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Your Body on a Chip: Functional Testing for Personalized Medicine

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Abstract:

Despite the well-known high prevalence of failure in drug development, recent advancements in tissue engineering and microfabrication have helped to create microphysiological systems (MPS), or "organs-on-chips," which mimic the function of human organs. These "tissue chips" might be used for toxicity and drug screening tests, which could revolutionize the early phases of the drug development process. Additionally, they may be utilized to simulate disease conditions, supplying new instruments for deciphering disease pathologies and causes and evaluating the efficacy of novel treatments. Future clinical trials on chips might be utilized to assess novel medicines in both populations and individuals, opening the door for precision medicine. Here, we'll discuss tissue chips' diverse potential and the difficulties in developing them.

INTRODUCTION

Organs-on-chips are microfluidic cell culture devices that precisely replicate the Physico-chemical microenvironment of tissues in the human body under dynamic, regulated settings (1). Therefore, the chips display tissue- and organ-level activities that are not seen in other, more straightforward in vitro cell systems. These microsystems have great potential for use in the present pharmaceutical research procedures to investigate the toxicity and efficacy routes in human tissues (2). They may also aid in creating personalized therapies and provide insight into disease processes, which bodes well for developing precision medicine (PM) (3). There is a growing understanding that in moreover to these uses in pharmaceutical and biomedical investigation, organs-on-chips can also be seen as controlled, physical representations of particular patients and can thus be used directly in the clinic to inform techniques for the treatment or prevention of disease (3). In this review, we will explain that the production of "personalized" organs-on-chips that accurately represent particular persons' genetics, physiology, and biometric factors is made possible by the regulated integration of person-specific cells, tissue samples, and culture conditions based on biometric data. We will provide samples of these customized products. Organs-on-Chip will demonstrate their potential to help create and assess treatment plans for specific patient populations or people. Introduction.

What exactly is a chip-based organ?

A bioengineered microdevice called an organ-on-a-chip, also known as a tissue chip, is typically no larger than a few square centimeters and is intended to imitate the fundamental functional unit of a human organ, such as the lung alveolus, proximal kidney tubule, or liver acinus (4). Microfluidic channels supply fluid to microfabricated chambers that mimic tissues' three-dimensional (3D) architecture and are filled with human cells. The channels enable the tissues to receive fluid perfusion. Their designed shapes and designs can mimic the biomechanical strain and shear pressures that human tissues experience in vivo. These instruments, sometimes referred to as MPS, are a part of a growing initiative by academia, the government, and the pharmaceutical industry to develop and make use of innovations that more accurately reflect human physiology and drug reactions (5-7).

Up to 60% of compounds are classified as failures owing to a lack of effectiveness, and a further 30%

pose unacceptable safety hazards from toxicity when compounds reach early clinical trials. This pressure is due to the ever-increasing costs and timetables for drug development (15 years and up to $2.8 billion) (8,9). Animals, in most circumstances, are not appropriate models for many human diseases because of the possibility that their reactions to medications may change depending on the species owing to physiologic variations (10). Therefore, the ideal models for illnesses that affect humans are based on human beings.

Models of several different organs, including the heart, liver, stomach, brain, muscle, and vasculature, have been created using tissue chips (3). Their ability to be stocked with human cells—whether from primary donors, differentiated induced pluripotent stem cells (iPSCs) from adult donors, or commercially accessible cell lines—is a significant benefit (3). Additionally, no other in vitro model has the potential to mimic hemodynamic and fluidic flow, as well as biomechanical stress. The lung-on-a-chip is one of the first and most commonly used tissue chips (11). This platform consists of two tiny chambers, one of which is filled with fluid and the other with air, and they are separated from one another by a thin membrane lined with lung cells (11). Two additional air-filled chambers are located next to the two main chambers. When a rhythmic vacuum is applied, the membrane between the two chambers stretches and relaxes, simulating the air-fluid interface of the human alveolus (11). Since then, this platform design has been adapted to simulate different systems, including gut peristalsis and the blood-brain barrier (BBB), lung infection, pulmonary edema, asthma, and chronic obstructive pulmonary disease (COPD) (12-14).

MPS platform designs vary greatly depending on the tissue they are intended to mimic. However, they all have three key design elements: 1) the cell arrangement in three dimensions to build a biologically accurate model, 2) incorporating microfluidic components for fluid exchange, and 3) tissues made up of different cell types, for as a liver chip made up of immunological, endothelial, and hepatocyte cells [3]. The capacity to represent the multicellular makeup of each human tissue in the systems and arrange the cells in scaffolds that simulate 3D tissue architecture is a significant benefit of employing MPS. Several preclinical assays for toxicity and efficacy screening can currently be performed on human cells. However, these assays frequently take place on two-dimensional (2D) monocellular layers in static cell culture plates, missing the multicellular, multidimensional, and fluid-exchange characteristics of tissues in vivo (15). Additionally, current developments in iPSC technology enable the production of a range of organ tissues from a single person's skin fibroblasts, adipose tissue, and blood cells, enabling the production of different organ tissue types from the same donor (15).

The accuracy of treatments is improved by using health data in precision medicine

The idea of precision medicine, in which each individual would receive customized treatment for the promotion, maintenance, and restoration of their health, is becoming more and more significant in medicine, toxicology, pharmacology, and biomedical science as a result of the growing recognition (16). Due to the current lack of "precision" in medicine, many patients receive treatments that are not in their best interests,

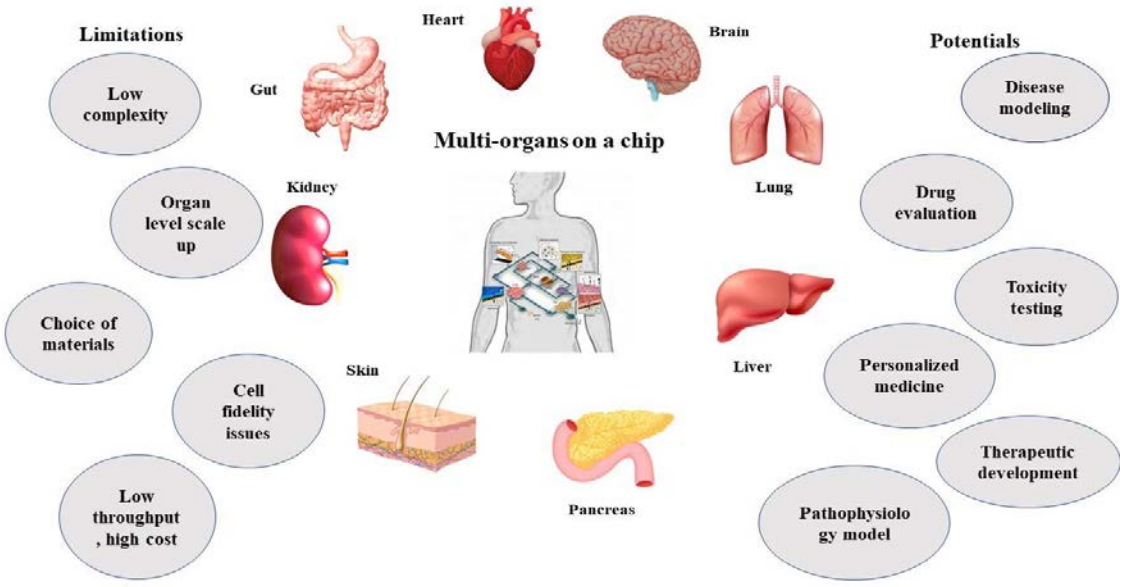

Fig 1. Several effective organ-on-a-chip applications and their accompanying functionalities. Preclinical to clinical translational precision biomedical applications are made possible by the organ-on-a-chip.

which results in ineffective healthcare (16). For example, for some medications with the most significant sales worldwide, the total number of users required for just one person to benefit from a drug's benefits ranges from 5 to 50 (16). Even more concerningly, several people are subjected to medical procedures that harm their health (17). Due to negative drug responses, millions of individuals are hospitalized annually, resulting in tens of thousands of fatalities (17). By creating techniques that can make therapy more accurate, hazardous and useless remedies can be avoided, potentially enhancing patient quality of life and lowering healthcare costs.

Linking an individual's health-related data to functional results in their response to specific therapies is the main difficulty in precision medicine. To determine which patients respond to which therapies, the typical method is to employ patient-specific data gathered from genomics, transcriptomics, imaging, biomarkers, and biometrics, followed by longitudinal studies using statistical and computational analysis. This strategy is thriving and has produced outstanding instances of precision medicine, such as choosing person-specific therapies for cancer and lung disease, which may be used in practice (18, 19).

Body-on-a-chip: multi organ system application

Clinical tests using chips for different patient subgroups

A benefit of an organ-on-a-chip is that it may be filled with human cells, either from the central organ or from iPSC sources. This enables the creation of an in vitro genotype model for a particular person. Particularly in multiorgan systems, as mentioned above, utilizing an individual's cells in MPS platforms enables secure and reusable techniques for researching disease causes, medication toxicity and effectiveness, off-target side effects, metabolic drug profiles, and more. Before a therapeutic is approved for use in a "first-in-human" clinical trial, these data and outcomes must be carefully described to minimize risk to any volunteer or patient. These data and results are essential for supplying information on the likely effects of drugs and therapeutics during development. However, some patient groups may not be eligible for clinical studies. For instance, individuals with certain aggressive tumors may need more time to engage in clinical trials before the disease progresses too far to get therapies or benefit from them.

Moreover, for patients with uncommon diseases, where a "one-size-fits-all" medication development procedure seldom helps distinct familial genetic variations, the availability of clinical trials and the difficulties of going to a facility where they can participate may rule out the possibility entirely. All patients may not even be able to sign up for a given experiment because of eligibility limitations, especially if they have already participated in studies. From a broad perspective, the idea of "clinical trials on chips"

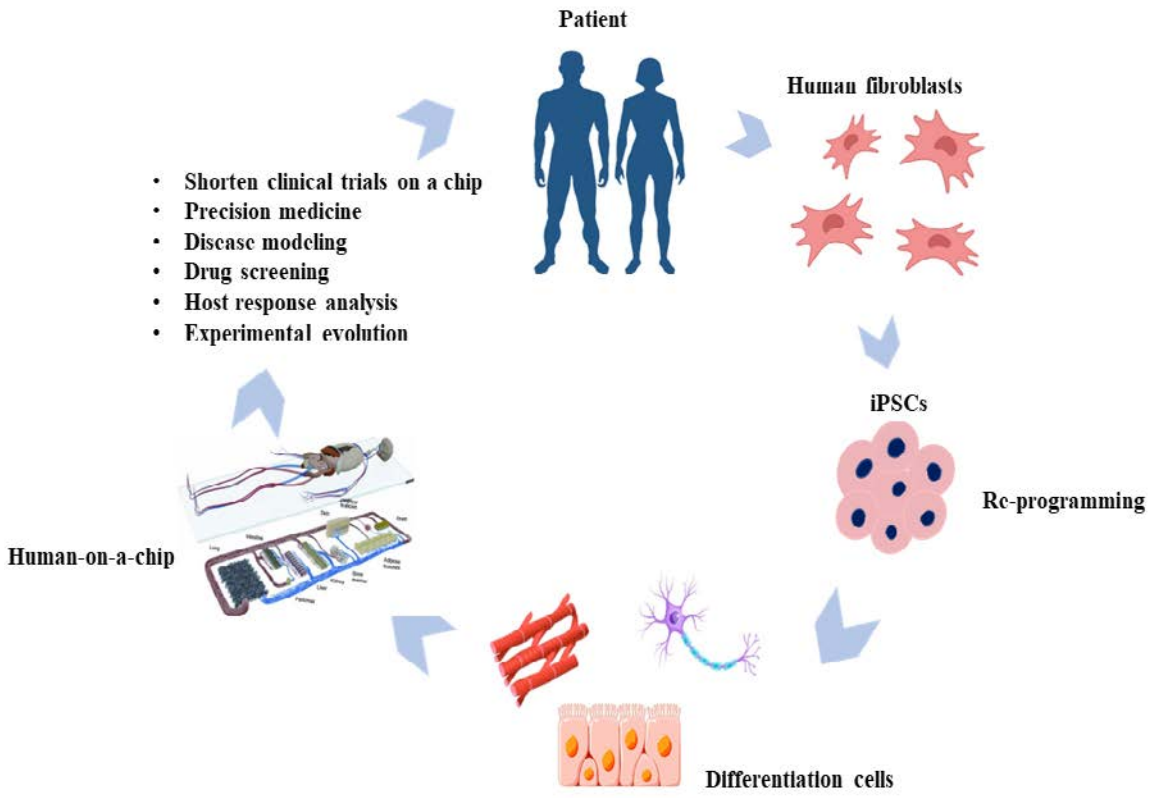

Fig2. Potential applications for tissue chip technology.

may be beneficial for particular patient subgroups, and modeling organs or humans on a chip would enable the monitoring of known toxicity profiles, for example, or the quantification of the efficacy of an experimental drug in vitro before administration to a patien.

Drug screening and testing

Studying the reactions of various organs and tissues to the administration of specific medications is essential in medicine, as we have covered throughout this paper. Several instances illustrate this idea (20). One typical chemotherapy drug is 5-FU, used to treat colorectal cancer. Unfortunately, 5-FU can have various adverse effects on patients, including intestinal cell destruction. Many prodrugs, including tegafur, have been developed to decrease toxicity. Tegafur and other prodrugs are inert when supplied; they only become active during metabolism, typically carried out by hepatocytes in the liver. Without using a metabolically active liver organoid, data from in vitro prodrug drug trials are probably utterly worthless. By creating a platform with a liver and other organoids or tumors, it would be possible to analyze the effects of the activated drug on the downstream tissues or tumors once the prodrug is metabolized (20).

Disease modeling

By simulating disease conditions on MPS, new perspectives on pathologies and therapeutic options are made possible. Using primary or induced stem cell sources or genetic techniques to create a disease phenotype that can be investigated in vitro against an isogenic background, diseases might be mimicked from patient donors.

Using tissue chips to study disease processes

Tissue chips have recently been used to represent various disease conditions. For example, when cultivated in 2D instead of 3D, malignant tumor cells exhibit various phenotypes. The microenvironment in which they are grown is a significant factor in determining their behaviors, including development and dissemination (21). The liver is an important site of metastasis for many carcinomas and a binding site for drug metabolism, making it an essential target for MPS cancer modeling. Metastatic spread is a significant cause of cancer-related mortality. According to Clark et al., human liver tissues are cultivated for many weeks in a liver bioreactor that can be seeded with carcinoma cells that multiply and form tiny tumors. Notably, the system demonstrated that some cancerous cells entered a dormant state within the systems when used to investigate the metastatic growth of breast cancer. As microtumors can lay dormant for many years before starting to grow again, this is a severe issue for human populations (22-24).

Tissue chips can be used to discover more about viral and bacterial infections. For instance, a "gut-on-a-chip" platform was recently utilized to show how human Caco2 intestinal cells react to infection with enterovirus coxsackievirus B1 in a two-chamber device similar in design to the lung-on-a-chip. Understanding the mechanisms of action of enteroviruses was made possible by the authors' demonstration of a viral entrance, replication, and inflammatory cytokine production in the epithelium. These two last examples show how data from MPS were verified against well-known clinical outcomes. It is a crucial step for the field since it demonstrates the value of platforms for understanding disease pathophysiology and developing new treatments (25-27).

Patients with rare diseases could benefit from tissue chip technology

Fewer than 5% of the over 7000 currently known rare diseases have viable pharmacological therapy, which makes treating people with rare diseases extremely difficult for various reasons (28). However, MPS technology may not only improve comprehension of several understudied illnesses but also offer platforms for evaluating repurposed current medications and new drug screening techniques for therapies that are already on the market (29). The use of a cardiac MPS to represent the uncommon illness Barth syndrome, which results in immunological deficiencies and cardiac and skeletal myopathy owing to a mutation in the TAZ gene, is one of the most well-known applications of MPS platforms to date (30, 31). Wang and colleagues used 'muscular thin films' (MTFs) of cardiac tissue on elastomeric thin films that 'twitch' in a measurable way as tissue contracts (30). The group created induced pluripotent stem cells (iPSCs) from two individuals with Barth syndrome, differentiated the cells into cardiomyocytes, and then implanted and cultivated the cardiomyocytes on the MTFs (30). The resultant MTFs exhibited decreased peak and twitch stress, which was consistent with the condition's pathophysiology. The scientists then demonstrated the effectiveness of gene editing methods on MPS by "rescuing" a normal phenotype in the tissue by introducing TAZ RNA (30). Several rare diseases, including Hutchinson-Gilford progeria syndrome, hereditary hemorrhagic telangiectasia (HHT), Rett syndrome, and Alpers-Huttenlocher syndrome, are currently being modeled in MPS platforms, to the authors' knowledge (32).

Tissue-based chips for precision medicine and clinical trials

MPS in illness research can advance our knowledge of a broad spectrum of diseases and pave the way for potentially game-changing discoveries about the causes and therapies of illness. The National

Institutes of Health (NIH) in the US are funding a program expressly for using the chips for disease modeling with an investment of around $75 M over the following five years. In other words, MPS might be helpful tools for efforts in precision medicine. In the future, the possibility of filling MPS with primary or iPS cells from patients provides opportunities for therapies that are personalized to an individual. Chips might be utilized to simulate patient-specific tissues for therapy evaluation. To represent pharmacogenetic variability within populations, tissues with known genetic polymorphisms might also be included in chips. Furthering this idea, tissue chips allow for population-wide variation by modeling genetic and environmental characteristics, such as gender and exposure to toxins or infectious agents. Together, these opportunities pave the door for clinical trials on chips in the future, allowing for testing both new and existing medications on MPS. It might speed up the medication development process by precisely simulating population variance throughout preclinical and early clinical trial stages, lowering the attrition rate of potential treatments (33-35).

What kind of cells is suitable for tissue chips?

Cell sourcing is one problem that the profession is now confronting. Currently, researchers have access to primary tissues from donors, commercially accessible immortalized cell lines, and iPSC sources (from both commercial and donor sources) (36). There are benefits and drawbacks to each of them. For instance, readily accessible cell lines from the marketplace make it easier for researchers to do their work. Nevertheless, because of their prolonged culture, these immortalized lines could have experienced considerable genetic or epigenetic drift (37). For MPS seeding, primary tissues from donors are preferred, especially for rare disorders. They may only be accessible in small quantities or from populations with diseases, and they are challenging to acquire from trim population levels. Depending on how long it takes after death for tissues to become accessible, it may be brutal or immoral to obtain healthy tissues, or they may come from compromised dead donors. Additionally, some tissues are challenging to get from donors (i.e., nervous system tissue) (38).

Stem cells, significantly induced pluripotent ones, are the most promising cell sources for MPS platforms. Reprogramming different tissue types from skin fibroblasts or blood cells (to form iPSCs) offers exceptional prospects and numerous advantages over primary cells (39). Technological advancements now provide renewable cell sources for several tissues. First, many tissue types may be produced by people, and they are known as isogenic tissues since they all have the same genetic makeup (40). Another benefit is that with the development of gene editing methods like CRISPR, the production and genetic treatment of tissues for monogenic or Mendelian illnesses is achievable in ways that were before unimaginable. Last but not least, the development of iPSCs offers hope for validating other population-wide investigations like genome-wide association studies (GWAS) (41).

What materials use to create MPS?

The materials used to make chips offer great design flexibility, but they also significantly impact functioning. Since it is transparent and flexible, polydimethylsiloxane (PDMS) is the perfect material for MPS creation (42). It is also inexpensive and straightforward to work within the lab (for example, through soft lithography). However, it is gas permeable and absorbs hydrophobic medicines as well. It might reduce the amount of medication that reaches tissues or pollute nearby microfluidic channels or cell-containing chambers (42).

Additionally, the hydrophobicity of the substance has been linked to poor cell adhesion in MPS devices, which might cause cell aggregation and obstruct fluidic flow. Researchers in a variety of methods have addressed this problem. These include plasma treating or coating the PDMS with proteins to limit cell attachment and medication loss and oxidizing it to produce a barrier layer of silicon dioxide on the surface (43, 44,45).

Outlook

The idea of customized organs-on-chips is anticipated to go from its current stage of academic proof-of-concept investigations to confirmation in the field of precision medicine during the next several years (46). Suppose personalized organs-on-chips can be shown to be helpful in guiding personalized treatment and preventative efforts. In that case, they are expected to become a crucial component of more extensive medical advancements toward a more predictive, preventive, personalized, and participative field. A more thorough understanding of disease mechanisms will result from a customized organ-on-chip that includes a disease-related parameter determined by observational studies, followed by an experimental functional comparison with matched, person-specific control organs-on-chips (46). This comparative analysis could then lead to the discovery of new drug targets and biomarkers. Finally, because organ-on-chip systems are dynamic, it may be feasible to create organs-on-chips whose cell culture settings may be changed under precise control when more recent or updated health information becomes available. This might be crucial for evaluating how well lifestyle modifications for prevention work (46).

Key challenges

Because organs-on-chips are helpful in the preclinical clinical domains, MPS technology offers significant promise for PM initiatives. However, before the tools can fully realize their promise, there are still issues to be solved, as with every new technology. An NIH-funded industry that has established two Tissue Chip Testing Centers for platform validation initiatives is tackling some of these difficulties, including technology transfer across laboratories and the validation of assay results from chips. The necessity for dependable, mature cell sources to fill the chips and the development of platform accessibility to a larger population are the two more significant problems. Individualized chips can be made for each person using iPS-derived cells for cell sources. However, not every tissue in the body presently has a differentiation pathway.

According to our predictions, highly complex single- or multiorgan systems should continue to be helpful in examining disease pathologies and drug mechanisms. However, human training and implementation costs for these sophisticated systems are higher. The physical challenges of scaling organs and tissues appropriately, perfusing tissues with the proper blood mimic to supply tissue-specific nutrients, and linking organs with functional vascularization while utilizing the appropriate type of endothelial cell are among the technical challenges facing the linkage of systems.

CONCLUSION

As instruments for modeling disease pathologies and profiling pharmacological and therapeutic effects in vitro, tissue chips provide previously unheard-of chances to comprehend disease processes and treatment effects. By creating "subpopulations-on-chips" and, eventually, "you-on-a-chip," these technologies will support PM efforts in various ways. The field of PM is still in its infancy. Nevertheless, if patient populations are modeled ex vivo or therapies are evaluated in people with particular genetic variants and genotypes, the ability to employ tissue chips to mimic organ systems in specific individuals will probably entail more awareness in the coming years. This intriguing and quickly developing subject has much promise to help biomedical researchers and the pharmaceutical industry understand how to cure some of the most common and difficult-to-treat ailments of our day.

Conflict of interest

There are no conflicts of interest to declare.

REFERENCES

1.Van Den Berg, Albert, et al. "Personalised organs-on-chips: functional testing for precision medicine." Lab on a Chip 19.2 (2019): 198-205.

2.Esch, Eric W., Anthony Bahinski, and Dongeun Huh. "Organs-on-chips at the frontiers of drug discovery." Nature reviews Drug discovery 14.4 (2015): 248-260.

3.Low, Lucie A., and Danilo A. Tagle. "'You-on-a-chip' for precision medicine." Expert Review of Precision Medicine and Drug Development 3.2 (2018): 137-146.

4.Low, L. A., and D. A. Tagle. "Tissue chips–innovative tools for drug development and disease modeling." Lab on a Chip 17.18 (2017): 3026-3036.

5.Sutherland, Margaret L., Kristin M. Fabre, and Danilo A. Tagle. "The National Institutes of Health Microphysiological Systems Program focuses on a critical challenge in the drug discovery pipeline." Stem cell research & therapy 4.1 (2013): 1-5.

6.Fabre, Kristin M., Christine Livingston, and Danilo A. Tagle. "Organs-on-chips (microphysiological systems): tools to expedite efficacy and toxicity testing in human tissue." Experimental biology and medicine 239.9 (2014): 1073-1077.

7.Livingston, Christine A., Kristin M. Fabre, and Danilo A. Tagle. "Facilitating the commercialization and use of organ platforms generated by the microphysiological systems (Tissue Chip) program through public–private partnerships." Computational and structural biotechnology journal 14 (2016): 207-210.

8. DiMasi, Joseph A., Henry G. Grabowski, and Ronald W. Hansen. "Innovation in the pharmaceutical industry: new estimates of R&D costs." Journal of health economics 47 (2016): 20-33.

9. Cook, David, et al. "Lessons learned from the fate of AstraZeneca's drug pipeline: a five-dimensional framework." Nature reviews Drug discovery 13.6 (2014): 419-431.

10.Seok, Junhee, et al. "Genomic responses in mouse models poorly mimic human inflammatory diseases." Proceedings of the National Academy of Sciences 110.9 (2013): 3507-3512.

11.Huh, Dongeun, et al. "Reconstituting organ-level lung functions on a chip." Science 328.5986 (2010): 1662-1668.

12.Huh, Dongeun, et al. "A human disease model of drug toxicity–induced pulmonary edema in a lung-on-a-chip microdevice." Science translational medicine 4.159 (2012): 159ra147-159ra147.

13.Benam, Kambez H., et al. "Matched-comparative modeling of normal and diseased human airway responses using a microengineered breathing lung chip." Cell systems 3.5 (2016): 456-466.

14.Herland, Anna, et al. "Distinct contributions of astrocytes and pericytes to neuroinflammation identified in a 3D human blood-brain barrier on a chip." PLoS One 11.3 (2016): e0150360.

15.Shi, Yanhong, et al. "Induced pluripotent stem cell technology: a decade of progress." Nature reviews Drug discovery 16.2 (2017): 115-130.

16.Schork, Nicholas J. "Personalized medicine: time for one-person trials." Nature 520.7549 (2015): 609-611.

17.Bouvy, Jacoline C., Marie L. De Bruin, and Marc A.

