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Table of Content

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Successful Treatment of Metastatic Lung Cancer with Fusion KIF RET Amplification Using Alectinib in the Absence of Selpercatinib and Pralsetinib: A Case Report and Literature Review

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Abstract

Background: The treatment landscape for advanced non-small cell lung cancer (NSCLC) has evolved significantly with the emergence of targeted therapies. Among these, tyrosine kinase inhibitors (TKIs) selective for the rearranged during transfection (RET) gene have shown promise. However, understanding resistance mechanisms and identifying effective treatments for patients progressing on RET-TKIs remains a challenge. **Case description:** we present a patient with metastatic lung cancer harboring a fusion involving KIF5B-RET amplification. Notably, this patient did not receive Selpercatinib or Pralsetinib, two RET-selective TKIs commonly used in RET-positive NSCLC. Instead, the patient was successfully treated with Alectinib, highlighting the potential efficacy of this alternative therapy. **Conclusion:** Our case report, provides a comprehensive overview of RET-positive advanced NSCLC, covering both therapeutic and molecular aspects. We compare clinical outcomes achieved with multikinase inhibitors (MKIs) and RET-selective TKIs, emphasizing the long-term resistance mechanisms. Additionally, we discuss unresolved issues and propose future pharmacological approaches.

Keywords: NSCLC, RET fusion, TKI, Drug resistance.

Introduction

Lung cancer continues to be the primary cause of cancer-related mortality globally, even with advancements in risk assessment, biological insights, immunological strategies, and the introduction of novel treatment modalities. Among lung cancers, non-small cell lung cancer (NSCLC) is the predominant type, constituting 84% of all lung cancer cases (1, 2).

The discovery of oncogenic activation in tyrosine kinases has revolutionized the treatment landscape for advanced non-small cell lung cancer (NSCLC). Significantly, mutations in the epidermal growth factor receptor (EGFR), rearrangements of the anaplastic lymphoma kinase (ALK) gene, and alterations in the c-ROS oncogene 1 (ROS1) gene have paved the way for targeted therapies in lung cancer.These findings have also prompted ongoing research to identify biomarkers and treatments applicable to other subsets of patients with advanced NSCLC (3).RET Gene fusions play a crucial role

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as oncogenic drivers in approximately 1% of non-small cell lung cancer (NSCLC) cases (4).

Recurrent rearrangements occur between RET and fusion partners such as CCDC6, KIF5B, and NCOA4. RET fusions account for about 1-2% of non-small cell lung cancer (NSCLC) cases and are more prevalent in younger patients and those who have never smoked. Overall, Brain metastases frequently occur in RET fusion-positive non-small cell lung cancers (NSCLC), with an approximate 46% prevalence. Break-apart FISH and NGS can identify RET rearrangements. Immunohistochemistry (IHC) is convenient but has poor sensitivity and specificity for RET detection in NSCLC. accurate identification of RET alterations is crucial for guiding treatment decisions in advanced patients (10- 12).

Patients with the KIF5B-RET genotype exhibit specific clinical features, showing that this fusion gene could serve as a more certain molecular marker in non-small cell lung cancer (NSCLC) (13).

The FDA has approved two RET inhibitors, Selpercatinib and Pralsetinib, for adult patients with advanced non-small cell lung cancer (NSCLC) harboring RET fusion-positive alterations. These agents are recommended as front-line treatment options for such patients, replacing immunotherapy or chemotherapy (14, 15).

Selpercatinib and Pralsetinib are FDA-approved RET-selective tyrosine kinase inhibitors (TKIs) with encouraging efficacy. In contrast, Cabozantinib, Vandetanib, Alectinib, and Sunitinib are multitargeted TKIs approved for other indications, but they are less potent than Selpercatinib and Pralsetinib (16- 22).

Immune checkpoint inhibitors (ICIs) have emerged as the standard treatment for patients with advanced non-small cell lung cancer (NSCLC) lacking driver mutations. Nevertheless, their effectiveness in treating advanced non-small cell lung cancer (NSCLC) with RET fusion remains a topic of debate. The IMMUNOTARGET trial published a modest response to ICIs in RET fusion-positive NSCLC, with an overall response rate (ORR) of 6.3% and a median progression-free survival (PFS) of 2.1 months. In contrast, real-world data from U.S. databases demonstrated favorable outcomes in patients with RET fusion-positive NSCLC who received ICI-based therapy, achieving a 53.8% ORR and a median PFS of 4.2 months. Given the limited and conflicting evidence, the use of ICIs in RET fusion-positive NSCLC remains uncertain (23- 27).

However, limited access to these drugs in certain clinical settings underscores the need to explore alternative treatment options. Here we present a case of successful management of metastatic lung cancer with fusion KIF-RET amplification using Alectinib as an alternative targeted therapy.

Case presentation

A 42-year-old nonsmoker woman presented with dyspnea, cough and weakness. In her chest CT scan, the mass lesion was reported in RLL and no evidence of metastasis on imaging's. In December 2023 she underwent CNB of the lung mass. The pathology showed necrosis and fibrosis and there was no evidence of malignant lesion. Based on the appearance of the lesion, the surgical team decided to perform resection and in January 2023 the patient underwent lobectomy. This time, the pathology was consistent with Adenosquamous carcinoma with necrosis. The subcarinal lymph node was also involved. The tumor was classified as pT2N2 (stage IIIA according to the TNM classification of the UICC). Imaging showed no evidence of metastatic disease. To decide on the treatment, the patient was subjected to the EGFR mutation panel test on a tissue sample. During this assessment, which was done by RT-PCR method, no mutation was detected. However, the evaluation of PDL1 expression with IHC showed 33%, which means significant PDL1 expression. Subsequently, the oncologist planned to treat her with a combination of pemetrexed and a platinum regimen. After 2 cycles of chemotherapy, in February 2023 the patient had a seizure. Brain MRI revealed a heterogeneous mass lesion in the frontal lobe (18*17*12 mm). Yet there was no evidence of other organ involvement. Due to the patient's lack of consent to surgery, we decided to do SRS. In this setting, the molecular profile of the patient was fully evaluated, which was positive for ROS1 amplification (20%) and RET rearrangement (67%) and negative for ALK gene rearrangement by FISH study and MET exon 14 by RT-PCR was also negative. Considering the unavailability of the medications and the inability of the patient to prepare the selected target drugs, as well as the suitable performance status and significant PDL1 expression, immunotherapy with pembrolizumab was added to the previous chemotherapy regimen which was continued up to a total of 6 cycles. Maintenance therapy with pembrolizumab was considered due to the lack of evidence for residual disease in the imaging. In July 2023, after receiving 4 cycles of pembrolizumab, evidence of disease progression was seen in the PET-CT scan in the form of mediastinal lymph node involvement and multiple brain lesions. The patient was a candidate for whole-brain radiotherapy. To choose the appropriate treatment plan, the patient's tissue sample was sent to another country (Turkey) for NGS. Regarding the NGS result that was positive only for fusion RET-KIF5b and negative for other biomarkers (such as ALK, ROS1, EGFR, BRAF, and HER2) it was decided to start RET inhibitor target therapy for the patient. It is noteworthy that the PDL1 expression was negative

in NGS despite being significantly positive in the patient's previous IHC test. Given the unavailability of Selpercatinib and pralsetinib due to logistical and financial constraints, the patient's treatment was initiated with Alectinib as a targeted therapy (1200 mg/day). After two weeks of treatment, she showed up with hemolytic anemia. Alectinib was temporarily stopped, and conservative treatment was performed. After a while, Alectinib started again with lower doses (600 mg/day) for 6months. Afterward, the patient underwent reevaluation with a PET scan. In the PET scan, the previous mediastinal lesions showed reduced uptake, with the only positive finding being increased uptake in the left humerus bone despite suboptimal dose administration of Alectinib (figure1). Furthermore the patient had no clinical symptoms. Overally, Considering that the patient's symptoms are controlled and imaging results was reasonable for this case, the decision was made to increase the Alectinib dose to 900 mg/day and for bone metastasis support, bisphosphonates were added to the patient's treatment.

Discussion

Fusion KIF RET amplification is a rare genetic alteration in lung cancer, occurring in approximately 1-2% of patients. (4) Based on LIBRETTO-431 and ARROW trial, first-line treatment with Selpercatinib and Pralsetinib has emerged as standard-of-care targeted therapies for this genetic alteration, demonstrating impressive response rates and prolonged progression-free survival in clinical trials (14, 28). However, access to these drugs may be limited in certain clinical settings (as was the case for this patient), necessitating the exploration of alternative treatment options. Alectinib, a potent and selective ALK inhibitor, demonestrates efficacy in treating ALK-positive non-small cell lung cancer and has also demonstrated activity against RET-altered tumors in preclinical studies. The successful outcome observed in this case highlights the potential efficacy of Alectinib as an alternative treatment for metastatic lung cancer with fusion KIF RET amplification when access to specific targeted therapies is limited. While Selpercatinib and Pralsetinib are considered standard-of-care options for this genetic alteration, the use of Alectinib in this context warrants further investigation and consideration as a viable treatment strategy (29).

Given the rarity of fusion KIF RET amplification, personalized treatment approaches tailored to individual patient needs and available resources are crucial in optimizing clinical outcomes. In addition to the high cost and lack of access to these medicines, the inconsistency of the results in the diagnostic modalities (ex NGS, FISH, PCR, etc.) renders it difficult to make an appropriate treatment decision (30).

In this case, the discrepancy between next-generation sequencing (NGS) and immune-histochemistry (IHC) results regarding PD-L1 expression influenced the treatment decision. Immunotherapy was chosen as the first-line treatment in the metastatic setting. Previous studies have highlighted the uncertainty surrounding immune checkpoint inhibitors (ICIs) in these patient subtypes. The efficacy of immune checkpoint inhibitors (ICIs) is notable, especially in patients with high PD-L1 and who have not undergone any prior therapy. Their effectiveness is comparable to that observed in unselected populations (23- 27).

The IMMUNOTARGET study reported a poor response of RET fusion-positive non-small cell lung cancer (NSCLC) to immune checkpoint inhibitors (ICIs). Before the approval of selective targeted agents, certain patients with RET fusion-positive lung cancer were treated with immune checkpoint inhibitors (ICIs)(31).

Conclusion

in the absence of access to Selpercatinib and Pralsetinib, Alectinib demonstrated remarkable efficacy in treating metastatic lung cancer with fusion KIF RET amplification. This case underscores the importance of exploring alternative therapeutic options and individualized treatment approaches to optimize patient outcomes, particularly in resource-constrained settings. Further research and clinical experience are warranted to validate the role of Alectinib in this context and inform treatment decisions for patients with similar genetic alterations. As precision medicine continues to evolve, the need for accessible and effective targeted therapies for rare genetic alterations remains a critical priority in improving outcomes for patients with metastatic lung cancer.

Statements and Declarations

Consent to participate and Publication

The patient provided informed consent for participation in this case report and publication. She understood the purpose, risks, and benefits of sharing her medical information for scientific and educational purposes. Confidentiality was maintained, and her initials were used to protect privacy.We seek to share this unique case to contribute to medical knowledge and improve patient care.

Availability of data and materials

availability of data and materials is subject to institutional policies, patient consent, and legal constraints. Researchers should follow established protocols and guidelines when requesting access to clinical information.

