immune system's warning systems and so-called 'missile defenses' are called macrophages. Macrophages will raise the alarm by releasing cytokines into the circulatory system when they find a pathogen. When a cell surrounds and kills another cell or organism, the process is known as phagocytosis [44].

Neutrophils are also innate immune cells which also have small life span. Neutrophils are the first cell type to be drawn to inflammatory areas. They can then change phenotypic and produce a number of subpopulations with various cell functions. Additionally, neutrophils can interact with other immune cells directly or indirectly through cytokines and chemokines to modify innate and adaptive immune responses. We still don't fully comprehend these neutrophil subpopulations, but the instances that follow make it very evident that they do exist as real inflammatory subsets [44].

To sum up, the innate immunity is comprehensively complicated, and the humoral immunity is tightly interconnected with cellular part. Some leukocytes have multiple functions with in different levels. The innate immunity is capable to face almost any microbial challenge with various molecular induceable arsenal that sustains integrity and healthy state.

Vaccination

The adaptive immunity consists both of cellular and humoral (antibodies) particles that helps to neutralize the pathogen in short as well as long term perspectives due to formation memory cells. The adaptive immunity requires time to select and expand the virus-specific cells from the large variability pools of naïve B cells and T cells for further specification in molecular structures and sequences – priming.

Table 2 – Shows the consistent adaptive immunity with cellular and humoral part with aduaquate functions with high specifity and efficassy when innate immunity get overwhelmed with infectiom

Acute immunity	**Humoral**	**Cell**	**Cell**	**Cell**
	Antibody	$CD4^+$ T-cells	$CD8^+$ T-cells	B-cells
Functions	Identification of epitops of intrest, forms cellular memory	Have Helpers and Effectors activities	Kill the infected cells	Production of Antbodies

When SARS-COV2 infection proceeds, the viral genome rapidly gets integrated into host protein and replication machinery, the race with time starts and the innate immunity has to withstand with viral load till the adaptive immunity get proliferated and properly differentiated to charge already circulating virions and virus seized cells. The innate immunity tries to manage the viral infections by its own by activating the immune response type I and type III interferons that are supposed to delay the intercellular viral infections and till the viral load gets the critical values and starts to alarm dendritic cells to call the adaptive immunity to help [43.].

In average situations of SARS-COV2, a so called 'simple' model appears to cause the temporal

delay in innate immunity response which is enough to launch the asymptomatic infection that occurs roughly in 40% percent of COVID-19 cases and the T-cells with antibodies get formed relatively rapidly to control the occurred viral load and infection rates [44.]. The presence in blood stream of COVID-19 patients the T-cells and antibodies in sufficient amounts is signaling that the positive resolution of COVID-19 took place [45.].

To sum up, the timely and accurately activated the adaptive immunity with humoral as well as cellular response brings the resolution from severe COVID-19 infection and its clinical outcome. Thus, ignoring the importance of innate immunity that charges the first viral invasion with specific delaying responses on viral replication as well as translation can grant a vital time to withstand rapidly increasing viral load. And thankfully, a significant part of COVID-19 infections run in asymptomatic manner with enough T-cells and secreted by B-cells – Antibodies. So, to ensure such scenario the innate and adaptive immunity must balance between magnitude levels and inpatient time. Ideally, the innate immunity holds the primary viral invasion long enough not only to control the viral load increase with the same immune intensity but also to provide time for B-Cell and T-cells to be released in blood stream from the lymph nodes. However, the clinical practices faced with relatively ineffective innate immunity so the viral load got significantly higher than the primary defense could withstand but then the adaptive immunity was in average capable to clear the COVID-19 infection. In severe cases the viral load was not even opposed by innate immunity response or the reaction was so excessive (The cytokinin shock – overreaction of macrophages and neutrophiles) that it only harmed and benefited the further viral invasion. In bad situations, the time line of innate immunity overlaps with adaptive and does not stop the exponential growth of virus production and viral load seriously dominates over the number of antibodies and the T-cells magnitude levels so their amount is critically low to fight effectively both the intercellular circulation of virions and infected cells as well causing serious health damage or even death.

Immunoglobulins (IgGs, IgA and IgM)

The vaccination is a process in which it is tried to cause the immune response as save as it only possible and as antigen – the response causing particle could be applied in our case alive but weakened viruses, viral structural proteins like spike protein or even based on mRNA (viral genome) vector vaccines and etc. There are always risks to face during the vaccination despite the multiple clinical approval procedures and trials.

Table 3 – Shows five main immunoglobulin or antibodies (Ab) classes properties and importance. GI*- gastrointestinal Secretions, GU*- glucagon secretion.

Class	Percentage in total	Features and purposes
IgG	~75%	Found in blood and lymph, active against Bacteria and their metabolites (toxic agents), viruses, increases **phagocytosis,** cross placenta and **active in second response**
IgA	~15%	Saliva, tears, bronchial, GI, prostatic, and vaginal secretions. Provides the local protection on surfaces, has anti-viral potential Prevents the absorption of antigens from food and protects against the **respiratory infections** as well as against GI and GU infections
IgM	~10%	Found in blood and lymph, levels go down during stress, the first antibody produced during t**he primary response, high** concentration in initial stage of infection. The level of IgM reduces within one week.
IgE	~less than 1%	Found in mast cells and neutrophils, involved instant hypersensitive response.
IgD	~less 0.1%	Found in blood and lymph, unknown functions

The B-cells and antibodies are the major players of adaptive immunity in antigen of interest triggered immune response – called seroconversion in a hope to get vaccination or so-called memory cells. The primary response or seroconversion leads within 5 days the increased amount of IgM – titter, and IgG-titters appears first only after 14 days, so the increased amount IgG demonstrates either the past infection or the vaccination active event [42].

The brightest correlations of seroconversion on IgG illustrated the Spike and Nucleocapsid viral structure protein. The clear tendency was

shown SARS-COV2 was neutralized by receptor binding domain (RBD) with over 90% frequency in COVID-19 cases antibodies [46]. Spike IgG, IgA and IgM start to develop simultaneously during the viral infection [47]. Receptor binding domain (RBD) of Spike protein of SARS-COV2 is the mile stone of virus neutralizing dogma both in vaccine or medicine design, to deny such important aspect of viral defense is not productive, however the virus neutralization follows also outside the cells thanks to antibodies, the infected cells can be also killed directly by antibodies [48].

Almost complete COVID-19 neutralizing antibodies run the seroconversion in Spike range [49]. In this extent it makes sense to claim that almost all neutralizing antibodies come from naïve or virgin B-cells, not from pre-existing cross-reactive memory B-cells [50]. As a result, the epitopes which are capable to neutralize the SARS-COVID-2 on RBD domain, especially those who corresponds highly likely to the ACE2 receptor binding footprint (or ACE2 – like repertoire which mostly found in the lung tissue) strives about to be effectively immunogenic and easily detected by antibodies. At the same time, it would be fair to state that the substantial fraction among recovered patients from COVID-19 of antibody titter is considerably low [51]. It means that most effective period of immunogenic response lasts relatively short and secondary response takes time to be activated. Thus, the most active period of viral fighting is timely limited and so-called memory cells are built to stand against repeated infection and viral load exposure and of course till the next B-cell proliferation takes place.

Fc-receptor-associated protective immunity against SARS – COV2 (lung tissue)

Many studies showed that humoral responses and neutralizing antibodies alone were not enough to overcome successfully the COVID-19 viral infection, especially, in severe cases or even among deceased patients [52]. Despite the fact that there is still no direct evidence that Fc-mediated (dependent) effector activity is responsible for effective protective immunity against SARS-COV-2, some studies showed that deceased patients had very reduced Fc-dependent antibody effector activity [53].

A variable fragment (Fab) that mediates antigen binding and a constant fragment (Fc) that mediates downstream effector functions by interacting with Fc-receptors on (innate) immune cells or with C1q, the recognition molecule of the complement system, are the two structural regions that make up an antibody. Through a number of immune effector mechanisms, such as **antibody-dependent cell-mediated cytotoxicity (ADCC)** and antibody-dependent cellular phagocytosis, the contact with Fc-receptors can cause the death of virus-infected cells (ADCP). Complement-dependent cytotoxicity may result from complement-mediated antibody activation (CDC). Complement activation and Fc-receptor interactions can both have a variety of immunomodulatory effects [54].

The Fc domain of antibodies that are linked to viral proteins on the surface of virus-infected cells activates Fc gamma receptors (*FcRs*) on innate effector cells, inducing ADCC. Infected cells are killed as a result of this interaction, which causes the release of cytotoxic granules that contains perforins and granzymes [55]. Natural killer (NK) cells, neutrophils, monocytes, and macrophages are just a few of the innate effector cells that can engage in ADCC in a lab setting. However, NK cells, which solely express *FcRIIIA*, are believed to be the most significant in vivo contributors to ADCC. ADCC has been acknowledged as a crucial mode of action for therapeutic monoclonal antibodies (mAbs) that target tumor cells in the field of tumor immunology [56].