Koopmanschap. “Epidemiology of adverse drug reactions in Europe: a review of recent observational studies.” Drug safety 38.5 (2015): 437-453.
18.Ashley, Euan A. “Towards precision medicine.” Nature Reviews Genetics 17.9 (2016): 507-522.
19.Woodruff, Prescott G., et al. “Current concepts in targeting chronic obstructive pulmonary disease pharmacotherapy: making progress towards personalised management.” The Lancet 385.9979 (2015): 1789-1798.
20.Skardal, Aleksander, Thomas Shupe, and Anthony Atala. “Organoid-on-a-chip and body-on-a-chip systems for drug screening and disease modeling.” Drug discovery today 21.9 (2016): 1399-1411.
21.Low, L. A., and D. A. Tagle. “Tissue chips–innovative tools for drug development and disease modeling.” Lab on a Chip 17.18 (2017): 3026-3036.
22.Clark, Amanda M., et al. “Liver metastases: Microenvironments and ex-vivo models.” Experimental Biology and Medicine 241.15 (2016): 1639-1652.
23.Clark, Amanda M., et al. “A microphysiological system model of therapy for liver micrometastases.” Experimental biology and medicine 239.9 (2014): 1170-1179.
24.Ghajari, Ghazal et al. “The association between testicular toxicity induced by Li2Co3 and protective effect of Ganoderma lucidum: Alteration of Bax & c-Kit genes expression.” Tissue & cell vol. 72 (2021): 101552.
25.Kim, Hyun Jung, et al. “Human gut-on-a-chip inhabited by microbial flora that experiences intestinal peristalsis-like motions and flow.” Lab on a Chip 12.12 (2012): 2165-2174.
26.Villenave, Remi, et al. “Human gut-on-a-chip supports polarized infection of coxsackie B1 virus in vitro.” PloS one 12.2 (2017): e0169412.
27.Ghajari, Ghazal, Arefe Heydari, and Masoud Ghorbani. “Mesenchymal stem cell-based therapy and female infertility: limitations and advances.” Current Stem Cell Research & Therapy (2022).
28.Fajgenbaum, David C., et al. “The collaborative network approach: a new framework to accelerate Castleman’s disease and other rare disease research.” The Lancet Haematology 3.4 (2016): e150-e152.
29.Low, Lucie A., and Danilo A. Tagle. “Tissue chips to aid drug development and modeling for rare diseases.” Expert opinion on orphan drugs 4.11 (2016): 1113-1121.
30.Wang, Gang, et al. “Modeling the mitochondrial cardiomyopathy of Barth syndrome with induced pluripotent stem cell and heart-on-chip technologies.” Nature medicine 20.6 (2014): 616-623.
31.Blumenrath, Sandra H., et al. “Tackling rare diseases: Clinical trials on chips.” Experimental Biology and Medicine 245.13 (2020): 1155-1162.
32.de Mello, Camilly P. Pires, et al. “A human-on-a-chip approach to tackling rare diseases.” Drug discovery today 24.11 (2019): 2139-2151.
33.Sun, Wujin, et al. “Engineering precision medicine.” Advanced Science 6.1 (2019): 1801039.
34.Haque, Muhammad R., et al. “Organ-chip models: opportunities for precision medicine in pancreatic cancer.” Cancers 13.17 (2021): 4487.
35.Piri-Gharaghie, Tohid, Abbas Doosti, and Seyed Abbas Mirzaei. “Fabrication and characterization of pcDNA3. 1 (+) location within chitosan/nanoparticles complexes for enhanced gene delivery.” Iranian Journal of Biotechnology 20.3 (2022): 88-100.
36.Nestor, Colm E., et al. “Rapid reprogramming of epigenetic and transcriptional profiles in mammalian culture systems.” Genome biology 16.1 (2015): 1-17.
37.Musah, Samira, et al. “Directed differentiation of human induced pluripotent stem cells into mature kidney podocytes and establishment of a Glomerulus Chip.” Nature protocols 13.7 (2018): 1662-1685.
38.Horvath, Peter, et al. “Screening out irrelevant cell-based models of disease.” Nature reviews Drug discovery 15.11 (2016): 751-769.
39.Workman, Michael J., et al. “Enhanced utilization of induced pluripotent stem cell–derived human intestinal organoids using microengineered chips.” Cellular and molecular gastroenterology and hepatology 5.4 (2018): 669-677.
40.Cochrane, Amy, et al. “Advanced in vitro models of vascular biology: human induced pluripotent stem cells and organ-on-chip technology.” Advanced drug delivery reviews 140 (2019): 68-77.
41.Warren, Curtis R., et al. “Induced pluripotent stem cell differentiation enables functional validation of GWAS variants in metabolic disease.” Cell Stem Cell 20.4 (2017): 547-557.
42.Low, Lucie A., et al. “Organs-on-chips: into the next decade.” Nature Reviews Drug Discovery 20.5 (2021): 345-361.
43.Yan, Mou, et al. “On-chip valley topological materials for elastic wave manipulation.” Nature Materials 17.11 (2018): 993-998.
44.Markov, Dmitry A., et al. “Variation in diffusion of gases through PDMS due to plasma surface treatment and storage conditions.” Biomedical microdevices 16.1 (2014): 91-96.
45.Tan, Say Hwa, et al. “Oxygen plasma treatment for reducing hydrophobicity of a sealed polydimethylsiloxane microchannel.” Biomicrofluidics 4.3 (2010): 032204.
46.Pun, Sirjana, Li Cai Haney, and Riccardo Barrile. “Modelling Human Physiology on-Chip: Historical Perspectives and Future Directions.” Micromachines 12.10 (2021): 1250.

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Histomorphometric Study Based on Personal Medicine Effect of Propiconazole on Bone Growth Plate of Male Gerbil

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Abstract:
Propiconazole is a systemic fungicide from the triazole group used to control a wide range of diseases. This poison causes cellular, genetic and metabolic damage in animals. A bone is a hard tissue whose content is constantly changing. Longitudinal growth of the bone occurs through the growth plate, which is a cartilaginous structure at the end of the body's long bones. During puberty, while the growth plate closes (ossifies), the longitudinal growth of the bone stops. This study aimed to investigate propiconazole's effect on growth plate width changes (including the area of proliferating cells and the area of hypertrophied cells) in immature rats. This experimental study was conducted on 12 immature male Wistar rats randomly divided into control and propiconazole groups. The treatments were done by oral gavage for 28 days. On the 28th day, the dead animals and the left leg femur were separated for histomorphometric studies of the growth plate width of the femoral epiphysis. Investigations were carried out by (Rasband Wayne, 40g.1. ver, ImageJ, USA, NIH), and the significance of the results was done by ANOVA analysis of variance and Tukey's test. The width of the growth plate in the propiconazole group had a significant decrease compared to the control group ($P = 0.0126$), which is a decrease in the width of the proliferating area ($P < 0.001$) and an increase in the width of the hypertrophied area ($P = 0.016$). Propiconazole leads to a decrease in the width of the growth plate of the femoral epiphysis of immature rats. It can be a factor in disrupting the process of longitudinal bone growth and premature closure of the growth plate.

INTRODUCTION

Bone tissue is an active and dynamic tissue whose internal microscopic structure is constantly changing and transforming by bone cells (osteoblasts and osteoclasts), plate growth, (remodeling structure). Cartilage is highly organized between the epiphysis and bone (1). The diaphysis is at the end of the long bones of the body, which is divided into horizontal areas of chondrocytes (chondrocytes) in different stages of differentiation (proliferating cells and hypertrophied cells) (2, 3).

Longitudinal bone growth results from the proliferation and differentiation of chondrocytes of the growth plate, which affects the genetic factors, hormones, growth factors, environment, and nutrition. Growth plates are closed with puberty (the matrix of chondrocytes becomes bone), and the longitudinal growth of bone ends (4-6). Growth plates in mice remain open for an extended period until after sexual maturity and perhaps throughout the natural life span of the animal. Propiconazole is a systemic fungicide from the triazole group, which is used to control a wide range of fungal diseases in agriculture, such as rice sheet blight, wheat rust disease, and wheat spike Fusarium (3, 7).

Propiconazole causes toxicity in animals and types of tissues, leaves a wide range of biochemical effects in non-lethal doses, and can cause cellular, genetic and environmental damage (8, 9). Few studies have been done on the effect of propiconazole on the bone and cartilage tissue of the skeletal system (10-12). During the analysis of propiconazole on chicken and quail embryos, the teratogenic effects of this poison on cartilage and bone growth have been reported (13,

19). Also, in the study of a family that was mistakenly exposed to this poison at home, it was found that propiconazole, in addition to neurotoxicity and endocrine, had destructive effects on the development of the skeletal system of the children of this family, including delayed calcification He pointed out the delay in bone growth, cyst growth in bones, pathological fractures and lack of response to bone grafting in the children of this family (14-18).

In response to the question of whether contact with propiconazole poison can lead to premature closure of the growth plate, followed by delay or reduction of longitudinal bone growth, the present study aims to investigate the effect of propiconazole poison on the epiphyseal cartilage (growth plate) of rats. It was done prematurely.

MATERIALS AND METHODS

Provision of animals

In this experimental study, 12 four- to five-week-old Wistar male desert rats (average weight 100 gr) were obtained from the Faculty of Pharmacy of Tehran University and transferred to the animal research laboratory.

Grouping and storage conditions

Mice were randomly divided into two groups of six, control and propiconazole. All the experiment stages were carried out following the principles of bioethics in the case of laboratory animals. During the research period, the rats were placed under the same and standard conditions in terms of light (12 hours of light and 12 hours of darkness), proper ventilation and temperature (22±2 ° C), and enough water and traditional food (chaw rat standard) in Their choice was made.

Determination of drug concentration

The concentration of propiconazole used in this study was chosen as 30 kg/mg based on previous studies (11, 17, 18). The injection of 30 kg/mg dose and even doses of 25, 15, and 10 kg/mg led to the death of animals. The reason for this could be the young age and immaturity of the tested mice; as a result, they could not tolerate the dose they consumed. Therefore, the dose of 5 kg/mg, the non-lethal dose of propiconazole, were considered in this research.

Preparation of medicinal dilutions

Propiconazole 95% from Shanghai Tosco Chemical Co., Shanghai (China) was used to prepare the dosage. Dilution was done using the formula C2V2=C1V1 and corn oil as a solvent. The volume of propiconazole 0.5 ml with a dose of 5 mg/kg for the propiconazole group and the same volume of corn oil for the control group was administered by oral gavage for 28 days at 10 am (20, 21).

Sampling of tested animals

After completing this period, the animals were killed in a desiccator following ethical principles. The left leg's femur was immediately removed for histological studies (growth plate histomorphometry) to fix the cells in 10% formalin for at least 24 hours. The time was set. In order to soften the bone tissue (decalcification), a 7% nitric acid solution was used for five days and nights, and the solution was replaced daily. After the decalcification, it was used to remove the effect of nitric acid from 5% sodium sulfate solution and to remove the effect of sodium sulfate from running water for 24 hours (22, 32).

Histopathological study

In order to investigate the tissue changes resulting from the treatments, histomorphometric studies were performed on the slides prepared from the growth plate tissue of the left leg's epiphysis region of the femur and with the help of hematoxylin-eosin (E&H) staining. In this staining, which is a general staining, the nuclei appear blue to purple, and the cytoplasm and connective fibers appear pink (30, 31).

Measurement of epiphyseal growth plate width

In order to measure the width of the epiphyseal growth plate in each group, the width in six tissue sections different from that group and in three regions of each section using ImageJ software, version 1.40g (Wayne Rasband, USA, NIH) and on the prepared photographs. The average of these values was measured from microscopic sections and considered each group's width of the epiphyseal growth plate. The proliferating cells' width and the area of hypertrophied cells on the growth plate were also measured in the same order in each group (23).

Statistical analysis

One-way ANOVA was used for statistical calculations using GraphPad Prism® software, version 6 (La Jolla, CA 92037, USA). The averages were calculated as SEM±Mean, and their comparison was made with Tukey's multiple comparisons test. A significance level of $P<0.05$ was considered. Also, the graphs were drawn with the help of Microsoft Office Excel 2007 software.

RESULTS

Investigation of growth plate width

The average width of growth plaque, proliferating area, and hypertrophied area in the propiconazole group was 0.4278, 0.2317, and 0.2383 mm, respectively. These values in the control group were 0.9200, 0.3417, and 0.1950, Table (1).

Investigating the average width of the epiphyseal growth plate

Examining the average width of the epiphyseal

Table 1. The average width of the growth plate, the area of proliferating cells of the growth plate, and the area of hypertrophied cells of the growth plate in the propiconazole group and the control group.

Variables	Propiconazole group (standard deviation ± mean)	Control group (standard deviation ± mean)	P value
Plaque growth width (mm)	4278,1212 ± 0.0	9200,3787 ± 0,0	0126,0
The width of the growing area of the growth plate cells (mm)	2317,03125 ± 0,0	3417,02401 ± 0,0	0001,0
The width of the area of hypertrophied cells of the growth plate (mm)	2383,02927 ± 0,0	1950,02258 ± 0,0	0166,0

growth plaque of the tested groups showed a significant decrease (P=0.0126) of the growth plaque width in the propiconazole group compared to the control group (Figure 1, 2, 3). The tested groups showed a significant decrease (P<0.0001) in the width of this area in the propiconazole group compared to the control group (P<0.0166). the width of this area was observed in the propiconazole group in comparison with the control group (Figure 3).

DISCUSSION

The data obtained from the calculation of the epiphyseal growth plate width of the tested groups showed that propiconazole significantly reduced the width of the growth plate (24). In the propiconazole study on chicken and quail embryos, the teratogenic effects of this poison on the growth of cartilage and bones were reported by researchers (24). The propiconazole teratogenic effects include the reduction of the growth of the skeletal elements of the leg and wing and the reduction of calcification in the leg bones (24). Bone and delayed bone calcification due to contact with poisons were reported in children exposed to this poison. Few studies have been done concerning the toxic effect of external factors on growth plate closure and with mechanisms different from the effect of propiconazole (25).

Researchers showed that in desert rats subjected to intensive and long-term treatment with warfarin (from birth to eight months), excessive mineralization of the growth plate led to fusion and complete closure of the growth plate of the tibia and the stop of longitudinal growth (26). Height in these animals has become. Warfarin, along with defects in the synthesis of coagulation factors related to vitamin K in the liver, causes a decrease in the amount of mineralization inhibitory protein BGP, which requires vitamin K for synthesis), leading to excessive mineralization and closure of growth plaques. While the increase of serum BGP in kidney patients or rats without kidney causes the growth plates to remain open (27).

Propiconazole causes a deficiency in the vitamin K function and a decrease in the level of BGP, followed by a disturbance in the inhibition of mineralization. Also, propiconazole has a destructive effect on the liver and hepatocytes. Propiconazole causes The fusion of the growth plate and a decrease in its width. This effect is different in each mouse compared to another mouse, which indicates the difference in the response of the body of people and is a personal medical approach. Of course, this hypothesis is against the oxidative effect of propiconazole on bone and cartilage cells (28). Growth plate areas have distinct morphological and biochemical characteristics controlled by growth factor signaling pathways. The destructive effect of propiconazole on mitochondrial membrane transmission, vacuolation, and swelling of mitochondria in the liver and heart of desert mice, destruction of cytochrome P450 and microsomes in human liver, changes in liver enzymes and biochemical indicators of hepatocytes has been proven (29).

Mitochondria are the first organelles that are affected by propiconazole toxicity. Mitochondrial changes caused by propiconazole are an indicator of increasing the cell›s need for energy to overcome toxicity effects (27-29). Considering the significant presence of mitochondria in the growth plate's multiplying area and these organelles' role in energy production, it can be hypothesized that the reduction of energy in these cells due to oxidative damage caused by propiconazole causes disruption in the process. Proliferation and the following stages of cell differentiation for the transition from proliferative state to mature and hypertrophied state. The destruction of other cell organelles membranes, which leads to a decrease in the synthesis of the intercellular matrix, can be a reason for the decrease in the width of the proliferating area in the present study. The hypertrophied area's width increase

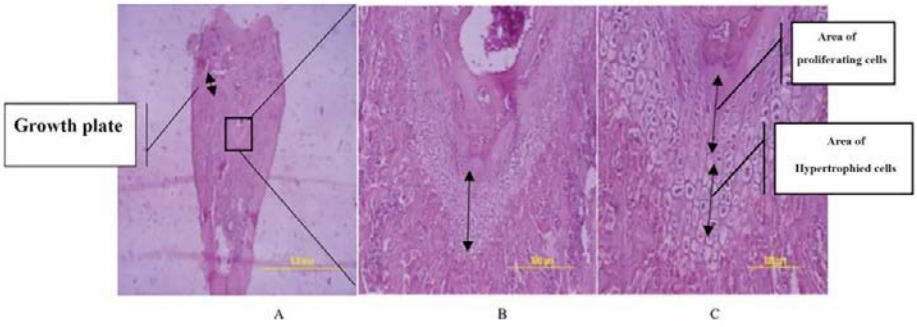

Fig1. The growth plate of the lower femoral epiphysis of a rat and its different areas with E&H staining. A) growth plate with 10X magnification. B) magnification 100X. C) the region of proliferating cells and hypertrophied cells, 200X magnification.

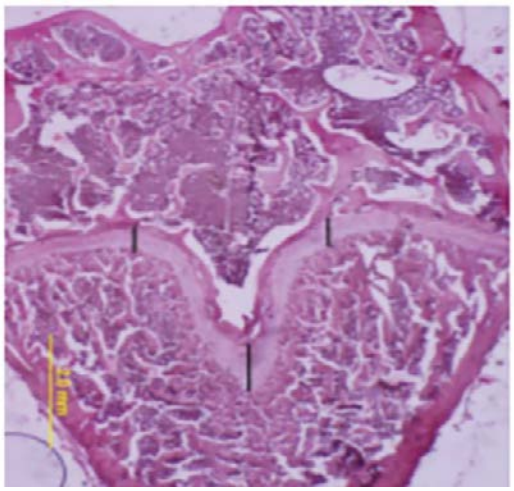

Fig2. The growth plate cartilage of rat femoral epiphysis with E&H staining. Three selected areas for calculating the average growth plate width in the tested groups are marked with dark lines.

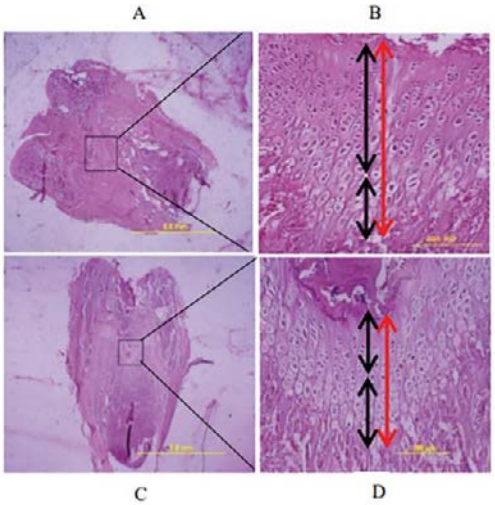

Fig3. A-D) tissue sections of the epiphyseal growth plate and proliferating and hypertrophied areas (one sample from six selected samples from each group) with hematoxylin-eosin staining. A, B) control group. C, D) Propiconazole group.

in the treatment with propiconazole can be attributed to this poisoning effect on the maturation and apoptosis of chondrocytes in this area and the disturbance in the process of chondroclastogenesis and calcification of the growth plate. In this case, propiconazole exerts its destructive effect by destroying the cell membrane of organelles (30).

Using transgenic mice with defects in vitamin D absorption, researchers stated that the width of the growth plate of the tibia increased in these mutants. In the present study, propiconazole, while destroying osteoclasts and chondroclasts and inhibiting osteo/

chondroclastogenesis, has increased the hypertrophied area's width (31, 32). Achieving more accurate results requires conducting experiments at the cellular and molecular level (including measuring serum malondialdehyde levels as the final product resulting from lipid peroxidation of cells in bone tissue, measuring fluctuations in calcium, phosphorus, vitamin D metabolites, parathormone, sex hormones (FSH), LH, Estradiol (Testosterone), measuring biochemical markers of bone changes including osteocalcin, osteoprotegerin and alkaline phosphatase of bone tissue and bone resorption markers, measuring bone mineral deposition rate (MAR) Apposition Mineral and estimating the amount of spongy bone that develops along the growth plate should be done by three-dimensional color maps to observe the decrease or increase in the thickness of this area, which shows the degree of calcification of the growth plaque (32).

CONCLUSION

Considering the histopathological effects of treatment with propiconazole in rat bone tissue, it is possible to point out the possibility of such cytotoxicity in farmers and people who are in regular contact with this compound and the need to take care and observe protective coatings to prevent the poison from entering the body. The body will lead to bone disorders and reduced or delayed skeletal growth due to their natural tendency to explore the surrounding environment by putting various objects in their mouths and being contaminated by direct contact with contaminated surfaces, floors, and air. In addition, the physiological characteristics of children, such as the high consumption of water, food, and air per unit of their body surface, can aggravate the risk and harm. It is also necessary to protect pregnant mothers. Based on the results from the histomorphometric studies, it can be concluded that propiconazole leads to a decrease in the width of immature rats' growth plates of the femoral epiphysis. Propiconazole can disrupt the longitudinal bone growth process and cause the growth plate to premature closure.

REFERENCES

1.Upledger J. Taming osteoporosis. Massage Today 2005;5(11):36-41.

2.Fattahi E, Jorsaraei SG, Gardaneh M. The effects of carbaryl on the pituitary-gonad axis in male rats. Iran J Reprod Med 2012; 10(5):419-24.

3.Battaglin W, Sandstrom M, Kuivila K, Kolpin D, Meyer M. Occurrence of azoxystrobin, propiconazole, and selected other fungicides in US streams, 2005–2006. Water Air Soil Pollut 2011; 218: 307–22.

4.Hester S, Moore T, Padgett WT, Murphy L, Wood CE, Nesnow S. The hepatocarcinogenic conazoles: Cyproconazole, epoxiconazole, and propiconazole induce a common set of toxicological and transcriptional responses. Toxicol Sci 2012; 127: 54–65.

5.Allen JW, Wolf DC, George MH, Hester SD, Sun G, Thai SF, et al. Toxicity profiles in mice treated with hepatotumorigenic and nonhepatotumorigenic triazole conazole fungicides: Propiconazole, triadimefon, and myclobutanil. Toxicol Pathol 2006;34 (7): 853-62.

6.Taxvig C, Hadrup N, Boberg J, Axelstad M, Bossi R, Bonefeld-Jørgensen EC, et al. In vitro-in vivo correlations for endocrine activity of a mixture of currently used pesticides. Toxicol Appl Pharmacol 2013;272(3):757-66.

7.Barton HA, Tang J, Sey YM, Stanko JP, Murrell RN, Rockett JC, et al. Metabolism of myclobutanil and triadimefon by human and rat cytochrome P450 enzymes and liver microsomes. Xenobiotica 2006; 36: 793–806.

8.Tully DB, Bao W, Goetz AK, Ren H, Schmid JE, Strader LF, et al. Gene expression profiling in liver and testis of rat to characterize the toxicity of triazole fungicides. Toxicol. Appl. Pharmacol 2006; 215: 260–73.

9.Skolness SC, Blanksma J, Cavallin J, Churchill E, Durhan K, Jensen R, et al. Propiconazole Inhibits Steroidogenesis and Reproduction in the Fathead Minnow (Pimephales promelas). Toxicological Sciences 2013;132(2), 284–97.

10.Li ZH, Zlabek V, Grabic R, Li P, Randak T. Modulation of glutathione-related antioxidant defense system of fish chronically treated by the fungicide propiconazole. Comp Biochem Physiol C Toxicol Pharmacol 2010;152(3):392-8.

11.Keramati V, Jamili S, Ramin M. Effect of diazinon on catalas antioxidant enzyme activity in liver tissue of rutilus rutilus. Fisher Aquatic Sci 2010;5(5):368-76.

12.Messarah M, Amamra W, Boumendjel A, Barkat L, Bouasla I, Abdennour C, et al. Ameliorating effects of curcumin and vitamin E on diazinon-induced oxidative damage in rat liver and erythrocytes. Toxicol Ind Health 2013;29(1):77-88.

13.Oksay T, Nazıroglu M, Ergun O, Dogan S, Ozatik O, Armagan A, et al. N-acetyl cysteine attenuates diazinon exposure-induced oxidative stress in rat testis. Andrologia 2013;45(3):171-7.

14.Meneely GA, Wyttenbach CR. Effects of the organophosphate insecticides diazinon and parathion on bobwhite quail embryos: skeletal defects and acetylcholinesterase activity. J Exp Zool 1989;252(1):60-70.

15.Yilmaz N, Yilmaz M, Altuntas I. Diazinon-induced brain toxicity and protection by vitamins E plus C. Toxicol Ind Health 2012;28(1):51-7.

16.Salehi M, Jafari M, Asgari A, Saleh Moghaddam M, Salimian M, Abbasnejad M, et al. Study of diazinon Effect on antioxidant enzymes and lipid peroxidation in rat's brain. RJMS 2010;17(70):15-23.
17.Misawa M, Doull J, Uyeki EM. Teratogenic effects of cholinergic insecticides in chick embryos. III. Development of cartilage and bone. J Toxicol Environ Health 1982;10(4-5):551-63.
18.Dahlgren JG, Takhar HS, Ruffalo CA, Zwass M. Health effects of diazinon on a family. J Toxicol Clin Toxicol 2004;42(5):579-91.
19.Ogutcu A, Uzunhisarcikli M, Kalender S, Durak D, Bayrakdar F, Kalender Y. The effects of organophosphate insecticide diazinon on malondialdehyde levels and myocardial cells in rat heart issue and protective role of vitamin E. Pesticide Biochem Physiol 2006;86(9):93-8.
20.Saberi M, Gholizadehmoghadam S, Sharifzadeh M. Assessment of diazinone-induced oxidative stress on memory acquisition in male rats. Daneshvar Med 2010;17(87):19-28.
21.Baniadam A, Esmaeilzadeh S, Razi Jalali M, Khazali MR. Histopathological and paraclinical study of autogenous cancellous bone and bone marrow grafting for filling of segmental bone defect. Jundishapur Sci Med J 2006;5(1):412 20.
22.Kiernan JA. Histological and Histochemical Methods: Theory and Practice. 4th ed. Bloxham, UK: Scion, 2008.
23.Parfitt AM, Drezner MK, Glorieux FH, Kanis JA, Malluche H, Meunier PJ, et al. Bone histomorphometry: standardization of nomenclature, symbols, and units. Report of the ASBMR Histomorphometry Nomenclature Committee. J Bone Miner Res 1987;2(6):595-610.
24.Azarni M, Minaeii B, Mozaffari Z, Tahamtani Y. Histopathological effects of leaf extracts of Trigonella foenum-graecum on the development of the fetal rat long bone tissue. J Dev Biol 2008;1(1):50-8.[Persian]
25.Price PA, Williamson MK, Haba T, Dell RB, Jee WS. Excessive mineralization with growth plate closure in rats on chronic warfarin treatment. Proc Natl Acad Sci U S A 1982;79(24):7734-8.
26.Lee CS, Chen J, Wang Y, Williams JK, Ranly DM, Schwartz Z, et al. Coordinated tether formation in anatomically distinct mice growth centers is dependent on a functional vitamin D receptor and is tightly linked to three-dimensional tissue morphology. Bone 2011;49(3):419-27.
27.Ogutcu A, Uzunhisarcikli M, Kalender S, Durak D, Bayrakdar F, Kalender Y. The efects of organophosphate insecticide diazinon on malondialdehyde levels and myocardial cells in rat heart tissue and protective role of vitamin E. Pesticide Biochem Physiol 2006;86(2):93-8.
28.Panda DK, Miao D, Bolivar I, Li J, Huo R, Hendy GN, et al. Inactivation of the 25-hydroxyvitamin D 1alpha-hydroxylase and vitamin D receptor demonstrates independent and interdependent effects of calcium and vitamin D on skeletal and mineral homeostasis. J Biol Chem 2004;279(16):16754-66.
29.Piri-Gharaghie T. Polycystic ovary syndrome and genetic factors influencing its development: A review article. Personalized Medicine Journal. 2021 Dec 1;6(23):25-9.
30.Piri-Gharaghie T, Doosti A, Mirzaei SA. Identification of Antigenic Properties of Acinetobacter baumannii Proteins as Novel Putative Vaccine Candidates Using Reverse Vaccinology Approach. Applied Biochemistry and Biotechnology. 2022 Jun 7:1-23.
31.Ghajari G, Moosavi R. Evaluation of the effects of diazinon toxin on some reproductive parameters in male rats.2022.
32.Yadollahi A, Ghajari G. Transgenic induction in Sesamum indicum with recombinant pBI121 expression construct containing CYP81Q1 and aroA genes using Agrobacterium tomfacensis. Agricultural Biotechnology Journal. 2022 Sep 23;14(3):223-42.