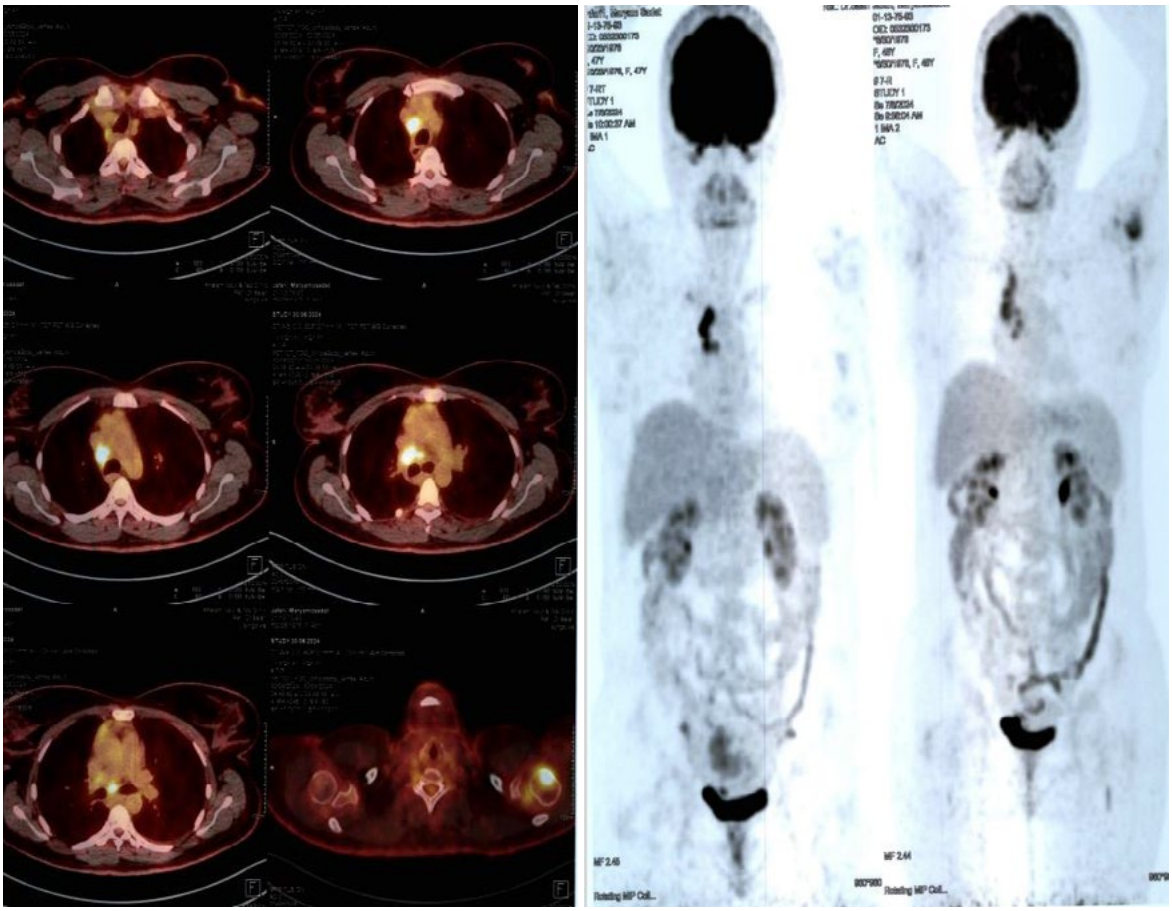

Fig 1. The patients PET scan rsult.

Ethics approval and consent to participate

Ethical considerations guided decision-making, ensuring patient autonomy, safety, and adherence to best practices.

Conflicts of Interest

The Authors affirm that they have no conflict of interest.

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Authors' contributions

Sina Salari: conceptualization; data curation; editing and review. Maedeh Mataji: investigation and writing. Soodeh Ramezaninejad: investigation and writing.

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Examining the Trajectory of Health Behavior in Smokers as a Pre-Awareness Determinant in the Field of Personalized Medicine

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Abstract

Smoking is widely recognized as a significant factor in the global disease burden, leading to 4 million deaths annually throughout the globe. Various research has shown that the prevalence of smoking worldwide is on the rise among both males and females, particularly among the younger demographic. Behavior modification is a primary objective in public health initiatives that prioritises preventive measures before the occurrence of illness. Utilizing innovative personalized medicine programs may serve as a significant prognostic determinant in modifying the behavior of smokers and enhancing societal health. Thus, this review research presents an appropriate framework for personal medical applications that may effectively alter smokers' health and health behavior.

Keywords: Personalized medicine, Smoking, Health behavior.

Introduction

Smoking is recognized as a contributing contributor to the global illness burden, resulting in the annual deaths of 4 million individuals worldwide (1). Various research has shown that the global incidence of smoking is increasing in both genders, particularly among the younger population. According to the World Health Organization, it is projected that by 2030, the global death toll from smoking will exceed 10 million individuals (2). This significant loss of life will primarily impact individuals in their middle-aged and productive years, reducing their life expectancy by 20 to 25 years. The age range of individuals between 35 and 61 years old constitutes the demographic makeup of society (3). Suppose the current pattern of tobacco use continues. In that case, it is projected that out of the 1.3 billion smokers worldwide, there will be around 450 million fatalities caused by smoking during the next fifty years (4).

Modification of conduct

Behavior modification is a primary objective in public health strategies that prioritizes prevention before illness (5). This is particularly crucial in nations with low and moderate incomes. Due to meticulous evaluation in recent decades, the cost-effectiveness of healthcare expenses and the advantages of healthcare treatments have been thoroughly examined. The user's text is enclosed

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in tags. Behavior change in public health is synonymous with communicating Behavior change and its impact on society (4, 5).

Context

A multitude of health disorders and circumstances may be attributed to hazardous Behaviors, such as alcohol use, substance abuse, tobacco use, dangerous driving, excessive eating, or engaging in unsafe sexual activity (6). An essential inquiry into health Behaviors pertains to the methods of accurately forecasting and altering, or embracing and maintaining, health Behaviors. Thankfully, people have a fundamental ability to govern their actions (6, 7). Self-regulation may abolish unhealthy Behaviors and can be replaced with health-promoting Behaviors such as physical exercise, weight management, proper diet, oral and dental cleanliness, condom usage, and accident avoidance. Changing health Behavior encompasses the intentional and self-driven processes that lead to the cessation of health-endangering Behaviors and the adoption and maintenance of health-promoting Behaviors (8).

Evolved Behavior change programs include diverse activities and strategies that target individual, community, and environmental factors influencing human Behavior. Behavior modification is a recently coined phrase in the field of health promotion that should be distinct from the notion of Behavior modification, which has a distinct definition in clinical psychiatry (9).

A novel idea gaining traction in the US healthcare system is that implementing little modifications is more feasible and easier to handle (10). It is unnecessary to implement radical and significant modifications to one's lifestyle to see its advantages; even a tiny amount is preferable to none. The subject of nutrition and exercise demonstrates the advantages of making tiny and moderate adjustments (11). For instance, the many phases of Behavior modification, including physical activities, have the potential to enhance life span, manage body weight, and bolster mental well-being (11, 12). In addition, they mitigate the likelihood of specific ailments such as type 2 diabetes, cardiovascular illnesses, and some forms of cancer (7, 12).

Hypotheses

Behavior modification programs often priorities the use of several behavior changes theories that were developed throughout the 1980s (13). These ideas are significantly similar in their definition of individual activities as a catalysts for change. Behavior modification programs, or interventions, often concentrate on implementing activities that assist people or groups in diminishing risk Behaviors and vulnerability (14). Some examples of theories in this context include metatheoretical theory (stages of change), Behavior change model, theory of rational Behavior, health belief model, theory of planned Behavior, diffusion of innovation, and health action process approach. Since the late 1990s, a concentrated effort has been made to integrate many health Behaviors change theories into a cohesive, comprehensive, unified theory (15).

Equipment

Care groups consist of 10 to 15 volunteer health educators who meet regularly in the community. *Barrier assessment* is a rapid evaluation method used in Behavior change initiatives to identify the factors influencing Behavior (16). Community-based disinfection is a Behavior modification technique used in the disinfection industry to address open defecation in rural parts of developing nations. Using shame, disgust, and peer pressure after an initial stimulation phase results in the prompt establishment and sustained utilization of the toilet (17).

Behavior change communication

Behavior change communication refers to using strategic communication techniques to promote and encourage individuals to adopt or modify new behaviors (18). *Behavior change communication*, a targeted method that emphasizes communication, is a powerful tool for promoting safety and well-being (19). It is assumed that certain forms of communication can guide people and communities to choose Behaviors that enhance their safety and well-being. Initially implemented in initiatives aimed at preventing HIV and TB, the scope of behavior change communication has now expanded to include any kind of communication that seeks to assist people and groups in modifying their behavior. They choose options that have a beneficial impact on their well-being, such as immunization, screening for cervical cancer, and the use of disposable needles (20).

The Johns Hopkins Centre for Communication Programs is a global leader in health-related communication Behavior modification programs, making a significant impact in poor nations. Their initiatives span a wide range of health issues, from reproductive health and family planning to malaria, HIV, and AIDS (21). The dissemination of global media via mass communication channels has been a game-changer, facilitating the adoption of beneficial Behaviors in Burkina Faso, the Democratic Republic of Congo, and Mozambique (22). The center's work also inspires curious adolescents to seek information, supporting initiatives aimed at decreasing the incidence of HIV/AIDS among

young people in Botswana (23). The reasons for the failure of health education activities are listed in Table 1, providing valuable insights for future initiatives.

Five factors affecting health-related behavior and conditions

Five elements influence health-related Behavior and conditions. Health behaviors are integral components of a broader social framework. Supportive reforms in the whole system are necessary for sustainable improvements in health behaviors. Table 2 shows five factors affecting health-related behavior and conditions (24). Theories may be classified into two distinct categories:

1)Explanatory theories elucidate the underlying causes of an issue and facilitate the identification of modifiable components that contribute to the problem.

2)Theories of transformation facilitate the development of health treatments.

Factors influencing smoking cessation

Smoking cessation Behavior is a complex issue that involves several dimensions (25). Factors associated with smoking cessation may be categorized into personal, societal, and environmental aspects. Research has shown that individual characteristics are associated with the act of quitting smoking (25). Multiple studies have shown that the longevity of smoking cessation varies across individuals. When designing smoking cessation programs, it is advisable to include treatments that involve the concurrent use of individual counselling, nicotine replacement medication, active monitoring, and follow-up for those who are prepared to stop smoking (26). Smoking is the primary avoidable factor leading to cardio-pulmonary disorders and is responsible for one-third of all fatalities in North America. This habit significantly harms society and individuals, resulting in millions of dollars in healthcare costs (27).

Approximately 45 million Americans in the United States persist in smoking, and smoking is responsible for 500,000 avoidable deaths in this nation. Out of the whole global population, a staggering 744 million individuals are now awaiting their demise as a result of their smoking habits (28). This Behavior leads to the unfortunate loss of about 7 million years of life. Of the people who smoke every day, 81% started smoking their first pack before the age of 38 (29). Additionally, it is projected that almost half of the teenagers who presently smoke will continue to do so until they pass away from lung disease. They will persist in

Table 1. The reasons for the failure of health education activitie.

Row	Failure reasons
1	Societal customs and expectations
2	Disseminating material that contradicts the objectives of education
3	The intended modifications in Behavior are not attainable
4	Inadequate selection of the target population
5	Insufficiency of the instructional approach used with the specific Behavior and target demographic

Table 2. Factors affecting health-related behavior and conditions

Row	Factors
1	Intrapersonal variables refer to individual characteristics and traits.
2	Interpersonal elements include interactions and relationships between individuals.
3	Organizational factors pertain to the structure and functioning of institutions.
4	Society factors encompass the broader cultural and societal influences.
5	Public policy aspects relate to government regulations and actions.

experiencing mortality due to smoking (27-29).

Smokers' Behavioral shift trend

When providing individual counselling in smoking cessation programs, it is essential to consider each individual's unique traits and characteristics (30). This includes individuals who are in the stage of preparing to quit smoking and have sufficient motivation to do so (30). Smokers benefit from being given priority in smoking cessation intervention programs. It is essential to take into account nicotine addiction and the symptoms of withdrawal in any smoking cessation program (31). Engaging in telephone follow-up and offering help to navigate challenging circumstances related to smoking, particularly during the first days of stopping, via the provision of social support decreases the likelihood of unsuccessful smoking cessation. The inter-theoretical model, also known as TTM, has proven to be a highly effective tool in health education for smoking cessation (32). The Prochaska approach, developed by Prochaska et al. in the early 1980s, has been widely adopted globally for over 17 years. It has been particularly successful in addressing a variety of health-related behaviors, with a strong focus on smoking cessation (33).

This model offers a framework for understanding Behavior modification and serves as a foundation for assessing individuals' preparedness for change and interventions for actual Behavior modification. This significant psychotherapy model operates on the idea that individuals do not make abrupt decisions to alter their Behavior. Instead, Behavior change is seen as a progressive process that can be split into several parts and phases. People undergo these steps in order to transform (30-33).

The inter-theoretical model (TTM) is composed of four key structures: stages of change, processes of change, self-efficacy, and decision-making balance. In the pre-contemplation stage, individuals are not yet considering a behavior change within the next month. This could be due to a lack of knowledge or information about the desired behavior, past negative experiences, or a lack of motivation. Understanding these stages is crucial for effective behavior change interventions (30-33).