The assimilation of virus-antibody complexes or virus-infected cells that are coated with antibodies by phagocytic cells is known as ADCP or opsonophagocytosis. Phagocytic cells such as monocytes, macrophages, neutrophils, eosinophils, and dendritic cells (DCs) express *FcRI, FcRII*, and *FcRI*, which are all capable of mediating immune complex absorption. The type of cell, stage of development, and degree of FcR expression all affect how effectively effector leukocytes can phagocytose. As a result of ADCP, immune complexes are eliminated from the infected host by being transported to lysosomes where they are processed for antigen presentation on Major Histocompatibility Complex (MHC) molecules on the cell surface. It's interesting to note that certain viruses have taken use of this method to infect phagocytes by evading lysosomal breakdown. However, this mechanism is mostly induced during the bacterial infection, in the matters of viral infections, the ADCP needs to be clarified [57].

The complement system has several parts and uses various paths to activate its effector functions. Complement plays a crucial role in antibody-mediated defense against viral infection, according to studies in complement-deficient mice. There

have been numerous proposed methods for this complement-enhanced defense. So, first of all, the steric hindrance of bound antibodies may increase when complement components are fixed to virus-antibody complexes to directly enhance the neutralization capacity of antibodies [58]. Another potential mechanism is complement-dependent opsonization of virus-infected cells, which results in phagocyte uptake later on. In an in vivo mouse model, complement has additionally been demonstrated to enhance the CD4(+) T cell response in the presence of respiratory syncytial virus (RSV) immune serum [59-64].

To sum up, complement activation and Fc-receptor interactions can have a variety of immunomodulatory effects on different levels. The Fc domain of antibodies that are linked to viral proteins on the surface of virus-infected cells activates Fc gamma receptors (FcRs) on innate effector cells, inducing ADCC. Natural killer (NK) cells, neutrophils, monocytes, and macrophages are just a few of the innate effector cells that can engage in ADCC in a lab setting. Phagocytic cells such as monocytes, macrophages, neutrophils, eosinophils, and dendritic cells (DCs) express *FcRI, FcRII*, and *FcRI*, which are all capable of mediating immune complex absorption. As a result of ADCP, immune complexes are eliminated from the infected host by being transported to lysosomes where they are processed for antigen presentation on major histocompatibility complex (MHC) molecules on the cell surface. There have been numerous proposed methods for this complement-enhanced defense. Another potential mechanism is complement-dependent opsonization of virus-infected cells, which results in phagocyte uptake later on.

Modern methods to produce immune modulators and SARS-COV2

Viruses are special ‘microorganisms’ that need to be treated specially, not like Bacteria or Protista or even various parasite worms. Virus infections are usually swift, aggressive and extremely virulent. The successful infection proceeds successfully if the virus concentration alternatively viral load is high enough to cause the infection that is why HIV for example can be pestilent if the infection runs through blood or in utero from mother to child. In other ways HIV-positive person does not demonstrate the social threat, because the virus load in his fluids like salvia or sweat is not sufficient to trigger HIV-infection. SARS-COV2 in striking contrast can infect airborne in the person-person way relatively easy. Alone this difference demonstrates clearly how complicated is the pathology and biological nature of viral infection and in order to prevent viruses to succeed the modern scientific idea should offer new methods to modulate, reinforce or improve the protective immunity against viral invasion. In this article we consider two sides of the viral nature, one how effectively fight them and how to use them as drug delivery system. The polyclonal technologies in antibody production have some weighty advantages. First and foremost, it is price, with reasonable and rational immunization with properly prepared antigen can produce enough number of antibodies from blood serum by protein purification methods that were proven by time. The physical methods mostly based on **photometrical methods such as ELISA or flow cytometry and electrochemical, optical or piezoelectric immunosensors (biosensors).** However, despite the solid advantage, polyclonal antibodies have low level of sensitivity on particular antibodies and lower specialization on particular reaction or antigen of interest, instead it can react on various reactions and epitopes in some extent it could be considered as a reasonable upper hand in methodology. The monoclonal antibodies are powerful tool both in research and in clinical approach as reliable agent, nonetheless, is very expensive method to produce highly specified antibodies, as laboratory animals mostly used rabbits for advantageous reasons for instance, still an animal must be killed to get plasma cells or lymph nodes. Thus, recombination technologies became the main headliner with a help which there is no need to kill the lab animal, by collecting blood samples and get from the blood centrifugation **the PBMC** (peripheral blood of mononuclear cells) to isolate the B-cells and their m-RNA. This technology allows us to magnify the production rates of monoclonal antibodies through recombination for yeast, phage etc. essays. The hybridoma technology offers the immortality potential of adenoma cell to produce almost unlimited rates of monoclonal antibodies in HAT-media.

Corona virus belongs to IV-group of viruses of Baltimore classification. Corona virus infection can be easily neutralized by conventional IgG produced by B-cells as secondary immunity response or by eventually by monoclonal heavy chain nano antibody (VHH) derived from the blood of camelids or sharks, still the role of virus neutralization agent must be confirmed clinically.

Polyclonal antibodies

Polyclonal antibodies or immunoglobulins refer to a mixture of IgG-molecules which are secreted against particular; each antibody or IgG (IgG_2-IgG_4) recognizes different epitopes that brings the further polyclonal properties for IgGs:

- They are produced by multiple clones of B-white blood cells
- Heterogeneous antibody population
- Interaction with various epitopes on the same antigen of interest
- Increased likelihood for cross reactivity for similar antigens
- *'Lot to lot'* or side by side variability
- The expense of production is relatively reasonable.

In laboratory allowed condition work with living organisms, polyclonal antibody production represents the simplest way in comparison to other more sophisticated ways like monoclonal approach [65]. Since our work bases and will be based in the future in SARS-COV2 pathogens derived antigen, there will be no need to worry about to add adjuvants to avoid hypersensitive reaction or response or development of tolerability.

The most important part of polyclonal antibodies production is the animal choice, mostly rabbits are the most convenient polyclonal antibody producer in terms of maintenance and produced number of antibodies [66]. Polyclonal antibodies can be arranged also against whole microorganisms; their planning has also been detailed utilizing rabbits immunized by peptides such as two parts – bacteriocin and pediocin PA-1 from *Pediococcus acidilactici* conjugated to the carrier protein keyhole limpet hemocyanin [67] and parts of human chaperonin 10 bound to ovalbumin [68]. Small molecules like organophosphates could be also applied with several additional modifications, however it could be used only as a fact of appearance ignoring the cross-linking reaction and minimal specification and in general the polyclonal assay could easily run either false positive or false negative reaction due to its nature – poly epitope reaction, still animals in polyclonal antibodies production could be used as specialized inbreeds. For instance, particular specificity of polyclonal antibodies can be improved when specific pathogen-free (SPF) animals are utilized. The safe framework of SPF creatures is *"tabula rasa"*. On the other hand, SPF creatures may be inclined to tall mortality due to the naivety of their immune framework. Antibodies can be recognized concurring to the number of B-lymphocyte lines that create them. Polyclonal antibodies are created from distinctive B-lymphocyte lines as a blend of immunoglobulins. Numerous decontamination strategies have been created for the generation of crude antibodies (it means it is needed to be humanized in order to be used in clinical trials or experiments) over the past decades. Since the structures of immunoglobulins are normal proteins, current strategies for protein decontamination are reasonable too for the refinement of immunoglobulins. For case, gel chromatography is one helpful strategy for isolating IgG from the IgM display in polyclonal counter acting agent tests. Precipitation by ammonium sulfate is able to separate the isotypes of immunoglobulins [69]. Proteins A, G and L are exceptionally common and commercially provided either free or bound to underpins such as agarose. Antibodies can be essentially filtered by strong stage extraction utilizing as it were a framework with the capturing protein A, G, or L [70]. Another appropriate bio-ligand is a counter acting agent particular against either an entire Ig gather or to one course or subclass of immunoglobulins. Compounds arranged by natural blend such as mercaptoethyl pyridine are effective options to naturally determined ligands. To sum up, the polyclonal antibodies mostly produced *in vivo*, production time is relatively short (2-3 months), have large variability if we use batch to batch approach – detect easy and fast some substances with lower specify but robust. Last but not least, the cross reactivity is possible and this probability level depends on purification level.

Monoclonal antibodies

Monoclonal antibodies are homogeneous population of antibodies which are produced by a single clone of plasma B-cells. This fact brings a lot of advantages like predictable reaction response, high specificity both on particular epitope and antigen of interest which is why monoclonal antibodies are so highly valued in oncological and recombination technology studies. Nothing is so precise as monoclonal IgG for instance in detection and in further neutralization of viruses or viral particles in adaptive immunity that also enables to build up firm long-term protection via consolidation of so-called immune memory cells. The monoclonal antibody is a product of a clone of B lymphocytes. A specific problem is a preparation of recombinant antibodies [70.] true genetic manipulation and the producer cell is applied could have different origins.