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SARS-CoV2: A perspective on genetic and protein structure, function and potent treatments with a comparison with other coronaviruses

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Abstract:
Severe acute respiratory syndrome (SARS) coronavirus 2 (SARS-CoV2) leading to COVID-19 has initiated a catastrophe for humans since December 2019. Genetic and protein similarities between SARS-CoV and SARS-CoV2 offer the same treatments for both types of virus. However, there are some sequence or structural differences between SARS-CoV2 and SARS-CoV as well as other coronaviruses that make difficulties in discovering drugs and vaccines against this novel type of virus. Therefore, it is vital to recognize protein and genetic structures of SARS-CoV2 to discover drugs which directly target this strain of coronavirus. This review presents a perspective on SARS-CoV2, it's genetic and protein structures with a brief comparison with other coronaviruses as well as summarizing some immune responses activated against SARS-CoV2. In addition, it introduces the novel strategies to combat with COVID-19 that would be potentially effective on SARS-CoV2.

INTRODUCTION

Coronavirus is one of the most crucial infectious agents causing respiratory, gastrointestinal, liver and central nervous system infections in humans and vertebrates such as birds, bats, mice and other wild animals (1). Coronaviruses as the members of the Coronaviridae family and the Coronavirinae subfamily have a single RNA strand and spike glycoproteins protruded from the virus envelope (2). The Coronaviridae family is classified into four groups, α, β, δ, and γ, also β type is divided into four types A, B, C, and D (Table 1) (1). Previously, Sever acute respiratory syndrome (SARS) and Middle east respiratory syndrome (MERS) caused pandemics from animal to human and from human to human in 2002/2003 and 2012, respectively (3). Recently, a novel Coronavirus, 2019-nCoV, or SARS-CoV2, which leads to acute and severe respiratory symptoms and COVID-19 disease, has broken out firstly in Wuhan, China and then spread out in other provinces/regions of China and many countries in other continents where reported high rates of death (1, 3). Compared to SARS-CoV, SARS-CoV2 is responsible for higher mortality in people with age of over 60 as well as people with diabetes and/or hypertension (4). Recognition of genetic and protein structures of the SARS-CoV2 and identification of the differences between coronaviruses can be effective in discovering novel drugs and strategies against SARS-CoV2. Recently, researchers have attempted to figure out a novel drug or vaccine for treatment of COVID-19. This review presents a perspective on SARS-CoV2, it's genetic and protein structures with a comparison with other coronaviruses as well as detecting immune responses activated during infection and summarizing the novel discoveries on SARS-CoV2 that may be effective for COVID-19 therapy.

Source of SARS-CoV2

Genetic comparison of SARS-CoV2 with coronaviruses derived from five wild animals, Paguma larvata, Paradoxurus Hermaphroditus, Civet, Aselliscus stoliczkanus and Rhinolophus sinicus demonstrated less than 75% homology in terms of total genome sequence, ORF1a, ORF1ab and spike (S) protein. However, compared to Bat-Coronavirus RaTG13, SARS-CoV2 indicated more than 96% similarity in terms of total genome sequence, ORF1ab, Nucleocapsid (N) and S proteins (5-7). It has also been shown that various species of animals, exception of Rat and mice, are involved in the 2019-nCoV infection as intermediate hosts (8). The conserved structure of the SARS-CoV2 receptor in humans and animals such as fish, amphibians, birds, reptiles and mammals suggests that these creatures can be identified as the hosts of the virus (9).

Table 1. Coronaviridae family and several types of coronaviruses in human

Coronaviridae	Types of Coronaviruses		Features
α Coronaviruse		HCOV-229E HCOV-NL63	Mild cold
β Coronaviruse	A	HCOV-OC43 HCOV-HKU1	Mild cold
	B	SARS-COV SARS-COV2	Acute respiratory syndrome
	C	MERS-COV	Acute respiratory syndrome
	D	-	-
δ Coronaviruse		-	-
γ Coronaviruse		-	-

SARS-CoV2 entry to host cell

SARS-CoV2 implicates the specific receptor and several co-receptors for cell entry. It uses angiotensin converting enzyme 2 (ACE2), as a surface receptor and also dipeptidyl peptidase 4 (DPP4) and mammalian glutamyl aminopeptidase (ENPEP) as co-receptors for binding, membrane fusion, and cell entry. S glycoprotein which have been protruded from the virus envelope also mediates binding and membrane fusion (10) whereas MERS tends to bind to DPP4 / CD26 as co-receptors on the surface of respiratory tract cells and lymphoid tissues for binding, membrane fusion, and cell entry (11-13). Totally, various strains of coronaviruses implicate different types of receptors for entry into the host cells as SARS-CoV, SARS-CoV2 and HCoV-NL63 use ACE2, and MERS-CoV and other coronaviruses use aminopeptidase N (ANPEP) and DPP4 (4).

Structure and Function of ACE2

The specific function of ACE2 is defined by it's structure which is consisted of several domains with several amino acid sequences (14). Figure 1A shows the protein structure of ACE2.

The ACE2 receptor and co-receptors are highly expressed in lung, liver, kidney, heart, stomach, intestine, ileum and oral cavity epithelial cells (10, 15)15</style>. In recent studies, the ACE2 receptor has also shown high expression in cholangioyte, myocardial, and bladder cells (4). Despite these tissues expressing ACE2 highly, the primary target of the SARS-CoV2 is AT2 cells in the lung tissues (10). The critical role of ACE2 is angiotensin maturation, a peptide hormone involved in regulating blood pressure. ACE2 also acts as a chaperone for the membrane trafficking of the B0 AT1 amino acid transporter. This transporter is implemented in transferring neutral amino acids to intestinal cells in a sodium-dependent pathway (16). Moreover, ACE2 recruits B0AT1 aminoacid transporter to form ACE2 / B0AT1 complex which binds to the S protein for membrane fusion and virus entry into the host cells (17). Therefore, this proposes that B0AT1 may be considered as a potential therapeutic target to design drugs.

Structure and function of SARS-CoV2 S glycoprotein

S glycoprotein of SARS-CoV2 binds to the various host cell targets such as ACE2, CD26, Ezrin, Cyclophilin, and other adhesion molecules (18). S glycoprotein is a trimer with 1273 amino acid which is consisted of two subunits in each monomer, subunit1 (S1) in N-ter and subunit2 (S2) in protein C-ter, that are

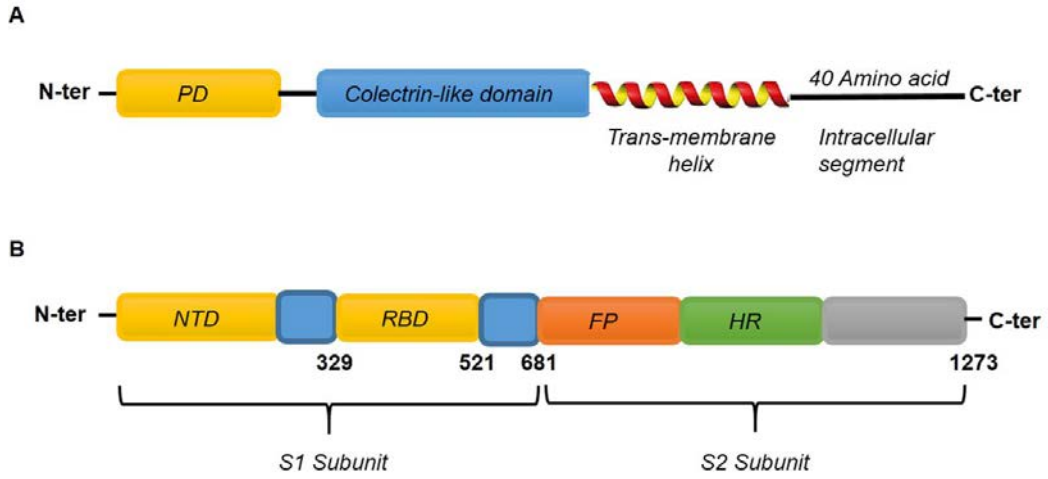

Fig1. The protein structure of ACE2 and Spike protein. A) The protein structure of ACE2, B) The protein structure of Spike protein.

effective in binding and fusing the virus into the host cell, respectively. Each monomer is about 180KD and contains a receptor binding domain (RBD) (Figure 1B) (19, 20). RBD domain of S subunit has two up and down conformations; the up and the down modes are related to the available and unavailable configuration of the receptor, respectively. Initially, subunit S1 is bonded to the ACE2 and thus changing the conformation causes S1 to shed off and S2 to convert to the stable conformation (21, 22).

To better understand, when the S1 subunit is bonded to the ACE2, the Heptad Repetition 1 (HR1) and HR2 domains of S2 subunit interact together and make six-helix bundle which brings the host cell membrane and the virus closer to fuse and enter the host cell. In HR1 domain, there are several mutations that increase the HR1 affinity to interact with HR2. For this reason, the affinity of the SARS-CoV2 is higher than other coronaviruses to interact with the host cell membrane (23).

Activation of S protein occurs in two stages of cleavage. In the first step, the RBD of S1 subunit binds to the peptidase domain (PD) of ACE2, directly. Thus, changing the protein conformation causes S1 and S2 to separate and S2 is then exposed and broken down by peptidase. Following this, protein reaches to a stable conformation which is necessary for membrane fusion and viral infection (16). In the second step, S protein is subjected to more cleavage by one or more host cell proteases such as Furin, trypsin, cathepsins, transmembrane protease serin protease-2 (TMPRSS-2) and TMPRSS-4 or human airway trypsin-like protease (HAT). Studies on SARS-CoV2 indicate that the virus enters the host cell through endocytosis which is mediated by TPC2, PIKfyve, and cathepsin L. Compared to SARS-CoV, S protein of SARS-CoV2 is less stable (24). Research on S protein recommends using spike glycoprotein as a primary target for vaccine and therapeutic antibodies.

SARS-CoV2 Genome and proteins

Coronaviruses have the largest genome (26-32Kb) among RNA viruses that it is almost 29.8 kb to 29.9 kb in SARS-CoV2. SARS-CoV2 has a single RNA strand with a 5' cap and a 3' poly A structure. 5' region of RNA encodes Orf1ab polyprotein which is cleaved into non-structural proteins (nsps 1-16) and 3' region of RNA encodes structural proteins such as spike (S), envelope (E), membrane (M) and nucleocapsid (N) proteins as well as encoding accessory proteins such as Orf3a, Orf6, Orf7a, Orf7b, Orf8, HE protein, Orf3a/b and Orf4a/b protein which play a critical role in the virus replication and contagious (1, 7, 25). Majority of the SARS-CoV2 genome encodes more than 20 types of proteins which are required in RNA replication and gene transcription in infected cells. Table 2 presents the several types of proteins encoded by both SARS-CoV2 and SARS-CoV. Once virus enters the host cells, it begins to mutate, generating changes that mostly occur in five major genes including genes encoding N, S, Orf8, Orf3a, and Orf1ab proteins. Furthermore, 42% of the mutations are well-known as a non-synonymous mutation (5). Several 3'-5'-exoribonuclease enzymes which provide proofreading ability for coronaviruses,

Table 2. Types of proteins encoded by both SARS-COV2 and SARS-COV.

Protein name	Function	References
Nsp1-nsp16	Protease, Replicase, Ribonuclease, Ribose methyltransferase	(*57*)
Spike (S) protein	Membrane fusion, entry into host cell	(*25*)
Nucleocapsid (N) protein	Structural protein	(*25*)
Membrane (M) protein	Structural protein	(*25*)
Envelope (E) protein	Structural protein	(*25*)
Orf1a/ab	Polyprotein cleaved into nsp1-nsp16	(*25*)
Orf3a/b	Pathogenicity, Inhibition of IFNβ expression, Genome maintenance, Virus replication	(*17*)
Orf4a/b	Genome maintenance, Virus replication	(*58*)
Orf6	Production of type I IFN and inhibition of signaling	(*59*)
Orf7a	Increase in NF-κβ, JNK, p38 MAP kinase, Inhibition of host translation, Induction of apoptosis and cell cycle arrest	(*59*)
Orf7b	No known function	(*59*)
Orf8a/b	Trigger intracellular stress pathways	(*59*)
HE protein, 3a/b and 4a/b protein	Genome maintenance, Virus replication	(*58*)

distinguish them from other RNA viruses (1).

One of the most crucial conserved proteins in coronaviruses is main protease (M^{pro}) or 3C-like protease ($3CL^{pro}$) with 306 amino acids. M^{pro} is required to replicate and process the viral functional proteins (2). Primary role of the M^{pro} is cleavage and activation of two viral polyproteins called PP1A and PP1AB as well as cleaving 11 sites in Replicase 1ab. The cleavage sites identified by M^{pro} are Leu-Gln (Ser, Ala, Gly) (presents the cleavage site) (26). In addition, research on the three-dimensional structure of SARS-CoV2 protease has shown nearly 96% similarity with SARS-CoV protease. Also, the cleavage site of M^{pro} has been conserved among the proteases in different types of coronaviruses. This suggests that inhibitors of the SARS-CoV protease could have a similar effect on SARS-CoV2 M^{pro} or 3C-like protease. Among well-known protease inhibitors, Ladipasvir and Velpatasvir can be hypothetically effective on SARS-CoV2 without any side effects (27). In addition, creating some alterations in chemical structure of protease inhibitors in order to protect host cell proteases from cleavage, can provide a vital step to modulate a safe anti-SARS-CoV2 drug with the least side effects. For instance, Peptidomimetic α-ketoamide endures some alterations in it's chemical structure through hiding the P2-P3 amide bond in the pyridone ring. Moreover, in this molecule, less hydrophobic groups are replaced by hydrophobic groups to increase solubility and reduce binding ability to plasma proteins (26).

Additionally to the catalytic residues His 41 and Cys 144 of the protease, research on the active site of $3CL^{pro}$ protease in SARS-CoV has found four novel main amino acids (PDEV) for protease activity that mutations and changes in these amino acids can greatly reduce protease activity. This proposes that intra-molecular dynamic changes in protein-substrate interaction may impair protease activity. Discovering novel and important amino acids in the active site of proteases can provide more effective sites for the design and development of protease inhibitors (28).

Comparing SARS-CoV2 genome and proteins with other coronaviruses

SARS-CoV2 has almost 89% and 96% nucleotide identity with SARS-CoV and Bat coronavirus, respectively (16). Genomic analysis has detected that slow codons and slow di-codons (codons without cognate tRNA gene) are lower in both SARS-CoV2 and SARS-CoV, thus the rate of protein synthesis is faster in comparing with other coronaviruses (29). In spite of high homology, comparing SARS-CoV2 and SARS-CoV genetic sequence, identified six regions of difference (RD), RD1, RD2 and RD3 (448nt, 55nt, and 278nt, respectively) in the ORF1ab coding gene, RD4 and RD5 (315nt and 80nt, respectively) in the S protein coding gene, and RD6 (214nt) in ORF8 and ORF7b coding genes (30). These different regions can be considered as diagnostic molecular markers and implicated to develop novel drugs against SARS-CoV2. Research on sequences, genome variations, and comparison of SARS-CoV2 with other β-coronaviruses, MERS, and SARS, identified at least two hyper-variable genomic hotspots in the SARS-CoV2. In spite of the low rate of variation in SARS-CoV2, one of the genome variations (C / U) is Ser / Leu84 in the protein encoded by ORF8. Comparison of ORF8-S with ORF8-L has shown that ORF8-S leads to some structural alterations in protein C-ter. It can also provide a new phosphorylation target for host cell Ser / Thr kinases. The other genome variation is a synonymous variation (U / C) encoding Serin 2839 in a protein encoded by ORF1ab (31). Furthermore, comparing the SARS-CoV2 genome with Bat-coronavirus and SARS-CoV also confirmed the replacement of a Ser to Gly in position 543 of the nsp2 protein encoded by ORF1ab. This variation plays a vital role in increasing the contagious ability of the SARS-CoV2. The other identified protein variation occurring near phosphatase protein and playing a key role in replication of the viral genome, is a prolin located at 192 of the nsp3 in SARS-CoV2 instead of polar and apolar amino acids in Bat-coronavirus and SARS-CoV, respectively. This variation recommends a potent strategy to diagnose and distinguish SARS-CoV2 from SARS-CoV (32). Identification of the amino acid differences in the protein sequences of SARS-CoV2 could help to discover novel antiviral drugs and strategies to combat with COVID-19 (31).

Comparing the sequences of S1 and S2 subunits in SARS-CoV2 S glycoprotein and SARS-CoV shows 91% and 55% similarity in S2 and S1 subunit, respectively. On the one hand, amino acid different regions in the S2 subunit of viruses are included 677-690, 877-884 and 930-943, whereas 570-1278 region is considered as a similar one. On the other hand, the amino acid differences in the S1 subunit of the SARS-CoV2 and SARS-CoV are located at 01-550 region (18). Moreover, four insertion sequences in SARS-CoV2 have been identified in comparing with SARS-CoV that three sequences are common in Bat coronavirus RaTG13 S glycoprotein. These four insertion sequences are GTNGTKR in subunit S1, YYHKNNKS in subunit S2, GDSSSG in subunit S3, and QTNSPRRA in subunit S4 (33)Protein structure and sequence reanalysis of 2019-nCoV genome refutes snakes as its intermediate host and the unique similarity between its spike protein insertions and HIV-1. Also, there are some differences in glycosylation sites in two strains of virus which can lead to diverse responses of SARS-CoV2 to anti-SARS-CoV drugs (18). In spite of these differences, there are several similar sequences

Table 3. Types of variations in SARS-COV2 in comparing with SARS-COV and MERS-COV.

Gene/Protein name	Variation type	Variation	Variation effect	References
ORF8	Non-synonymous	84Ser/Leu	Structural deviation in C-ter Novel phosphorylation site for Ser/Thr kinase of host cells	(*31*)
ORF1ab	synonymous	Serin 2839	-	(*31*)
ORF1ab/Nsp2	Non-synonymous	543Ser/Gly	High ability of contagious	(*32*)
Nsp3	Non-synonymous	192Prolin/Polar, Apolar AA	Possible role in virus replication	(*32*)
S1 subunit	Insertion	GTNGTKR	-	(*33*)
S2 subunit	Insertion	YYHKNNKS	-	(*33*)
S3 subunit	Insertion	GDSSSG	-	(*33*)
S4 subunit	Insertion	QTNSPRRA	-	(*33*)
RBD	Non-synonymous	Glycyl loop/ prolyl loop	-	(*9*)
RBD	Insertion	F486	Strong interaction of RBD with ACE2	(*9*)
N protein	Non-synonymous	37S/P ،215 G/S ،243 G/S 267A/Q	-	(*5*)
S protein	Insertion	680PRRA	Proteolytic cleavage of S protein by cellular protease High ability of contagious	(*5*)

that provide conserved structures between SARS-CoV2 and SARS-CoV, offering the same targets with different molecular interactions for both viruses.

Although comparing RBD domain of SARS-CoV2 and SARS-CoV has shown 28% variations in amino acid sequences, they are structurally too similar. A sequence difference in RBD domain of both viruses is a loop of Glycyl residues in SARS-CoV2 instead of prolyl residues in SARS-CoV. Furthermore, phenylalanine F486 in RBD domain of the SARS-CoV2 leads to strong interaction with ACE2 receptor through interacting phenylalanine with hydrophobic pocket in ACE2. It can be the other reason for greater affinity of SARS-CoV2 to bind to the host cell in comparing with SARS-CoV. Therefore, the usage of antibodies and inhibitors which prevent RBD from binding to the ACE2 can be applied as a combating strategy against SARS-CoV2 (9).

Comparing N proteins of SARS-CoV2 and Bat-coronavirus has shown four different amino acids in 37S / P, 215 G / S, 243 G / S and 267A / Q, respectively. Also, 33 different amino acids are located at the regions of 439-449 and 482-505 in SARS-CoV2 S protein. An insertion peptide (PRRA), which is located at amino acid 680 of S protein in the SARS-CoV2, may be involved in contagious ability of the virus and in proteolytic cleavage of the S protein mediated by cellular proteases. In addition, 103 different amino acids have been known in 919-1227 region of Orf1ab protein (5). Table 3 presents types of variations in SARS-CoV2 in comparing with SARS-CoV and MERS-CoV.

Despite the amino acid differences in the S protein, there are no structural differences in the protein between both strains of virus. This suggests that inhibitors of SARS-CoV S protein could also be used as inhibitors of SARS-CoV2 S protein. Although there are some similar epitopes in both viruses, there are numerous antigenic differences between them (34). These variances could provide an opportunity to develop novel and more effective drugs and vaccines against SARS-CoV2. The similar epitopes are well-known as RISNCVADY, CVADYSVLY, RSFIEDLLF, RVDFCGKGY, MTSCCSCLK, VLKGVKLHY, whereas different epitopes are KTSVDCTMY, STECSNLLL, ECSNLLLLQY, and LTDEM. In addition, diverse glycosylation sites in S glycoprotein of SARS-CoV2 are NGTK, NFTI, NLTT and NTSN, whereas common and conserved glycosylation sites are NITN, NGTI, NFSQ, NESL, NCTF and NNTV in both types of viruses (34).

Immune responses to SARS-CoV2

Once entering the host body, the virus is detected by the innate immune system through C-type-lectin-like receptors, Toll like receptors (TLRs), NOD-like receptor (NLR), and RIG-I-like receptor (RLR). It also activates either inflammatory factors or the synthesis of INFs, however, the viral N protein causes the virus to escape from the host's immune response. The critical role of C3a and C5a complement factors has also been demonstrated in combating against viruses (17). In the next step, CD4+ T and CD8+ T adaptive immune response cells are activated to secrete antibodies and

cytokines and kill the virus directly. However, SARS-CoV2 escapes from the adaptive immune system through inducing apoptosis in T cells. Consequently, high levels of immune responses and high levels of free radicals produced by immune cells can damage lungs and other organs, which thus lead to death (17). Cytokine storm is an over-activated and severe form of inflammatory and immune response in which a large amount and variety of cytokines such as TNFα, IL-1β, IL-2, IL-6, IFNα, IFNβ, IFNγ and monocyte chemoattractant protein-1 (MCP1) are involved in combating against infectious agents like SARS and MERS. In response to the cytokines, immune cells produce large amounts of free radicals, which can lead to severe lung damage (17).Analyzing various cells in particular cells around the viral target also demonstrates the key role of macrophages in immune defense during COVID-19 disease through triggering chemokine signaling pathways and phagocytosis which are mediated by interacting with ACE2-expressing cells (10) Immunoinformatics studies on viral epitopes of SARS-CoV2 have identified five and three epitopes in glycoproteins for detection by cytotoxic T cell (CTL) and sequential B cell, respectively, as well as identifying five epitopes for discontinuous B cell detection. CTL epitopes are able to attach to MHC class I through multiple interactions which mediate activation of the immune responses. Consequently, Some epitopes may be recommended as targets for developing SARS-CoV2 vaccines (35).Additionally, during a virus attack, cell death or apoptosis occurs in order to kill infected cells as an antiviral defense mechanism. It has been shown that the expression level of myeloid cell leukemia 1 (MCL1), a positive apoptotic regulator, which regulates mitochondrial apoptotic responses, greatly increases during coronavirus infection. Therefore, by interacting with Orf7a, MCL1 can trigger apoptosis in infected host cell and induce death in both host cell and virus (11). In addition, there are some defense mechanisms regulated by host cells that help to immune cells indirectly to deal with infectious agents. For instance, eukaryotic translation elongation factor 1 alpha 1 (EEF1A1) playing a critical role in transmitting tRNA to the ribosome, is down-regulated during β-coronaviruses infection (11). The other down-regulated protein, GRAIL, is an E3 ubiquitin ligase that indirectly triggers an antiviral immune response to RNA viruses. TMPRSS2 is the other protein which is down-regulated in SARS-CoV infection as well as in other β-coronaviruses and plays a vital role in interaction with the viral S glycoprotein (11).

Drugs and vaccines against SARS-CoV2

Recently, many efforts have been implemented to develop novel drugs such as remdesivir, GS- 441524, protein inhibitors and vaccines for combating against coronaviruses (36, 37). However, various types of vaccines targeting SARS-CoV2, may have several advantages, disadvantages, and challenges. Overall, antiviral vaccines are included in the whole virus, the recombinant protein, and the nucleic acid vaccines (4).

One major challenge with making coronavirus vaccines is the adverse immune responses such as eosinophilic infiltration and infection which may occur after immunosuppression of the entire virus or complete spike protein. Also, efforts have been made to develop a vaccine against SARS-CoV2, similar to Ebola or Flu vaccines, which use the adenovirus as a vector. Totally, these vaccines consisted mainly of SARS-CoV2 proteins and codon deoptimization technology has also been used to reduce virus activity (4). There are both upsides and downsides in using the whole virus vaccine. One of the most important beneficial aspects is stimulation of TLRs such as TLR3, TLR718 and TLR9, whereas one problem with this vaccine is it's greater infectivity. Protein vaccine is one of the other types of vaccines which either creates immune responses against spike proteins of the virus in order to prevent them from binding to the ACE2 receptor, or is a recombinant S protein in seudo-virus nanoparticles. The other type of protein vaccine which has recently been tested, is subunit vaccine that includes a subunit of S protein with only one RBD. One positive point of subunit vaccine in particular RBD-based vaccine, is minimizing unwanted immune reactions as well as high level of safety. Today, DNA and RNA vaccines with various modifications are being studied to improve the activity of nucleic acid vaccines in humans (4).