During the contemplation stage, individuals intend to adopt a specific Behavior within the following month and carefully consider the pros and cons of that behavior. Individuals intend to adopt a specific behavior during the preparation stage within the next month (34). They actively seek to plan, acquire the required equipment, and make preparations to change their behavior successfully. During the action stage, individuals successfully adopt the intended behavior, lasting less than one month after the Behavior change. During the maintenance stage, the last step, individuals successfully adopt the desired behavior for a period beyond one month and develop a sufficient level of self-efficacy when confronted with enticing circumstances. One fundamental premise of this model is that at each of the aforementioned steps, there is a potential for deviation, mistake, and regression, leading to a return to the former behavior (35). The following are the seven phases related to smoking Behavior: During the pre-contemplation stage, individuals persist in smoking and have no intention of quitting within the following month. During the contemplation stage, individuals engage in smoking Behavior while also contemplating the possibility of quitting smoking during the next month. During the preparation phase, both persons persist in smoking but have intentions to quit smoking within the next month. The action phase refers to the period when individuals have just stopped smoking, having done so for less than one month. The maintenance phase, on the other hand, pertains to those who have successfully abstained from smoking for more than one month (36). Figure 1 displays the cancer risk variables linked to smoking, as well as personalized medical support.

Behavioral patterns Change processes refer to overt and covert actions individuals employ to modify their desired behavior (37). These processes encompass 34 activities, which can be categorized into two groups: cognitive processes (such as increasing knowledge, experiencing dramatic relief, re-evaluating the environment, and engaging in self-liberation) and Behavioral processes (including social liberation, reverse conditioning, stimulus control, reinforcement, and establishing auxiliary relationships). Cognitively, an individual autonomously gets information, but in Behavioral processes, information is obtained from the surrounding environment (36, 37).

The conducted investigations revealed that pre-action phases (pre-contemplation, meditation, and preparation) are emphasized in using processes, whereas maintenance and action stages rely more on Behavioral processes. Individuals' movements are determined by their actions (36, 37). Properly executing these actions in the correct sequence enables progression to the next phase. Conversely, incorrect or insufficient execution of these actions will disrupt the progression to the next phase. Self-efficacy, a key component of the phases of the change model, plays a significant role in individuals' ability to successfully modify and sustain desired behaviors (38). It is the individual's confidence in their capacity to carry out a certain behavior. In the context of sustaining behavioral changes, particularly when confronted with circumstances that may lead to a relapse in behavior, a strong sense of self-efficacy is essential. For instance, an individual who is attempting to stop smoking needs a strong sense of self-efficacy to resist the temptation to smoke in

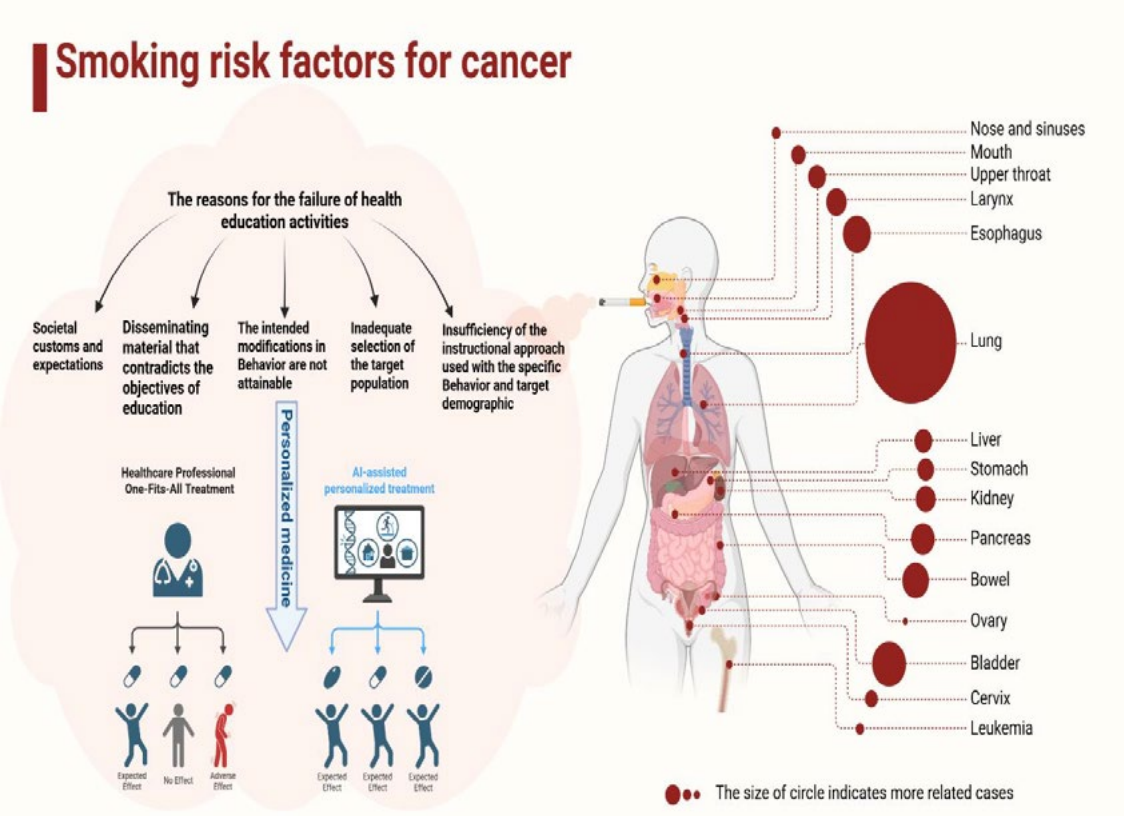

Fig1. The cancer risk factors associated with smoking along with personalized medical assistance.

circumstances that may trigger a relapse, such as social gatherings and financial difficulties (38, 39).

Decisional balancing, a crucial component of the metatheoretical model, is a significant framework for understanding the deliberate process of Change. It involves individuals evaluating the advantages and disadvantages of the intended course of action. In the context of behavior change, this scenario is seen as a decision-making equilibrium, when individuals cognitively assess the positive features or advantages and the negative aspects or barriers of specific behavior. They then carefully study and evaluate the rationale for either adopting or not adopting the behavior change. This concept is highly relevant in our research and practice, as it helps us understand the thought process behind behavior change decisions.

Conclusion

The fundamental objective of public health efforts is to implement preventative interventions to modify the behavior of smokers before the onset of illness. Implementing cutting-edge personalized medicine programs may be crucial in altering smokers' behavior and enhancing societal well-being.

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Eskandar Hoseinnzhad Lazarjani, and Seyedeh Sahar Ebrahimi Hosseini were involved in the conceptualization, design, and writing of the manuscript.

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Meta-Analysis Study: Age Variations in PBSC Expression in COVID-Positive Individuals

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Abstract

Autoimmune disorders are complex conditions that result from a combination of genetic and environmental causes and currently have no recognized therapy. Various therapeutic strategies may be used in various illnesses to promote remission or, at the very least, alleviate the symptoms. For customized therapy to be implemented, it is necessary to identify groups of individuals who are generally similar and share pathogenic signaling pathways. Therefore, research about autoimmune disorders mainly focuses on identifying new biomarkers, uncovering novel targets for therapy and agents, and understanding the processes involved in developing various disorders. We are just at the nascent phase of implementing tailored therapy for autoimmune illnesses. Hence, this research delved into the examination of several autoimmune illnesses and the impact of personalized therapy on their progression.

Keywords: Genomic analysis, Autoimmune disorders, Personalized medicine.

Introduction

The COVID-19 pandemic has profoundly impacted global health, economies, and communities. In January 2020, the World Health Organization declared it a public health emergency and, in March 2020, a pandemic. Millions of cases and countless deaths have been recorded globally. To address this unprecedented situation, experts, scientists, and healthcare professionals have dedicated themselves to research and the dissemination of knowledge through publications. They aim to unravel the mysteries of the virus, its spread, and potential ways to combat or prevent it (1). Researchers continue to analyze the COVID-19 virus in light of the global pandemic. A recent study explored age differences in immune response to the virus by examining peripheral blood stem cells (PBSC) in individuals who tested positive for COVID-19. This study focused on understanding how age influences the immune system's response to the virus, particularly in older adults, who may face higher risks of severe outcomes (2). This study aimed to understand the effects of age on the immune response to COVID-19 and identify risk factors for severe illness in older adults. Researchers collected and combined data from multiple studies measuring a specific immune cell marker (PBSC) in COVID-19 patients of varying ages. By comparing PBSC levels between younger and older individuals, they searched for relationships with disease severity to identify potential differences in immune function and risk factors across age groups (3). This study revealed that older people infected with COVID-19 have

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higher levels of PBSC in their blood compared to younger people. This suggests that age may influence the immune response to the virus. These findings support previous research showing that age affects immune function and vulnerability to infections (4). The study found that higher levels of a protein called PBSC in the blood were linked to worse COVID-19 symptoms and outcomes, especially in older adults. This suggests that PBSC levels could be used as a marker to assess the severity of the disease in older individuals with COVID-19. The study highlights the importance of age-related differences in PBSC expression in COVID-19 patients. Age plays a crucial role in how the immune system responds to the virus. Understanding this link helps us identify those vulnerable to severe COVID-19. Studies should investigate the reasons behind age-related differences and explore treatments that target peripheral blood stem cell (PBSC) levels in older patients with COVID-19. It's essential to know how age affects the immune system's response to COVID-19 to create specific treatments and find people who are at higher risk of getting very sick from it. Peripheral blood stem cells (PBSCs) are important for how the immune system works, so they are being studied in COVID-19 research. Differences in PBSC expression based on age could give us important information about how likely someone is to get sick and how bad their symptoms will be. Researchers can combine multiple studies into a meta-analysis to see how age affects PBSC levels in people with COVID-19. Looking at differences in PBSC levels between different age groups may help connect them to how severe the disease is and how it turns out. Understanding how PBSC expression changes with age can help us learn more about how different age groups respond to the illness and make better clinical decisions when treating COVID-19. This study adds to what we know about how the immune system responds to COVID-19 and shows that age is an important factor in how the disease gets worse. Understanding how the body's peripheral blood stem cells (PBSCs) change with age in COVID-19 patients could lead to new treatments and ways to identify those at higher risk. However, more research is needed to consider other factors that could affect the immune response, such as existing health conditions, genetics, and environmental factors. Long-term studies could also help track how PBSC expression changes in COVID-19 patients and how this change relates to the severity of their illness. Studying immune cell changes in COVID-19 patients based on age and other factors can improve ways to predict outcomes and adjust treatments for each patient. Additionally, understanding how these immune cells interact with other immune cells during COVID-19 can reveal how immune responses affect disease severity. This knowledge can help develop treatments that target these specific immune mechanisms. Teamwork between researchers from different fields is vital to uncovering the intricate immune responses to COVID-19 and creating better ways to prevent and treat the disease. Continued research into the levels of peripheral blood stem cells (PBSCs) in COVID-19 patients, especially as it relates to their age, is valuable. It can help us better understand how the immune system responds to the virus and lead to improved treatments for people of all ages (5).