The monoclonal antibodies in general have further characteristics that seriously distinguish them from polyclonal ones:

- The genetical clone of one B-cells
- The primary Nucleotide (**mRNA**) should and could be isolated either from blood (PBMC), lymph nodes or spleen from animals.
- Antibody population represents a high value of appliance – like identical lots in laboratory working flow
- Low cross reactivity
- Clear interaction with particular epitope on antigen
- High price of studies

So, monoclonal antibodies are produced by a same clone plasma B-cell or isolated intact plasma cells from spleen or lymph node tissue. Population of the antibodies (IgG for instance) is homogeneous. Monoclonal antibodies interact solely with specified epitope on antigen that allow them to have very low cross reactivity and have identical lots, nevertheless this approach is much more expensive and more sophisticated than polyclonal methodology. However, in case of hybridoma, the production life span is significantly higher than *in vivo* way and modified (fused) cells and they are capable to produce pricy monoclonal antibodies for six and more moths [71].

Hybridoma

Monoclonal antibodies are identical antibodies produced by hybridized cloning of immortalized B cells derived from a parent cell. Hybridoma technology was used by Kohler and Milstein in 1975 to produce monoclonal antibodies [72]. Hybridoma technology is effective mostly to produce precisely to treat cancer and produce the antidots, such as anti-snake venom. The antigen is a key factor for production. The small mammals like mice, rats and rabbits are the main model organisms. The blood streams immunization enables to produce monoclonal B-cells (antibody secreting cells) and spleen is the main organ to extract for synthesis/ cultivation plasma cells/B-cells.

The main problem of plasma cells/spleenocytes/ isolated B-cells. Hybridoma innovation produces monoclonal antibodies particular to antigens. These cell lines can moreover be cryopreserved for a long period of time. Hybridoma innovation has brought about the generation of an assortment of diverse monoclonal antibodies with specificity for a particular antigen.

Antigen particles incorporate chemicals, hormones, inner and outside structures of microbes, infections, and eukaryotic cells. Monoclonal antibodies created by this strategy are exceedingly particular antibodies, which are determined from a single parental B cell clone [73]. Analysts for the most part favor hybridoma innovation, for monoclonal counter acting agent generation over other strategies to preserve a helpful, cost-effective, and boundless generation of monoclonal antibodies [74]. Out of the several techniques developed over years to produce monoclonal antibodies (single lymph cell amplification or by culturing strategies), hybridoma technology is one of the most important and most commonly used [75].

Main steps of Hybridoma technology

To begin with, **immunization** includes infusing the research facility animals like rabbits or mice with a chosen antigen against which the antibodies are raised through an arrangement of infusions over a period of a few weeks to invigorate B cell separation into plasma B cells and memory B cells. Once an adequate number of antibodies are made within the animal serum taking after many weeks of immunization, the alive subject is died [76].

Isolation of B- cells is tightened strongly with a biomaterial like the spleen which is evacuated in aseptic conditions to confine the enacted B-cells. This strategy is performed utilizing ***density gradient centrifugation***. The nearness of antibodies within the Serum is recognized utilizing strategies like ELISA or ***flow cytometry***. The serum contains the actuated B lymphocytes (that create a counteracting agent). The enacted B lymphocytes are at that point combined with myeloma cells.

To prepare myeloma cells a few weeks before the ***cell fusion*** takes place, metastatic tumor cells are incubated in 8-azaguanine to urge non-functional hypoxanthine-guanine phosphoribosyltransferase (HGPRT) qualities within the myeloma cells. Non-functional HGPRT can halt the get-together of nucleotides from the rescue pathway and makes the metastatic tumor cells touchy to HAT media as the favoured strategy in hybridoma innovation [76].

Cell fusion is that the method within which the activated B lymphocytes area unit consolidated with HAT-sensitive malignant tumour cells. This step is performed by natural action of freshly obtained activated B-cells with HAT-sensitive malignant tumour cells in a very fusion-promoting media. synthetic resin glycol (PEG) is employed during this procedure PEG helps within the fusion of cells by promoting the fusion of the cell wall of

the malignant tumour cells with the cell wall of the antibody-producing cells, so giving rise to a cell with over one nucleus, forming heterokaryon. Another technique used for fusion is electrofusion, within which cells area unit consolidated beneath the result of an electrical field. This technique is additional deficient than the previous technique [76,77].

Hybridoma **selection** starts in the PEG-containing media, cells area unit amalgamated to create somatic cell cells however even foremost economical fusion methodology can permit the formation of solely concerning one to twenty of amalgamated somatic cells. Therefore, there are a unit variety of unfused cells inside the media [72]. This step permits the choice of the amalgamated cells from all the unfused cells. this is often achieved by incubating the cell mixture followed by culturing for 10–14 days in HAT media (a choice media). HAT medium contains hypoxanthine-aminopterin-thymidine. Aminopterin gift in HAT media blocks the ability of cells to synthesize nucleotides by the Delaware novo synthesis pathway. Hypoxanthine and nucleoside permit cells with purposeful hypoxanthine-guanine phosphoribosyltransferase (HGPRT) genes to survive through salvage pathways. thanks to a restricted lifetime, unfused B cells decease inside some days. Unfused malignant growth cells die as a result of the shortage of the hypoxanthine-guanine phosphoribosyltransferase (HGPRT) cistron. The presence of aminopterin blocks their ability to synthesize nucleotides through the Delaware novo pathway [78]. Therefore, the remaining viable cells left within the media area unit the hybrid cells; these hybrid cells have the power to grow and divide on HAT media as a result of they need purposeful HGPRT cistron from the B lymphocytes, that makes them HGPRT positive, and thus, they will grow in unlimited concentration on HAT media [76].

The further screening needs HAT- somatic cells selection that are transferred to enzyme-linked-immunosorbent serologic assay plates, wherever every well home one somatic cell this is often achieved victimization the limiting dilution methodology [76]. The genes of the lymph cell lineage gift within the somatic cell cells turn out a selected protein with a selected epitope; this protein is thought as a "monoclonal protein." There is also alternative hybridomas gift in alternative wells manufacturing antibodies specific to a different epitope for identical matter. once the separation and isolation of various hybridomas, screening is performed for choosing hybridomas that turn out the required associate degree antibodies targeting specific epitopes for a matter [79].

Functional genome

The SARS-COVID2 consists of viral genome: fourteen open reading frames (ORFs), two-thirds of which encode **sixteen nonstructural proteins (nsp 1–16)** that make up the **replicase complex** [80,81]. The rest encodes the nine accessory proteins (ORF) and four structural proteins: spike (**S**), envelope (**E**), membrane (**M**), and nucleocapsid (**N**), of which Spike enables the SARS-CoV entry into cytosol of target cell [81]. As any virus of these type, the Spike protein is the most variable and due to this capacity, the SARS-COVs are capable to penetrate the various cell membrane types of mammals [80-81].

A s viral genome of *beta* strain containing about 31,3kB in total. The polyprotein regions (pp) or so-called opened reading frames (ORFs) are mostly represented in the viral genome for replicase genes serving, the fragments of which are defined as non-structural proteins or NSPs. The most appealing regions to impact on are **nsp5** and **nsp12** which are crucial for viral replication. The structural genes encode for further purposes: spike (**S**), envelope (**E**), membrane (**M**), and nucleocapsid (**N**), and with auxiliary or accessory proteins among them [82].

Drug antiviral activity

The COVID19 pandemic caused the huge problems to national health care (NHC) worldwide. The first response on such epidemical spread was how to treat the infected patients, in order to ensure the clinical effect and people around the globe stormed the pharmacies to get paracetamol which is effective against fever, others were claiming anti flu drugs, hoping to get the therapeutical effects and some even bought out the antibiotics, considering that it would help too. Covid 19 is single stranded positive RNA ((+) ssRNA) corona virus that attaches to host cell receptor (ACE2) receptor via spike glycoprotein in a combination of surface protease (TMPRSS2). This virus relies heavily on **replicase targets** such as: RNA – dependent RNA polymerase (RdRp), Helicase, Exonuclease and Endoribonuclease. None of above mentioned claimed drugs could not handle the fast-increasing viral load and could bring neither therapeutic nor prophylactic (preventive) effect. Since then, the scientist worldwide launched the rally to find the best drug against anti-viral activity with replication-inhibiting feature that could ease the patients' infection spread.

The viral infections are hard to fight without harming the host cells, because viral genome uses the cell host machinery to replicate and assembly themselves into the new copies. The viral load is fully dependent on the assembly rates take a COVID19, one infected host cell can produce over 10 thousand of new corona viruses till the cell burst.