A recent study on COVID-19 vaccine confirmed that the usage of vaccine adjuvant toll like receptor agonists, which had previously been an efficient adjuvant in the SARS-CoV vaccine, could be combined with S proteins as a potent effective vaccine for COVID-19 without eosinophilic infiltration with cytokine Th1 / 17 responses, whereas S proteins attached to gold nanoparticles as adjuvants can lead to eosinophilic infiltration as well as creating strong cytokine/ IgG responses (38).

Recently, numerous studies on synthetic vaccines in particular synthetic epitope vaccines have highly been implemented. A highly conserved amino acid sequence among coronaviruses like SARS-CoV2, is KRSFIEDLLFNKVV, which is also attributed to the region around a cleavage site in the SARS virus. This amino acid sequence is critical for the virus entry and can be suggested as a synthetic epitope vaccine (39).

Different studies have reported variety of potent treatments and methods for COVID-19 therapy. The best treatment for patients with severe symptoms is cytokine storm treatment or immunosuppression such as using Corticosteroids, Tocilizumab and Anti-IL-6. Although, usage of steroids may have many side

effects, say, avascular osteonecrosis, a low dose is recommended (17). Furthermore, it has been reported that mesenchymal stem cells (MSC) implantation which are well known as strong immunomodulatory cells, have been resulted in improvement of disease through increasing peripheral lymphocytes, decreasing C reactive protein (CRP), as well as elevating activity of cytokine-secreting immune cells such as CXCR3 + CD4 + T, CXCR3 + CD8 + T CXCR3 + NK cell, increasing CD14 + CD11c + CD11bmid regulatory DC cells and TNFα and decreasing IL-10. MSCs are ACE2- and TMPRSS2^{-}, therefore, they can be considered as safe and effective cells for treatment of COVID-19 pneumonia patients (15, 40). Recently, research has been applied to design and manufacture antibodies to treat COVID-19. Although, various monoclonal antibodies used for SARS-CoV cannot be used and effective for this novel type of virus, recently, an antibody isolated from SARS patients called CR3022 may target either receptor binding sites or conserved epitopes in both SARS-CoV and SARS-CoV2. The function of CR3022 depends on the conformation of RBDs in the S glycoprotein. Once at least two RBDs of trimeric S protein are in the up conformation, epitope will be available for CR3022 (41). SARS-CoV antibodies such as m396 and CR3014 target the various epitopes on RBD of S glycoprotein in ACE2-binding site, however, they cannot be attached to SARS-CoV2 S protein. This suggests that there are some potent sequence or structural differences between RBDs of SARS-CoV and SARS-CoV2 (42). In addition, a recent study on the treatment of COVID-19 has shown that the usage of high-dose intravenous immunoglobulin (IVIG) can be introduced as a satisfactory treatment (43).

On the other hand, it has been reported that HIV-1 protease inhibitors such as Lopinavir, sequinavir, ritonavir can have a strong interaction with activate site of SARS-CoV2 protease. Among the twenty compounds classified in these three groups, five compounds are hypothetically the main compounds that target the SARS-CoV2 main protease. IDs of these compounds are 444745, 444663, ZINC1014061061, ZINC1014061081, 444743 in the ZINC database (44). The other antiretroviral agent, atazanavir has exhibited greater anti-SARS-CoV2 activity in comparing with lopinavir through inhibiting SARS-CoV-2 replication and proinflammatory cytokine production (IL-6 and TNFα), alone or in combination with ritonavir (45). Various studies have been implemented to design and synthesize drugs targeting M^{pro}. Recently, two designed and synthesized components (11a and 11b) have depicted potent anti-SARS-CoV2 and pharmacokinetic activity in vivo (46). In addition, drug designing, virtual drug screening and high-throughput screening recommended ebselen as the other candidate anti-SARS-CoV2 drug which needs to be approved clinically (47).

Moreover, studies have indicated that cyclosporin A inhibits virus replication through binding to cell cyclophilins and inhibiting it's cis-trans peptidyl-prolyl isomerization activity. Cyclophilin A is also involved in binding virus N proteins to cells, thus the usage of cyclophilin A inhibitors such as CSA derivatives including Alisporivir and NIM811 can inhibit protein N interaction with cyclophilin A (48) suggesting cyclophilin A inhibitors can be investigated for treatment of COVID-19 as well as other coronaviruses. Previous studies have exhibited that the Pan-corona virus inhibitor, EK1, targets the HR1 domain of S2 subunit in coronavirus, such as SARS-CoV and MERS-CoV. Recent study on SARS-CoV2 has shown high inhibitory activity (120/240 fold) of EK1 peptide against membrane fusion and viral infections of SARS-CoV2, SARS-CoV and MERS-CoV through binding to cholesterol in order to form EK1C4 lipopeptide (23).

It has recently been reported that protein kinase inhibitors can be recommended as antiviral drugs for the treatment of coronavirus due to the activation of multiple signaling pathways in the host cells during virus's growth. Therefore, protein kinases which are activated by virus or infected cells, may be used as targets for developing anti-COVID-19 drugs. Also, recent studies have exhibited the activation of hydrophobic derivatives of vancomycin, teicoplanin, as well as aglycon or pseudo-aglycon against several viruses such as HIV, HCV, influenza viruses (A / H1N1, A / H3N2), flaviviruses as well as coronaviruses through targeting protein kinases (49).

A number of antiviral drugs are well-known as protease inhibitors, integrase inhibitors, and polymerase inhibitors. Among these drugs, protease inhibitors such as tyrannavir, indinavir, atazanavir, darunavir, ritonavir and amprenavir could, as indicated by in silico virtual screening, inhibit virus replication and are thus suggested as potential effective drugs for COVID-19 (2). Macrolides such as erythromycin, clarithromycin and azithromycin also have anti-inflammatory and antibacterial effects as well as antiviral impacts. Previous studies have confirmed that Macrolides have antiviral effects on Rhinovirus, influenza, Zika and Ebola viruses, all of which are respiratory viruses. Studies on COVID-19 patients have indicated that the usage of azithromycin or macrolides in combination with hydroxychloroquine could represent a potential antiviral drug-combination for the treatment of COVID-1 and that hydroxychloroquine alone could have antiviral effects against SARS-CoV-2 (50). One of the drawbacks of using hydrochloroquine which was previously used as an anti-malarial drug and also

Table 4. Types of drugs and therapy suggested to be used against coronaviruses in particular SARS-COV2

Type of drug	Type of virus	Target	Function	Reference
Corticosteroide Tocilizumab Anti-IL-6	SARS-COV2	Immune response	Suppression of cytokine storm	(*17*)
CR3022	SARS-COV2 SARS-COV	ACE2 binding site	Inhibition of virus entry to cells	(*41*)
m396 CR3014	SARS-COV	RBD epitopes in ACE2-binding site	Inhibition of virus entry to cells	(*42*)
Intravenous immunoglobulin (IVIG)	SARS-COV2	Immune response	Suppression of immune response	(*43*)
Lopinavir Sequinavir Ritonavir	HIV-1 SARS-COV2	Protease	Inhibition of virus replication	(*44*)
Cyclosporin A		cyclophilin	-Suppression of N protein interaction with cyclophilin - Inhibition of virus replication	(*48*)
EK1	SARS-COV MERS-COV	HR1 domain of S2 subunit	Inhibition of membrane fusion	(*23*)
EK1C4 lipopeptide	SARS-COV2 SARS-COV MERS-COV	HR1 domain of S2 subunit	Inhibition of membrane fusion	(*23*)
Vancomycin Teicoplanin Aglycon Pseudo-aglycon	HIV ،HCV A/H1N1 A/H3N2 coronaviruse flavivirus	Protein kinases	Inhibition of virus entry and replication	(*49*)
Tipranavir Indinavir Atazanavir Darunavir Ritonavir Amprenavir	Coronaviruses (SARS-COV2)	Protease	Inhibition of virus replication	(*2*)
Macrolides (Erythromycin Clarithromycin Azithromycin) + Hydroxychloroquine	SARS-COV2	Immune response	Anti-inflammation	(*50*)
Ginkgolic acid (GA)	HIV ،Ebola Influenza EBV coronaviruses	Viral proteins	Inhibition of virus replication	(*52*)

for autoimmune diseases is creating cardiac toxicity that leads to arrhythmia as well as liver and kidney disorders in high-risk individuals. In addition, the risk of overdose is higher in patients with renal and hepatic disorders than in other populations (51). The other suggested drug is Ginkgolic acid (GA) found in the leaves and fruits of Ginkgobiloba. It has shown antiviral impacts against HIV, Ebola, Influenza, and EBV viruses through inhibiting virus's replication. Therefore, GA could be suggested as an effective drug for combating with coronaviruses. Furthermore, GA has previously displayed anti-cancer effects through inhibiting lipogenesis, reducing the expression of proteins involved in metastasis, suppressing sumoylation, and inhibiting fatty acid synthesis (52). Table 4 summarizes several types of drugs and therapies proposed to be used against coronaviruses in particular SARS-CoV2.

Personalized medicine in COVID-19

Personalized medicine in COVID-19 can provide an opportunity for prevention, diagnosis, treatment and management of this disease in the public health. Personalized medicine can detect the susceptibility of everyone to COVID-19 infection with highly variable symptoms. Patients with COVID-19 experience the variable symptoms ranging from mild to severe and progressive infections. Identifying susceptible patients to severe infection can help to better management of COVID-19 pandemic. Personalized medicine in COVID-19 may be lead to design novel therapeutic and preventive strategies according to individual profiles. For this purpose, sequencing of SARS-CoV2 genome along with detection of genome variation of patients can propose the best approach to combat with COVID-19 (53). Individual Factors associated to disease severity are divided to two categorizes,

non-genomics/clinical and genomics factors. Non-genomics or clinical factors include age, gender, BMI, diabetes, hypertension and smoking, whereas genomic factors include variations in chromosomes 1 (1q22.1), 2 (2p21.1), 3 (3p21.1–3), 6 (6p21.1), 8 (8q24.13), 9 (9q34.1–2), 12 (12q24.1–2), 17 (17q21.3), 19 (19p13.1–3) and 21 (21q21–q22) which can be specific for different ethnics or phenotypes. For instance, 3p21 locus variants are present in 30% and 8% of people in South Asia and Europe, respectively that are associated to severe inflammation and infection. In addition, 3p21.31 locus comprises SLC6A20 gene coding for a transporter regulated by ACE2, and other loci may have indirect association with COVID-19 severity (53, 54).

In other hand, variations in SARS-CoV2 are estimated to accumulate at rate of about 1–2 variations per month. Sequencing of viral genome has reported huge variability which affects the severity of infections in patients with COVID-19. For example, D614G variant in spike protein and ORF8 deletion increases and reduces infectivity of the virus as well as mortality of patients, respectively (55, 56). Variations in RNA polymerase can also increase the replication mistakes that could result in resistance to antiviral treatments. Therefore, personalized medicine in COVID-19 may be lead to design novel therapeutic and preventive strategies according to individual profiles in order to better management of COVID-19 pandemic.

CONCLUSION

Recently, SARS-CoV2, the novel strain of coronaviruses has initiated a catastrophe for humans all over the world with sever acute respiratory syndrome and high rate of death. It is essential to study on genetic and protein structure of SARS-CoV2 to discover potent drugs, antibodies and vaccines in order to treat COVID-19. High similarity to SARS-CoV proposes the same treatments, although some differences in genetic and protein structures make difficulties in discovering therapy. SARS-CoV2 uses subunit S1 and S2 for binding and membrane fusion in order to entry to host cells through binding to ACE2. SARS-CoV2 genome encodes several proteins such as nsps, structural proteins and accessory proteins which have been involved in structure, activity, replication and transmission of virus. There are several variations in genetic and protein sequences in SARS-CoV2 in comparing with SARS-CoV and MERS-CoV. These variations recommend a possible mechanism to distinguish SARS-CoV2 from SARS-CoV and MERS-CoV and could help to discover novel antiviral strategies to treat COVID-19. Moreover, recently, variety of publications have proposed several novel vaccines, drugs and antibodies such as anti-inflammatory factors, anti-ACE2, anti-S protein and protease inhibitors to combat with SARS-CoV2.

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REFERENCES

1.Chen Y, Liu Q, Guo D. Emerging coronaviruses: genome structure, replication, and pathogenesis. Journal of medical virology. 2020; 92:418-423.

2.Liu X, Wang X-J. Potential inhibitors against 2019-nCoV coronavirus M protease from clinically approved medicines. Journal of Genetics and Genomics. 2020.

3.Chen J. Pathogenicity and transmissibility of 2019-nCoV—a quick overview and comparison with other emerging viruses. Microbes and infection. 2020.

4.Chen W-H, Strych U, Hotez PJ, Bottazzi ME. The SARS-CoV-2 vaccine pipeline: an overview. Current tropical medicine reports. 2020;1-4.

5.Li C, Yang Y, Ren L. Genetic evolution analysis of 2019 novel coronavirus and coronavirus from other species. Infection, Genetics and Evolution. 2020;104285.

6.Lu R, Zhao X, Li J, Niu P, Yang B, Wu H, Wang W, Song H, Huang B, Zhu N. Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. The Lancet. 2020; 395:565-574.

7.Paraskevis D, Kostaki EG, Magiorkinis G, Panayiotakopoulos G, Sourvinos G, Tsiodras S. Full-genome evolutionary analysis of the novel corona virus (2019-nCoV) rejects the hypothesis of emergence as a result of a recent recombination event. Infection, Genetics and Evolution. 2020; 79:104212.

8.Wan Y, Shang J, Graham R, Baric RS, Li F. Receptor recognition by the novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS coronavirus. Journal of virology. 2020; 94.

9.Chen Y, Guo Y, Pan Y, Zhao ZJ. Structure analysis of the receptor binding of 2019-nCoV. Biochemical and biophysical research communications. 2020.

10.Qi F, Qian S, Zhang S, Zhang Z. Single cell RNA sequencing of 13 human tissues identify cell types and

receptors of human coronaviruses. Biochemical and biophysical research communications. 2020.
11.Guzzi PH, Mercatelli D, Ceraolo C, Giorgi FM. Master regulator analysis of the SARS-CoV-2/human interactome. Journal of Clinical Medicine. 2020; 9:982.
12.Iwata-Yoshikawa N, Okamura T, Shimizu Y, Kotani O, Sato H, Sekimukai H, Fukushi S, Suzuki T, Sato Y, Takeda M. Acute respiratory infection in human dipeptidyl peptidase 4-transgenic mice infected with Middle East respiratory syndrome coronavirus. Journal of virology. 2019; 93:e01818-01818.
13.Te N, Vergara-Alert J, Lehmbecker A, Pérez M, Haagmans BL, Baumgärtner W, Bensaid A, Segalés J. Co-localization of Middle East respiratory syndrome coronavirus (MERS-CoV) and dipeptidyl peptidase-4 in the respiratory tract and lymphoid tissues of pigs and llamas. Transboundary and emerging diseases. 2019; 66:831-841.
14.Lan J, Ge J, Yu J, Shan S, Zhou H, Fan S, Zhang Q, Shi X, Wang Q, Zhang L. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature. 2020;1-6.
15.Shetty AK. Mesenchymal stem cell infusion shows promise for combating Coronavirus (COVID-19)-induced pneumonia. Aging and disease. 2020; 11:462.
16.Yan R, Zhang Y, Li Y, Xia L, Guo Y, Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science. 2020; 367:1444-1448.
17.Yi Y, Lagniton PN, Ye S, Li E, Xu R-H. COVID-19: what has been learned and to be learned about the novel coronavirus disease. International journal of biological sciences. 2020; 16:1753.
18.Vankadari N, Wilce JA. Emerging COVID-19 coronavirus: glycan shield and structure prediction of spike glycoprotein and its interaction with human CD26. Emerging microbes & infections. 2020; 9:601-604.
19.Liu Z, Xiao X, Wei X, Li J, Yang J, Tan H, Zhu J, Zhang Q, Wu J, Liu L. Composition and divergence of coronavirus spike proteins and host ACE2 receptors predict potential intermediate hosts of SARS-CoV-2. Journal of medical virology. 2020; 92:595-601.
20.Shang J, Ye G, Shi K, Wan Y, Luo C, Aihara H, Geng Q, Auerbach A, Li F. Structural basis of receptor recognition by SARS-CoV-2. Nature. 2020;1-4.
21.Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh C-L, Abiona O, Graham BS, McLellan JS. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science. 2020; 367:1260-1263.
22.Tai W, He L, Zhang X, Pu J, Voronin D, Jiang S, Zhou Y, Du L. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cellular & molecular immunology. 2020;1-8.
23.Xia S, Liu M, Wang C, Xu W, Lan Q, Feng S, Qi F, Bao L, Du L, Liu S. Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors a high capacity to mediate membrane fusion. Cell research. 2020; 30:343-355.
24.Ou X, Liu Y, Lei X, Li P, Mi D, Ren L, Guo L, Guo R, Chen T, Hu J. Characterization of spike glycoprotein of SARS-CoV-2 on virus entry and its immune cross-reactivity with SARS-CoV. Nature communications. 2020; 11:1-12.
25.Khailany RA, Safdar M, Ozaslan M. Genomic characterization of a novel SARS-CoV-2. Gene Reports. 2020;100682.
26.Zhang L, Lin D, Sun X, Curth U, Drosten C, Sauerhering L, Becker S, Rox K, Hilgenfeld R. Crystal structure of SARS-CoV-2 main protease provides a basis for design of improved α-ketoamide inhibitors. Science. 2020; 368:409-412.
27.Chen YW, Yiu C-PB, Wong K-Y. Prediction of the SARS-CoV-2 (2019-nCoV) 3C-like protease (3CL pro) structure: virtual screening reveals velpatasvir, ledipasvir, and other drug repurposing candidates. F1000Research. 2020; 9.
28.Zhou J, Fang L, Yang Z, Xu S, Lv M, Sun Z, Chen J, Wang D, Gao J, Xiao S. Identification of novel proteolytically inactive mutations in coronavirus 3C-like protease using a combined approach. The FASEB Journal. 2019; 33:14575-14587.
29.Yang C-W, Chen M-F. Composition of human-specific slow codons and slow di-codons in SARS-CoV and 2019-nCoV are lower than other coronaviruses suggesting a faster protein synthesis rate of SARS-CoV and 2019-nCoV. Journal of Microbiology, Immunology and Infection. 2020.
30.Xu J, Zhao S, Teng T, Abdalla AE, Zhu W, Xie L, Wang Y, Guo X. Systematic comparison of two animal-to-human transmitted human coronaviruses: SARS-CoV-2 and SARS-CoV. Viruses. 2020; 12:244.
31.Ceraolo C, Giorgi FM. Genomic variance of the 2019-nCoV coronavirus. Journal of medical virology. 2020; 92:522-528.
32.Angeletti S, Benvenuto D, Bianchi M, Giovanetti M, Pascarella S, Ciccozzi M. COVID-2019: the role of the nsp2 and nsp3 in its pathogenesis. Journal of medical virology. 2020.
33.Zhang C, Zheng W, Huang X, Bell EW, Zhou X, Zhang Y. Protein structure and sequence reanalysis of 2019-nCoV genome refutes snakes as its intermediate host and the unique similarity between its spike protein insertions and HIV-1. Journal of proteome research. 2020; 19:1351-1360.
34.Kumar S, Maurya VK, Prasad AK, Bhatt ML, Saxena SK. Structural, glycosylation and antigenic variation between 2019 novel coronavirus (2019-nCoV) and SARS coronavirus (SARS-CoV). VirusDisease. 2020;1-9.
35.Baruah V, Bose S. Immunoinformatics-aided identification of T cell and B cell epitopes in the surface glycoprotein of 2019-nCoV. Journal of Medical Virology. 2020; 92:495-500.
36.Amirian ES, Levy JK. Current knowledge about the antivirals remdesivir (GS-5734) and GS-441524 as therapeutic options for coronaviruses. One Health. 2020;100128.
37.Prajapat M, Sarma P, Shekhar N, Avti P, Sinha S, Kaur H, Kumar S, Bhattacharyya A, Kumar H, Bansal S. Drug targets for corona virus: A systematic review. Indian journal of pharmacology. 2020; 52:56.
38.Sekimukai H, Iwata-Yoshikawa N, Fukushi S, Tani H, Kataoka M, Suzuki T, Hasegawa H, Niikura K, Arai K, Nagata N. Gold nanoparticle-adjuvanted S protein induces a strong antigen-specific IgG response against severe acute respiratory syndrome-related coronavirus infection, but fails to induce protective antibodies and limit eosinophilic infiltration in lungs. Microbiology and Immunology. 2020; 64:33-51.

39.Robson B. Computers and viral diseases. Preliminary bioinformatics studies on the design of a synthetic vaccine and a preventative peptidomimetic antagonist against the SARS-CoV-2 (2019-nCoV, COVID-19) coronavirus. Computers in biology and medicine. 2020;103670.
40.Leng Z, Zhu R, Hou W, Feng Y, Yang Y, Han Q, Shan G, Meng F, Du D, Wang S. Transplantation of ACE2-mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. Aging and disease. 2020; 11:216-228.
41.Yuan M, Wu NC, Zhu X, Lee C-CD, So RT, Lv H, Mok CK, Wilson IA. A highly conserved cryptic epitope in the receptor binding domains of SARS-CoV-2 and SARS-CoV. Science. 2020; 368:630-633.
42.Tian X, Li C, Huang A, Xia S, Lu S, Shi Z, Lu L, Jiang S, Yang Z, Wu Y. Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. Emerging microbes & infections. 2020; 9:382-385.
43.Cao W, Liu X, Bai T, Fan H, Hong K, Song H, Han Y, Lin L, Ruan L, Li T. High-dose intravenous immunoglobulin as a therapeutic option for deteriorating patients with coronavirus disease 2019. In: Open forum infectious diseases (Oxford University Press US, 2020; pp. ofaa102.
44.Ortega JT, Serrano ML, Pujol FH, Rangel HR. Unrevealing sequence and structural features of novel coronavirus using in silico approaches: The main protease as molecular target. EXCLI journal. 2020; 19:400.
45.Fintelman-Rodrigues N, Sacramento CQ, Lima CR, da Silva FS, Ferreira AC, Mattos M, de Freitas CS, Soares VC, Dias SdSG, Temerozo JR. Atazanavir, alone or in combination with ritonavir, inhibits SARS-CoV-2 replication and proinflammatory cytokine production. Antimicrobial Agents and Chemotherapy. 2020; 64.
46.Dai W, Zhang B, Jiang X-M, Su H, Li J, Zhao Y, Xie X, Jin Z, Peng J, Liu F. Structure-based design of antiviral drug candidates targeting the SARS-CoV-2 main protease. Science. 2020; 368:1331-1335.
47.Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, Zhang B, Li X, Zhang L, Peng C. Structure of M pro from SARS-CoV-2 and discovery of its inhibitors. Nature. 2020;1-5.
48.Ma-Lauer Y, Zheng Y, Malešević M, von Brunn B, Fischer G, von Brunn A. Influences of cyclosporin A and non-immunosuppressive derivatives on cellular cyclophilins and viral nucleocapsid protein during human coronavirus 229E replication. Antiviral Research. 2020; 173:104620.
49.Cozza G, Fortuna M, Meggio F, Sarno S, Kubbutat M, Totzke F, Schaechtele C, Pinna L, Olsufyeva E, Preobrazhenskaya M. Hydrophobic Derivatives of Glycopeptide Antibiotics as Inhibitors of Protein Kinases. Biochemistry (Moscow). 2018; 83:1222-1230.
50.Ohe M, Shida H, Jodo S, Kusunoki Y, Seki M, Furuya K, Goudarzi H. Macrolide treatment for COVID-19: Will this be the way forward? BioScience Trends. 2020.
51.Perinel S, Launay M, Botelho-Nevers É, Diconne É, Louf-Durier A, Lachand R, Murgier M, Page D, Vermesch R, Thierry G. Towards optimization of hydroxychloroquine dosing in intensive care unit COVID-19 patients. Clinical Infectious Diseases. 2020.
52.Borenstein R, Hanson BA, Markosyan RM, Gallo ES, Narasipura SD, Bhutta M, Shechter O, Lurain NS, Cohen FS, Al-Harthi L. Ginkgolic acid inhibits fusion of enveloped viruses. Scientific reports. 2020; 10:1-12.
53.Dopazo J, Maya-Miles D, García F, Lorusso N, Calleja MÁ, Pareja MJ, López-Miranda J, Rodríguez-Baño J, Padillo J, Túnez I. Implementing personalized medicine in COVID-19 in andalusia: An opportunity to transform the healthcare system. Journal of Personalized Medicine. 2021; 11:475.
54.Schmiedel BJ, Rocha J, Gonzalez-Colin C, Bhattacharyya S, Madrigal A, Ottensmeier CH, Ay F, Chandra V, Vijayanand P. COVID-19 genetic risk variants are associated with expression of multiple genes in diverse immune cell types. Nature communications. 2021; 12:1-12.
55.Li Q, Wu J, Nie J, Zhang L, Hao H, Liu S, Zhao C, Zhang Q, Liu H, Nie L. The impact of mutations in SARS-CoV-2 spike on viral infectivity and antigenicity. Cell. 2020; 182:1284-1294. e1289.
56.Young BE, Fong S-W, Chan Y-H, Mak T-M, Ang LW, Anderson DE, Lee CY-P, Amrun SN, Lee B, Goh YS. Effects of a major deletion in the SARS-CoV-2 genome on the severity of infection and the inflammatory response: an observational cohort study. The lancet. 2020; 396:603-611.
57.Graham RL, Sparks JS, Eckerle LD, Sims AC, Denison MR. SARS coronavirus replicase proteins in pathogenesis. Virus research. 2008; 133:88-100.
58.Mousavizadeh L, Ghasemi S. Genotype and phenotype of COVID-19: Their roles in pathogenesis. Journal of Microbiology, Immunology and Infection. 2020.
59.Narayanan K, Huang C, Makino S. SARS coronavirus accessory proteins. Virus research. 2008; 133:113-121.

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The Emerging Role of Personalized Medicine in Immunotherapy for Ovarian Cancer

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Abstract:
The ability of immunotherapy to treat ovarian cancer is currently limited, however evaluating sensitive/resistant target treatment subpopulations based on stratification by tumor biomarkers may enhance this ability. These indicators include the number of tumor mutations, PD-L1, tumor-infiltrating lymphocytes, a lack of homologous recombination, and intratumoral heterogeneity of neoantigens. The use of these indicators to choose the best candidates for ovarian cancer treatment is one of the future directions. In addition to reviewing innovative treatments and study designs including tumor biomarkers that improve the chances of immunotherapy success in ovarian cancer, this paper also analyzes the function of immunotherapy in ovarian cancer.