The COVID-19 pandemic caused by the novel coronavirus, SARS-CoV-2, has imposed an unprecedented global health crisis, affecting millions of lives worldwide. While the severity of COVID-19 varies widely among individuals, emerging evidence suggests that age plays a significant role in disease susceptibility and outcomes. Understanding the intricate interplay between age and immune responses is crucial for elucidating the pathogenesis of COVID-19 and guiding therapeutic interventions (6, 7). PBSCs, crucial parts of our immune system, play a vital role in fighting infections. They have the special ability to transform into different types of immune cells, like T cells and B cells, which work together to protect our bodies from invaders. By studying how PBSCs change in COVID-19 patients of various ages, we can better understand why people respond differently to the virus depending on their age (8). While many studies have looked at how COVID-19 affects immune cells, we still don't know exactly how ageing affects the expression of PBSCs. Some studies have found that getting older is linked to changes in the number and quality of PBSCs, which could make the immune system weaker and make people more likely to get sick. However, other studies suggest that ageing could trigger mechanisms that make up for these changes and strengthen some parts of the immune response in older adults. To better understand how PBSC (peripheral blood stem cell) expression varies based on age in people with COVID-19, we need to combine all the existing studies. This will help us see if there are any patterns or differences. To do this, we need to perform a meta-analysis where we carefully examine and analyze all the published research on PBSC expression in COVID-19 patients from different age groups. This will give us a more complete picture of the situation and help us draw more reliable conclusions. We will combine information from various studies to better understand how PBSC populations change with age. We will then investigate how these changes may affect how COVID-19 progresses and the results of treatment. Our findings could lead to new treatments and vaccines that are specifically designed for older people, who are more likely to suffer severe effects

from COVID-19. This research aims to enhance knowledge about the complex relationship between age, immunity, and COVID-19. By doing so, it aims to guide the development of more effective interventions to address the ongoing global pandemic. Studying the expression of PBSC in people who have tested positive for COVID-19 is important because it helps us learn how the immune system responds to the virus at different ages. This knowledge is vital for developing treatments and vaccines that work well for people of all ages. Examining PBSC expression levels can reveal possible indicators for determining how severe COVID-19 is and how well patients will do. Researchers can find important elements that affect the outcome of the disease and aid in making clinical choices by examining these fluctuations. Additionally, a thorough meta-analysis of this subject can aid in building a better grasp of COVID-19's disease-causing processes and point to potential areas of future research. By examining the levels of PBSC expression in COVID-19 patients of varying ages, researchers can gain valuable insights that can lead to better treatment approaches and public health measures to combat this ongoing pandemic. Examining the levels of peripheral blood stem cells (PBSC) in people with COVID-19 is crucial for comprehending how the immune system responds and developing treatments to fight the infection. Here are several key reasons for the importance of such studies: immune response characterization: PBSCs play a crucial role in the immune system, being a source of various immune cells. Examining the behavior of a specific cell type, called PBSCs, in people infected with COVID-19 reveals important information about the body's immune response against the virus. This analysis helps identify specific markers, called biomarkers, that are linked to the COVID-19 infection. Understanding these markers provides valuable insights into how the body fights the virus at the cellular level. These molecular markers can provide valuable information about the seriousness, progression, and response to treatment for COVID-19. They enable early detection and personalized treatment plans. Furthermore, understanding these markers can help in the design of targeted therapies specifically for COVID-19. Researchers can develop new treatments that alter the immune system to strengthen defenses against viruses by understanding how cells and molecules work together in the immune response. Insights into PBSC expression can also guide the creation of better vaccines. Vaccine Development, Insights into the production of proteins by a specific type of blood cell (PBSC) can guide the creation of vaccines. By understanding how the immune system reacts to the COVID-19 virus at a cellular level, scientists can design vaccines that trigger a strong and effective immune response, protecting people from infection more effectively. Long-Term Immune Memory: Studying PBSC expression can also provide valuable information about how long-term immune memory develops after a COVID-19 infection. This knowledge is essential for understanding how long immunity lasts and whether booster shots or other vaccination strategies are necessary to maintain protection. COVID-19 affects different people in varying ways, with some experiencing more severe symptoms than others. Researchers can study how proteins found in blood cells change in response to COVID-19 (PBSC expression). This could help us better understand why some people get sicker than others, based on factors like their genes or other health problems they may already have. Tracking the presence and amount of PBSC proteins during an infection provides valuable information for predicting the outcome. By detecting patterns in these levels that indicate how the infection progresses or clears up, healthcare professionals can make more accurate forecasts about the patient's health. This knowledge allows them to adjust treatment plans based on the expected course of the infection, ensuring the best possible care for each individual. Techniques utilized in the systematic review and synthesis of research findings have focused on how the gene expression patterns in peripheral blood stem cells differ between COVID-19-positive and negative individuals. Scientists have been studying how the gene expression patterns in the stem cells found in the blood of COVID-19 patients differ from those of non-infected individuals. This research study combined data from multiple studies to analyze how the gene expression patterns in peripheral blood stem cells of COVID-19-positive patients vary with age. The study aims to identify age-specific differences in gene expression within these cells.

Materials and Methods

Researchers studied immune responses in 76 COVID-19 patients from Hong Kong and Atlanta, as well as 69 healthy individuals. They found that COVID-19 patients had lower levels of immune molecules called MHC-II and proinflammatory cytokines in myeloid cells. Additionally, they observed reduced signaling activity in plasmacytoid dendritic cells, leading to impaired production of interferon-α, an important immune defense molecule. The study also revealed elevated levels of inflammatory proteins like EN- RAGE2, TNFSF143, and oncostatin4 M in the blood of COVID-19 patients. These levels were linked to increased disease severity and bacterial byproducts in the bloodstream. The researchers conducted single-cell analysis and found a reduction in type I interferons, lowered levels of HLA-DR on immune cells, and temporary expression

of genes activated by interferons in patients with severe COVID-19. The researchers examined genetic data (RNA-seq) from blood stem cells to study how gene expression changed with age in COVID-19 patients. They used specific statistical methods (gene set enrichment analysis and single sample gene set enrichment analysis) to compare gene expression patterns in different types of COVID-19 patients. By analyzing the patterns of genes that were turned on (upregulated) and turned off (downregulated), they identified biological pathways that were disrupted in these patients. Age plays a crucial role in the gene activity patterns observed in blood stem cells from COVID-19 patients (figure 1).

1.These variations provide insights into the age-dependent immune response to the virus, potentially guiding the development of tailored treatments. Researchers studied gene activity in blood stem cells from COVID-19 patients of different ages. They found age-based variations in gene activity patterns, indicating that age influences how the immune system responds to COVID-19. By analyzing the activity of these genes, they identified specific pathways that may be affected differently in different age groups. This suggests that age may contribute to the severity of COVID-19 symptoms. Age plays a crucial role in how gene expression changes in stem cells from the blood of COVID-19 patients. These differences have implications for comprehending the body's immune response to the virus and may lead to new treatments. Scientists examined the activity of genes in immune cells from people infected with COVID-19, considering how gene activity changes with age. They found specific gene patterns that were different in people of different ages. By analyzing these patterns, they identified biological pathways that were affected by the virus in

2.extracellular newly identified receptor for advanced glycation end-products binding protein

3.The protein encoded by this gene is a member of the tumor necrosis factor (TNF) ligand family.
4.Oncostatin M, also known as OSM, is a protein that in humans is encoded by the OSM gene

Fig 1. Changes in gene activity based on age in blood stem cells in patients with COVID-19.

different ways depending on age. Researchers discovered that COVID-19-positive patients' peripheral blood stem cells exhibited age-specific changes in gene activity. This suggests that age influences the immune response to COVID-19 and could affect disease severity. This comprehensive analysis provides insights into the immune reactions of COVID-19 patients and highlights the differences between healthy individuals. Scientists investigated the immune reactions of people with COVID-19 and healthy individuals. They found that in COVID-19 patients, certain immune cells (myeloid cells) had lower levels of a protein (human leukocyte antigen class DR) and signaling molecules (proinflammatory cytokines). Additionally, another type of immune cell (plasmacytoid dendritic cells) had problems with certain signaling pathways (mammalian target of rapamycin signaling) and the production of an antiviral protein (interferon-α). The study revealed higher levels of inflammatory proteins (EN-RAGE, TNFSF14, and oncostatin M) in the blood of COVID-19 patients. These levels were linked to the severity of the disease and the presence of bacterial components in the blood. This suggests that the immune system's response to the virus and the underlying mechanisms of COVID-19 may vary across different age groups (figure 2). This extensive study analyzed changes in gene activity within blood stem cells from individuals infected with COVID-19. The results indicate that gene activity patterns vary with age, suggesting that age influences how the immune system responds to the infection. This research emphasizes the significance of including age when analyzing the immune response and severity of illness in COVID-19 patients. By studying the genetic activity in stem cells from the blood of COVID-19 patients, particularly looking at how this activity differs based on age, the study aims to understand the impact of age on the immune response to the virus. The study revealed that people of different ages showed distinct patterns of gene activity, suggesting that age plays a role in how the immune system reacts to COVID-19. This knowledge helps us comprehend how age affects the immune response to COVID-19 and might shed light on the factors that contribute to the severity of the disease.

Discussion and Conclusion

This research study combines existing data to enhance our knowledge about how the amount of a specific protein in the blood (PBSC) changes with age in people with COVID-19. It highlights the critical role of age in predicting how severe the disease

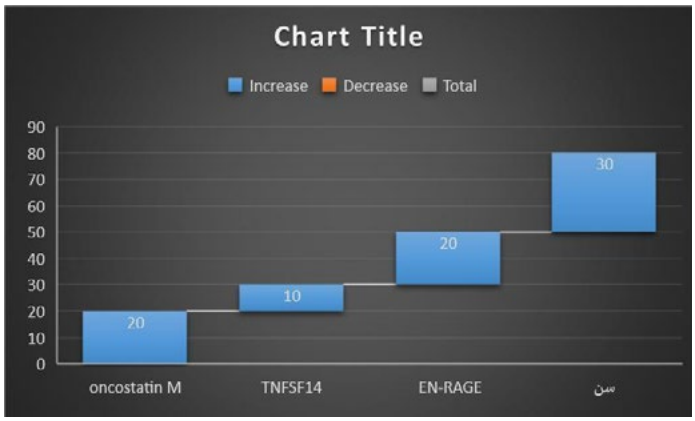

Fig 2. Changes in protein activity based on age in blood stem cells in patients with COVID-19

will be and who is at risk for serious problems. This information is important for developing specific strategies and individualized treatments for older adults with COVID-19. This large-scale study provides valuable insights into how age affects PBSC levels in people with COVID-19. It highlights the critical role of age in assessing the severity of the disease and determining who is at higher risk of developing serious complications. Our study shows that age significantly affects how immune cells, called PBSCs, respond to COVID-19. These age-related differences in PBSC behavior are important for understanding how the disease progresses and how it is treated. It highlights the need to consider age when evaluating how severe a person's COVID-19 is and when developing treatments that are tailored to individual patients. Older adults have different immune profiles than younger adults, specifically in the expression of PBSCs. This means that older adults may need different treatments for COVID-19 to address their specific immune needs and reduce the risk of complications. Personalized medicine approaches that take into account age-related differences in PBSC expression are crucial for managing COVID-19 in older patients. Customizing medical treatments based on each person's immune system response can be beneficial, especially for older adults. This is because, as we age, the expression of proteins called PBSCs changes. By considering these age-related differences in PBSCs, treatments can be designed to enhance their effectiveness and lead to better health outcomes for older individuals. Our research highlights the intricate relationship between age, immune system responses, and how COVID-19 develops. This knowledge is essential for creating personalized treatments and specific interventions tailored to individual patients. It is especially important for elderly individuals, who are at a higher risk of developing severe complications. Ongoing research should delve deeper into why PBSC levels vary with age and how this affects COVID-19 treatment. This knowledge will enhance patient care and mitigate the pandemic's impact. A comprehensive analysis combined data from various studies that examined PBSC expression in COVID-19-positive individuals of different ages. Researchers studied data to compare amounts of a protein called PBSC in younger and older people with COVID-19. They looked for patterns and links between PBSC levels and how severe the disease was. The analysis found that PBSC levels changed significantly with age in people with COVID-19. Older adults have more PBSC expression than younger adults, indicating that age affects how the body's immune system responds to the virus. This aligns with earlier studies that found age-related variations in immune response and vulnerability to infectious diseases. Additionally, the study revealed a link between higher levels of PBSC expression and increased disease severity and worse outcomes in elderly individuals. This indicates that PBSC expression levels could be a potential indicator for monitoring disease progression and severity in COVID-19-positive individuals, particularly in older age groups. It's crucial to consider how age-related changes in PBSC (peripheral blood stem cells) affect people with COVID-19 (9).

1.Weaker Immune Response: These changes can weaken the overall immune response, making individuals more likely to get infected and develop more severe symptoms due to decreased defense against SARS-CoV-2.

2.Inflammation and Cytokine Imbalance: COVID-19 triggers an excessive inflammatory response with a disruption in cytokine balance (known as a "cytokine storm"). Age-Related Changes in Stem Cells and COVID-19 Severity as we age, our bone marrow stem cells (PBSCs) undergo changes that can affect COVID-19 disease severity.

3.These changes may: increase inflammation: PBSCs can release signaling molecules that worsen inflammation, contributing to the immune system's

overreaction in COVID-19.