To understand how effectively to fight and to treat the viral infection, we need to embrace the viral life cycle that consist of further stages: Attachment to the host (animal) cell receptor. The most animal specific viruses have the additional lipid membrane called **envelope** with protein **spikes** that serves to attach a target cell, in SARS-COV2 genome by the way they belong to the structural protein's cohort. Viral entry (endocytosis, fusion). Release of genome (uncoating). Replication of viral genome. Proteins synthesis or processing, assembly. Release of new viruses.

Antiviral drugs

Represents almost 60 years development of antiviral drugs in USA since early 60s in 20th century. Unfortunately, only with virus discoveries, the research studies on antiviral drugs took place allowing viral infection either to spread or adapt to human immunity and genome, causing pharmacology industry many problems in strategy establishment to fight the viral invasion. Thus, only few drugs were approved to fight them effectively. To make things worse, the pharmacology and science were being aimed only against persistent viral diseases that require long and expensive therapy just to drag down the viral load in host cells, furthermore viruses are difficult to treat without serious side effects. During many decades the antiviral medicine production has created four main groups. They are 1) Anti-influenza, 2) Anti-HIV drugs, 3) Anti-hepatitis and 4) Anti-herpes.

Favipiravir is pyrazine analog T-705 and capable inhibitor of influenza viral RNA polymerase [83]. Favipiravir's metabolite (Favipiravir RTP (ribofuranosyl 5'-triposhpate interacts with viral RNA dependent polymerase (RdRp). It is assumed that the antiviral effect can be downgraded in the appearance of purine nucleotides ATP and GTP. In addition, this metabolite can be identified as a 'false' purine by the viral RdRp [84]. The previous *in-vitro* studies showed that SARS-COV2 Vero E6 infected cells tolerable cytotoxic response, namely half-cytotoxic concentration (CC_{50}) was at the level of 400μM and above [85]. Thus, it became clear that Favipiravir could be used at high concentrations to serve as safe and effective medicine against COVID19 infection.

Ribavirin is well-known antiviral drug with clear RNA and DNA replication interfering guanosine analog. The RNA-polymerase is no single target, but also thanks to it structure it prevents RNA caping during the RNA strand maturing that is heavily dependent on natural guanosine that keeps RNA from degradation [86]. According to some studies, no significant cytotoxicity was detected at ribavirin concentrations of 31.3 μg/mL in Vero cells model [87]**.** The clinical experience during the pandemic showed that the patients in worsening cases were given 400mg every 8hours in addition methylprednisolone administration to decrease the progressive viral load activity [88]. High specialization of ribavirin drug made doctors to pair with either IFN-α2a or IFN-α2b (interferon) to cover the therapeutic threshold to stop viral replication [89]. In 2003, in Canada, the ribavirin therapy with a dose of 500mg every 8 hours for 4-6days long was also combined with a corticosteroid in 40% of SARS patients [90]. So, Ribavirin is universal antiviral agent that could be taken solely or in a combination with either antiviral compound like interferon or excessive immune suppressors like corticosteroid in a worsening clinical dynamic.

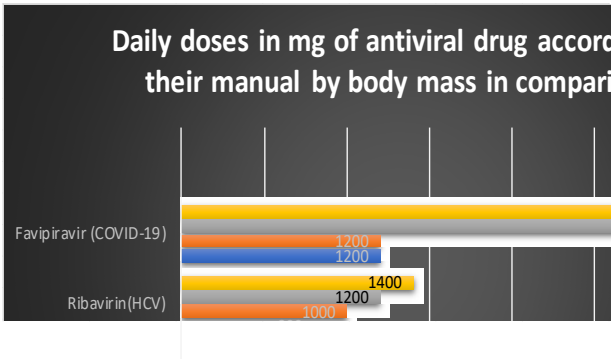
Tenofovir belongs both for anti-HIV drugs and Antihepatitic drug according to the producer's manual. Tenofovir represents the reverse transcriptase inhibitors or nucleoside reverse transcriptase inhibitors (NRTIs) are structural analogues of nucleic acids which competitively inhibit the reverse transcription by causing the chain termination after they got involved into viral DNA. This viral DNA-incorporation causes so-called 'lethal mutagenesis'. Tenofovir is also used as antiviral drug against chronic hepatitis B as nucleotide analogue. Tenofovir inhibits the HBV (hepatitis B virus) polymerase by competing with natural substrate for in cooperation with growing viral DNA-strand causing as in HIV (human immune deficit virus) chain termination, subsequently stalls the reverse transcription and synthesis of viral DNA. Tenofovir is yet another nucleotide analogue that was initially designed to inhibit the HIV (human immunogenicity virus) reverse transcriptase by interfering the ATP-Polymerization in the growing nucleic acid chain [9,10]. Tenofovir was also assumed to be effective against COVID-19 as it showed the tendency to dock the RNA- dependent RNA-polymerase and silence its activity in replication as well as in transcription and translation

of structural and accessory proteins making virions assembly almost impossible [91]. Tenofovir that is used in our study is for oral administration medicine in a form of disoproxil furmate (TDF) has many side effects if it is used in high dosage, such as renal toxicity, bone density degradation etc. [92]. *In-vitro* studies suggest that at concentrations under 100μM, tenofovir does not inhibit the viral replication in VeroE6 cells at the multiple infections in a so-called preventive way, when tenofovir was administered 1h prior to infection and up to 48h post infection. In the discussion of results, researchers came to idea that tenofovir in ATP-forms require the activation by host kinase and any cell type has probably the proper kinase activity to launch the tenofovir antiviral features and was suggested to try a study on human airway epithelial cells [93.95].

Dexamethasone according to **medicine producer's manual** dexamethasone is synthetic glucocorticoid (GCS), a methylated derivative of fluoroprednisolone. Provision of anti-inflammatory, anti-allergic, immunosuppressive action, increased sensitivity of beta-adrenergic receptors to endogenous catecholamines. The anti-inflammatory effect is linked to decreased capillary permeability, stabilization of cell membranes (especially lysosomal) and organelle membranes, inhibition of eosinophil and mast cell release of inflammatory mediators, induction of lipocortin formation, and reduction in the number of mast cells that produce hyaluronic acid.It acts on all stages of the inflammatory process: it inhibits the synthesis of prostaglandins (Pg) at the level of arachidonic acid (lipocortin inhibits phospholipase A2, inhibits the liberation of arachidonic acid and inhibits the biosynthesis of endoperoxides, leukotrienes, which contribute to inflammation, allergies, etc.), the synthesis of "pro-inflammatory cytokines" (interleukin 1, tumor necrosis factor alpha, etc.); increases the resistance of the cell membrane to the action of various damaging factors. The immunosuppressive effect is brought on by lymphoid tissue involution, inhibition of lymphocyte proliferation (especially T-lymphocyte proliferation), suppression of B-cell migration and interaction between T- and B-lymphocytes, inhibition of cytokine release from lymphocytes and macrophages (interleukin-1, 2; interferon gamma) [94]. And decreased antibody production. The antiallergic effect develops as a result of a decrease in the synthesis and secretion of allergy mediators, inhibition of the release of histamine and other biologically active substances from sensitized mast cells and basophils, a decrease in the number of circulating basophils, T- and B-lymphocytes, mast cells; suppression of the development of lymphoid and connective tissue, reducing the sensitivity of effector cells to allergy mediators, inhibition of antibody formation, changes in the body's immune response. It is worth to mention that 0.5 mg of dexamethasone is equivalent to roughly 3.5 mg of prednisone (or prednisolone), 15 mg of hydrocortisone, or 17.5 mg of cortisone, depending on the degree of glucocorticoid action. According to WHO data, dexamethasone should be used in severe cases of COVID-19 cases, especially, if a patient is dependent on live supporting systems.

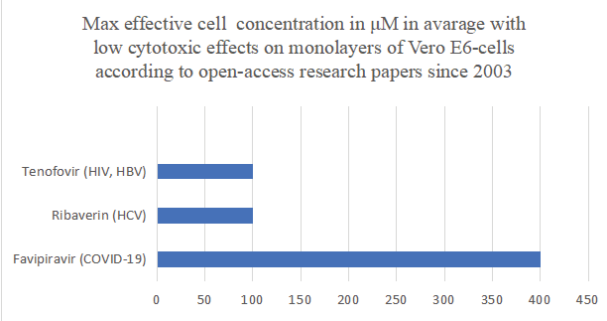
Effective doses of antiviral effect of Favipiravir, Ribavirin and Tenofovir for humans (in vivo) for Vero E6 cells (in vitro)

Toxicity has a definition as the amount or degree of a substance required to be poisonous. Toxicity depends on the amount and concentration involved, frequency of use, interactions of the person receiving the substance of interest, and individual reaction of the person [96].