INTRODUCTION

Ovarian cancer is the tenth most prevalent cancer among female patients and the fifth largest cause of cancer-related mortality in women in the United States, where 22,240 individuals are diagnosed with it each year (1). Currently, debulking surgery combined with platinum-taxane maintenance chemotherapy is the first-line standard of treatment (2, 3). After receiving front-line treatment, 60–70% of patients with optimum debulking (1 cm residual disease) and 80–85% of patients with inadequate debulking (>1 cm residual disease) may experience a cancer recurrence, bringing the five-year survival rate down to roughly 45%. Front-line maintenance treatment advancements have attempted to increase this time frame (4, 5, 6). More efficient maintenance treatment is required since it has been demonstrated to effectively prolong progression-free survival (PFS) with bevacizumab or PARP inhibitors, but not overall survival (OS) (7). Currently, the majority of clinical studies concentrate on targeted strategies, including more recent initiatives to add immune therapies to the landscape of ovarian cancer treatment (7).

Through a variety of methods, including immunostimulatory cytokines, tumor antigen vaccines, and monoclonal antibodies that target inhibitory ligands generated by tumor cells, immunotherapy improves the antitumor immune response. The latter strategy focuses mostly on immunological checkpoint inhibition (ICI). Immune checkpoints such as programmed death receptor-1 and its ligand (PD-1:PD-L1) and cytotoxic T-lymphocyte associated protein 4 and its ligand (CTLA-4:B7/CD80) are used to identify pathogens from self-cells (8). A T-lymphocyte searches for epitopes that are compatible with its T-cell receptor (TCR) affinity when it comes into contact with a peripheral cell to identify whether it is a pathogen or a self-cell. T-cells recognize the epitope as a self-cell when immunological checkpoints like PD-L1 are present (9). When immunological checkpoints are absent, the T-cell recognizes the target as pathogenic, which triggers the killing response. Immune checkpoints are upregulated by cancer cells, which reduces the local immune response and enables immune evasion. By binding CTLA-4, PD-1, or PD-L1, ICIs prevent the immune checkpoint interaction between the tumor and T-cell, thereby restoring T-cell cytotoxicity (10). The importance of immunotherapy in ovarian cancer is discussed in this paper, along with innovative treatments and research designs including tumor biomarkers that improve the chances of immunotherapy effectiveness in ovarian cancer.

Molecular profiling

Even though ICIs can result in long-lasting responses in certain patients, there is still a small percentage of patients who do not react, such as those whose tumors express PD-L1. Therefore, molecular profiling that indicates immunogenic phenotypes is increasingly used to determine the indication for immunotherapy, and new knowledge is exploding in this area. PD-1, PD-L1, and tumor mutational burden (TMB) are

immunophenotype indicators (11, 12). Other indicators include homologous repair deficient and proficient (HRD, HRP) phenotypes and elements governing the tumor microenvironment (TME), such as the characteristics of lymphocytes that infiltrate the tumor (TILs). These elements together provide a picture of the immunogenicity of the cancer cell itself, the immune system's capacity to reach the tumor, and the capacity of immune cells to carry out lethal operations (13, 14).

TMB

The amount of nonsynonymous mutations found in a tumor sample is known as the tumor mutational load, which also indicates the degree of genomic instability and the possibility that neoepitopes would emerge on the cell surface (15). Neoepitopes are cancer-specific proteins that are expressed on the cell surface and therefore accessible to the immune system. Different research teams have established different TMB criteria; generally speaking, TMB is split into high and low categories, with TMB high being defined as >10 mutations/Megabase of DNA. A TMB-high phenotype denotes a high level of mutant proteins that may be expressed as neoepitopes on the cell surface (16).

It has been shown in both preclinical and clinical efficacy trials showing the TMB-high phenotype predicts response to treatment with ICIs in solid tumors. A monoclonal antibody that targets PD-1, pembrolizumab, was recently authorized for treatment in any TMB-high (10) tumor, regardless of histology (17, 18, 19).

Consideration of other markers that may increase a patient's likelihood of responding to immunotherapy (e.g., combined TMB-high and PD-L1-high) or predict resistance to immunotherapy despite the TMB-high phenotype (e.g., combined TMB-high and high neoantigen intratumoral heterogeneity) generally improves TMB's predictive ability (ITH). According to estimates, 45% of ovarian cancers express PD-1/PD-L1 highly, which is defined as more than 10% of tumor cells in a tissue sample expressing PD-L1 on their surface (20, 21, 22). ICIs were created to stop this checkpoint-mediated immunosuppression, which is immunoinhibitory when PD-1/PD-L1 expression is high. Independent of TMB status, elevated PD-1/PD-L1 expression predicts responsiveness to ICIs. However, the two markers work better together to predict response than they do alone. Single biomarkers have been utilized as treatment indications up until now, but taking into account mixed biomarkers may increase the accuracy of choosing individuals who are most likely to respond to immunotherapy, particularly in cases of ovarian cancer (23, 24).

HRD

Homologous repair deficit (HRD) is a biomarker that predicts how cancers will react to platinum chemotherapy and poly-(ADP ribose) polymerase (PARP) inhibitors. It defines malignancies with inadequate reaction to DNA damage. 41–50% of epithelial ovarian cancers are thought to be HRD, and more than one in four people with ovarian cancer have germline mutations in HRD genes (25, 26). 25.7% of people with ovarian cancer have somatic or germline mutations in their BRCA genes. BRCA mutant cells are called HRD cells. HRD is linked to cancer genes that run in families, such as germline BRCA mutations in breast and ovarian cancer and mismatch repair (MMR) in Lynch syndrome. Surprisingly, a family history of cancer is linked to a higher objective response rate (ORR), disease control rate (DCR), the median time to treatment failure (MTTF), and median overall survival (OS) after ICIs are given. This makes us wonder if HRD may be the link between ICIs and this response in familial cancers. Some genes, like RAD51, BARD1, and TP53, that are part of the homologous recombination pathway, also show that DNA can't be fixed. This is called a "gene signature." Pembrolizumab, which is a PD-L1 inhibitor, works well on tumors with MMR (26, 27, 28). So, pembrolizumab was approved as the first treatment for colorectal tumors that lack MMR. But tumors with BRCA1/2 mutations haven't responded to avelumab or other immunotherapies. In this group, immune checkpoint inhibitors have been less successful than expected. HRD causes more tumors to grow, more mutations to happen in the tumors, and then more tumor neoantigens to be expressed. Compared to HRP tumors, HRD tumors have more immunophenotype markers like TMB-high, more CD3+ and CD8+ TILs, and higher levels of PD-1/PD-L1 (29, 30). Even though HRD is common in ovarian cancer, TMB is lower than would be expected. In a study of breast cancer samples, it was found that HRD tumors had more PD-L1 expression, which was linked to the activation of the STING pathway. The same study found that CXCL10 and CCL5 expression was 3.5 to 11.9 times higher than in HRP tumors and that CXCL10 and CCL5 expression, not neoantigen expression, was linked to the increased recruitment of peripheral blood mononuclear cells (PBMCs) (31). Dunphy et al. went into more detail about the non-canonical, antigen-independent recruitment of NK cells, M1-macrophages, T- and B-cells to the ovarian TME via CXCL10 and CCL5. The same group saw that the ATM-TRAF6-mediated "alternate STING pathway" made IL-6 and TGF-beta and brought in regulatory T-cells (Tregs) and protumor M2-macrophages. This ATM-TRAF6 also helps to raise the level of PD-L1 even more, which helps to explain why the immune system doesn't react much to ovarian cancer. So, immunomodulation in HRD tumors is controlled by the STING pathway and the alternative-

STING pathway. Depending on the molecular profile of the tumor, the immune response can be either boosted or slowed down (32, 33).

Tumor-infiltrating lymphocytes

Quantification of tumor-infiltrating lymphocytes (TIL), which measures immune cell infiltration of the tumor microenvironment (TME) and predicts a good response to immunotherapy, is another biomarker that may predict response. T-cells, NK cells, and, more recently, B cells' roles in the TME have been brought to people's attention. Different findings about how TILs affect survival and prognosis have made it harder to analyze (14, 34). For example, a study of the TME in melanoma found that the aggressiveness and stage of the tumor were linked to the number of TILs. In the same study, high levels of CD69+, which is a sign of activated lymphocytes, were linked to survival, showing that the number and quality of TILs are important for predicting prognosis. When it comes to ovarian cancer, the presence of TILs is linked to a longer PFS and OS. TILs in ovarian cancer are a good sign, especially when CD8+ T-cells are present, no matter what stage the tumor is in (35, 36, 37). Westergaard et al. found that the TIL profile of ovarian tumors is similar to that of melanoma, but there are more CD4+ T-cells than CD8+ T-cells in ovarian tumors. Most of these T-cells had the phenotype CD45RO+CCR7-CD62L, which is typical of effector memory T-cells (38).

Clinical trials of immune therapy for ovarian cancer

Even though ovarian cancer has a lot of HRD tumors with suspected high TMB, more CD8+ TILs, and high expression of tumor antigens that can trigger anti-tumor responses on their own, the first immunotherapy attempts were mostly not very successful (6, 39, 40). In the JAVELIN Ovarian 100 trial, avelumab, a PD-L1 inhibitor, was used as maintenance therapy for stage III/IV epithelial ovarian cancer that hadn't been treated before. Arms included chemotherapy followed by avelumab, avelumab plus chemotherapy followed by avelumab, and chemotherapy followed by watching as a control. The study was stopped before it was finished because the limits of futility had been set and there was no improvement in PFS compared to the control group (11.1 months, 11.0 months, and 10.2 months, respectively across arms) (40). Similarly, IMagyn050/GOG3015/ENGOT-OV39 compared atezolizumab, a PD-L1 inhibitor, to placebo plus paclitaxel, carboplatin, and bevacizumab in patients with advanced epithelial ovarian cancer. Researchers found no significant difference in median PFS between the PD-L1 positive group and the placebo group (18.5 months vs. 19.5 months, HR = 0.92) or between the PD-L1 positive group and the placebo group (19.5 months vs. 18.5 months) (20.8 months vs. 18.4 months, HR 0.80). Both of these studies came up with negative results, which suggests that using checkpoint inhibitors in ovarian cancer may need more biomarker efficacy analysis to find a potentially sensitive population (41).

In the KEYNOTE-158 trial, patients with solid tumors like ovarian, endometrial, and gastric cancer that had been treated with standard chemotherapy and were found to have cytologically confirmed high microsatellite instability (MSI-H) and mismatch repair deficiency (dMMR) were given pembrolizumab as a single treatment. The patients were thought to have a high number of tumor mutations because at least one of the four mismatch repair proteins, MLH1, MSH2, MSH6, and PMS2, was missing from their tumors. Also, high microsatellite instability was found in two of the five allelic loci shifts of BAT25, BAT26, Di 5S346, Di 2S123, or Di 17S250. Pembrolizumab was given every three weeks at a dose of 200 mg for two years or until the disease got worse. Five of the 15 people with ovarian cancer who had been resistant to treatment in the past had an objective response, and three of them had a full response. Since the study is still going on, more results are still to come. A total of 223 people took part in the study. 23 of them (9.9%) reported a full response, and 57 of them (24.5%) reported a partial response. It was said that the ORR was 34.3% (95% CI, 28.3–40.8). The good results of this study are one reason why the FDA approved pembrolizumab for use in metastatic MSI-H/dMMR solid tumors, like ovarian cancer, in May 2017. The results of the study as a whole, which include other kinds of tumors, are also important (42).

In the KEYNOTE- 100 trial, pembrolizumab was also tested on people with ovarian cancer that came back. In this trial, the first 100 people who signed up were used to figure out the cut-off score for PD-L1 (CPS). From this group of patients, CPS scores between 1 and 10 were used to figure out how well the treatment worked. Patients with recurrent ovarian cancer were split into two groups. Cohort A was made up of people who had one to three previous lines of therapy and had gone 2 to 12 months without platinum. Patients in Cohort B had four to six lines of therapy before and went three months without platinum. In both groups, a higher CPS score was linked to a better response, and responses were seen 6 months later. But antitumor activity was said to be small (43).

The MIMOSA study was a phase III, double-blind, placebo-controlled, multicenter trial that looked at the effects of abagovomab maintenance therapy on ovarian cancer patients in their first clinical remission. Abagovomab is a murine monoclonal antibody that goes after the CA-125 antigen that is found in tumors. Patients in the study had stage III or stage IV ovarian cancer and were in complete clinical remission after surgery and chemotherapy with platinum and taxanes.

Abagovomab or a placebo was given once every two weeks for the first six weeks, and then once every four weeks until the disease came back, or for up to 21 months after the last patient was randomly assigned. Out of the 888 patients who were studied, 81.5% had the serous papillary subtype, 85.9% were in stage III, and 80.9% had a cancer antigen 125 35 U/mL after the third cycle. The study treatment was used for a mean of 449.7 days. When the tumor size was broken down into two groups (1 cm and >1 cm), there was no improvement in RFS (HR = 1.099; 95% CI = 0.919–1.315; p = 0.301). In the same way, no benefit was shown in OS (1.150, 95% CI, 0.872–1.518; p = 0.322). At two years, 80% of people in both groups were still alive. The SE was 1.71 in the abagovomab group and 2.43 in the placebo group. At the last visit, the median level of anti–anti-idiotypic antibody was 493,000.0 ng/mL, which shows that a strong immune response was achieved. The trial found that maintenance therapy with abagovomab in the first remission does not extend RFS or OS. The treatment was safe, and there was a measurable immune response. The study was eventually stopped because the main goal wasn't reached (RFS) (44).

Clinical trials in progress

Even though immunotherapies aren't effective in treating ovarian cancer in previous trials, researchers are still looking for biomarkers that can predict how subgroups of ovarian cancer patients will respond to immunotherapies. There are also studies going on that combine immune therapies with other treatments. Many trials are going on right now to study the effects of different immunotherapies on ovarian cancer. Here, we'll look at some ongoing trials that use biomarkers to sort patients by how well they respond to treatment, which is listed in Table 1.

These ongoing phase I, II, and III trials have shown that immunotherapy, which is often used in combination with other treatments, makes ovarian cancer patients respond better. It is important to do more research on biomarkers and how they relate to increased and decreased response. Immunotherapy has a lot of potential as a treatment for ovarian cancer, and some patients do seem to respond to it. However, more research needs to be done before we can fully understand its potential.

The current state of personalized immunotherapy

For immunotherapy to be successful, the TME must activate an anti-tumor T-cell response. To do this, a variety of strategies, including dendric cells, autologous tumor vaccines, and other combination treatments, are now being researched.

Dendritic cells

Pulsing autologous dendritic cells (DCs) with a tumor peptide is one method. The kidneys, testis Sertoli cells, and ovarian granulosa cells all ordinarily express the chosen peptide, Wilms' tumor protein 1 (WT1) (45, 46). However, a study of 100 patients revealed that 78% of EOC tissues expressed WT1, and WT1 was linked to tumors with higher grades and staging (p = 0.006 and p = 0.002, respectively). The Wilms tumor protein 1 (WT1), which is associated with a poor prognosis in ovarian cancer with a 5-year survival rate of 47%, was studied in phase I/II clinical research. In individuals with ovarian, breast, and gastric cancer who presented with a WT1 mutation, vaccination with autologous DCs produced a substantial CD8+ T-cell activation against WT1 ($p < 0.05$) (47, 48). After therapy, seven out of ten patients (70%) had stable disease, with three of the seven patients having reported tumor-shrinking on a CT scan. Two out of ten (20%) patients had indicated limited response. The therapy was deemed to be safe and well tolerated since all adverse events were grade 1 or 2 (46). This research illustrates the necessity for customized vaccines that aim to target certain mutations in patient subgroups to achieve better results.

Autologous vaccines

Autologous vaccines may potentially be essential for the evolving function of immunotherapy in the treatment of cancer. Autologous vaccines are one such combination that may simultaneously reduce tumor evasion and increase immune responses to target tumor cells. In one pilot research, six late-stage, chemotherapy-resistant ovarian cancer patients received combination treatment (49). Surgery, ex vivo expanded autologous TILs, IL-2, and nivolumab, an anti-PD-1 antibody, were administered after ipilimumab, an anti-CTLA4 antibody. The median progressive-free survival was found to be 86 days, with a range of 84 to 342 days in the study's outcomes of one patient with partial response and five with stable illness after 12 months. These findings were contrasted with earlier findings obtained without the use of ipilimumab, which showed that ipilimumab enhanced the success rate of ex vivo expanded autologous TILs by causing an uptick in CD8+ T-cell activity (50, 51).

Overall, the research emphasizes the positive outcomes of ICIs and autologous vaccination combination treatment. The natural next step is to use ICIs in conjunction with Vigil, an additional autologous vaccination that trains T-cells to the pertinent clonal tumor neoantigens and enhances peripheral circulating CD3+/CD8+ T-cells. In a phase I study, women with relapsed ovarian cancer were given the combination of Vigil and atezolizumab. The time of administration was discovered by the investigators to be crucial for both safety and effectiveness. Predating atezolizumab

Table 1. Some clinical trials using biomarkers.

Trial Name	References	Short Description	Experiment Arms/Cohorts	Biomarker Stratification
KEYNOTE-158	[43]	People with advanced solid tumors took part in a Phase II, two-arm, open-label study of pembrolizumab and predictive biomarkers.	Arm 1: Pembrolizumab 200 mg Arm 2: Participants failed at least one line of therapy and have TMB high.	TMB high
CT03428802	ClinicalTrials.gov	Phase II, single-arm, open-label trial studying the use of pembrolizumab in people with metastatic, recurrent, or locally advanced solid tumors and genetic mutations.	Arm 1: Pembrolizumab and lab biomarker analysis	Response rate will be divided into groups based on the type of mutatio (POLE and POLD1 vs. BRCA1/2). PD-L1 expression, the presence of PD-1/PDL-1 polymorphisms, and th presence of immunoregulatory gene mutations will be used to divide patients into groups with different clinical outcomes (via deep sequencing)
DUO-O	ClinicalTrials.gov	Phase III, randomized, double-blind, placebo-controlled, multicenter trial looking at the use of durvalumab with chemotherapy and bevacizumab, followed by maintenance durvalumab, bevacizumab, and olaparib in advanced ovarian cancer.	Arm 1: Platinum-based chemotherapy with bevacizumab and durvalumab placebos, followed by maintenance bevacizumab, durvalumab placebo, and olaparib placebo Arm 2: Platinum-based chemotherapy with bevacizumab and durvalumab followed by maintenance bevacizumab, durvalumab, and olaparib placebo Arm 3: Platinum-based chemotherapy with bevacizumab and durvalumab followed by maintenance bevacizumab, durvalumab, and Olaparib tBRCAm Cohort: Platinum-based chemotherapy with bevacizumab and durvalumab followed by maintenance bevacizumab, durvalumab, and olaparib (bevacizumab is optional)	Somatic BRCA mutation status
V3-OVA	ClinicalTrials.gov	A single-arm, open-label Phase II trial is being done to study the use of the V3-OVA vaccine in ovarian cancer.	Arm 1: V3-OVA vaccine (containing ovarian cancer antigens	The effect on the level of tumor markers in the blood will be measure as a secondary outcome (including CA-125)
AdORN	ClinicalTrials.gov	A Phase I/II, single-arm, open-label trial is being done to study the use of atezolizumab with neoadjuvant chemotherapy in patients with newly diagnosed advanced-stage epithelial ovarian cancer before and after cytoreductive surgery.	Arm 1: Atezolizumab, carboplatin, and paclitaxel (and optional bevacizumab)	PFS will be divided into groups base on how much PD-L1, tumor-infiltrating lymphocytes, immune checkpoint receptors, cytokines, and gene expression profiles are expressed. Each of these subsets will be divide into even smaller groups based on th BRCA mutation status and the tumo mutation profile.
OLAPem	ClinicalTrials.gov	A single-arm, open-label Phase II trial is being done to study the use of olaparib alone and olaparib and pembrolizumab together to treat ovarian cancer.	Arm 1, Cohort 1: Olaparib before surgery Arm 1, Cohort 2: Olaparib and pembrolizumab before surgery	Biomarkers (germline mutations), changes in tumor-infiltrating lymphocytes, and the number of mutations in the tumor will be used t determine the therapeutic effect.

with Vigil improved effectiveness and lowered atezolizumab-related side effects associated with therapy. OS was not achieved in the first treatment arm of Vigil and was 10.8 months in the first arm of atezolizumab (HR 0.33). It was also shown in this study that Vigil may have a greater therapeutic benefit in BRCA wild-type patients (NR in Vigil first vs. 5.2 in atezolizumab first HR 0.16, p = 0.027). Continued research is necessary, according to the limited cohort of patient's clinical outcomes and safety profiles (51).

Combined treatment approaches

Research studies examining the effects of autologous DC vaccination combined with ICIs and chemotherapy should also be taken into account. One included three therapy groups in a phase I investigation of women with recurrent ovarian cancer. The autologous DC vaccination that had been pulsed with tumor cells was administered as monotherapy to the first group. The autologous DC vaccine was also administered in combination with bevacizumab to the second group. The findings showed that following immunization, CD4+, and CD8+ T-cell numbers considerably increased. Two of the 25 patients who received treatment were indicated to have shown a partial response, while 13 patients reported stable illness for a median of 14 months after immunization (52). The data were further examined based on vaccination response, which was identified by T-cell identification of tumor cells. The 2-year survival rate among the 11 out of 25 people who reacted to the vaccination was 100%; whereas, the 2-year survival rate among the non-responders was 25%. This research is essential in demonstrating how the proper patients with the best probability of responding to the medication might be crucial in improving results. Determining the elements that influence therapy response is thus quite helpful and should be investigated going forward. The study's emphasis on the advantages of cyclophosphamide, bevacizumab, and the autologous DC vaccination in combination is another crucial point. Eight out of ten patients were found to have responded to the vaccination in the group receiving cyclophosphamide in combination, compared to three out of 12 in the group receiving just the vaccine. A rise in TGF-β was seen after vaccination and again after cyclophosphamide injection, related to the responsiveness of the vaccine, according to further serum studies (52). It is necessary to do further study to show the full impacts of autologous vaccinations, which might have additional advantages when combined with already fully studied and effective medicines to give customized medicine.

These trials show promising outcomes for cancer vaccination. Additionally, a promising next step to make tumors sensitive to the neoantigen repertoire is the combination of immunotherapy and vaccination (53). This approach has to be developed further, as does the understanding of the biomarkers that predict response. A major part of how the body reacts to immunotherapies may be played by autophagy. Elevated MHC-II levels are linked to a better prognosis and overall survival in ovarian cancer (53).

One way for cells to produce more neoantigens for MHC-II presentation is via autophagy. One mechanism for the complicated control of autophagy includes BRCA1/2 (54). Compared to BRCA1/2 wild-type cells, autophagy is increased in BRCA1/2 mutant cells. Autophagy inducers in combination with immunological treatments, such as checkpoint inhibitors and autologous tumor vaccines, may thus be a sensible strategy in BRCA wild-type malignancies (55). However, autophagy inhibitors may also be employed to make tumors more sensitive to chemotherapy or to improve their response. In reaction to stress, particularly chemotherapy, autophagy is increased. Autophagy inhibitors may reduce cell viability by collaborating with chemotherapy, according to preclinical investigations. Context may affect whether autophagy inducers or inhibitors are used (56).

CONCLUSIONS

Early research suggested that ovarian cancer may be immunogenic as a result of several causes, including homologous repair inadequacy brought on by widespread BRCA mutation. However, compared to the majority of other immunogenic tumor forms, such NSCLC and melanoma, ovarian cancer immunotherapies have had less effectiveness. To increase the effectiveness of immunotherapy application to ovarian cancer, many strategies are being modified. These include choosing individuals based on immune profile, such as MSI-H/dMMR, HRD, and combining ICI with other therapies. Further study is required to properly describe immunological features common to ovarian cancer, identify optimum response indicators, and improve the patient selection for treatment. To reliably predict response, more than one biomarker could be required due to the complicated immunological landscape of ovarian cancer. It is vital to do deeper investigation of effectiveness and risk since combinatorial therapies seem to be an optimistic alternative for optimizing therapeutic benefit.