4.Impair tissue repair: PBSCs are essential for healing and regenerating damaged tissues. However, their function may decline with age, slowing down recovery in COVID-19 patients. This can lead to longer hospital stays, an increased risk of complications, higher chances of lasting health problems in older individuals as we age, and changes in the body's immune system that can affect how well it responds to infections. This includes the production of antibodies, which are essential for fighting off viruses like SARS-CoV-2. Research suggests that certain immune cells may be less efficient at producing antibodies in older individuals, which could impact the effectiveness of vaccinations in this population. It's essential to understand how age affects PBSC expression and its role in COVID-19 to create specific treatments for different ages. Age significantly influences the severity and progress of the disease, so it's important to consider it when treating COVID-19. Different age groups may require different approaches to exploring age-related differences in PBSC expression and their impact. As we grow older, our body produces fewer peripheral blood stem cells (PBSCs) that help strengthen our immune system. Researchers believe that for people who have COVID-19, these changes related to age may impact how their body fights the virus. As people age, their immune systems have a harder time recovering and rebuilding. This can make it take longer for them to get over COVID-19. Compared to younger people, older people's immune systems have a weaker response to COVID-19. Because of this, older people are more likely to have severe symptoms, take longer to recover, and have serious complications from COVID-19. Changes in immune cell levels, called PBSCs, in people of different ages, can affect how their immune systems fight COVID-19. Studying these changes could help diagnose, predict, and treat the disease. However, more research is needed to understand the exact role they play and how they are affected by age. A recent study found that younger COVID-19 patients had higher PBSC levels than older patients, which suggests that age may influence the immune response to the virus.

Results

The results reveal the importance of age in understanding COVID-19's development and treatment. There is a need to account for age in evaluating disease severity and outcomes. The significance of further investigating the causes of age-related differences in immune responses The potential for age-specific therapies to improve COVID-19 outcomes. Statistical analysis shows a clear link between age and levels of certain immune proteins in COVID-19 patients. Expression of Primitive Blood-Stem Cells Based on Age as people age, there are noticeable changes in how their immune cells (PBSCs) respond to COVID-19. Compared to younger people, older individuals have different patterns of PBSC activity. This suggests that age plays a role in shaping the immune system's response to COVID-19 at the cellular level. Variations in Numerical Measures by Age Groups There are distinct variations in the levels of peripheral blood stem cells (PBSCs) in different age groups when it comes to COVID-19. A detailed analysis has revealed specific ages at which major changes in PBSC levels occur, helping us better understand the important stages in the immune response to the disease as we age. Correlation with Disease Severity Research suggests that age-related changes in the levels of specific proteins (PBSCs) may be linked to the severity of COVID-19. Older people who have abnormal PBSC patterns could be more likely to experience serious symptoms or health problems caused by COVID-19. Clinical Implications: The study's findings have practical significance in healthcare settings. Medical professionals can leverage this knowledge to customize treatment plans and interventions specific to the immune responses of different age groups. This information can guide decisions about vaccination schedules, treatment options, and tailored medical care for patients with COVID-19 across various age demographics. Public Health Considerations: Studying how the expression of PBSC varies with age is important for developing public health plans. This information can help guide vaccinations, public health programs, and how resources are used to meet the unique needs of different age groups.

Suggestions for further research

This study's findings provide opportunities for future research. Further studies can explore the biological processes behind age-related changes in PBSC levels. They can also investigate the wider impact on the immune system's performance and reaction to viral infections in older individuals.

Limitations and Caveats

The study's results have some potential weaknesses, including differences in how studies were done, the types of patients included, and the number of participants. These limitations should be kept in mind when understanding the results, and they can help plan future research to fill in any gaps or answer any questions. In summary, our analysis reveals age-related differences in PBSC expression that greatly impact our understanding of COVID-19 immune responses. These insights highlight the complex relationship between age and immunity, enabling tailored treatment strategies for COVID-

positive individuals of different ages.

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Personalized Neoantigen Therapy Is an Innovative Strategy for Combating Cancer

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Abstract

Immunotherapies that use the immune system to eliminate tumor cells have shown substantial therapeutic effectiveness in several types of human malignancies. Several investigations have emphasized the importance of neoantigens in the recognition of cancer cells by innate T lymphocytes. The identification of neoantigens, which are altered proteins that are selectively produced in tumor cells and not in healthy cells, has resulted in the development of enhanced cancer vaccines. Neoantigen targeting may stimulate anti-tumor T-cell reactions to eliminate tumors while sparing healthy cells from harm. Significant progress in DNA sequencing and computational biology has enabled the identification and development of potent neoantigens for application as therapeutic cancer vaccines. Therapeutic customized vaccines that target neoantigens have demonstrated encouraging outcomes in the field of cancer therapy. Therefore, this study aims to introduce neoantigens and their use in cancer immunotherapy.

Keywords: Neoantigens, T-cell, DNA sequencing, Cancer therapy

Introduction

Detrimental stimuli, such as ultraviolet radiation, ionizing radiation, and carcinogens, can lead to various types of genetic alterations, such as single-nucleotide mutations, insertions or eliminations gene fusion, frameshift mutations, fundamental mutations, or the integration and clonal expansion of the tumor-associated virus genome within the human genome. Specifically, these genetic changes can result in somatic cell carcinogenesis (1). In recent decades, immunotherapy has shown significant promise in the management of malignancy. Cancer cells generate mutated proteins that may be identified by the body's immune system as antigens, leading to subsequent activation of cellular and humoral immune system reactions. Non-synonymous mutations may generate mutant peptides that are not identical to the original peptides (2). Human (HLA) molecules can recognize these mutated peptides and trigger T-cell responses. These mutated peptides are referred to as neoantigens. Due to the lack of influence from thymus selection or central tolerance, probably, T lymphocytes with a high avidity for leukocyte antigen neoantigens probably exist. Hence, the study topic of immunotherapy for malignant tumors, specifically targeting these non-synonymous mutant proteins, is attracting considerable attention (3).

Cancer immunotherapy encompasses a wide array

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of treatment strategies that seek to use the immune system to create specific and effective immune responses against tumors. Cancer cells possess a collection of somatic mutations that accumulate over time and occur at different rates. These mutations can trigger an immunological response and be identified by the immune system (4). Nevertheless, tumors can avoid detection by the immune system through multiple means, including interfering with the presentation of antigens, manipulating checkpoint pathways, infiltrating the tumor with cells that suppress the immunological response, and increasing the production and release of cytokines that inhibit the immune system. The study of cancer immunotherapy has revived due to an improved comprehension of the complex processes that control the inhibition of the immune system caused by tumors. Neoantigens originate from genetic abnormalities in tumor cells, such as chromosomal translocations, somatic point mutations, and insertions and deletions (indels). Most mutations occur inside introns, and some of these changes result in mistakes during splicing. The altered genes undergo transcription and translation to generate modified peptides, which are then broken down and presented by MHC molecules for identification by T-cells, leading to the activation of T-cell immune responses (5). The functional significance of cancer neoantigens may be ascribed to three factors: (1) the number of mutations in the tumor (tumor mutation burden or TMB) and the presence of neoantigens, (2) the display of neoantigens by major histocompatibility complex (MHC) molecules, and (3) the identification of cancer neoantigens by T-cells. The conventional approach for treating malignant tumors involves surgical intervention, in conjunction with either radiation or chemotherapy (6). Nevertheless, these treatment options often come with undesirable adverse reactions. In recent decades, tumor immunotherapy has shown promising results in terms of its remarkable effectiveness in treating cancer. Due to advancements in DNA sequencing and bioinformatics computations, neoantigens have emerged as attractive candidates for cancer immunotherapy because of their highly immunogenic nature. Furthermore, neoantigen-based vaccinations have shown promise in cancer treatment, mainly via enhancing T-cell responses. Additionally, neoantigens have demonstrated efficacy in immune checkpoint blockade treatment. Thus, neoantigens have the potential to function as prognostic indicators and complementary goals in cancer immunotherapy (7). The objective of this study was to offer a comprehensive summary of the current advancements in the categorization, evaluation, and medical use of neoantigens for the treatment of cancer.

Introduction of neoantigens

The genesis of cancer is a multifaceted phenomenon. Genetic alterations result in changed internal signaling, leading to cellular activity changes and malignant tissue development. Ultimately, both organs and the whole body are impacted. Tumor immunology originated more than a century ago with the first discovery that antibodies might be generated in response to malignancies (8). This discovery provided evidence for the notion that cancers were considered “alien” or not native to the body. Extensive research has been conducted to discover specific targets inside tumors that might trigger an immune response against the tumor. There are two primary categories of cancer antigens. Tumor-associated antigens (TAAs), characterized by their elevated expression on cancerous cells compared to normal cells, are mostly found in tumor cells (9, 10). Cancer neoantigens, also known as tumor-specific antigens (TSAs), result from genetic modifications that cancer cells undergo throughout the development of cancer or via epigenetic processes. Tumor-specific neoantigens, which are produced by somatic mutations, may be detected by T-cells and have an impact on how patients respond to immunotherapy. Following transcription and translation, the peptide containing neoantigens undergoes processing by the antigen-processing apparatus. It is subsequently transferred onto the major histocompatibility complex (MHC) for presentation on the cell surface. In contrast to TAAs, cancer neoantigens are not influenced by central immunological tolerance. They exhibit greater immunogenicity and have a higher affinity for major histocompatibility complex class II (MHC-II), resulting in a robust tumor immune response (11). Not every somatic mutation creates neoantigens, and only a mere 10% of the non-synonymous mutations found in cancer cells can form antigenic peptides. Immunological neoantigens must possess several characteristics. Initially, the somatic mutations must modify the protein expression. Subsequently, the neoantigens may be appropriately digested and deposited onto MHC complexes, enabling them to be identified by the TCR of responsive T cells (12). Furthermore, every tumor has several distinct clonal/trunk and sub-clonal neoantigens. The presence of a large number of clonal/trunk neoantigens, but not sub-clonal neoantigens, is linked to a higher likelihood of tumor recurrence and worse survival rates in patients with early-stage NSCLC. The use of next-generation sequencing (NGS) technology and computational bioinformatics has facilitated the identification of the genetic characteristics of individual tumors, the discovery of potential neoantigens, and the analysis of immune system profiles for personalized oncology in a fast and cost-efficient manner (13).

T cells and their T cell receptors (TCRs) have a

crucial function in adaptive immune responses directed against cancer cells. During the process of tumor-mediated evasion (TME), T-cells produce a wide variety of TCR repertoires by rearranging genes in response to tumor antigens. The TCR repertory exhibits more variability in tumors compared to non-tumor tissues in different cancer (14). Due to the heterogeneity of neoantigens in the environment, each tumor in different patients has a distinct and diverse T cell repertoire, making it distinct. As the tumor advances, the quantity and variety of neoantigens also change (15). Furthermore, the process of eliminating tumors also results in the release of extra neoantigens and TAAs. The TCR repertoire becomes more diverse as tumor growth advances, due to the rise in both neoantigens and TAAs in both the tumor microenvironment (TME) and sentinel lymph nodes. Individuals exhibiting more TCR diversity demonstrate enhanced clinical responses to immune checkpoint inhibitors (ICIs) compared to patients with lesser TCR diversity in cases of lung and cervical cancer. The clonality and variety of T-cell repertoires to neoantigens differ in tumors, non-tumor tissues, and peripheral blood, and can change over the course of cancer development (16). Effective anti-tumor immune responses necessitate the proper display of tumor antigens and a tumor microenvironment (TME) that is abundant in capable immune cells. The presence of immune cells in tumors can differ both among various tumors and within the same tumor. This variation is due to different mechanisms that cause malfunction in the presentation of neoantigens, which are more prevalent in specific immune microenvironments. A comprehensive meta-analysis was conducted on more than 1000 cases treated with IPI, where exome/transcriptome data was analyzed (17). The study found that the level of clonal TMB, but not sub-clonal TMB, was the most influential factor in predicting the response to IPI treatment. The presence of immune cells differed both among various cancers and within individual tumors, with different modes of failure in presenting neoantigens being more prevalent in specific immunological microenvironments. The tumor sites that were invaded by the immune system showed ongoing immunoediting, which involved either the loss of heterozygosity in human leukocyte antigens or the hypermethylation/depletion of expressed neoantigens. Therefore, the present neoantigen vaccines are specifically engineered to focus on several clonal neoantigens to enhance the immune response to cancer in each unique patient (18).