Graph 1 – Since only Favipiravir or also known in the market as Fabiflu is specialized medicine against SARS-COV2 infection, its dose is so high and is designed to administer within 10 days for minor or moderate stages of COVID19 infection. Unlike Fabiflu, Ribavirin for instance was designed to slow down the hepatitis C replication up to 72 weeks, taking a drug twice a day at least. Tenofovir is suggested by its manual to take once a day one tablet that contains 300mg of tenofovir also for a long period of time under strict physician prescription and control according to manufacturers' manual and according to publications [83-95].

Effective doses of antiviral effect of Favipiravir, Ribavirin and Tenofovir for humans (invitro) Vero E6 cells



Graph 2 – Vero E6 -model also shows the toxicity edge for monolayers cells tenofovir and ribavirin could be and dose control plays a significant role not only to gain absolute viral RNA/DNA-replication silencing but also to minimize the negative side effect impact on contacting cells and tissues

Effective concentrations (EC_{10}, EC_{50}, EC_{90}), concertation efficacy of three drugs (Inhibition activity)

Half maximal viable concentration (**EC_{50}**) may be a degree of the concentration of a medicate, counter-acting agent (*e.g.,* antibodies), or toxicant which actuates a reaction *midway* (halfway) between the pattern and *the greatest value* after an indicated introduction time, saying it differently, EC_{50} can be specified as the concentration needed to obtain a 50% drug, antibody or toxicant effect.

$$\mathbf{pEC_{50}} = -\log_{10}(\mathbf{EC_{50}}) \quad (1)$$

There is a wide run of EC_{50} (1) values of drugs; they are regularly at any value from *nM to mM*. Thus, it is frequently more common sense to allude to the logarithmically transformed **pEC_{50}** values rather than EC_{50}. The term "potency" refers to the EC_{50} value. The lower the EC_{50} value, **the lower** the drug concentration required to achieve 50% of the maximal effect and **the higher** the potency. The EC_{10} and EC_{90} concentrations to induce 10% and 90% maximal responses respectively.

However, viral replication is needed to be stopped utterly, even 90% of replication silencing or 'breaking' is not enough to achieve the therapeutic effect of antiviral medication. Thus, so called old drugs like **ribavirin** and **tenofovir** are designed to be administrated for a long period of time and in relatively moderate concentration to inhibit the viral replication activity in host cells. **Ribavirin** and **tenofovir** are the antivirals for a long run drug therapy for primary purposes, but the increased concentration for 10 days prescribed therapy like **favipiravir** can be either reasonable risk for a cheap and effective alternative or 'side-effect disaster' for a chance to fight COVID-19 for instance or influenzas. To make things worse, the effective concentrations (EC_{10}, EC_{50}, EC_{90}) measure was heavily criticized already in 2003 due its 'vagueness' [97].

To support the idea of the vagueness of this measure methodology, A study for antivirals as individual run and as a drug combination effectiveness were made in Japan to show how E_{50} values span *in vitro* studies, the difference between minimum and maximum values are in average 40 times [98]. Thus, the values of E_{10} and E_{90} also demonstrated the wide range of 'runaway' values with data infirmity in its veracity.

To sum up, to fight the viral replicase of fast developing SARS-COV2 (i.e., its intercellular spread), 100% silencing is required and to gain this, physicians prescribe either high drug doses withing 10days in average with a particular drug like T-705 (favipiravir) or a combination of drugs like ribavirin with corticosteroids (such as: dexamethasone), or even 300mg tenofovir daily up to one week period, yet not at critical phase of COVID-19 infection.

Lethal mutagenesis as a purpose

The lethal mutagenesis characterization for virus existence and since any virus after entrance into the host cytosol consists of genetical information (mRNA), the lethal error rates in replicating itself plays critical role, so that the threshold line between extinction and survival is very thin and as proof-reading-important for viral survival as for pharmacology to target viral replicating machinery in a host cell. **[104].**As mRNA -Virus, COVID-19 has basically two ways to fight against, namely vaccination and drug intervention. The drug intervention of these antivirals is mostly bound with **RdR-** inhibition to reach lethal mutagenesis of viral infection. When the viral genomic RNA (gRNA) ingests itself in the host cell it has relatively unstable single stranded positive genomic RNA that requires to be replicated as soon as possible to be able to replicate new genomic RNA for structural proteins synthesis and assembly, furthermore, after replicating itself the **'original'** genomic RNA craves to build the sub-genomic RNAs (sgRNA) via transcription, these sg-RNAs (with caped mRNA, as in eucaryotic cells) are essential for translation in expressing the structural proteins that go to viral

assembly as well as **newly** replicated gRNA. As a result, inhibiting or interfering the viral replicase represents a serious arsenal in antiviral therapy that allows us to insert mutated gRNA or damaged gRNA into assembly process, providing so called extinction by fatal error in viral genome during and after replication [99,100,105].

The lethal mutagenesis of Ribavirin

As it was already mentioned ribavirin was invented roughly 40yeras ago and showed antiviral efficacy not only in human but also in animal lines. As guanosine analog it goes to host kinase as ribavirin triphosphate and pairs either with **cytidine** or **uridine-**triphosphate and mimics the purine nucleobase, causing the serious mutations during replicase and causes the lethal mutagenesis as anti-viral therapy reducing the viral load rates [99-101, 103]. In 2019 a new drug against influenzas was developed-**molnupirovir** having the same RdR-inhibiting properties as ribavirin has, and it showed the promising results during Covid-19 pandemic. Both drugs are **nucleoside-inhibitors**. Unlike ribavirin, molnupirovir is **pyrimidine analog**. It is worth to mention that ribavirin is much cheaper and more carefully observed drug than *molnupirovir* demonstrating the similar effectiveness. Nevertheless, during pandemic crisis in 2003 and 2019 the treatment was combined either with other drugs or so-called adjuvants like interferons and corticosteroids to achieve maximum outcome from treatment, and ribavirin was a classic example for these combination lines with acceptable survival as well as recovery rates among mild and moderate patients with SARS and SARS-COV2 infection [105].

The lethal mutagenesis of Favipiravir

Favipiravir is yet another effective nucleoside -inhibitor with proven wide-spectrum viruses that strongly rely on RdR. In countries like India and Japan, favipiravir showed the high rates of clinical effectiveness and relatively low cytotocity as well as side-effect potential. Along with ribavirin it was mainly prescribed for mild or moderate patient with 9-14days inpatient background. [102,105]. ***Favipiravir*** has also a good response on host RNA dependent replicase kinase that enables favipiravir as effective lethal mutagenesis causer not only in SARS-CoV2 populations but also against deadly Ebola, commonly known influenza and terrifying rabies which makes it valuable asset as antiviral medicine [99-105].

The lethal mutagenesis of Tenofovir

The most cytotoxic drug among our antiviral drug list (only 300mg oral administration is allowed daily). Tenofovir belongs too to the nucleoside -inhibitor that incorporates with RdR and makes viral DNA synthesis not viable and shouts down the virulence potential. Originally, it was designed against HIV and Hepatitis B viral invasion [103,105].

Steroids save lives in critical and sever cases of SARS-COV2 infection, nevertheless, it has its cost – bone infarct development

As it was already mentioned the immune system in humans is responsible for 'overdefensive' response on viral invasion causing huge tissue damage independently on age group casing severe pneumonia as 'final act' of immunity – so called '*cytokinetic storm'* which probably was the main reason of lethal outcomes during Spanish influenza pandemic after WWI. All three antiviral drugs a clinically prescribed for patients with **mild or moderate** state of viral infection, reducing the viral load through lethal mutagenesis, enabling us to achieve ideally the viral extinction. In severe cases, doctors mostly betake steroids to calm the overreacted immune response that could be lethal if it is not stopped and here comes corticosteroids in combination with antiviral therapies like with ribavirin already in 2003. The antiviral effect was so high and effective that WHO (world health organization) recommends dexamethasone as additional and safe medicine to fight COVID-19 infection in mild and moderate patients care,

Before 2019, the steroids' side-effects were studied among many patients with passivated immunity. The bone infarct mostly caused by chronic appearance of immune passivation as well as during other health destructive patterns like alcohol misusage and chronic smoking The doses risk mostly started from 500mg of **corticosteroids** of daily administration for 1-3months [106]. However, the low doses up to 100mg a day showed the low risk, somewhere between 2-3%. The dexamethasone has 4mg/ml interveinal administration protocol during the COVID-19 treatment and only a physician makes a decision on the effective dose. WHO recommends to use in mild stages of infection 15-20mg a day as auxiliary therapy option. But what happens with severe cases is still not clear and everything is highly individual and intense steroids therapy was inevitable to fight the progressing pneumonia and other signs of acute COVID-19 complications [1.8,14].