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REFERENCES

1. Torre, Lindsey A et al. "Ovarian cancer statistics, 2018." CA: a cancer journal for clinicians vol. 68,4 (2018): 284-296.
2. Siegel, Rebecca L., Kimberly D. Miller, and Ahmedin Jemal. "Cancer statistics, 2019." CA: a cancer journal for clinicians 69.1 (2019): 7-34.
3. Howlader, Nadia, et al. "Differences in breast cancer survival by molecular subtypes in the United States." Cancer Epidemiology, Biomarkers & Prevention 27.6 (2018): 619-626.
4. Winarto, Hariyono, et al. "Overall Survival and Related Factors of Advanced-stage Epithelial Ovarian Cancer Patients Underwent Debulking Surgery in Jakarta, Indonesia: A Single-center Experience." Open Access Macedonian Journal of Medical Sciences 10.B (2022): 265-280.
5. Perez-Fidalgo, J. Alejandro, et al. "Systemic treatment of newly diagnosed advanced epithelial ovarian cancer: From chemotherapy to precision medicine." Critical Reviews in Oncology/Hematology 158 (2021): 103209.
6. Morand, Susan, et al. "Ovarian cancer immunotherapy and personalized medicine." International Journal of Molecular Sciences 22.12 (2021): 6532.
7. González-Martín, Antonio, et al. "Niraparib in patients with newly diagnosed advanced ovarian cancer." New England Journal of Medicine 381.25 (2019): 2391-2402.
8. Salmaninejad, Arash, et al. "PD-1/PD-L1 pathway: Basic biology and role in cancer immunotherapy." Journal of cellular physiology 234.10 (2019): 16824-16837.
9. Tumeh, Paul C., et al. "PD-1 blockade induces responses by inhibiting adaptive immune resistance." Nature 515.7528 (2014): 568-571.
10. Granier, Clemence, et al. "Mechanisms of action and rationale for the use of checkpoint inhibitors in cancer." ESMO open 2.2 (2017): e000213.
11. Keenan, Tanya E., Kelly P. Burke, and Eliezer M. Van Allen. "Genomic correlates of response to immune checkpoint blockade." Nature medicine 25.3 (2019): 389-402.
12. Conway, Jake R., et al. "Genomics of response to immune checkpoint therapies for cancer: implications for precision medicine." Genome medicine 10.1 (2018): 1-18.
13. Pellegrino, Benedetta, et al. "Homologous recombination repair deficiency and the immune response in breast cancer: a literature review." Translational oncology 13.2 (2020): 410-422.
14. Paijens, Sterre T., et al. "Tumor-infiltrating lymphocytes in the immunotherapy era." Cellular & molecular immunology 18.4 (2021): 842-859.
15. Meléndez, Bárbara, et al. "Methods of measurement for tumor mutational burden in tumor tissue." Translational lung cancer research 7.6 (2018): 661.
16. Choucair, Khalil, et al. "TMB: a promising immune-response biomarker, and potential spearhead in advancing targeted therapy trials." Cancer gene therapy 27.12 (2020): 841-853.
17. Yarchoan, Mark, Alexander Hopkins, and Elizabeth M. Jaffee. "Tumor mutational burden and response rate to PD-1 inhibition." New England Journal of Medicine 377.25 (2017): 2500-2501.
18. Yarchoan, Mark, et al. "PD-L1 expression and tumor mutational burden are independent biomarkers in most cancers." JCI insight 4.6 (2019).
19. Hellmann, Matthew D., et al. "Tumor mutational burden and efficacy of nivolumab monotherapy and in combination with ipilimumab in small-cell lung cancer." Cancer cell 33.5 (2018): 853-861.
20. Chan, Timothy A., et al. "Development of tumor mutation burden as an immunotherapy biomarker: utility for the oncology clinic." Annals of Oncology 30.1 (2019): 44-56.
21. McGranahan, Nicholas, et al. "Clonal neoantigens elicit T cell immunoreactivity and sensitivity to immune checkpoint blockade." Science 351.6280 (2016): 1463-1469.
22. Benvenuto, Monica, et al. "Tumor antigens heterogeneity and immune response-targeting neoantigens in breast cancer." Seminars in Cancer Biology. Vol. 72. Academic Press, 2021.
23. Alsaab, Hashem O., et al. "PD-1 and PD-L1 checkpoint signaling inhibition for cancer immunotherapy: mechanism, combinations, and clinical outcome." Frontiers in pharmacology 8 (2017): 561.
24. Ansell, Stephen M., et al. "PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma." New England Journal of Medicine 372.4 (2015): 311-319.
25. Keung, Man Yee T., Yanyuan Wu, and Jaydutt V. Vadgama. "PARP inhibitors as a therapeutic agent for homologous recombination deficiency in breast cancers." Journal of clinical medicine 8.4 (2019): 435.
26. Moschetta, M., et al. "BRCA somatic mutations and epigenetic BRCA modifications in serous ovarian cancer." Annals of Oncology 27.8 (2016): 1449-1455.
27. Elvin, Julia Andrea, et al. "Comprehensive genomic profiling (CGP) with loss of heterozygosity (LOH) to identify therapeutically relevant subsets of ovarian cancer (OC)." (2017): 5512-5512.
28. da Cunha Colombo Bonadio, Renata Rodrigues, et al. "Homologous recombination deficiency in ovarian cancer: a review of its epidemiology and management." Clinics 73 (2018).
29. Konstantinopoulos, Panagiotis A., et al. "Homologous recombination deficiency: exploiting the fundamental vulnerability of ovarian cancer." Cancer discovery 5.11 (2015): 1137-1154.
30. Frey, Melissa K., and Bhavana Pothuri. "Homologous recombination deficiency (HRD) testing in ovarian cancer clinical practice: a review of the literature." Gynecologic oncology research and practice 4.1 (2017): 1-11.

31.Manchana, Tarinee, Natacha Phoolcharoen, and Patou Tantbirojn. "BRCA mutation in high grade epithelial ovarian cancers." Gynecologic oncology reports 29 (2019): 102-105.
32.Cortellini, Alessio, et al. "Family history of cancer as surrogate predictor for immunotherapy with anti-PD1/PD-L1 agents: preliminary report of the FAMI-L1 study." Immunotherapy 10.8 (2018): 643-655.
33.Dunphy, Gillian, et al. "Non-canonical activation of the DNA sensing adaptor STING by ATM and IFI16 mediates NF-κB signaling after nuclear DNA damage." Molecular cell 71.5 (2018): 745-760.
34.Plesca, Ioana, et al. "Characteristics of tumor-infiltrating lymphocytes prior to and during immune checkpoint inhibitor therapy." Frontiers in Immunology 11 (2020): 364.
35.Mosier, Jenna A., et al. "Cancer cell metabolic plasticity in migration and metastasis." Clinical & Experimental Metastasis 38.4 (2021): 343-359.
36.Clarke, Blaise, et al. "Intraepithelial T cells and prognosis in ovarian carcinoma: novel associations with stage, tumor type, and BRCA1 loss." Modern Pathology 22.3 (2009): 393-402.
37.Hwang, Wei-Ting, et al. "Prognostic significance of tumor-infiltrating T cells in ovarian cancer: a meta-analysis." Gynecologic oncology 124.2 (2012): 192-198.
38.Westergaard, Marie Christine Wulff, et al. "Tumour-reactive T cell subsets in the microenvironment of ovarian cancer." British journal of cancer 120.4 (2019): 424-434.
39.Frey, Melissa K., and Bhavana Pothuri. "Homologous recombination deficiency (HRD) testing in ovarian cancer clinical practice: a review of the literature." Gynecologic oncology research and practice 4.1 (2017): 1-11.
40.Barber, Emma, and Daniela Matei. "Immunotherapy in ovarian cancer: we are not there yet." The Lancet Oncology 22.7 (2021): 903-905.
41.Ledermann, Jonathan A., et al. "Avelumab in combination with and/or following chemotherapy vs chemotherapy alone in patients with previously untreated epithelial ovarian cancer: results from the phase 3 javelin ovarian 100 trial." SGO 2020 Annual Meeting on Women's Cancer. SGO, 2020.
42.Moore, Kathleen N., and Sandro Pignata. "Trials in progress: IMagyn050/GOG 3015/ENGOT-OV39. A Phase III, multicenter, randomized study of atezolizumab versus placebo administered in combination with paclitaxel, carboplatin, and bevacizumab to patients with newly-diagnosed stage III or stage IV ovarian, fallopian tube, or primary peritoneal cancer." International journal of gynecologic cancer 29.2 (2019).
43.Marabelle, Aurelien, et al. "Efficacy of pembrolizumab in patients with noncolorectal high microsatellite instability/mismatch repair–deficient cancer: Results from the phase II KEYNOTE-158 study." Journal of Clinical Oncology 38.1 (2020): 1.
44.Matulonis, U. A., et al. "Antitumor activity and safety of pembrolizumab in patients with advanced recurrent ovarian cancer: results from the phase II KEYNOTE-100 study." Annals of Oncology 30.7 (2019): 1080-1087.
45.Sabbatini, Paul, et al. "Abagovomab as maintenance therapy in patients with epithelial ovarian cancer: a phase III trial of the AGO OVAR, COGI, GINECO, and GEICO—the MIMOSA study." Journal of clinical oncology 31.12 (2013): 1554.
46.Zhang, Wen, et al. "Phase I/II clinical trial of a Wilms' tumor 1-targeted dendritic cell vaccination-based immunotherapy in patients with advanced cancer." Cancer Immunology, Immunotherapy 68.1 (2019): 121-130.
47.Sarivalasis, Apostolos, et al. "A Phase I/II trial comparing autologous dendritic cell vaccine pulsed either with personalized peptides (PEP-DC) or with tumor lysate (OC-DC) in patients with advanced high-grade ovarian serous carcinoma." Journal of translational medicine 17.1 (2019): 1-10.
48.Han, Yun et al. "Wilms' tumor 1 (WT1) promotes ovarian cancer progression by regulating E-cadherin and ERK1/2 signaling." Cell cycle (Georgetown, Tex.) vol. 19,20 (2020): 2662-2675.
49.Salvatorelli, Lucia, et al. "Wilms tumor 1 (WT1) protein: Diagnostic utility in pediatric tumors." Acta histochemica 117.4-5 (2015): 367-378.
50.Thomas, Sunil, and George C. Prendergast. "Cancer vaccines: a brief overview." Vaccine Design (2016): 755-761.
51.Kverneland, Anders Handrup, et al. "Adoptive cell therapy in combination with checkpoint inhibitors in ovarian cancer." Oncotarget 11.22 (2020): 2092.
52.Rocconi, Rodney Paul, et al. "A phase I combination study of vigil and atezolizumab in recurrent/refractory advanced-stage ovarian cancer: Efficacy assessment in BRCA1/2-wt patients." (2020): 3002-3002.
53.Wang, Peipei et al. "Beyond Tumor Mutation Burden: Tumor Neoantigen Burden as a Biomarker for Immunotherapy and Other Types of Therapy." Frontiers in oncology vol. 11 672677. 29 Apr. 2021,
54.Tanyi, Janos L., et al. "Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer." Science translational medicine 10.436 (2018): eaao5931.
55.Morand, Susan, et al. "BRCA1/2 mutation status impact on autophagy and immune response: Unheralded target." JNCI Cancer Spectrum 4.6 (2020): pkaa077.
56.Jeda, Ali Salimi, et al. "Autophagy Modulation and Cancer Combination Therapy: A Smart Approach in Cancer Therapy." Cancer Treatment and Research Communications (2022): 100512.

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Treatment Insights from Long COVID Syndrome Emerging as Neuropsychiatric Exacerbations in Autism Spectrum Disorder

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Abstract:
Even if the symptoms during the acute phase are minimal, COVID-19 not only results in severe respiratory problems but also long-term consequences. Significant long-term consequences are now being identified as neurological and neuropsychiatric problems. The onset of neuropsychiatric symptoms brought on by a lengthy COVID might be challenging to detect and treat in patients with behavioral problems, such as those with autism spectrum disorders (ASD). In this article, we describe three instances of ASD that showed a substantial worsening of neuropsychiatric symptoms after exposure to COVID-19 and subsequent difficulties controlling the post-COVID neuropsychiatric symptoms. The therapy intended to target COVID-19-induced immune reaction was delayed because Case 1 caught SARS-CoV-2 in the early phases of the epidemic. Case 2 had a verified COVID-19 exposure but showed no symptoms during the acute phase, however, she later had severe neuropsychiatric symptoms. Case 3 had a challenging course, in part because of underlying immunological dysregulation and the past use of many immunomodulating drugs. Significant variations in peripheral blood monocytes' generation of inflammatory and counter-regulatory cytokines were seen in cases 1 and 3, for which serial blood samples were taken. The instances discussed here show how COVID-19 has a significant impact on neuropsychiatric symptoms in ASD patients as well as how challenging it is to treat long-term COVID side effects.

INTRODUCTION

Our healthcare system was severely disrupted by the severe acute respiratory disorder coronavirus 2 (SARS-CoV-2)-which caused the coronavirus disease 2019 (COVID-19) epidemic. Both children and people with intellectual disabilities were affected, as was obvious (1, 2). It was shown that COVID-19 patients frequently experienced a long-term sequela that affected several organ systems, termed the "long COVID syndrome," as the pandemic expanded (3, 4). Both children and young people are impacted by this illness. Due in part to the absence of a precise case definition, the frequency of protracted COVID disorder in children is not yet fully characterized (5, 6).

A typical symptom of protracted COVID disorder is the cognitive disturbance, sometimes known as "brain fog," which is recorded in one out of every four to five post-COVID-19 individuals (7). Along with other neuropsychiatric symptoms such as anxiety, restless sleep, exhaustion, and melancholy mood, this disease is characterized by deficits in attention, concentration, memory, speed of information processing, and executive function (7, 8). Such persistent post-COVID-19 neuropsychiatric symptoms may have a major negative impact on quality of life (QOL) and academic and occupational performance.

Long-term COVID syndrome patients often exhibit cognitive deficits that are similar to the disorder of cancer therapy-related cognitive impairment (CRCI), which is commonly recognized as a side effect of intrathecal methotrexate injection (9). Chronic fatigue syndrome (CFS) and CRCI have several characteristics (3, 10). Microglial cells have a role in the systemic inflammation brought on by modest levels of lipopolysaccharide (LPS) (9), which may result in reactive microglia in the white matter and monocyte/macrophage lineage cells being attracted to the brain (11,12).

It may be challenging to discern between the long-term effects of the above-mentioned neurological impairment in children and the psychological effects of the COVID-19 epidemic (5). COVID has enhanced parenting stress, which can lead to behavioral issues (13). This is especially true for parents of children with autism spectrum disorder (ASD), where it has been linked to increased levels of anxiety and depression as well as the development of more maladaptive behaviors in the child (14). It is even more challenging to discern between COVID-19-induced abnormal behavior and pre-existing neuropsychiatric problems if brain failure brought on by COVID-19 affects children who already have an intellectual handicap or other pre-existing neuropsychiatric illnesses, such as ASD.

ASD is a complicated developmental disability that is marked by poor social interaction, repetitive or restricting activities, and a high prevalence of coexisting diseases (15). It is anticipated that identification and treatment may be difficult for children with ASD who have brain damage as a long-term side effect of COVID-19. Such neuropsychiatric and neurological problems brought on by COVID-19 may be brushed off as typical ASD symptoms, losing the chance to treat COVID-19-induced neuro-inflammation and alleviate behavioral problems (16).

This article recounts three ASD individuals who had significant and long-lasting changes in their neuropsychiatric symptoms after taking COVID-19, changes that were difficult to treat. These instances highlight how challenging it is to treat the long-term neuropsychiatric effects of COVID-19 disease and the requirement for therapeutic approaches that specifically target COVID-19-induced immunological response.

MATERIALS AND METHODS

Research Topics

At Azad university organization, all individuals participated in the monocyte cytokine pattern evaluation methodology for ASD that was authorized by the institutional review board. Informed permission papers with parental signatures were acquired in every instance.

Profiles of monocyte cytokines

Monocytes from peripheral blood (PBMo) were extracted. PBMCs were separated using a Ficoll-Hypaque ultracentrifugation separation method. Using magnetic beads tagged with anti-CD3, CD7, CD16, CD123, and glycophorin A, PBMo was further purified from PBMCs (monocyte **MojoSort™ Isolation Kits**, BioLegend's, MA, USA, Cat. No. 480019)

PBMo were grown overnight at a frequency of 5×105/mL with or without innate immune system stimuli such as LPS, zymosan, CL097, and candida heat extraction as a source of ß-glucan. PBMos were incubated for 24 hours in RPMI 1640 containing additives with LPS (0.1 µg/mL, GIBCO-BRL, Gaithersburg, MD, USA), zymosan (50 µg/mL, Sigma-Aldrich, USA), C097 (liquid derivative of imidazoquinoline, 20 µM, InvivoGen, USA), and candida heat extractHCKA, heat killed candida albicans (107 cells/mL, InVivogen, CA) as a source of β-glucan. the number of cytokines (IL-1ß, IL-6, IL-10, IL-23, TNF-, sTNFRII, IL-12p40, TGF-ß, and CCL2) in the growth supernatant was determined by ELISA (eBiosciences, San Diego, California). ELISA protocol was performed according to manufacturer's instrument.

RESULTS

Presentation of a Case

Case 1

The first case was a 25-year-old boy with ASD who was monitored at an allergy/immunology center. He arrived with recently developed extreme weariness, broad muscle problems, and increasing fluctuation of behavioral symptoms associated with an autism spectrum disorder. This came after a minor respiratory illness that had happened one month before the Tehran COVID-19 lockdown began. He had previously received diagnoses for common variable immunodeficiency, multiple seizures from intractable epilepsy, and ASD (level III) (CVID). Immunomodulatory medications were used to manage his refractory seizures (sirolimus and anakinra). Supplemental immunoglobulin (Ig) administered during biweekly subcutaneous (SQ) infusion helped manage recurrent bacterial infections and bacterially-induced seizure complexes. Additionally, he was taking azithromycin three times per week as part of a prophylactic program. This was brought on by a history of widespread cholesteatoma exacerbated by severe mastoiditis, which necessitated numerous surgical resections. Around the same time, his mother began to have similar respiratory problems, severe exhaustion, and "brain fog." It was determined that this patient and his mother both had COVID-19 due to the presence of SARS-CoV-2 antibodies. After each immunological insult (usually a microbial infection), he had a history of escalating behavioral symptoms (irritability, mood swings, anger, self-harming activities, and disrupted sleep), and even cognitive decline. He was administered a variety of anti-inflammatory treatments (NSAIDs, oral steroid burst, etc.), as well as maintenance immunomodulating pharmaceuticals provided to control his seizures since it was believed that his illness was related to post-infectious neuroinflammation. He and his mother both proceeded to have chronic exhaustion, "brain fog," joint and muscular pain, and dysautonomia problems, which were not alleviated by the anti-inflammatory drugs to which he had previously reacted. He and

his mother were severely affected by these problems, which harmed their quality of life. Mycophenolate was tested, however, it only partially relieved symptoms. Colchicine (0.6 mg dose) was initiated to inhibit such signaling pathway activation as a result of a better knowledge of neuropsychiatric symptoms as a long-term sequela of COVID-19 and stimulation pathways via SARS-CoV-2. He and his mother both reacted well to colchicine, with a significant improvement in long-term COVID problems. Both his mother's and his symptoms improved six months after taking colchicine. According to his mother, he did not, however, fully revert to his prior baseline. We were able to identify his deteriorating neuropsychiatric problems as long-term COVID-19 sequelae in his instance because of the comparable symptoms that his mother had experienced.

Case 2

Case 2 came into the clinic for the first time at the age of 14 as a result of repeated illnesses. After a noticeable delay in progress led to the diagnosis of ASD at the age of 36 months, she began having seizures at the age of 40 months. These seizures began as partially complicated seizures but later turned into secondarily generalized seizures. Many antiepileptic medications were unable to stop her seizures (AEDs). Between the ages of 6 and 11, she saw positive results with a modified Atkin's diet and stopped having seizures. The onset of puberty did, however, cause a recurrence of clinical seizure complexes. Her neurologist had recommended a high dosage of valproate at the time of her first presentation (375 mg in the morning, 1,125 mg at lunchtime, and 2,250 mg at bedtime). By the time she was 12 years old, she had developed a persistent respiratory illness. She was evaluated for secondary immunosuppression brought on by AED usage because valproate-induced hypogammaglobulinemia has been documented (18). Pneumococcal vaccination responses were diminished, according to our early workup (PPV23). Her recurring respiratory infections were under control, and the frequency of her seizures reduced thanks to further SQ Ig. After that, she and every member of the family caught COVID-19. Due to her complete COVID-19 vaccination before the COVID-19 exposure, the patient didn't exhibit any symptoms. Her valproate dosage was gradually tapered down beginning at the age of 15. Her mother didn't notice any significant acute symptoms; such as taste loss. The individual could verbally convey all of his or her symptoms. She had severe exhaustion, a lack of appetite, and weight loss (>20 lbs over 2 months from the beginning of neuropsychiatric symptoms) two to three weeks after developing COVID-19. She was prescribed celecoxib 100 mg bid for two weeks before switching to colchicine 0.6 mg bid since it was determined that she had been exposed to COVID-19 before the commencement of her neuropsychiatric symptoms. After using colchicine for three months, her neuropsychiatric problems progressively improved over 10 to 12 weeks, in part because of her gastrointestinal (GI) issues (mainly loose stool). After contracting COVID-19, she did not have any seizure clusters. Her neuropsychiatric problems significantly increased when colchicine was stopped, manifesting as extreme mood swings and tantrums that were managed by increasing the dosage of valproate. Celecoxib is still needed for her every

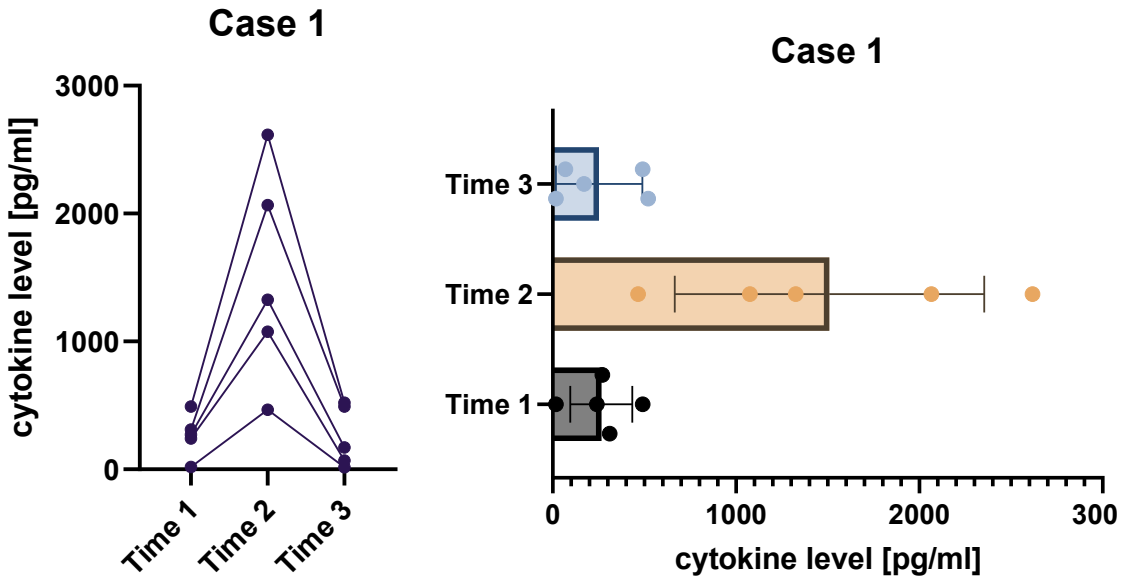

Fig 1. Changes in spontaneous generation of TNF-α, IL-1ß, IL-12, TGF-ß, and IL-10 by purified peripheral blood monocytes in Cases 1 at different time point. time point 1: before COVID-19, time point 2: after suffering from COVID-19, and time point 3: after colchicine treatment.

two weeks to treat behavioral symptoms and flare-ups brought on by modest immunological shocks such as microbial infection. Her prior monocyte cytokine pattern suggested high responses to innate immunity simulating viral infection, which should be successfully counter-regulated by celecoxib, even though we did not study it after her SARS-CoV-2 exposures. Celecoxib worked well to reduce small flare-ups.

Case 3

Case 3 involves a boy patient with ASD who first visited our pediatric allergy/immunology department at the age of 6 with worries about changing maladaptive behaviors that were often brought on by immunological insults. He was given an ASD diagnosis when he was 3 years old, but the conventional ASD therapies have not worked for him. No symptom alleviation was offered by the hematopoietic stem cell transplant he had at the university a year before his first visit. He has since received high-dose intravenous immunoglobulin (IVIg) therapy for autoimmune encephalitis (AE). But thorough AE testing came up empty on any AE-related autoantibodies. Later, SQIg was substituted for IVIg to reduce post-infectious neuroinflammation. Several therapies were explored when he arrived at this facility, including several neurotropic medicines, antioxidants, and immunomodulating drugs (mycophenolate, mTOR inhibitor, etc.). A novel discovery of vEEG anomalies led to the selection of the mTOR inhibitor sirolimus (19). Following a transient symptomatic alleviation with sirolimus, the vEEG returned to normal. No harmful gene variations were found after extensive genetic testing, including whole exome and genome sequencing (WES and WGS). An extensive immunological evaluation with an emphasis on neuroinflammation at another hospital produced no notable outcomes. At age 9, the vEEG changed once again due to the reappearance of clinically proven epileptic activity. The purpose of starting Anakinra was to limit seizure activity while providing transient symptom relief. However, it became very challenging to control his behavioral problems. Then, everyone in the family had COVID-19, and his behavioral problems became worse. Around that time, a vEEG showed generalized epileptic activity. Other healthcare professionals treated him for many months for seronegative AE with an oral steroid bolus (dexamethasone 12 mg/day 3 every month) without any symptomatic alleviation. Since his most recent deterioration of behavioral issues was probably brought on by COVID-19, baricitinib, a JAK1/2 inhibitor, was begun and helped to alleviate some symptoms. Due to increasing epileptic activity after ceasing sirolimus post-COVID-19, sirolimus was restarted. It had been stopped after acquiring COVID-19. With the help of baricitinib, sirolimus, and mycophenolate, his health stabilized, even though he continued to have flare-ups of neuropsychiatric symptoms after each immunological assault. A brief treatment of colchicine helped to normalize his behavioral problems due to a recent immunological assault. Then, for a brief period, minocycline was used in the hope that inhibiting indoleamine 2, 3-dioxygenase (IDO) function would provide further symptomatic relief for the acute worsening of his neuropsychiatric symptoms, which were ostensibly brought on by a viral illness. It did

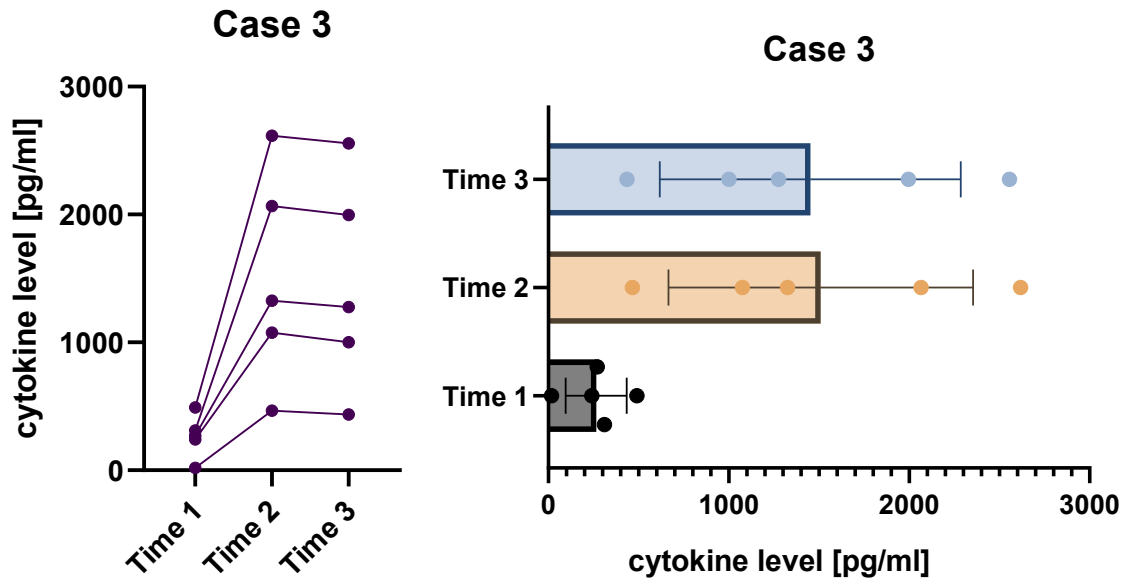

Fig 2. Changes in spontaneous generation of TNF-α, IL-1ß, IL-12, TGF-ß, and IL-10 by purified peripheral blood monocytes in Cases 3 at different time point. time point 1: before immune activation, time point 2: after immune activation by viral infection (not SARS-CoV-2), time point 3: after suffering from COVID-19.

alleviate certain symptoms..