Neoantigen classification

Neoantigens may be categorized into two groups: shared neoantigens and individualized neoantigens. Shared neoantigens are mutant antigens that are found in several cancer patients but do not exist in the normal genome. Neoantigens that are both shared and very immunogenic may be investigated for possible use as broad-spectrum therapeutic cancer vaccines for people with the same mutant gene. Personalized neoantigens are mutant antigens that are distinct from most neoantigens and vary totally from person to person (19). Therefore, the medicine for individualized neoantigen production must be precisely tailored to each patient, resulting in a personalized treatment (20). Neoantigens, with potent immunity, may decrease the likelihood of immune evasion by tumor cells. However, the presence of certain mutations results in distinct kinds and amounts of neoantigens among people of the same tumor, leading to evident individual heterogeneity. Consequently, the use of neoantigens in tumor immunotherapy will likely be tailored to individual patients (21). Personalized cancer vaccines may be used alone or in conjunction with other treatments to enhance the potency and longevity of the anti-tumor response, enhance survival rates and quality of life, and eventually improve the result of cancer therapy for patients. The next trend in the treatment of cancer patients will be determined by the feasibility, security, and immunogenicity of customized cancer vaccines. In the near future, it is anticipated that personalized cancer vaccinations will allow the majority of patients to get very accurate therapy (22).

Neoantigen diagnosis

While neoantigens have shown promising results in tumor treatment, the pool of neoantigens that exhibit immunogenicity is limited, making it challenging to accurately anticipate and compare their effectiveness. Thus, the neoantigen domain requires enhanced algorithms and verified techniques to precisely anticipate and identify highly immunogenic neoepitopes, ensuring their reliability. Currently, the accuracy of predicting tumor neoantigens is a pressing issue. When developing a tumor neoantigen prediction algorithm, many parameters must be taken into account. These elements include HLA type, expression analysis, mutation analysis, prediction of peptide processing, TCR binding strength, MHC affinity, PMHC stability, and the source of tumor neoantigens. Additionally, it encompasses the evaluation of T cell recognition, TCR analysis, and immune cell analysis to gauge the T cell response. To screen neoantigens and evaluate T cell responses, next-generation sequencing is commonly used. However, there are also additional approaches such as high-resolution and tandem mass spectrometry, as well as in silico methods for peptide estimation (23, 24). It is important to note that prediction algorithms based on machine learning and artificial intelligence

require ongoing training with reliable datasets. The type, quality, and quantity of the data used for training can significantly impact the accuracy of these algorithms (25). Hence, the ongoing collection of datasets, particularly the verified tumor neoantigen information, is of utmost importance in enhancing algorithm precision (26). The Tumor Neoantigen Selection Alliance (TESLA) was established via collaboration between the Park Institute for Cancer Immunotherapy (PICI) and the Cancer Research Institute (CRI). TESLA collaborates with 36 prominent biotechnology, pharmaceutical, academic, and non-profit research teams, including the National Cancer Center (NCC), PICI, Memorial Sloan-Kettering Cancer Center (MSKCC), MD Anderson Cancer Center, and over 30 other leading neoantigen research organizations. The consortium's objective is to develop algorithms and standards for worldwide neoantigen testing, collaborate to forecast more accurate targets for anticancer treatments, and promote the study and implementation of tailored tumor vaccines. Scientists at TESLA have identified algorithmic models and fundamental factors that might improve the prediction of neoantigens. Their research successfully predicted 75% of validated neoantigen targets and filtered out 98% of invalid neoantigen targets. These results were published in reputable cell publications (27, 28).

Utilization of neoantigens in clinical trials

The anticipated tumor-specific neoantigens are significant targets for therapeutic customized immunotherapies, such as a vaccination or a cellular therapy product.

Cancer vaccines

Personalized vaccinations may be created using several methods, such as synthetic long peptide (SLP), DNA, RNA, and DC, as well as viral and bacterial components (29). The findings obtained from many clinical studies including neoantigen vaccinations in individuals diagnosed with melanoma or glioblastoma are quite promising. Carreno et al. first discovered that the DC vaccination enhanced existing neoantigen-specific immunity and triggered previously unnoticed neoantigen-induced T-cell responses in three patients with advanced melanoma. HLA-A*02:01 molecules digest and express neoantigens. In 2017, two consecutive clinical trials were reported that validated the efficacy of tailored neoantigen vaccines for the treatment of melanoma patients (30). Ott et al. administered a vaccine to six patients, targeting specific tumor neoantigens that were expected to be present. The clinical trial identifier for this study is NCT01970358. Out of the six patients, four did not have their tumors come back during the next 25 months after receiving the immunization. The other two patients who had tumor recurrence were able to achieve full tumor remission after undergoing anti-PD-1 treatment (31).

An additional study revealed that eight individuals at high risk had long-lasting T-cell responses produced by neoantigens. It is promising that over four years after receiving vaccinations, all individuals are still alive, and six of them do not have any ongoing illness. Sahin et al. developed the first personalized RNA mutanome vaccines for 13 patients diagnosed with stage melanoma (NCT02035956). During the follow-up period, eight patients did not have any recurrence of tumors. Out of the five patients with metastatic illness, two had objective answers, and one had a full response when treated with a combination of an RNA vaccine and a PD-1 inhibitor (32). The promising findings suggest that the tailored neoantigen vaccination strategy is viable for immunologically "cold" cancers characterized by a low tumor mutation load. A recent vaccination research conducted in Washington, D.C. for ovarian cancer demonstrated encouraging clinical results, with no significant negative effects. Vaccination enhanced the activation of T cells in response to neoantigens, hence inducing a wide-ranging immune response against tumors (33, 34). Consistent with prior research, the EpiGVAX vaccination, which is based on neoantigens, enhanced the body's ability to fight against tumors in colorectal cancer. Ott et al. recently carried out a clinical study (NCT02897765) where they coupled a tailored neoantigen-based vaccination called NEO-PV-01 with nivolumab in patients suffering from advanced melanoma, non-small cell lung cancer, and urothelial carcinoma. Neoantigen-specific T cells were identified in all immunized individuals, and no significant adverse effects were reported. The method described here stimulates the proliferation of T lymphocytes, which may effectively suppress tumor development and eliminate tumor cells, resulting in potential advantages for therapeutic applications (35).

Assisted cell therapy

Adoptive cell therapy is another neoantigen-targeted therapeutic strategy. Patients get ex vivo expansion of natural or engineered T cells injected into them to boost T cell responses and eliminate cancer cells. Adoptive T cell treatments involve the adoptive transfer of TILs, T cells genetically modified to produce a chimeric antigen receptor (CAR) or a T cell receptor (TCR), and other immune cells such as natural killer cells (36). Neoepitopes produced by somatic mutations were recognized by personal TILs in gastrointestinal malignancies. Adoptive transfer of T lymphocytes targeted against oncogenic mutations has been shown in many trials to be able to induce tumor regression in metastatic breast,

colorectal, cervical, and cholangiocarcinoma (37). Neoantigen-reactive CD4+ TILs administered by Rosenberg et al. in 2014 to a patient with metastatic cholangiocarcinoma (NCT01174121) completely regressed the tumor. The discovery provided proof that epithelial carcinoma regression may be mediated by CD4+ T lymphocytes against neoantigens. Then, in TILs from a patient with metastatic colorectal cancer, CD8+ T cells were identified that could selectively target mutant KRAS G12D (NCT01174121) (38). There was objective remission of all lung metastases after infusion of the HLA-C*08:02–restricted TILs. One lesion that worsened nine months later was found to have lost chromosome 6, which encodes the HLA-C*08:02 MHC class I protein (38). Moreover, in cervical carcinoma linked to the virus, therapeutic TILs against mutant neoantigens produced immunodominant antitumor T cell responses rather than against antigens of the human papillomavirus (HPV), leading to total tumor regression. Metastatic breast cancer patients were also observed to benefit from adoptive treatment (NCT01174121). The individual had long-lasting tumor reduction over 22 months after receiving TILs against four neoantigens (SLC3A2, KIAA0368, CADPS2, and CTSB). These studies all provided evidence in favour of the essential function of adoptive transfer of neoantigen-based TILs in immune therapy (39, 40).

Challenges

Although there have been recent improvements, there are still several obstacles to overcome in implementing tailored neoantigen-based vaccines or adoptive cell transfer. An urgent problem that requires attention is the costly and labor-intensive manufacturing process (39). While the expense of genome sequencing has dropped, the identification of neoantigens and adherence to good manufacturing procedures still incur significant costs. The whole duration from obtaining the patient's sample to administering the vaccination was around 3 to 5 months. It is crucial to expedite the manufacturing turnaround time, particularly for patients suffering from metastatic illness. Standardizing these cell-based investigations is challenging and requires a substantial quantity of cells. Therefore, it may be necessary to use high-throughput and unbiased computational algorithms to choose neoantigens (41). Another potential challenge might arise in the prediction and confirmation of neoantigens. Although there are now computational algorithms and laboratory validation methods (such as tetramers or multimers, ELISpot) being utilized to select neoantigens, more efforts are required to better optimize these processes. Strategies include enhancing the ability to forecast MHC-peptide binding and creating extensive datasets and novel algorithms. Furthermore, tumor heterogeneity is prevalent and may arise from multiple causes, such as 1) natural mutations occurring during tumor development, 2) modulation of tumor microenvironments or loss of neoantigens, and 3) the presence of numerous lesions or a single tumor originating from diverse subclones. Tumor heterogeneity may diminish the precision of antigen clone prediction in heterogeneous tumor masses. Hence, it is essential to meticulously examine advantageous mutations (42). Moreover, the successful implementation of individualized immunotherapy that specifically targets several clonal neoantigens may face the challenge of tumor heterogeneity. Another obstacle is the task of establishing precise immune biomarkers that can accurately forecast antitumor immunity and potential survival advantages. While immune-related response criteria (irRC) aim to assess the impact of immunotherapy in clinical settings, they may not comprehensively capture all aspects of clinical responses. In addition, the T-cell responses generated by neoantigen-based therapy may not immediately result in long-lasting clinical responses. Therefore, it is feasible to detect immune response indicators in a methodical manner (43).

Concluding remarks and outlook

New research suggests that tumor neoantigens are crucial for the effectiveness of cancer immunotherapies and the body's immune response against tumors. Precision medicine, namely tailored or public neoantigen vaccination, signifies state-of-the-art progress and potential for cancer therapy. Neoantigens, which originate from diverse and fluctuating origins, have been scientifically shown to be unique to tumors and extremely capable of triggering an immune response. Furthermore, they can create long-lasting immunological memory that protects against cancer. Neoantigen-focused cancer treatments have advanced significantly in recent years, including the discovery, forecasting, and evaluation of neoantigens, as well as therapeutic alternatives. Neoantigen vaccines have shown significant safety and effectiveness in several forms of cancer via clinical studies, and numerous current investigations are further exploring their potential. Neoantigen vaccines, which use mRNA as a delivery method and target public neoantigens, are a novel and promising technique. These vaccines have shown enhanced clinical characteristics and drugability, making them very attractive for the advancement of precision cancer immunotherapy. Nevertheless, there are still obstacles to overcome in the development of neoantigen vaccines, since some elements need further optimization to get more favorable clinical outcomes. Neoantigen-targeted treatments are projected to become more feasible,

affordable, and successful due to many factors. These include a decrease in production turnaround time, a reduction in manufacturing cost, enhanced detection of immunogenic neoantigens, advancements in computational methods, and the identification of more effective treatment biomarkers. Neoantigen-based treatments can transform cancers that lack an immune response into tumors that elicit a strong immune response. Thus, it is justified to investigate the combination of different immunotherapies, such as checkpoint blockade therapies or traditional treatments like chemoradiotherapies, kinase inhibitors, anti-angiogenesis therapies, and others. Therefore, it is reasonable to believe that personalized medicines based on neoantigens might soon be extensively used in the treatment of different types of cancer.

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Authors' Contribution

Parisa Sanaati and Neagar Pour Naghshband were involved in the conceptualization, design and writing of the manuscript draft. The authors read and confirmed the final manuscript.