Conclusion

All above mentioned aspects of COVID-19 virus and its spread potential are based on its structure like receptor binding capacities and host cell invasion procedures that fully depend on functional genome both of non-structural (***nsps***) or poly-protein regions and of course the structural proteins synthesis which play the most important role in viral assembly. The innate immunity takes the first defense line that allows to withstand the intense viral load growth. The adaptive immunity is the most powerful arsenal against human to human – spreading way, that neutralize viruses or viral particles not only intercellular but also intracellular way. The vaccination against SARS-CoV2 show high rates of resistance and survival rates among hospitalized patients and temporary protection for healthy individuals. Apart vaccination, the biotechnological approaches like poly- and mono-clonal antibody synthesis can offer a big deal of advantages to comprehend the viral infection patterns and set the sophisticated strategy lines to neutralize the viral infection, nevertheless, polyclonal and monoclonal antibodies studies bare upper hand therapy strategies as well as serious drawbacks that cannot be ignored freely. The hybridoma technologies, for example can offer the high rates of monoclonal immunoglobins that could be used in recombination science, yet only in lablotory scales. The pharmacological therapies also give some solutions in antiviral drug development. By comprehending the nature of viral replication, the lethal mutagenesis concept began to be one of the main directions of COVID-19 spreading fighting policy in clinical practice. The combination of drugs like using wide range nucleoside – analogs (Ribavirin, Tenofovir and Favipiravir) with corticosteroids like dexamethasone showed the marvelous effect in mild and moderate stages of COVID-19 – infection severeness. Three discussed drugs form a basis of antiviral therapy for many years because they were primarily used against so called static and long-lasting viral infections like Hepatitis B and C, HIV and even against Ebola-virus. These drugs represent the real antiviral effect on COVID-19 virus replication, causing so called **viral extinction** through lethal mutagenesis. However, as it was already discussed the steroids can have profound negative effect on human health in long term perspectives as side-effect(s) and one of them is the *bone infarct*. Thus, any practicing physician has to weigh the risks of steroids involving in antiviral therapy.

Acknowledgments

This article is a part of doctorate research-scientific work and this laboratory practice was conducted at Republican State Enterprise on the right of economic management «Research Institute for Biological Safety Problems» of the Ministry of Health of the Republic of Kazakhstan.

This work was carried out within the framework of the grant funding project on the topic: AP09058338 “STUDY OF ANTI-VIRAL ACTIVITY OF DRUGS AGAINST SARS-COV-2 VIRUS IN VITRO AND CONDUCTING MOLECULAR-EPIDEMIOLOGICAL ANALYSIS OF CIRCULATING COVID-19 STRAINS “under targeted funding for 2021–2023 with the support of the Science Committee of the Ministry of Science and Higher Education of the Republic of Kazakhstan.

Reference

1. World Health Organization. 2020. Coronavirus disease (COVID-19) weekly epidemiological update and weekly operational update. https://www.who.int/ emergencies/diseases/novel-coronavirus-2019/situation-reports/. Retrieved 15 October 2022.

2. Jia Y, Shen G, Nguyen S, Zhang Y, Huang K-S, Ho H-Y, Hor W-S, Yang C-H, Bruning JB, Li C, Wang W-L. 2020. Analysis of the mutation dynamics of SARS-CoV-2 reveals the spread history and emergence of RBD mutant with lower ACE2 binding affinity. BioRxiv. https://doi.org/10.1101/2020.04.09.034942.

3. Chiara M, Horner DS, Gissi C, Pesole G. 2020. Comparative genomics suggests limited variability and similar evolutionary patterns between major clades of SARS-CoV-2. BioRxiv. https://doi.org/10.1101/2020.03.30.016790.

4. Ivan Dorp L, Acman M, Richard D, Shaw LP, Ford CE, Ormond L, Owen CJ, Pang J, Tan CCS, Boshier FAT, Ortiz AT, Balloux F. 2020. Emergence of genomic diversity and recurrent mutations in SARS-CoV-2. Infect Genet Evol 83:104351. https://doi.org/10.1016/j.meegid.2020.104351.

5. Walls AC, Park Y-J, Tortorici MA, Wall A, McGuire AT, Veesler D. 2020. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 181:281–292.e6. https://doi.org/10.1016/j.cell.2020.02.058.

6. Zhugunissov K, Zakarya K, Khairullin B, Orynbayev M, Abduraimov Y, Kassenov M, Sultankulova K, Kerimbayev A, Nurabayev S, Myrzakhmetova B, Nakhanov A, Nurpeisova A, Chervyakova O, Assanzhanova N, Burashev Y, Mambetaliyev M,

Azanbekova M, Kopeyev S, Kozhabergenov N, Issabek A, Tuyskanova M, Kutumbetov L. 2021. Development of the inactivated QazCovid-in vaccine: protective efficacy of the vaccine in Syrian hamsters. Front Microbiol 12:720437. https://doi.org/10.3389/fmicb.2021 .720437.

7. Johns Hopkins University. 2019. COVID-19 data repository by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University. https://github.com/CSSEGISandData/COVID-19. Retrieved 15 October 2022.)

8. Gorbalenya, A.E. et al. (2020) The species severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat. Microbiol. 5, 536–544.

9. 2.Lu, R. et al. (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395, 565–574.

10. Zhang, Y.Z. and Holmes, E.C. (2020) A genomic perspective on the origin and emergence of SARS-CoV-2. Cell 181, 223–227.

11. Perlman, S. and Netland, J. (2009) Coronaviruses post-SARS: update on replication and pathogenesis. Nat. Rev. Microbiol. 7, 439–450.

12. Zhang W, Cao S, Martin JL, Mueller JD, Mansky LM. Morphology and ultrastructure of retrovirus particles. AIMS Biophys. 2015; 2(3): 343-369.

13. Wyatt R, Sodroski J. The HIV-1 envelope glycoproteins: Fusogens, antigens, and im- munogens. Science. 1998; 280(5371): 1884-1888.

14. Wang, D. et al. (2020) Clinical characteristics of 138 hospitalzed patients with 2019 novel coronavirus-infected pneumonia in Wuhan, China. JAMA 323, 1061–1069.48

15. Li, Q. et al. (2020) Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. N. Engl. J. Med. 382, 1199–1207

16. Chan, J.F.W. et al. (2020) A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to person transmission: a study of a family cluster. Lancet 395, 514–523.

17. Clinical management of COVID-19 15th September 2022, WHO p.35- 62.

18. Burashev Y. et al. Coding Complete Genome Sequence of the SARS-CoV-2 Virus Strain, Variant B.1.1, Sampled from Kazakhstan, 2022, *Microbiology Resource Announcements.* doi: https://journals.asm.org/doi/10.1128/mra.01114-22

19. Wu, F. et al. (2020) A new coronavirus associated with human respiratory disease in China. Nature 579, 265–269.

20. Hoffmann, M. et al. (2020) SARS-CoV-2 cell entry depends on ACE2 and **TMPRSS2** and is blocked by a clinically proven protease inhibitor. Cell 181, 271–280.

21. Perlman, S. and Netland, J. (2009) Coronaviruses post-SARS: update on replication and pathogenesis. Nat. Rev. Microbiol. 7, 439–450.

22. Snijder, E.J. et al. (2006) Ultrastructure and origin of membrane vesicles associated with the severe acute respiratory syndrome coronavirus replication complex. J. Virol. 80, 5927–5940.

23. Wu, H.-Y. and Brian, D.A. (2010) Subgenomic messenger RNA amplification in coronaviruses. Proc. Natl. Acad. Sci. U. S. A. 107, 12257–12262.

24. Shailendra K. Saxena and Sai V. Chitti (2016) Molecular Biology and Pathogenesis of Retroviruses. Advances in Molecular Retrovirology, 9-24.

25. Varmus H. Retroviruses. Science. 1988; 240(4858): 1427-1435.

26. Coffin JM. Structure and classification of retroviruses. In: Levy, JA. The Retroviridae (First edition). New York: Plenum; 1992. p. 20. ISBN 0-306-44074-1.

27. Rabson AB, Graves BJ. Synthesis and processing of viral RNA. In: Coffin JM, Hughes SH, Varmus HE, editors. Retroviruses. Cold Spring Harbor (NY): Cold Spring Har- bor Laboratory Press; 1997.

28. Bharat TA, Davey NE, Ulbrich P, Riches JD, de Marco A, Rumlova M, Sachse C, Ruml T, Briggs JA. Structure of the immature retroviral capsid at 8 Å resolution by cryo -electron microscopy. Nature. 2012; 487(7407): 385-389].

29. Clinical management of COVID-19 15th September 2022, WHO p.110

30. Clinical management of COVID-19 15th September 2022, WHO p.113-115

31. Marilyn J. Roossinck, Viren, https://doi.org/10.1007/978-3-662-61684-0, ISBN 978-3-662-61684-0 (eBook), pp.28

32. Kanneganti, T. D. Intracellular innate immune receptors: life inside the cell. Immunol. Rev. 297, 5–12 (2020).

33. Rui, Y. et al. Unique and complementary suppression of cGAS–STING and RNA sensing—triggered innate immune responses by SARS-CoV-2

34. Franz, K. M., Neidermyer, W. J., Tan, Y. J., Whelan, S. P. J. & Kagan, J. C. STING-dependent translation inhibition restricts RNA virus replication. Proc. Natl Acad. Sci. USA 115, E2058–E2067 (2018).