Monocyte Cytokine Profiling Variations Prior to and Following COVID-19

For Cases 1 and 3, it was possible to compare the monocyte cytokine patterns before and after being infected with SARS-CoV-2. In Case 1, the most striking observation was a rise in the spontaneous generation of inflammatory (IL-1ß), tumor necrosis factor-α (TNF-α), and IL-12) and counter-regulatory (IL-10 and TGF-ß) cytokines 14 months after COVID-19 disease (time point 2). 4 months after commencing colchicine, these increases recovered to their baseline levels (time point 3). In Case 3, there was a considerable aggravation of his neuropsychiatric symptoms that increased the endogenous production of inflammatory cytokines (IL-1ß, IL-10, IL-12, and TGF-ß) before COVID-19 treatment (time point 2). Eight months after being exposed to COVID-19, a similar rise in monocyte inflammatory cytokines was still present (time point 3). The TNF-α synthesis also rose spontaneously after COVID-19. For both Cases 1 and 3, it seems that COVID-19 had less of an impact on the generation of these mediators in reaction to innate immunity cues. Significant variations in IL-6 serum concentrations throughout the antepartum and postoperative phases have been linked to an increased risk of ASD, according to the research (20). In the situations that are being discussed, we have analyzed IL-6 generation but have not seen any notable alterations. The fact that plasma IL-6 concentrations reflect synthesis from numerous cellular origins, not only monocytes, and that they have a lengthy half-life in the serum may be related to this.

DISCUSSION

According to reports, the clinical presentation of protracted COVID illness often includes neurological and neuropsychiatric symptoms including “brain fog.” Even if one only has modest COVID-19 symptoms, this may still happen after getting over the acute period (21). In ASD participants for whom the early stages of COVID-19 were either moderate or asymptomatic, the examples described here demonstrate a range of clinical symptoms of long-term COVID-19 aftereffects. According to certain theories (21, 22), a percentage of people with ASD may have neuroinflammation that is brought on by intricate interplay between hereditary and environmental variables. Such ASD patients should have neuroinflammation flare-ups in response to an immunological stimulation that activates the systemic immune system; this condition is likely to happen in conjunction with a viral disease. Due to a strong and persistent immunological activation, the formation of COVID-19 and its long-term repercussions have been found to impact various organ subsystems, such as the brain (23–25). Therefore, COVID-19 is anticipated to have a significant impact on the level of neuroinflammation in ASD patients who already have an inflammatory component. However, when the COVID-19 epidemic first started, this wasn’t first understood. In our experience, several ASD patients had thorough examinations for ailments like AE and other autoimmune diseases that have symptoms of ASD. Case 1 had an autoimmune examination because of his ongoing lethargy and musculoskeletal problems. The fact that his mother had identical clinical symptoms after getting COVID-19 at about the same period as the patient in his instance, however, gives us a hint for identifying the severe, long-lasting consequences of COVID-19. We were able to identify the ongoing in vivo activation of monocytes and macrophages thanks to the rise in spontaneous monocyte inflammatory cytokines (Figure 1) as well. We weren’t yet sure which immunomodulating drugs would be effective in managing his condition. Others’ research on the immunological activation caused by COVID-19, however, highlighted the significance of the signaling pathways that activate type I IFNs, which then activate the inflammasome systems (23, 24, 26). These results prompted us to think of using inflammasome blockers to modulate the downregulation stimulation of cells of the monocyte/macrophage lineage. A study with inhibitors of generated phagocytic cells was deemed reasonable given that this individual has been receiving treatment with an mTOR blocker (sirolimus), despite reports of the continued presence of stimulated T and B cells (26). Additionally, colchicine has been shown to have a positive effect on COVID-19, however, most of these publications only addressed the acute phase of the disease (27–29). Even one year after COVID-19, we saw a rise in the spontaneous generation of monocyte cytokines, which led us to believe that colchicine should be tested. He did show positive reactions. The significance of carefully choosing immunomodulating drugs appropriate for each condition may be highlighted by this example.

Despite the patient in Case 2 being asymptomatic and in the acute period of COVID-19, we were able to identify the likelihood of protracted COVID-19 immediately. Given her subsequent hypogammaglobulinemia, which was probably brought on by the high dosage of valproate (17), and Case 1’s positive clinical outcomes with colchicine, she also was given colchicine and had a positive clinical outcome. However, this participant’s neuropsychiatric issues became worse after the colchicine was stopped because of GI difficulties. This example also demonstrates the potential for even asymptomatic COVID-19 to potentially have serious neurological consequences, necessitating the potential need for an extended course of immunomodulating medications that target protracted COVID.

Among the most challenging examples, we came

across was Case 3, which is depicted in this article. Despite a trial of many immunomodulating drugs, the patient's immune system was in this instance already noticeably active in vivo, as seen in Figure 1. He most likely had neuroinflammation that was made worse by COVID-19. After he developed COVID-19, we used baricitinib, a JAK1/2 inhibitor, to disrupt downstream type 1 IFN signal transduction given the past usage of other immunomodulating drugs that targeted different signaling pathways. In extreme COVID-19 patients, baricitinib has been shown to have positive immunomodulating actions and has been suggested as a viable COVID-19 treatment drug (30, 31). Baricitinib produced some clinical results in the patient, but once the immune system was subsequently activated by infected contacts (not COVID-19), its pharmacological effects vanished (32-34). To suppress the post-COVID-19 immune reaction, colchicine was subsequently administered to have a wider anti-inflammatory impact. The patient appeared to gain from receiving therapy that included both colchicine and baricitinib. This serves as an example of a situation in which a combination of several immunomodulating drugs may be necessary (30, 35). In example 1, the individual was already receiving mTOR inhibitor therapy (sirolimus). The good response to colchicine that was seen may have benefited from this. Based on these three clinical studies, it is challenging to provide comprehensive management recommendations for extended COVID in ASD patients because of the well-known variability of ASD. Instead, the instances are offered to highlight the difficulties in treating ASD patients and the potentially severe consequences of COVID-19 on these individuals. The instances mentioned may point to the necessity for individuals with ASD to have customized care, particularly if immune-mediated inflammation contributes to their behavioral dysfunction, as it does in extended COVID-19 disorder.

CONCLUSION

Overall, the three instances show that, even though COVID-19's clinical signs are moderate or asymptomatic, the virus may have dramatic, long-lasting effects on immunological activation, which might manifest as a marked worsening of neuropsychiatric symptoms in ASD participants. Such late-onset, long-lasting impacts of COVID-19 may be readily disregarded in the ASD community due to pre-existing, challenging-to-treat ASD behaviors and inadequate expressive language. Treatment of ASD individuals with extended COVID disorder will need a deeper understanding of the condition and available therapy alternatives.

REFERENCES

1.Lai CC, Shih TP, Ko WC, Tang HJ, Hsueh PR. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and coronavirus disease-2019 (COVID-19): The epidemic and the challenges. International journal of antimicrobial agents. 2020 Mar 1;55(3):105924.

2.Piri GT, Beiranvand S, Doosti A, Ghadiri AH, Haji MS. A review of the epidemiology and clinical signs of SARS-COV-2.2021:103-120.

3.Piri Gharaghie T, Beiranvand S, Ghadiri A, Hajimohammadi S. A review of bioinformatics studies on the function of structural and non-structural proteins and the level of glycoprotein inhibiting Heme metabolism by SARS-CoV-2 virus. Jundishapur Scientific Medical Journal. 2022 May 22;21(2).

4.Korompoki E, Gavriatopoulou M, Hicklen RS, Ntanasis-Stathopoulos I, Kastritis E, Fotiou D, Stamatelopoulos K, Terpos E, Kotanidou A, Hagberg CA, Dimopoulos MA. Epidemiology and organ specific sequelae of post-acute COVID19: a narrative review. Journal of Infection. 2021 Jul 1;83(1):1-6.

5.Walsh-Messinger J, Manis H, Vrabec A, Sizemore, BS J, Bishof K, Debidda M, Malaspina D, Greenspan N. The kids are not alright: a preliminary report of post-COVID syndrome in university students. Journal of American College Health. 2021 May 7:1-7.

6.Kim JH, Levine BD, Phelan D, Emery MS, Martinez MW, Chung EH, Thompson PD, Baggish AL. Coronavirus disease 2019 and the athletic heart: emerging perspectives on pathology, risks, and return to play. JAMA cardiology. 2021 Feb 1;6(2):219-27.

7.Garg M, Maralakunte M, Garg S, Dhooria S, Sehgal I, Bhalla AS, Vijayvergiya R, Grover S, Bhatia V, Jagia P, Bhalla A. The conundrum of 'long-COVID-19: a narrative review. International journal of general medicine. 2021;14:2491.

8.Sharma S, Nehra A. Cognitive functions in the geriatric population. InResearch Anthology on Diagnosing and Treating Neurocognitive Disorders 2021 (pp. 123-146). IGI Global.

9.Badenoch JB, Rengasamy ER, Watson C, Jansen K, Chakraborty S, Sundaram RD, Hafeez D, Burchill E, Saini A, Thomas L, Cross B. Persistent neuropsychiatric symptoms after COVID-19: a systematic review and meta-analysis. Brain communications. 2022;4(1):fcab297.

10.Lim EJ, Lee JS, Lee EJ, Jeong SJ, Park HY, Ahn YC, Son CG. Nationwide epidemiological characteristics of chronic fatigue syndrome in South Korea. Journal of Translational Medicine. 2021 Dec;19(1):1-6.

11.Zarinnezhad A, Shahhoseini MH, Piri Gharaghie T. Evaluating the Relative Frequency of Fungal Infections in the Serum of Patients with Multiple Sclerosis and Healthy Subjects Using PCR. Biological Journal of Microorganism. 2021 Mar 21;10(37):37-50.

12.Piri-Gharaghie T. Polycystic ovary syndrome and genetic factors influencing its development: A review article. Personalized Medicine Journal. 2021 Dec 1;6(23):25-9.

13.Bucciarelli V, Nasi M, Bianco F, Seferovic J, Ivkovic V, Gallina S, Mattioli AV. Depression pandemic and cardiovascular risk in the COVID-19 era and long COVID syndrome: gender makes a difference. Trends in Cardiovascular Medicine. 2021 Oct 5.

14.De Giacomo A, Pedaci C, Palmieri R, Simone M, Costabile A, Craig F. Psychological impact of the SARS-CoV-2 pandemic in children with neurodevelopmental disorders and their families: Evaluation before and during

COVID-19 outbreak among an Italian sample. Rivista di psichiatria. 2021 Jul 1;56(4):205-10.
15.Jyonouchi H, Geng L, Rossignol DA, Frye RE. Long COVID Syndrome Presenting as Neuropsychiatric Exacerbations in Autism Spectrum Disorder: Insights for Treatment. Journal of Personalized Medicine. 2022 Nov 2;12(11):1815.
16.Baweja R, Brown SL, Edwards EM, Murray MJ. COVID-19 pandemic and impact on patients with autism spectrum disorder. Journal of Autism and Developmental Disorders. 2022 Jan;52(1):473-82.
17.Jyonouchi H, Geng L. Associations between monocyte and T cell cytokine profiles in autism spectrum disorders: Effects of dysregulated innate immune responses on adaptive responses to recall antigens in a subset of ASD children. International journal of molecular sciences. 2019 Sep 24;20(19):4731.
18.Eom TH, Lee HS, Jang PS, Kim YH. Valproate-induced panhypogammaglobulinemia. Neurological Sciences. 2013 Jun;34(6):1003-4.
19.Griffith JL, Wong M. The mTOR pathway in treatment of epilepsy: a clinical update. Future neurology. 2018 May;13(2):49-58.
20.Tenório MC, Graciliano NG, Moura FA, Oliveira AC, Goulart MO. N-Acetylcysteine (NAC): impacts on human health. Antioxidants. 2021 Jun 16;10(6):967.
21.Mantovani A, Morrone MC, Patrono C, Santoro MG, Schiaffino S, Remuzzi G, Bussolati G. Long Covid: where we stand and challenges ahead. Cell Death & Differentiation. 2022 Oct;29(10):1891-900.
22.Harden RN, Bruehl S, Stanton-Hicks M, Wilson PR. Proposed new diagnostic criteria for complex regional pain syndrome. Pain medicine. 2007 May 1;8(4):326-31.
23.Padda I, Khehra N, Jaferi U, Mosabbeh D, Atwal H, Musaji A, Bhullar S. Organ system effects and reinfection of COVID-19: A Review. Journal of Research in Clinical Medicine. 2021 Feb 17;9(1):6-.
24.Francistiova L, Klepe A, Curley G, Gulya K, Dinnyes A, Filkor K. Cellular and molecular effects of SARS-CoV-2 linking lung infection to the brain. Frontiers in Immunology. 2021 Aug 13;12:730088.
25.Pelle MC, Zaffina I, Lucà S, Forte V, Trapanese V, Melina M, Giofrè F, Arturi F. Endothelial Dysfunction in COVID-19: Potential Mechanisms and Possible Therapeutic Options. Life. 2022 Oct 14;12(10):1605.
26.Mehandru S, Merad M. Pathological sequelae of long-haul COVID. Nature immunology. 2022 Feb;23(2):194-202.
27.Jyonouchi H, Geng L, Rossignol DA, Frye RE. Long COVID Syndrome Presenting as Neuropsychiatric Exacerbations in Autism Spectrum Disorder: Insights for Treatment. Journal of Personalized Medicine. 2022 Nov 2;12(11):1815.
28.Bonaventura A, Vecchié A, Dagna L, Tangianu F, Abbate A, Dentali F. Colchicine for COVID-19: targeting NLRP3 inflammasome to blunt hyperinflammation. Inflammation Research. 2022 Feb 3:1-5.
29.Mansouri N, Marjani M, Tabarsi P, von Garnier C, Mansouri D. Successful treatment of Covid-19 associated cytokine release syndrome with colchicine. a case report and review of literature. Immunological investigations. 2021 Nov 17;50(8):884-90.
30.Piri-Gharaghie T, Doosti A, Mirzaei SA. Identification of Antigenic Properties of Acinetobacter baumannii Proteins as Novel Putative Vaccine Candidates Using Reverse Vaccinology Approach. Applied Biochemistry and Biotechnology. 2022 Jun 7:1-23.
31.Beiranvand S, Piri-Gharaghie T, Dehganzad B, Khedmati F, Jalali F, AsadAlizadeh M, Momtaz H. Novel NAD-independent Avibacterium paragallinarum: Isolation, characterization and molecular identification in Iran. Veterinary Medicine and Science. 2022 May;8(3):1157-65.
32.Souod N, Kargar M, Doosti A, Ranjbar R, Sarshar M. Genetic analysis of cagA and vacA genes in Helicobacter pylori isolates and their relationship with gastroduodenal diseases in the west of Iran. Iranian Red Crescent Medical Journal. 2013 May;15(5):371.
33.Piri-Gharaghie T, Jegargoshe-Shirin N, Saremi-Nouri S, Khademhosseini SH, Hoseinnezhad-Lazarjani E, Mousavi A, Kabiri H, Rajaei N, Riahi A, Farhadi-Biregani A, Fatehi-Ghahfarokhi S. Effects of Imipenem-containing Niosome nanoparticles against high prevalence methicillin-resistant Staphylococcus Epidermidis biofilm formed. Scientific reports. 2022 Mar 24;12(1):1-3.
34.Azadbakht N, Doosti A, Jami MS. CRISPR/Cas9-mediated LINC00511 knockout strategies, increased apoptosis of breast cancer cells via suppressing antiapoptotic genes. Biological procedures online. 2022 Dec;24(1):1-5.
35.Ghajari G, Nabiuni M, Amini E. The association between testicular toxicity induced by Li2Co3 and protective effect of Ganoderma lucidum: Alteration of Bax & c-Kit genes expression. Tissue and Cell. 2021 Oct 1;72:101552.

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Opportunities and Challenges in Using Cancer Organoids Derived from Patients in Personalized Medicine

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Abstract:

A model system for precision medicine has been suggested using tumor organoids. Tumor organoids are unique for cancer research on a patient-by-patient basis because they are able to preserve properties of the original tumor. As a result, it is alluring to consider using tumor organoids to improve patient outcomes during clinical decision-making. Patient outcomes have a good correlation with tumor organoid responses to a variety of medicines in vitro. Before application in clinical cancer care can be considered, however, there are still significant obstacles to be overcome and large cohort prospective trials are desperately needed. Tumor organoids offer a lot of potential in preclinical research due to their unique traits and direct connection to patient data. Here, we have evaluated the most recent developments in the development and use of cancer organoids grown from patients for cancer biology research and customized treatment. We have concentrated on the potential of organoids as a platform for the discovery and creation of innovative targeted therapies for the most intractable malignancy, pancreatobiliary cancer.

INTRODUCTION

Human health is seriously threatened by cancer. The genetic makeup of many tumors has increasingly become known with the advancement of next-generation sequencing technologies. On the basis of the biological profile of malignancies, scientists throughout the world have been trying to develop molecular targeted treatments (1, 2). Nevertheless, only a few number of medications have received FDA approval following clinical studies (2). Treatment for cancer patients has changed over the past ten years, moving from treatment based on tumor type to treatment based on molecular features of a tumor or its microenvironment. This strategy, also known as precision medicine or individualized treatment, has improved the prognosis for many patients with advanced tumors and is being used in (neo-) adjuvant research (3).

Precision medicine has been driven by extensive tumor sequencing studies that resulted in the identification of various therapeutic targets. Although several breakthroughs based on DNA sequencing have been recorded, it is also evident that the majority of cancer patients still have an urgent demand for effective therapies (5). The emphasis on genetic abnormalities in a small number of coding areas has substantial drawbacks and fails to recognize or convey the disease's complexity. DNA sequencing has so far provided information on the factors that cause cancer that are rather well kept throughout the course of the disease, but it does not include additional modulators such epigenetic modifications or the impact of non-coding areas. These modulators are far more dynamic, and it is difficult to understand how they are relevant (6). Although cancer is a hereditary disorder, understanding the underlying malignant pathways at all cellular levels is important for developing effective therapies (6). It is obvious that there is a considerable need for a dynamic, adaptable model system that enables in-depth investigation of various aspects of tumor biology and accurately mimics the behavior of the primary tumor in patients. Ex vivo tumors that are alive and developed from individual patient have inspired a lot of interest in developing precision medicine. It is now feasible to grow tumors in three-dimensional (3D) structures on an individual basis according to the development of new technology. These so-called "organoids" are multicellular in vitro structures made from adult or embryonic stem cells that exhibit self-organization and self-renewal, as well as characteristics that match those of their origin tissue (7). In comparison to traditional 2D cell lines or patient-derived xenografts (PDXs), patient-derived cancer organoids (PDCOs) may be more appropriate for cancer research. In this article, we discuss PDCOs' present situation and potential for the future (8). We paid special attention to pancreatobiliary cancer organoids and their usage in personalized medicine. Our goal was to demonstrate their potential as a cancer model to better understand cancer biology and evaluate possible treatments in vitro, which would eventually lead to clinical applications and more

translational research.

Dimensional culture and organoid structure

An organoid is a 3D cell culture model that is similar to the source organs in the body. Additionally, an organoid is a population of cells that are specialized to an organ that are created from adult stem cells (AdSCs) or pluripotent stem cells (PSCs) (9). They can self-form similarly to the body by cell sorting and spatially constrained lineage differentiation. Currently, research including drug testing, disease modeling, and tissue regeneration and repair frequently employ animals as models; however, these models do not fully represent the physiological properties of the human body (10). The novel in vitro model organoid fills the gap between human beings and animal models by painstakingly recreating the cellular make-up and activity of a typical organism to mimic the physiological makeup of human organs (11). Organoid building benefits from personalization, rapid modeling, high-throughput genetic or pharmacological screening, and the potential for genetic modifications. In the fields of basic biology research, drug testing, and molecular medicine, organoids have emerged as a contemporary research hotspot with significant theoretical implications and promising future development (12). However, there are some disadvantages to organoid fabrication, including the inability to accurately replicate the in vivo microenvironment, insufficient vascularization, the slightly different size of organoid self-organization from that of normal organs, the lack of precise spatial ordering, and the lack of a recognized co-culture system with other cell types. Some of these drawbacks have been addressed by recent bioprinting methods (12).

Culture of organoids

Organoids are divided into two groups based on the features of their stem cells. Organoids made from pluripotent stem cells are the first, while organoids made from adult stem cells are the second. Both separate adult stem cells and organ-specific tissue pieces can be used to create organoids (13, 14). Cells may multiply in culture for a very long period while retaining their genetic stability and loyalty to their original tissue provided the right circumstances are met (15). The adult functioning tissues and somatic mutational processes in these cultures can be used as models for researching stem cell biology. Organoids are three-dimensional structures containing differentiated epithelial cells that have the capacity to self-organize and show important activities as mini organs when growth factors and suppressors are given to the media. With the identification of various niche factors that maintain specific microenvironments, it has become possible to culture organoids derived from various organs (7). Wnt, extracellular antagonist of BMP proteins (Noggin), inhibitor of TGF- type I receptor (A83-01), fibroblast growth factor, epidermal growth factor (EGF), epidermal growth factor (EGF), prostaglandin E2, nicotinamide, R-spondin, gastrin-I, and N-acetylcysteine amide are some of these niche factors. Organoids generated from a variety of organs, including the breast gland, bone, small intestine, stomach, colon, lung, liver, pancreas, prostate, fallopian tube, salivary gland, and tongue, have been described. Particularly, organoid technologies are adaptable (7). Theoretically, tiny tissue samples, which are normally taken by biopsy or from surgical specimens, can be used to start organoid cultures (7).

Cancer Organoid

Organoids offer enormous promise for use in many different contexts, including models for cancer progression, immunotherapy, and genetic illnesses. Among these, the available research suggests that organoids are preferable to 2D cell lines or PDXs as cancer models (16, 17). PDXs and cancer 2D cell lines, which are frequently employed as human cancer models, have major drawbacks despite having made substantial contributions to the field of cancer research. First, generating cell lines often has poor success rates, making the creation of cancer cell lines from primary tissue relatively ineffective (18). This is mostly because it can be difficult for cells to adapt to in vitro 2D growing circumstances. Additionally, because only a few clones are expected to persist and proliferate, the cultures occasionally fail to accurately recreate the genetic diversity of the original tumor. In order to examine the driver mutations necessary for tumor start and development, 2D cell lines may not be superior than organoids (19).

Fresh tissue from a patient is implanted into the subcutis or another orthotopic location in immunodeficient mice to produce PDXs. In contrast to 2D cultures, PDXs can accurately recreate the tumor microenvironment in a 3D structure, including the interaction of cancer cells with the stroma and the development of blood vessels [20]. However, PDX is a labor-intensive and expensive approach that has trouble engrafting and needs considerable resource inputs for its upkeep, making it unsuitable for high-throughput drug screening or genetic editing. Additionally, this method takes time and could need a tumor adaption particular to the host (the mouse) (20).

A number of cancer organoid cultures originated from humans have been created recently, and some information exploiting the characteristics of organoids has been presented (21-23). Depending on the kind of tumor, PDCO cultures have varying degrees of success. Organoid lines for colon, breast, lung, and liver malignancies were reported to be established with 90%, >80%, 70%, and 100% success rates, respectively. However, several tumor types or early-stage cancers, such as biliary tumors, intraductal papillary mucinous neoplasms of the pancreas, and ovarian tumors, are still challenging to culture and have poor chances of success. As described in more depth in following parts, cancer organoids have developed into a tool for research that integrates genetic editing methods with drug susceptibility testing, immunotherapy, and tumor microenvironmental investigations (22, 24, 25).

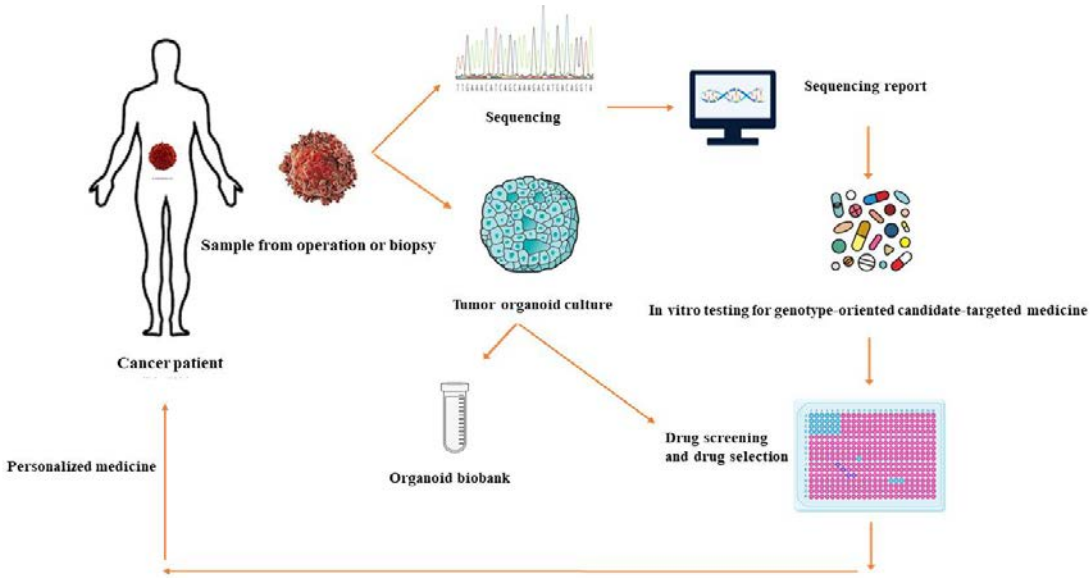

Fig 1. Organoid culturing and thorough genotyping are both components of the genotype-oriented, personalized medicine paradigm that we promote. Exome sequencing and organoid culture were combined in our model, which made it possible to find genotype-oriented targets for personalized medicine and evaluate the effectiveness of potential targeted medications in organoids.

Tumor organoid implementation in clinical decision making

Recent successes and future difficulties

In contrast to static sequencing data, tumor organoids keep the characteristics of the original tumor and provide the opportunity to investigate specific tumors as dynamic systems. MThe necessity for such a dynamic system is highlighted by the persistently high failure rate of medications evaluated in clinical trials, with success rates for cancer therapies in phase I–III clinical trials being as low as 3.4% (26). Tumor organoids may facilitate the transition from the bench to the bedside by providing a more flexible and individualized perspective on cancer biology and therapy response. Many scientists across the world have employed tumor organoids to address their scientific issues and generated a lot of data since the creation of the first organoid cultures made from stem cells (27). Long-term tumor organoid cultures may now be produced from a variety of human epithelial tissues, including the colon, liver, lung, pancreas, prostate, ovaries, bladder, breast, endometrial, esophagus, and others (27). In recent years, sizable live biobanks of tumor organoids have been developed, and this work has been widely described and debated by others. Along with such successes, further research on long-term organoid cultures indicates that organoids retain the phenotypic, genetic diversity, and mutational fingerprints of the original tumor sample. Furthermore, compared to other model systems, tumor organoids have better qualities. They fall between cell lines and patient-derived xenograft (PDX) models due to their biology. Transforming human malignancies into cell lines is far more difficult than doing it with organoids (28, 29).