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A Review of Vaccinia Virus as a New Therapeutic Target Against Cancer

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Abstract

The World Health Organization's smallpox eradication program extensively utilized the Vaccinia virus, which is today regarded as a viable vector for gene therapy due to its distinctive features. Vaccinia virus can specifically reproduce and spread effectively in cancer cells, leading to the destruction of the tumor. Furthermore, the fast generation of viral particles, the ability to infect a wide variety of hosts, the big genome size (about 200 kb), and safe handling make the vaccinia virus an appropriate choice as a vector for gene therapies. Oncolytic virotherapy (OVT) is a highly prospective modality for fighting cancer that involves the use of genetically modified viruses to reproduce specifically inside cancer cells and stimulate an immune response against the tumor. Oncolytic viruses not only destroy cancer cells but may also modify the surrounding tumor microenvironment and trigger a durable defense against the tumor. Vaccinia virus has gained attention as a promising contender because of its capacity to invade a diverse array of cancer cells. In this study, we introduce the vaccinia virus, its molecular mechanism and cell cycle, and its potential to destroy cancer cells.

Keywords: Gene therapy, Cancer, Viral vector, Vaccinia virus.

Introduction

The management of cancer is today a worldwide issue of great importance. Around 7 million people globally succumb to aggressive tumors every year, and it is projected that this figure will rise to 12 million by 2030 (1). The main approaches for treating cancer involve surgical intervention, radiation therapy, and chemotherapy. Nevertheless, even conventional therapeutic approaches have some restrictions. Conventional surgical therapy allows for the quick and direct removal of tumor tissue. However, if the resection is not thorough, there is a risk of residual tumors remaining. In addition, chemotherapy and radiation treatment lack specificity, since they not only target tumor cells but also kill healthy immune cells, resulting in a decrease in patient immunity (2). Hence, there is a need for novel and focused strategies to address these constraints in the management of malignancy. Recently, novel methods have arisen for creating new biological treatments for cancer, such as CAR-T cell therapy and oncolytic viruses (OVs), either on their own or in conjunction

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with conventional treatments. Over the course of more than three decades, OVs have demonstrated exceptional effectiveness in treating many forms of cancer (3).

Ovs exert their therapeutic properties via a complex interplay of sophisticated processes that are specifically engineered to target and destroy cancer cells preferentially. Upon injection, OVs selectively identify and invade cancerous cells (4). This precision is accomplished by using various genetic alterations that capitalize on the intrinsic weaknesses of cancer cells. One main method is the direct destruction of infected cancerous cells, where the multiplication of the virus inside the tumor leads to cell destruction, releasing new viruses and components from within the cell (5). Simultaneously, the transmission of viruses stimulates immunogenic cell death, which releases threat signals and facilitates the activation of immune system reactions against tumors. Once the immune system is mobilized and stimulated, it identifies and eradicates infected and uninfected adjacent cancerous cells, resulting in a widespread immune response against the malignancy. In addition, oncolytic viruses can trigger a series of changes in the tumor environment that affect the immune system. This leads to an increased presence and stimulation of immune cells, including cytotoxic T lymphocytes and natural killer cells. OVs may also hinder the blood flow to tumors by disrupting tumor blood vessels, hence adding to the anticancer impact (6). OVs can induce the production of therapeutic transgenes in the tumor microenvironment (TME) after infecting cancer cells, in addition to their main role. This characteristic enhanced the ability of the treatment to destroy cancer cells and is thus crucial for the subsequent integration of oncolytic therapy and immunotherapy. These methods enable scientists to manipulate the immune system in order to generate antitumor immunity via OV activation (7). The relative roles of each pathway are contingent upon the specific properties of the tumor cell, the viral vector, and the relationships among the virus, tumor environment, and host immune response. The several systems working together result in the specific removal of malignant cells by oncolytic viruses, offering a hopeful path for novel and focused cancer immunotherapies. OVs treatment involves several processes, including connections among cancer cells, viruses, and the immune system (8). OVs' anti-tumor action may be classified into two distinct groups: tumor cell death, which relies on the presence of the receptor on the cell, and the anti-viral response of the host cell. The second method enhances the development of a proinflammatory tumor microenvironment, promoting systemic anti-tumor immunity (9).

Various methods have been devised to modify viruses to make them oncolytic. These methods include reducing the activity of viral genes that are essential for proliferation in normal cells, as well as using promoters that are unique to certain tissues or tumors. According to reports, OVs reproduce more effectively in tumor cells because tumor cells lack proper antiviral type I interferon signaling (10). In general, oncolytic vaccinia virus (VV) can infect both normal and cancerous cells. However, with genetic alterations, it is possible to make these viruses specifically replicate in cancer cells. The VV is a kind of virus that has a protective outer layer and a double-stranded DNA genome that is around 190 kilobases in size (11). It has about 250 genes that code for proteins. The first secure and efficient human vaccination, finally eliminating smallpox. As VV replicates only in the cytoplasm of infected cells, there is no need to worry about the occurrence of mutagenesis inside the nucleus. Vaccinia is known to infect a diverse array of cells (12). The virus infiltrates host cells via an endocytic mechanism by penetrating the cell membrane. VV can accommodate up to 50 kilobases of foreign DNA and can concurrently produce numerous medicinal genes. VV undergoes a quick and destructive replication cycle, stimulating a strong immune system reaction and inflammation in the host (13). VV has become an appealing choice for genetic modification as an oncolytic drug due to recent progress in molecular biology. This article provides a comprehensive review of the vaccinia virus, its cell cycle, and various types of recombinant vectors. In addition, it provides a summary of the progress made in the use of this virus as an oncolytic virus in cancer treatment.

Biology of VV

VV is the most thoroughly studied member of the Poxviridae family, which is the largest and most complex group of animal viruses. The worldwide elimination of smallpox was accomplished with the widespread use of vaccinia virus, which is thought to have evolved from cowpox virus (14). The smallpox vaccination, first described by Edward Jenner in 1798, consists of live VV1 and is largely acknowledged as one of the most effective vaccinations in history. Smallpox is the only human disease that has been completely eradicated. In 1980, the World Health Organization (WHO) recommended the global cessation of smallpox vaccination, except for researchers who face a significant risk of contracting the poxvirus (15). The vaccinia virus genome is composed of two DNA strands organized in a linear shape, measuring about 200 kilobases in length. Inside this genome, there are about 250 potential genes. The virions have an exterior envelope and measure around 200 x 300 nm, displaying a unique rectangular shape. In 1980,

the World Health Organization (WHO) provided guidance The vaccinia life cycle has many unique features that distinguish it as a eukaryotic expression vector. The vaccinia genome can tolerate at least 25 kilobases (kb) of DNA without any detrimental effects on viral replication or assembly (16). Vaccinia virus undertakes complete reproduction inside the cytoplasm of the host cell. Consequently, it either brings in or regulates the production of its own polymerases and transcription factors. Moreover, the virus has a wide range of hosts, enabling it to infect a majority of established mammalian cell lines. The virus can be easily grown and purified in large quantities, and it presents a relatively minimal danger when handling (16).

Reproductive cycle of VV

Considering the size of the vaccinia virus, it is unsurprising that the replication and assembly process is complex. After binding to the host cell, it is believed that the viral envelope immediately fuses with the plasma membrane, resulting in the release of the core virion into the cytoplasm. Although there is little understanding of the uncoating mechanism, the transcription of early genes starts within one hour after infection. Upon entering the cell, the vaccinia virus undergoes DNA uncoating and initiates early transcriptions (17). The virus progresses through three separate phases of transcription, namely early, middle, and late stages, each of which is controlled by various promoters and transcription factors. The enzymes required for the initiation of early transcription are contained inside viral particles and are released upon viral entry. During the first stage of infection, the production of essential proteins required for the replication of the virus takes place (18). The virus introduces a DNA-dependent RNA polymerase into the cytoplasm, leading to the synthesis of early mRNA. The RNA molecule facilitates the synthesis of primary proteins involved in the removal of the viral DNA's protective coating, DNA replication, and the generation of intermediate mRNA by transcription. Afterwards, an intermediate form of mRNA is produced by transcription. This mRNA carries the necessary instructions for the development of late transactivators, which in turn leads to the synthesis of late mRNA. The mRNAs that are produced later are responsible for encoding structural proteins that have a function in the building of membranes (19). In addition, they encode crucial early transcription factors that are necessary for the integration of these proteins into the just-created viral particles. The virus may recruit and use certain cellular proteins throughout the transcription process. An instance of this is the cellular transcription factor YY1, which acts as a stimulator of the vaccinia virus late promoter (19).

VV DNA replication occurs inside mini-nuclei, which are enclosed by the endoplasmic reticulum in the cytoplasm. Replication takes place by the creation of many concatamers of the genome. The concatamers are then disassembled into individual genomes, which are then encased, along with the early transcription factors, inside membranes formed by the Golgi (19). The first stage in the formation of infectious particles entails the production of viral crescents, including lipids and viral proteins. The origin of these crescents is still unknown. Currently, it is hypothesized that the crescent consists of a single lipid bilayer that is not linked to cellular membranes. Afterwards, these crescents combine to create an immature virus that lacks the capability to induce infection. The

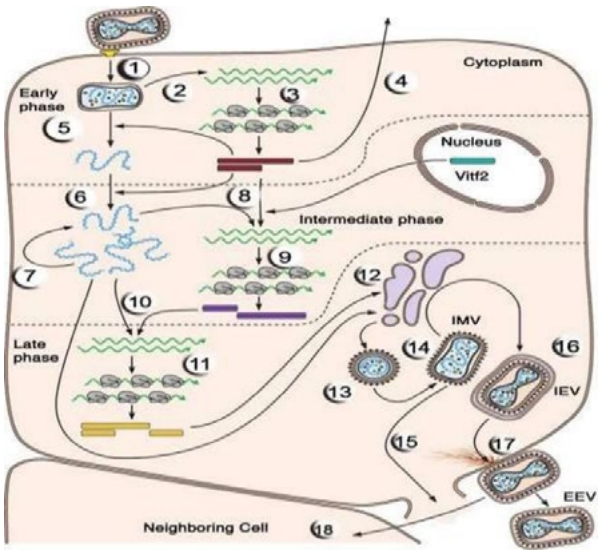

Fig1. The life cycle of the VV. The essential phases of the life cycle that are required for replication and serve as appropriate objectives for drug development include DNA synthesis, transcription, morphogenesis, and cell egress.

nascent virus undergoes maturation by compacting its core and degrading core proteins, leading to the creation of an intracellular mature virus (IMV) (19). The intracellular membrane vesicle (IMV) is then transported to specified locations where it obtains two additional membranes. The membranes in issue originate from the transGolgi network. The proteins encoded by a virus have modified them, causing them to become a part of the outer envelope of the EEV. Internal encapsulated viruses are conveyed to the cell surface, where the outer membrane fuses with the plasma membrane, thus exposing the virus to the cell surface. An intracellular enveloped virus (IEV) has four membranes that are tightly intertwined and can fuse with the plasma membrane. As a consequence of this fusion, virions with three membranes are released into the extracellular space (20).

VV promoters

VV promoters have a concise and straightforward structure, including a central core-initiator region that does not exceed a length of 30 base pairs. Extensive mutagenesis research has definitively found the optimum sequences that trigger both early and late gene transcription (22). Consequently, VV expression vectors have been created that have natural early and/or late promoters or appropriate regulatory sequences. Due to the conservation of several components of the viral transcription machinery within the Poxviridae, the insertion of most promoters into other genera's members leads to their activation. Foreign genes incorporating early promoters should avoid including the TTTTTNT sequence inside the open reading frame. This region serves as a termination signal for the early viral transcription machinery. Unlike early mRNAs, intermediate and late mRNAs do not have a fixed length, indicating that the termination of these gene classes is not dictated by the sequence (22).

Production of recombinant VV vectors

The procedure of incorporating exogenous genes into the vaccinia virus and then verifying the virus's capacity to faithfully express these genes at both the RNA and protein levels is scientifically captivating and engaging. Upon its creation, the recombinant virus has a multitude of applications. The objectives of this study are as follows: (1) to examine the processes involved in the expression of vaccinia genes; (2) to investigate the transcription, translation, and post-translation of foreign gene products without any disruption from natural factors; (3) to generate and separate specific and biologically significant gene products; (4) to carry out immunological analyses of clearly defined antigens; (5) to create live recombinant vaccines; and (6) to explore the possibility of using viruses as carriers for gene replacement therapy (23).