35. Sun, B. et al. Dengue virus activates cGAS through the release of mitochondrial DNA. Sci. Rep. 7, 3594 (2017).

36. Briard, B., Place, D. E. & Kanneganti, T. D. DNA sensing in the innate immune response. Physiology 35, 112–124 (2020).

37. Sun, L., Wu, J., Du, F., Chen, X. & Chen, Z. J. Cyclic GMP–AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. Science 339, 786–791 (2013)

38. Singh, K. K., Chaubey, G., Chen, J. Y. & Suravajhala, P. Decoding SARS-CoV-2 hijacking of host mitochondria in COVID-19 pathogenesis. Am. J. Physiol. Cell Physiol. 319, C258–C267 (2020).

39. Kanneganti, T. D. Intracellular innate immune receptors: life inside the cell. Immunol. Rev. 297, 5–12 (2020).

40. Viren, Walter Doerfer (2002), ISBN 3-596-15369-7, pp. 37-42.

41. Viren, Walter Doerfer (2002), ISBN 3-596-15369-7, pp. 42-49.

42. Viren, Walter Doerfer (2002), ISBN 3-596-15369-7, pp. 49-51.

43. Viren, Walter Doerfer (2002), ISBN 3-596-15369-7, pp. 49-52.
44. Viren, Walter Doerfer (2002), ISBN 3-596-15369-7, pp. 52-53.
45. Viren, Walter Doerfer (2002), ISBN 3-596-15369-7, pp. 54-58.
46. Viren, Walter Doerfer (2002), ISBN 3-596-15369-7, pp. 60-61.8.
47. Beyrau, M., Bodkin, J. V., and Nourshargh, S. (2012). Neutrophil heterogeneity in health and disease: a revitalized avenue in inflammation and immunity. Open Biol. 2:120134. doi: 10.1098/rsob.1201349.
48. Blanco-Melo, D., Nilsson-Payant, B.E., Liu, W.-C., Uhl, S., Hoagland, D., Møller, R., Jordan, T.X., Oishi, K., Panis, M., Sachs, D., et al. (2020). Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. Cell 181, 1036–1045.e9.
49. Oran, D.P., and Topol, E.J. (2020). Prevalence of Asymptomatic SARS-CoV-2 Infection: A Narrative Review. Ann. Intern. Med. 173, 362–367.11.
50. Grifoni, A., Weiskopf, D., Ramirez, S.I., Mateus, J., Dan, J.M., Moderbacher, C.R., Rawlings, S.A., Sutherland, A., Premkumar, L., Jadi, R.S., et al. (2020). Targets of T Cell Responses to SARS-CoV-2 Coronavirus in Humans with COVID-19 Disease and Unexposed Individuals. Cell 181, 1489–1501.e15.
51. Piccoli, L., Park, Y.-J., Tortorici, M.A., Czudnochowski, N., Walls, A.C., Beltramello, M., Silacci-Fregni, C., Pinto, D., Rosen, L.E., Bowen, J.E., et al. (2020). Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology. Cell 183, 1024–1042.e21.
52. Isho, B., Abe, K.T., Zuo, M., Jamal, A.J., Rathod, B., Wang, J.H., Li, Z., Chao, G., Rojas, O.L., Bang, Y.M., et al. (2020). Persistence of serum and saliva antibody responses to SARS-CoV-2 spike antigens in COVID-19 patients. Sci. Immunol. 5, eabe5511.
53. Lu, L.L., Suscovich, T.J., Fortune, S.M., and Alter, G. (2018). Beyond binding: antibody effector functions in infectious diseases. Nat. Rev. Immunol. 18, 46–61.15.
54. Gudbjartsson, D.F., Norddahl, G.L., Melsted, P., Gunnarsdottir, K., Holm, H.,Eythorsson, E., Arnthorsson, A.O., Helgason, D., Bjarnadottir, K., Ingvarsson,R.F., et al. (2020). Humoral Immune Response to SARS-CoV-2 in Iceland.N. Engl. J. Med. 383, 1724–1734.
55. Anderson, E.M., Goodwin, E.C., Verma, A., Arevalo, C.P., Bolton, M.J., Weirick, M.E., Gouma, S., McAllister, C.M., Christensen, S.R., Weaver, J., et al. (2020). Seasonal human coronavirus antibodies are boosted upon SARS-CoV-2 infection but not associated with protection. medRxiv.
56. Dan, J.M., Mateus, J., Kato, Y., Hastie, K.M., Faliti, C.E., Ramirez, S.I., Frazier, A., Yu, E.D., Grifoni, A., Rawlings, S.A., et al. (2021). Immunological memory to SARS-CoV-2 assessed for up to eight months after infection. Science, eabf406.
57. Reed Magleby, Lars F Westblade, Alex Trzebucki, Matthew S Simon, Mangala Rajan, Joel Park, Parag Goyal, Monika M Safford, Michael J SatlinImpact of Severe Acute Respiratory Syndrome Coronavirus 2 Viral Load on Risk of Intubation and Mortality Among Hospitalized Patients with Coronavirus Disease 2019 | Clinical Infectious Diseases | Oxford Academic (oup.com)
58. Zohar, T., Loos, C., Fischinger, S., Atyeo, C., Wang, C., Slein, M.D., Burke, J.,Yu, J., Feldman, J., Hauser, B.M., et al. (2020). Compromised humoral functional evolution tracks with SARS-CoV-2 mortality. Cell 183, 1508–1519.e12.
59. Van Erp, E. A., Luytjes, W., Ferwerda, G., & van Kasteren, P. B. (2019). *Fc-Mediated Antibody Effector Functions During Respiratory Syncytial Virus Infection and Disease. Frontiers in Immunology, 10.* doi:10.3389/fimmu.2019.00548 pp. 221.
60. Smyth MJ, Cretney E, Kelly JM, Westwood JA, Street SE, Yagita H, et al. Activation of NK cell cytotoxicity. Mol Immunol. (2005) 42:501–10. doi: 10.1016/j.molimm.2004.07.03422.
61. Van Erp, E. A., Luytjes, W., Ferwerda, G., & van Kasteren, P. B. (2019). *Fc-Mediated Antibody Effector Functions During Respiratory Syncytial Virus Infection and Disease. Frontiers in Immunology, 10.* doi:10.3389/fimmu.2019.00548 pp.2-3.23.
62. Van Erp, E. A., Luytjes, W., Ferwerda, G., & van Kasteren, P. B. (2019). *Fc-Mediated Antibody Effector Functions During Respiratory Syncytial Virus Infection and Disease. Frontiers in Immunology, 10.* doi:10.3389/fimmu.2019.00548 pp 4.
63. Kaul TN, Welliver RC, Ogra PL. Comparison of fluorescent-antibody, neutralizing-antibody, and complement-enhanced neutralizing-antibody assays for detection of serum antibody to respiratory syncytial virus. J Clin Microbiol. (1981) 13:957–62.25.
64. Kruijsen D, Bakkers MJ, van Uden NO, Viveen MC, van der Sluis TC, Kimpen JL, et al. Serum antibodies critically affect virus-specific CD4+/CD8+ T cell balance during respiratory syncytial virus infections. J Immunol. (2010) 185:6489–98. doi: 10.4049/jimmunol.1002645.].
65. Pohanka M, Pavliš O, Kroča M: ELISA detection of Francisella tularensis using polyclonal and monoclonal antibodies. Def Sci J 58:698–702, 2008a.
66. Morris TJ, Stanley EF: A simple method for immunocytochemical staining with multiple rabbit polyclonal antibodies. J Neurosci Methods 127:149–155, 2003.
67. Martínez JM, Martínez MI, Suárez AM, Hezzanz C, Casaus P, Cintas LM, Rodríguez JM, Hernández PE: Generation of polyclonal antibodies of predetermined specificity against pediocin PA-1. Appl Environ Microbiol 64:4536–4545, 1998.
68. Somodevilla-Torres MJ, Hillyard NC, Morton H, Alewood D, Halliday JA, Alewood PF, Vesey DA, Walsh MD, Cavanagh AC: Preparation and characterization of polyclonal antibodies against human chaperonin 10. Cell Stress Chaperones 5:14–20, 2000.
69. Bergmann-Leitner ES, Mease RM, Duncan EH, Khan F, Waitumbi J, Angov E: Evaluation of immunoglobulin purification methods and their impact on quality and yield of antigen-specific antibodies. Malar J 7:129, 2008.
70. Pohanka M: Evaluation of immunoglobulin production during tularemia infection in BALB/c mouse model. Acta Vet Brno 76:579–584, 2007.
71. Emanuel PA, Dang J, Gebhardt JS, Aldrich J, Garber EAE, Henrieta K, Stopa P, Valdes JJ, Schultz AD: Recombinant antibodies: a new reagent for biological agent detection. Biosens Bioelectron 14:761–770, 2000.
72. Köhler G, Milstein C: Continuous cultures of fused cells secreting antibody of predefined specificity. Nature 256:495–497, 1975.