The creation of a cancer cell monoculture from patient tissue has a reported success rate of about 26% for various cancer types. On the other hand, tumor organoids can be more easily established, less expensive to maintain, and do not require the use of experimental animals, in line with ethical goals regarding animal welfare (30). Tumor PDX models, however, continue to be the only system to capture individualized tumor growth in vivo, surrounded by and in crosstalk with the tumor microenvironment (TME) and parts of the immune system. All of the aforementioned benefits are crucial for clinical application and enable individualized tumor biology analysis by researchers (30).

The idea of taking a tumor specimen from a patient, producing and growing organoids, subjecting the patient-derived tumor organoids to a variety of medications, and then treating the patient with the best drug or combination of treatments appears to be within reach at this point. This would genuinely represent the best bench-to-bedside approach to cancer treatment (31). This strategy has been used by several organizations, including ours, and associations between organoid cultures before and after treatment and susceptibility to a certain medicine have been discovered. The hurdles that need to be overcome in order to properly harness the potential utility of tumor organoids for clinical decision making, however, have also been highlighted by humble experiences (32). The success rate of growing organoids varies between tumor types, allowing therapeutic translation to a minority of cancer patients even though it is higher than that of two-dimensional (2D) cultures. Resection vs biopsy, tumor cellularity, and the beginning material are all crucial elements in influencing the success of a culture, according to our experiences and those of others (33).

These variables differ significantly across different tumor types and are unquestionably a rate-limiting stage in the therapeutic use of organoids. Once a culture has been formed, the growth rate varies depending on

the tumor type, the intra- and inter-patient samples, and the culture itself. Slow-growing samples might cause a delay in making prompt decisions (34). It is not yet known whether they accurately reflect the features of the original tumor or if they could provide unintended biases in in vitro drug testing that rely on cellular growth. Additionally, cultivating organoids is time-consuming, and the necessity for a particular culture medium with a variety of growth factors is pricey and requires careful consideration based on the experimental outcome (35). ALK inhibitor A83-01 and p38 inhibitor SB202190 are two typical additives to organoid culture media that may interact with medications that target the same signaling pathway. Also a concern in prostate and non-small-cell lung cancer, contamination of normal epithelial cells can be damaging to the purity of organoid cultures. In addition to methodological difficulties, tumor organoids fail to include the TME, such as fibroblasts or immune cells, for instance (36). This has been somewhat addressed by the development of co-cultures, which are detailed below, but it still presents a challenge, particularly with regard to immunotherapy. It will be essential to find solutions to the existing issues if tumor organoids are to be used clinically.

View of pancreatobiliary cancer

PBC (pancreatobiliary carcinoma) is an incurable condition with a dismal prognosis. The lowest 5-year survival rate of all solid malignancies is found in those with pancreatic cancer, which is only 8%. Biliary cancer has a 5-year survival rate of about 25%, which is much lower than that for other gastrointestinal malignancies. Over the past few decades, PBC incidence and death rates have risen around the globe. To extend survival, curative resection with a negative surgical margin is required. The best therapeutic option is still surgical resection, however this has a major impact on the course of the disease. However, many patients with PBC have unresectable tumors when they are diagnosed, and thus have few chemotherapeutic alternatives available to them. Therefore, the creation of potent therapeutic medicines is critical (36, 37, 38). The emergence of next-generation sequencing technology has shed light on the genetic origins of PBC. Several molecularly targeted medications have been created recently, some of which have been administered to PBC patients. For instance, ivosidenib, an oral inhibitor of isocitrate dehydrogenase type 1, dramatically increased progression-free survival in patients with biliary cancer (IDH1) (39). Additionally, pre- and post-effectiveness chemotherapy's for PBC has been evaluated. Adjuvant chemotherapy is now the new standard of care for pancreatic cancer patients who have had resection; however, neoadjuvant chemotherapy may also be a normal procedure. In the past, prolonged surgery was used to treat PBC; today, multimodal therapy is used instead (40).

PBCs have distinctive mutational profiles compared with other cancers. TP53, SMAD4, KRAS, CDKN2A, and other gene alterations are often seen in pancreatic tumors. In particular, KRAS mutations are present in over 90% of pancreatic ductal adenocarcinomas, demonstrating the critical role aberrations in the RAS signaling pathway play in the development of pancreatic cancer (41). Additionally, in pancreatic tumors, other pathways and processes other than RAS signaling, including CDK, TGF-, SHH, JNK, and integrin signaling, are complicatedly changed, with some of the modifications frequently overlapping (42). Several different genes, including ARID1A, ELF3, ERBB2, IDH1, IDH2, KRAS, PIK3CA, and TP53, are mutated in biliary cancers. Biliary malignancies don't frequently exhibit distinctive somatic mutations; as a result, each tumor has a unique mutation pattern. Cancers with somatic mutations frequently present a target for molecularly targeted treatment. However, the variety of somatic mutations present in each patient makes the development of individualized therapy approaches necessary (42).

Organoid-derived pancreatic cancer

Compared to other malignancies, patient-derived PBC organoids have received fewer reports. Despite recent reports of a procedure for cultivating PBC organoids, the success rate of these cultures is still lower than that of other malignancies. There might be a number of variables that make it difficult to culture PBC organoids. First, there is a significant quantity of stroma and just a small number of live tumor cells in PBC resected tissues. This was particularly evident in samples of pancreatic cancer collected following neoadjuvant treatment (43). Second, as discussed in the previous section, PBC has different mutational patterns in comparison to other malignancies. These unique mutational profiles in PBC indicate that specific culture conditions are necessary to produce organoids by modifying growth factors to meet the mutational profile (43). We discovered that the cancer organoids that were grown from surgical specimens' tumor portions were mixed with normal epithelial organoids, and we demonstrated experimentally that it was required to remove the normal organoids in order to culture the cancer organoids. Organoids made from healthy epithelial cells had balloon-like structures and were devoid of primary tumor mutations, but organoids made from cancer cells had solid shapes and did, in fact, contain primary tumor mutations (44). A technique for the selective cultivation of pancreatic cancer organoids was described by Seino et al. by modifying the growth factors given to the culture media. Due of the high frequency of KRAS mutations in pancreatic ductal adenocarcinoma (PDAC), their technique calls for the organoids to first be put in an environment where EGF is deficient in order to enrich cancer organoids with an autonomously active EGFR-RAS pathway. To select probable TP53 or SMAD4 mutant organoids, respectively, organoids that are amenable to EGF removal are alternatively treated with Nutlin3 (an MDM2 inhibitor) or Noggin removal/ BMP4 (44).

As with biliary malignancies, our research and others' findings show that the mutations are not only unique from other cancers but also vary across

different tumors. This means that depending on the tumor, different culture conditions should be used. The presence of heterogeneity in a tumor, such as intraductal papillary neoplasm of the bile duct, is important and frequently poses significant challenges to culture (43, 45).

Recent research on PBC organoids has two main goals. The first is the use of cancer organoids in the screening of medicines. The second is to study cancer biology, including how tumor cells and their surrounding environment interact. Using a panel of 76 distinct therapeutic agents, Driehuis et al. developed a platform with 27 patient-derived pancreatic cancer organoids for high-throughput drug screening (46). Through the use of organoids and PDXs in treatment investigations, Hu et al. discovered SIRT5 to be a significant tumor suppressor in PDAC (47). Cholangiocarcinoma (CCA) organoids were examined for their unique 5- ALA-based photodynamic activity, and Fujiwara et al. proposed that this activity may be used as a diagnostic marker to distinguish CCA from non-tumor tissues (48). Research and therapy methods that target not just cancer cells but also the tumor microenvironment, such as fibroblasts or immune cells, may be necessary for PBC with an abundance of stroma. A co-culturing method for pancreatic organoids, fibroblasts, and T cells was developed by Tsai et al. They observed T cells invading the Matrigel, moving in the direction of the organoids, and spreading out at the boundary of Matrigel domes containing organoids (49). According to Koikawa et al., pancreatic stellate cells (PSCs) and organoids were co-cultured in direct contact and indirect contact systems. Their findings suggested that matrix metalloproteinase (MMP) 2, which binds to membrane type-1 MMP (MT1MMP) on PSCs, causes basement membrane breakdown and stromal invasion of organoids in direct contact with PSCs (50). Ohlund et al. were successful in co-culturing fibroblasts and pancreatic cancer organoids, and they were able to see how well they cooperated together in terms of growth morphology (51).

Personalized medicine

A treatment strategy based on a patient's DNA profile, environmental factors, and lifestyle is known as personalized medicine. The genetic profile of the patient's tumor should ideally serve as the basis for customized cancer treatment. Organoids created from surgically removed tumors of patients might potentially be highly helpful in developing tailored medication since they mimic the in vivo characteristics of the underlying tumor and enable in vitro biochemical testing. Personalized medicine systems that integrate thorough genotyping with organoid culture have, however, only been the subject of a few reports of effective implementation (52, 53).

Ideally, the tumor's genetic profile should serve as the basis for personalized cancer treatment. Finding genotype-oriented targets and choosing candidate-targeted treatments for cancers would be reasonable and effective (54). As an alternative, multidrug screening on cell lines originating from tumors may be useful to verify this impact. However, the generation of 2D cancer cell lines from primary tumor materials is relatively ineffective, and cancer cell lines do not always phenotypically mimic real malignancies (54). The organoid, often known as "a miniature of organ," inherits the gene alterations and characteristics of the parent tumor. Previous publications claim that even after prolonged cultivation of organoids, there is minimal genetic alteration. As a result, it is anticipated that organoids created and cultivated from recent tumor samples would be helpful in developing individualized treatment. Translational research on cancer should benefit greatly from using PDCOs as models (54).

The PDCO has been claimed to have some significant applications as a model for personalized medicine, which is predicted to benefit patients. The first is multidrug screening, like high-throughput screening. Organoids are ideal for multi-drug screening because, with the proper adjustments to the culture conditions, numerous clones may be produced quickly (46, 55, 56). High-throughput screening is acceptable for quickly identifying certain successful medications or finding novel compounds. Saito et al. performed a multi-drug screening of 339 therapeutically available drugs on biliary tract cancer organoids and experimentally showed that 22 substances, including antifungal agents, HMG-CoA reductase inhibitors, and dopamine D2 receptor agonists, inhibited organoid development (54). So, the benefit of multi-drug screening, such as high-throughput screening, is the discovery of novel medications and unexpected outcomes. In order to create an organoid from peritoneal diffused colorectal cancer, Narasimhan et al. conducted multi-drug screens. After conventional surgery and five cycles of chemoradiotherapy, they were able to discover a novel agent from the findings of drug screening that was clinically successful in patients with progressing illness (57). Four patients' main pancreatic cancer tumors and the organoids that were created from them were studied by Driehuis et al. for correlations in gemcitabine sensitivity. In all four cases, the sensitivity to gemcitabine was essentially same between original tumors and organoids, indicating that organoids can be used as a personalized medication in vitro testing paradigm (46).

Additionally, using organoids for the verification of genome-driven targeted treatments is particularly helpful for choosing individualized therapy for specific patients (46, 58, 59). According to certain biological pathways, Broutier et al. confirmed genomic alterations in original tumors and patient-derived cancer organoids. These pathways were the focus of the selection of potential targeted medications, and the effectiveness of those medications was examined in organoids (60).

Organoids have also been used in certain trials on immunotherapy. Nael et al. created a coherent group of endogenous syngeneic tumor-infiltrating lymphocytes (TILs) and primary tumor epithelial cells in co-culture. Immune checkpoint inhibition using anti-PD-1 and/or anti-PD-L1 increasing and activating

tumor antigen-specific TILs and inducing tumor cytotoxicity was successfully reproduced in human and mouse organoids (61). In order to investigate the cytotoxicity of lymphocytes made with a chimeric antigen receptor (CAR) in patient-derived colon organoids, Schnalzger et al. created a platform. They showed effective targeting in a variety of organoids utilizing CAR-engineered NK-92 cells that were targeted toward a common epithelial antigen, and CAR-NK-92 cells that were directed toward organoids expressing EGFRvIII were used to study tumor antigen-specific cytotoxicity. Finally, they presented a sensitive in vitro platform for a tailored assessment of CAR effectiveness and tumor specificity (62). Organoids have also been used in various customized medical models, albeit the number of reports is still fairly modest. Forsythe et al. tested the most effective treatment temperature and medication concentration for hyperthermic intraperitoneal chemotherapy in 23 instances using organoids obtained from appendiceal cancer or colorectal cancer (HIPEC). They came to the conclusion that the best perfusion protocol differed by patient and that organoid technology may provide a platform for customizing HIPEC settings to the level of each individual patient (63).

For the practical use of customized therapy with patient-derived cancer organoids, there are various obstacles to be addressed. First, it is essential to reduce the length of culture period and raise the success rate of organoids. Depending on the tumor, organoids grow at varying speeds and have varying degrees of success in culture (64). The second point is creativity in cultivating from the tiny sample of the following: If it is possible to grow cancer organoids from needle biopsy or liquid cytology samples, they can be used to test medications for neoadjuvant chemotherapy or even for resectable tumors that are not amenable to surgery. There have been a few reports on this strategy thus far, but they are relatively sparse (65). The third point deals with the impact of tumor heterogeneity, which is unavoidably a component of the tumor but does not entirely capture its characteristics. Even from a single-cell clone, a tumor is often a mass of heterogenic clones; the organoids must have only received some of the tumor's traits. Fourth, the candidate-targeted therapy approach using genomic profiling may not produce any therapeutic drugs that are clinically available (66). However, high-throughput multi-drug screening with non-anticancer agents may reveal unexpected drugs that are effective for some cancer cells with the above-mentioned distinct phenotypes, which could result in the discovery of new antitumor drugs. Moreover, investigations on radiation treatment using patient-derived organoids may offer substitutes, even if the number of patients is currently small.

CONCLUSIONS

We examined the technical advancement of cancer organoids and their present uses in cancer research and personalized treatment. In the study of cancer biology and translational medicine, PDCOs offer a lot of potential. It works well for evaluating potential targeted medications found through multi-omics analysis. Organoid-based research is now used in radiation treatment and immunotherapy as well as targeted medication screening. Furthermore, systems have been established that allow for the in vitro study of the tumor microenvironment employing cancer organoids in combination with immune cells and stromal cells, such as lymphocytes and fibroblasts, respectively. There are still certain hurdles to clear for therapeutic applications, such as speeding up, stabilizing, and simplifying methods for cultivating and studying cancer cells. Organoids are expected to grow in versatility in a variety of malignancies and provide a greater contribution to the study of cancer.

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REFERENCES

1.Dowden, Helen, and Jamie Munro. "Trends in clinical success rates and therapeutic focus." Nat. Rev. Drug Discov 18.7 (2019): 495-6.
2.Hwang, Thomas J., et al. "Failure of investigational drugs in late-stage clinical development and publication of trial results." JAMA internal medicine 176.12 (2016): 1826-1833.
3.Lee, Hye Won, et al. "Single-cell RNA sequencing reveals the tumor microenvironment and facilitates strategic choices to circumvent treatment failure in a chemorefractory bladder cancer patient." Genome medicine 12.1 (2020): 1-21.
4.Veninga, Vivien, and Emile E. Voest. "Tumor organoids: opportunities and challenges to guide precision medicine." Cancer Cell 39.9 (2021): 1190-1201.
5.Cobain, Erin F., et al. "Assessment of clinical benefit of integrative genomic profiling in advanced solid tumors." JAMA oncology 7.4 (2021): 525-533.
6.Li, Hongyi, et al. "Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects." Signal transduction and targeted therapy 5.1 (2020): 1-23.
7.Shiihara, Masahiro, and Toru Furukawa. "Application of Patient-Derived Cancer Organoids to Personalized Medicine." Journal of Personalized Medicine 12.5 (2022): 789.
8.Ben-David, Uri, et al. "Genetic and transcriptional evolution alters cancer cell line drug response." Nature 560.7718 (2018): 325-330.
9.Ren, Ya, et al. "Developments and opportunities for 3D bioprinted organoids." International Journal of Bioprinting 7.3 (2021).
10.Huch, Meritxell, and Bon-Kyoung Koo. "Modeling mouse and human development using organoid cultures." Development 142.18 (2015): 3113-3125.
11.Schutgens, Frans, and Hans Clevers. "Human organoids: tools for understanding biology and treating diseases." Annual Review of Pathology: Mechanisms of Disease 15 (2020): 211-234.
12.Wang, Qinying, et al. "Applications of human organoids in the personalized treatment for digestive diseases." Signal Transduction and Targeted Therapy 7.1 (2022): 1-30.

13.Assawachananont, Juthaporn, et al. “Transplantation of embryonic and induced pluripotent stem cell-derived 3D retinal sheets into retinal degenerative mice.” Stem cell reports 2.5 (2014): 662-674.
14.McCracken, Kyle W., et al. “Modelling human development and disease in pluripotent stem-cell-derived gastric organoids.” Nature 516.7531 (2014): 400-404.
15.Spence, Jason R., et al. “Directed differentiation of human pluripotent stem cells into intestinal tissue in vitro.” Nature 470.7332 (2011): 105-109.
16.Chen, Kevin G., et al. “Pharmacological analysis of CFTR variants of cystic fibrosis using stem cell-derived organoids.” Drug discovery today 24.11 (2019): 2126-2138.
17.Nozaki, Kengo, et al. “Co-culture with intestinal epithelial organoids allows efficient expansion and motility analysis of intraepithelial lymphocytes.” Journal of gastroenterology 51.3 (2016): 206-213.
18.Ben-David, Uri, et al. “Genetic and transcriptional evolution alters cancer cell line drug response.” Nature 560.7718 (2018): 325-330.
19.Ghajari, Ghazal, Arefe Heydari, and Masoud Ghorbani. “Mesenchymal stem cell-based therapy and female infertility: limitations and advances.” Current Stem Cell Research & Therapy (2022).
20.Ben-David, Uri, et al. “Patient-derived xenografts undergo mouse-specific tumor evolution.” Nature genetics 49.11 (2017): 1567-1575.
21.Broutier, Laura, et al. “Human primary liver cancer–derived organoid cultures for disease modeling and drug screening.” Nature medicine 23.12 (2017): 1424-1435.
22.Boj, Sylvia F., et al. “Organoid models of human and mouse ductal pancreatic cancer.” Cell 160.1-2 (2015): 324-338.
23.Broutier, Laura, et al. “Culture and establishment of self-renewing human and mouse adult liver and pancreas 3D organoids and their genetic manipulation.” Nature protocols 11.9 (2016): 1724-1743.
24.Van de Wetering, Marc, et al. “Prospective derivation of a living organoid biobank of colorectal cancer patients.” Cell 161.4 (2015): 933-945.
25.Kim, Minsuh, et al. “Patient-derived lung cancer organoids as in vitro cancer models for therapeutic screening.” Nature communications 10.1 (2019): 1-15.
26.Wong, Chi Heem, Kien Wei Siah, and Andrew W. Lo. “Estimation of clinical trial success rates and related parameters.” Biostatistics 20.2 (2019): 273-286.
27.Veninga, Vivien, and Emile E. Voest. “Tumor organoids: opportunities and challenges to guide precision medicine.” Cancer Cell 39.9 (2021): 1190-1201.
28.Kretzschmar, Kai. “Cancer research using organoid technology.” Journal of Molecular Medicine 99.4 (2021): 501-515.
29.Drost, Jarno, and Hans Clevers. “Organoids in cancer research.” Nature Reviews Cancer 18.7 (2018): 407-418.
30.Kodack, David P., et al. “Primary patient-derived cancer cells and their potential for personalized cancer patient care.” Cell reports 21.11 (2017): 3298-3309.
31.de Witte, Chris Jenske, et al. “Patient-derived ovarian cancer organoids mimic clinical response and exhibit heterogeneous inter-and intrapatient drug responses.” Cell reports 31.11 (2020): 107762.
32.Ooft, S. N., et al. “Prospective experimental treatment of colorectal cancer patients based on organoid drug responses.” ESMO open 6.3 (2021): 100103.
33.Huang, Ling, et al. “Ductal pancreatic cancer modeling and drug screening using human pluripotent stem cell–and patient-derived tumor organoids.” Nature medicine 21.11 (2015): 1364-1371.
34.Karthaus, Wouter R., et al. “Identification of multipotent luminal progenitor cells in human prostate organoid cultures.” Cell 159.1 (2014): 163-175.
35.Dijkstra, Krijn K., et al. “Patient-derived organoid models of human neuroendocrine carcinoma.” Frontiers in endocrinology 12 (2021): 627819.
36.Patel, Tushar. “Worldwide trends in mortality from biliary tract malignancies.” BMC cancer 2.1 (2002): 1-5.
37.Khan, Shahid A., et al. “Changing international trends in mortality rates for liver, biliary and pancreatic tumours.” Journal of hepatology 37.6 (2002): 806-813.
38.Carriaga, Marisa T., and Donald Earl Henson. “Liver, gallbladder, extrahepatic bile ducts, and pancreas.” Cancer 75.S1 (1995): 171-190.
39.Abou-Alfa, Ghassan K., et al. “Ivosidenib in IDH1-mutant, chemotherapy-refractory cholangiocarcinoma (ClarIDHy): a multicentre, randomised, double-blind, placebo-controlled, phase 3 study.” The Lancet Oncology 21.6 (2020): 796-807.
40.Unno, Michiaki, Tatsuo Hata, and Fuyuhiko Motoi. “Long-term outcome following neoadjuvant therapy for resectable and borderline resectable pancreatic cancer compared to upfront surgery: a meta-analysis of comparative studies by intention-to-treat analysis.” Surgery today 49.4 (2019): 295-299.
41.Biankin, Andrew V., et al. “Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes.” Nature 491.7424 (2012): 399-405.
42.Dalton, W. Brian. DNA damage during mitotic arrest: A novel, p53-regulated source of structural chromosome instability in human cells. Diss. Emory University, 2008.
43.Shiihara, Masahiro, et al. “Development of a system combining comprehensive genotyping and organoid cultures for identifying and testing genotype-oriented personalised medicine for pancreatobiliary cancers.” European Journal of Cancer 148 (2021): 239-250.
44.Seino, Takashi, et al. “Human pancreatic tumor organoids reveal loss of stem cell niche factor dependence during disease progression.” Cell stem cell 22.3 (2018): 454-467.
45.Jusakul, Apinya, et al. “Whole-Genome and Epigenomic Landscapes of Etiologically Distinct Subtypes of CholangiocarcinomaIntegrative Genomic and Epigenomic Analysis of Cholangiocarcinoma.” Cancer discovery 7.10 (2017): 1116-1135.
46.Driehuis, Else, et al. “Pancreatic cancer organoids recapitulate disease and allow personalized drug screening.” Proceedings of the National Academy of Sciences 116.52 (2019): 26580-26590.
47.Hu, Tuo, et al. “Metabolic rewiring by loss of Sirt5 promotes Kras-induced pancreatic cancer progression.” Gastroenterology 161.5 (2021): 1584-1600.
48.Fujiwara, Hiroaki, et al. “5-Aminolevulinic acid-mediated photodynamic activity in patient-derived cholangiocarcinoma organoids.” Surgical Oncology 35

(2020): 484-490.
49.Tsai, Susan, et al. “Development of primary human pancreatic cancer organoids, matched stromal and immune cells and 3D tumor microenvironment models.” BMC cancer 18.1 (2018): 1-13.
50.Koikawa, Kazuhiro, et al. “Targeting Pin1 renders pancreatic cancer eradicable by synergizing with immunochemotherapy.” Cell 184.18 (2021): 4753-4771.
51.Öhlund, Daniel, et al. «Distinct populations of inflammatory fibroblasts and myofibroblasts in pancreatic cancer.» Journal of Experimental Medicine 214.3 (2017): 579-596.
52.Grandori, Carla, and Christopher J. Kemp. “Personalized cancer models for target discovery and precision medicine.” Trends in cancer 4.9 (2018): 634-642.
53.Nussinov, Ruth, et al. “Precision medicine and driver mutations: computational methods, functional assays and conformational principles for interpreting cancer drivers.” PLoS computational biology 15.3 (2019): e1006658.
54.Saito, Yoshimasa, et al. “Establishment of patient-derived organoids and drug screening for biliary tract carcinoma.” Cell reports 27.4 (2019): 1265-1276.
55.Pauli, Chantal, et al. “Personalized In Vitro and In Vivo Cancer Models to Guide Precision MedicinePersonalized Cancer Models to Guide Precision Medicine.” Cancer discovery 7.5 (2017): 462-477.
56.Larsen, Brian M., et al. “A pan-cancer organoid platform for precision medicine.” Cell reports 36.4 (2021): 109429.
57.Narasimhan, Vignesh, et al. “Medium-throughput Drug Screening of Patient-derived Organoids from Colorectal Peritoneal Metastases to Direct Personalized TherapyFunctional Precision Medicine for Advanced Colorectal Cancer.” Clinical Cancer Research 26.14 (2020): 3662-3670.
58.Gilles, Maud-Emmanuelle, et al. “Personalized RNA medicine for pancreatic cancer.” Clinical Cancer Research 24.7 (2018): 1734-1747.
59.Tung, Kuei-Ling, et al. “Integrated chromatin and transcriptomic profiling of patient-derived colon cancer organoids identifies personalized drug targets to overcome oxaliplatin resistance.” Genes & diseases 8.2 (2021): 203-214.
60.Broutier, Laura, et al. “Human primary liver cancer–derived organoid cultures for disease modeling and drug screening.” Nature medicine 23.12 (2017): 1424-1435.
61.Neal, James T., et al. “Organoid modeling of the tumor immune microenvironment.” Cell 175.7 (2018): 1972-1988.
62.Schnalzger, Theresa E., et al. “3D model for CAR-mediated cytotoxicity using patient-derived colorectal cancer organoids.” The EMBO journal 38.12 (2019): e100928.
63.Forsythe, Steven D., et al. “Organoid Platform in Preclinical Investigation of Personalized Immunotherapy Efficacy in Appendiceal Cancer: Feasibility StudyImmunotherapy Efficacy in Appendiceal Cancer Organoids.” Clinical Cancer Research 27.18 (2021): 5141-5150.
64.Votanopoulos, Konstantinos I., et al. “Appendiceal cancer patient-specific tumor organoid model for predicting chemotherapy efficacy prior to initiation of treatment: a feasibility study.” Annals of surgical oncology 26.1 (2019): 139-147.
65.Nuciforo, Sandro, et al. “Organoid models of human liver cancers derived from tumor needle biopsies.” Cell reports 24.5 (2018): 1363-1376.
66.Pasch, Cheri A., et al. “Patient-derived cancer organoid cultures to predict sensitivity to chemotherapy and radiation.” Clinical Cancer Research 25.17 (2019): 5376-5387.

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