73. Shulman M, Wilde CD, Köhler G (1978) A better cell line for making hybridomas secreting specific antibodies. Nature 276(1978):269–270.

74. Parray HA, Shukla S, Samal S et al (2020) Hybridoma technology a versatile method for isolation of monoclonal antibodies, its applicability across species, limitations, advancement and future perspectives. Int Immunopharmacol. 85:106639. https://doi.org/10.1016/j.intimp.2020.1066.

75. Ghosh S, Wakchaure R (2013) Monoclonal antibodies: a tool in clinical research. J Indian Acad Clin Med 4:9–21. https://doi.org/10.4137/IJCM.S11968.

76. Ganguly S, Wakchaure R (2016) Hybridoma technology: a brief review on its diagnostic and clinical significance. Pharmaceut Biol Eval 3(issue 6):554–555.

77. Buck DW, Larrick JW, Raubitschek A, Truitt KE, Senyk G, Wang J, Dyer B (1984) Production of human monoclonal antibodies. In. Kennett RH, Bechtol KB and McKearn TJ (ed) Monoclonal Antibodies and Functional Cell Lines. Progress and Applications. New York: Plenum Press; pp 275-309.

78. Saeed AFUH, Awan SA (2016) Advances in monoclonal antibodies production and cancer therapy. MOJ Immunol 3(4):00099. https://doi.org/10. 15406/moji.2016.03.00099.

79. Parray HA, Shukla S, Samal S et al (2020) Hybridoma technology a versatile method for isolation of monoclonal antibodies, its applicability across species, limitations, advancement and future perspectives. Int Immunopharmacology. 85:106639. https://doi.org/10.1016/j.intimp.2020.106639.

80. Lu, R. et al. (2020) Genomic characterization and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395, 565–574.

81. Zhang, Y.Z. and Holmes, E.C. (2020) A genomic perspective on the origin and emergence of SARS-CoV-2. Cell 181, 223–227.) Perlman, S. and Netland, J. (2009) Coronaviruses post-SARS: update on replication and pathogenesis. Nat. Rev. Microbiol. 7, 439–450).

82. Roe et al., Journal of General Virology 2021;102:001558 DOI 10.1099/jgv.0.001558.

83. Joshi S, Parkar J, Ansari A, Vora A, Talwar D and Tiwaskar M: Role of favipiravir in the treatment of COVID-19. Int J Infect Dis 2021; 102: 501-8.

84. Delang L, Abdelnabi R and Neyts J: Favipiravir as a potential countermeasure against neglected and emerging RNA viruses. Antiviral Res 2018; 153: 85-94.

85. Wang M, Cao R, Zhang L, Yang X, Liu J and Xu M: Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in-vitro. Cell Res 2020; 30(3): 269-71.

86. Graci JD, Cameron CE. Mechanisms of action of ribavirin against distinct viruses. Rev Med Virol. 2006;16(1):37-48.

87. Myung J.1., Kye-Hyung Kim. In vitro antiviral activity of ribavirin against severe fever with thrombocytopenia syndrome virus. Korean J Intern Med. 2017 Jul; 32(4): 731–737.

88. Lee N, Hui D, Wu A, et al. A major outbreak of severe acute respiratory syndrome in Hong Kong. N Engl J Med. 2003;348(20):1986-1994.

89. Falzarano D, de Wit E, Martellaro C, Callison J, Munster VJ, Feldmann H. Inhibition of novel beta coronavirus replication by a combination of interferon-alpha2b and ribavirin. Sci Rep. 2013;3: 1686.

90. Koren G, King S, Knowles S, Phillips E. Ribavirin in the treatment of SARS: A new trick for an old drug? CMAJ. 2003;168(10):1289-1292.

91. Balzarini, J.; Holy, A.; Jindrich, J.; Naesens, L.; Snoeck, R.; Schols, D.; De Clercq, E. Differential antiherpesvirus and antiretrovirus effects of the (S) and (R) enantiomers of acyclic nucleoside phosphonates: Potent and selective in vitro and in vivo antiretrovirus activities of (R)-9 (2phosphonomethoxypropyl)-2,6-diaminopurine. Antimicrob. Agents Chemother. 1993, 37, 332–338.

92. Edited Tsai, C.-C.; Follis, K.E.; Sabo, A.; Beck, T.W.; Grant, R.F.; Bischofberger, N.; Benveniste, R.E.; Black, R. Prevention of SIV Infection in Macaques by (R)-9-(2-Phosphonylmethoxypropyl) adenine. Science 1995, 270, 1197–1199.

93. Elfiky, A.A. Ribavirin, Remdesivir, Sofosbuvir, Galidesivir, and Tenofovir against SARS-CoV-2 RNA dependent RNA polymerase (RdRp): A molecular docking study. Life Sci. 2020, 253, 117592.

94. Edited Li, G.; Yue, T.; Zhang, P.; Gu, W.; Gao, L.-J.; Tan, L. Drug Discovery of Nucleos(t)ide Antiviral Agents: Dedicated to Prof. Dr. Erik De Clercq on Occasion of His 80th Birthday. Molecules 2021, 26, 923.

95. Choy, K.-T.;Wong, A.Y.-L.; Kaewpreedee, P.; Sia, S.F.; Chen, D.; Hui, K.P.Y.; Chu, D.K.W.; Chan,M.C.W.; Cheung, P.P.-H.; Huang, X.; et al. Remdesivir, lopinavir, emetine, and homoharringtonine inhibit SARS-CoV-2 replication in vitro. Antivir. Res. 2020, 178, 104786.

96. Rob W. Brooker; Zaal Kikvidze. Importance: an overlooked concept in plant interaction research. Journal of Ecology 2008, 96, 703–708. https://doi.org/10.1111/j.1365-2745.2008.01373.x

97. Neubig, Richard R. (2003). "International Union of Pharmacology Committee on Receptor Nomenclature and Drug Classification. XXXVIII. Update on Terms and Symbols in Quantitative Pharmacology". Pharmacological Reviews. 55 (4): 597–606. doi:10.1124/pr.55.

98. Yousuke. A., et.al. Favipiravir (T-705), a broad-spectrum inhibitor of viral RNA polymerase. Proc. Jpn. Acad., Ser. B 93 (2017).

99. Perales, C., Gallego, I., de Ávila, A. I., Soria, M. E., Gregori, J., Quer, J., & Domingo, E. (2019). *The increasing impact of lethal mutagenesis of viruses. Future Medicinal Chemistry, 11(13), 1645–1657.* doi:10.4155/fmc-2018-0457

100. Perales, C., Martín, V., & Domingo, E. (2011). *Lethal mutagenesis of viruses. Current Opinion in Virology, 1(5), 419–422.* doi:10.1016/j.coviro.2011.09.001

101. Crotty, S., & Andino, R. (2002). *Implications of high RNA virus mutation rates: lethal mutagenesis and the antiviral drug ribavirin. Microbes and Infection, 4(13), 1301–1307.* doi:10.1016/s1286-4579(02)00008-4

102. Espy, N., Nagle, E., Pfeffer, B., Garcia, K., Chitty, A. J., Wiley, M., … Palacios, G. (2019). *T-705 induces lethal mutagenesis in Ebola and Marburg populations in macaques. Antiviral Research.* doi:10.1016.

103. McDaniel, Y. Z., Patterson, S. E., & Mansky, L. M. (2019). *Distinct dual antiviral mechanism that enhances hepatitis B virus mutagenesis and reduces viral DNA synthesis. Antiviral Research, 104540.* doi:10.1016/j.antiviral.2019.1045

104. Bull, J. J., Sanjuan, R., & Wilke, C. O. (2007). Theory of Lethal Mutagenesis for Viruses. Journal of Virology, 81(6), 2930–2939. doi:10.1128/jvi.01624-06. pp. 2937.

105. Xiaoying XuYuheng ChenXinyu LuWanlin ZhangWenxiu FangLuping YuanXiaoyan Wang. An update on inhibitors targeting RNA-dependent RNA polymerase forCOVID-19 treatment: Promises and challenges. Biochemical pharmacology 205 (2022). https://doi.org/10.1016/j.bcp.2022.115279.

106. Christian Powell, Christopher Chang, Stanley M. Naguwa, Gurtej Cheema, M. Eric Gershwin, *Steroid induced osteonecrosis: An analysis of steroid dosing risk.* Autoimmunity Reviews (2010) 724-743.