Homologous recombination is the most often used method for producing rVV. The cells are genetically modified by introducing a transfer plasmid that carries the foreign gene positioned next to a suitable VV promoter and surrounded by DNA obtained from VV (24). Subsequently, cells are infected with the wild-type VV, and recombination occurs between the transfer plasmid and homologous areas within the viral genome during replication. The recombined genome is then released from the cell in a packed form. A possible location for recombination was the VV thymidine kinase (Tk) gene, which allowed for selection based on a tk-negative phenotype using BrdU. To improve the efficiency of identifying and selecting recombinant viruses, other flag genes have been added to the transfer plasmids. One of these genes is the neomycin phosphotransferase enzyme (neo) gene from *Escherichia coli* (24).

Expression vectors

There have been two overarching categories of vaccinia expression systems delineated: (a) recombinant viruses, and (b) recombinant plasmids in which transient expression is contingent upon infection with the wild-type vaccinia virus.

Recombinant viruses

Recombinant vaccinia virus production has become a routine technique in several laboratories owing to its straightforward protocols. Firstly, a recombination plasmid is constructed, which contains a foreign gene located downstream of a vaccinia promoter region. This plasmid also has DNA that shares similarities with a dispensable segment of the vaccinia genome, serving as a site for gene integration. Multiple plasmid vectors with distinct viral promoters may be used to introduce genetic material into various sites within the viral genome. Afterwards, the recombination plasmid is extracted and then injected into host cells using transfection. Subsequently, the host cells are invaded by the vaccinia virus. Homologous recombination occurs between the plasmid and the viral genome, with the assistance of the flanking sequences of the plasmid. The infection's development is thereafter allowed, particularly if the plasmid contains a selectable marker. There are many ways available to screen offspring virions that come from unselected or selected transfections, each with its level of effectiveness. The resulting expressing plaques corresponding to recombinant viruses harboring the inserted gene may be later assessed (17, 25).

Recombinant plasmids

The transitory expression of the foreign gene may be achieved by using a plasmid that contains the gene controlled by a VV promoter. This plasmid is then

introduced into cells that have been superinfected with the normal form vaccinia virus. The promoter of the foreign gene is temporarily activated by substances generated by the superinfecting virus. At that point, the cells may be quickly collected and analyzed for protein production. An important advantage of this process is its efficiency; expression may be obtained quickly after cloning, unlike procedures that require the creation of recombinant viruses, which usually take around three weeks. Nevertheless, the levels of expression are somewhat lower in absolute terms when compared to those produced by a recombinant virus. To tackle this difficulty, a heterologous system was used, which consisted of T7 RNA polymerase and bacteriophage T7 promoters. The expression levels obtained with this approach were 10-20 times higher than those acquired by transient expression and a vaccinia promoter that is similar in nature. After introducing plasmids with the T7 promoter upstream of the target gene into cells, these plasmids are then infected with a vaccinia recombinant that carries the T7 RNA polymerase gene. With a high degree of promoter specificity, this highly efficient monomeric enzyme specifically identifies the T7 promoter on the plasmid and starts the production of the matching mRNA. Increased amounts of gene expression may be achieved by combining the target gene and the polymerase on separate recombinant viruses (26, 27).

OVs activity mechanism in cancer cells

Tumor cells undergo constant viral replication, which utilizes the resources, energy, and response sites of the host cells, destroying the tumor cells. In addition, the offspring virus that is produced can infect cancer cells on the periphery, resulting in a constant increase in the effectiveness of the treatment against the malignancy. Vaccinia virus has potent cell lysis activity, resulting in the production of both cell death signs and viral death signals (28). Concurrently, the immune system's response is exposed to tumor-associated antigens and virus-associated antigens at the infection location, which triggers the related inflammatory reactions. Therefore, the inhibition of the immune system in the local area is overcome, allowing the body to generate a targeted immunological response. In addition, the immune response may be delivered to the host via tumor-associated antigens, resulting in immunological impacts occurring locally. Furthermore, VV can invade and infect the vascular endothelial cells inside tumors, leading to their programmed cell death and subsequent breakdown of the tumor's blood vessels. This process indirectly facilitates the programmed cell death of tumor cells (29).

Alteration of OVs

The VV vectors currently employed in oncolytic anticancer experiments consist of the Wyeth strain, Western Reserve (WR) strain, Lister strain, Copenhagen strain, and vaccinia virus Tian Tan strain (VTT) (1). The toxicity and host range of these strains of VV varies due to the global viral evolution that occurred after smallpox vaccination. Enhancing the virus's ability to specifically target tumors and effectively destroy them is crucial for the success of oncolytic viral therapy. To selectively stimulate the growth of VV in tumor cells while sparing normal cells, the genes required for replication in normal cells are often removed, but they are retained in tumor cells. TK plays a crucial role in the production of vaccinia virus DNA. The production of TK is often reduced in normal cells but elevated in rapidly dividing tumor cells (30).

The TK-deleted VV has a particular capacity to infect cancerous tissue. In contrast, its infectivity and replicability are significantly diminished in most normal cells due to the loss of the TK gene (31). When normal cells are infected with the vaccinia virus, it triggers antiviral responses, which result in the generation of antiviral substances or the onset of death. These systems can control the activity of infected cells and nearby cells by causing a halt in the cell cycle, encouraging cell death, preventing protein production, and triggering an immune response. These mechanisms can hinder or halt the reproduction and spread of viruses. The JX-594 virus is a variant of the Vaccinia virus (VACV) that produces granulocyte-macrophage colony-stimulating factor (GM-CSF) and lacks the TK domain. It has been used to eradicate metastases in solid tumors, namely stage II liver malignancies (32). The modified vaccinia Ankara (MVA) strain is a variant of the Turkey vaccinia virus Ankara. It has undergone 500 natural passages in chicken embryo fibroblasts (CEF), resulting in the loss of genes associated with immune evasion and determining the spectrum of hosts it may infect. MVA is capable of effectively expressing exogenous genes or antigens and eliciting a robust immune response. Additionally, it may be used in animals with weakened immune systems (33).

Oncolytic VV encoding immunostimulatory genes

As our understanding of immune defense strategies in tumor tissues continues to grow, researchers have enhanced the anti-tumor immune response of oncolytic VV by introducing immunostimulatory genes into its genome. These genes encode for factors such as cytokines, chemokines, and co-stimulatory molecules, which promote anti-tumor immune activity (12). Cytokines, in particular, are water-soluble proteins that play a role in regulating both innate and adaptive immunity, including both inflammatory and anti-inflammatory responses.

Utilizing pro-inflammatory cytokines is a common method to bolster Oncolytic virotherapy by attracting and stimulating immune cells while suppressing immunosuppressive cells. GM-CSF is a highly used cytokine that enhances Oncolytic virotherapy. The process involves the recruitment of both DCs and NK cells to facilitate the maturation of DCs. This, in turn, triggers the activation of anti-tumor immune responses. T-VEC, a herpes simplex virus (HSV) equipped with granulocyte-macrophage colony-stimulating factor (GM-CSF), was granted approval in the United States specifically for the treatment of melanoma in 2015 (34). JX-594, a genetically engineered vaccinia virus that incorporates the GM-CSF gene, has shown encouraging anti-tumor efficacy in clinical studies. IL-21 enhanced the effectiveness of oncolytic VV in fighting tumors by boosting the number of CD8+ T-cells that can kill cancer cells (35, 36).

Certain pro-inflammatory cytokines, such as IL-12, IL-23, and IL-15, when combined with oncolytic VV, have shown a more potent anti-tumor immune response. This is achieved by boosting the activation and cytotoxicity of T-cells and NK cells, as well as raising the generation of IFN-γ. Additional cytokines that have been used in the modification of oncolytic viruses, such as adenovirus and HSV, may further augment the anti-tumor impact of oncolytic VV with deliberate planning. It is important to consider the evaluation of real exposure to prospective payloads and the handling of safety concerns that may result from these payloads when designing oncolytic VV (37, 38).

Due to the significant impact of anti-inflammatory cytokines (such as TGF−β and IL-10) on the host's immune system, they additionally enhance the effectiveness of oncolytic virotherapy as a treatment. Delgoffe and colleagues discovered that the use of oncolytic VV to deliver a TGFβ inhibitor may effectively counteract the immunosuppressive tumor environment. This is achieved by limiting the immunosuppressive effects of TGF−β and enhancing the sensitivity to INF-γ. IL-10 is an acknowledged immunosuppressive cytokine that hinders the synthesis of pro-inflammatory cytokines including IL-12 and INF-γ (39). Hickman and colleagues showed that the production of IL-10 in the immediate area following VV infection restricted the reproduction and spread of VV. A further investigation conducted by Wang and colleagues shown that IL-10 has the potential to improve the effectiveness of oncolytic VV in treating pancreatic cancer. This is achieved by reducing the immune reaction to the virus and extending its presence in tumors (40).

Chemokines are cytokines that are released and function as chemotactic agents, attracting immune cells to tumor lesions and facilitating anti-tumor immune responses. Bartlett's team showed that oncolytic VV carrying CCL5 or CXCL11 genes induced a strong anti-tumor immune response and improved treatment effectiveness by recruiting activated immune cells such T (Th1) and NK cells. In general, chemokine receptors, such as CXCR4, play a role in activating the immune system. However, an imbalance in the control of the signaling pathway might contribute to the formation and spread of tumors. CXCR4 antagonist-armed oncolytic VV shown the capacity to modulate the tumor microenvironment (TME) by reducing the formation of immunosuppressive myeloid-derived suppressor cells (MDSCs) and regulatory T-cells (Tregs) inside the tumor. In addition to immune stimulators, several genes linked to other signaling pathways have been discovered to stimulate anti-tumor immunity. As an example, the presence of a DNA-dependent activator of IFN-regulatory factors (DAI), which is a sensor for double-stranded DNA located in the cytoplasm, was increased by an oncolytic VV. This resulted in a stronger activation of the innate immune system and an improved immune response against tumors. Collectively, the use of oncolytic VV that carry genes capable of stimulating anti-tumor immune responses can enhance the effectiveness of anti-tumor activity (41, 42).

Oncolytic VV delivery pathway

Thus far, all the authorized oncolytic viruses (OVs) have been administered by injection directly into the tumor, which limits their effectiveness for tumors that are challenging to treat using this method or have already spread to other parts of the body. Creating intravenous oncolytic viruses (OVs) is crucial for expanding the range of medical uses for OVs. The analysis of 97 distinct clinical trials documenting OV investigations conducted between 2000 and 2020 revealed that the predominant method used was intratumoral administration, utilized in 48 trials (49.5%), followed by intravenous administration, employed in 34 trials (35%). During the clinical studies, intravenous oncolytic VV demonstrates favorable safety and promising anti-tumor efficacy. Unlike adenoviruses, oncolytic VV may be administered intravenously in a single dose since the majority of human bodies do not have preexisting neutralizing antibodies. Nevertheless, after oncolytic VV therapy, the production of neutralizing antibodies took place, which restricted the recurrent systemic administration (43).

Lately, scientists have been focused on creating various methods to achieve effective tumor therapy by continuous intravenous infusion of oncolytic VV. Ferguson et al. discovered that temporary

suppression of PI3Kδ, using the PI3Kδ-selective inhibitor IC87114 or the clinically approved idelalisib (CAL-101), before administering a tumor-targeting VV intravenously, can hinder the virus from attaching to systemic macrophages. This inhibition is achieved by disrupting signaling pathways including RhoA/ROCK, AKT, and Rac. However, the internalization of the virus by the macrophages is not affected. Consequently, this approach enhances the effectiveness of intravenous administration of oncolytic VV to cancers (44).

Furthermore, the use of COX-2 inhibitor medication may strengthen the long-lasting protective anti-tumor effects produced by oncolytic VV by preventing the production of neutralizing antibodies against oncolytic VV infection. This allows for the recurrent administration of oncolytic VV (45). A separate study conducted by McCart's team revealed that pretreatment with CP40, a complement inhibitor, resulted in a significant 10-fold increase in the infectious titer in the blood shortly after the JX-594 infusion. This increase was achieved by preventing the neutralization of oncolytic VV, which is advantageous for the repeated intravenous administration of oncolytic VV (46). Oncolytic VV expressing human CD55 protein may enhance viral survival by shielding against complement-mediated lysis and avoiding neutralization by VV-specific antibodies, hence boosting intravenous effectiveness. All the aforementioned methods are both theoretically and practically viable with the use of antiviral inhibitors before the intravenous administration of oncolytic VV. The findings indicate that the use of VV coating might potentially enhance the systemic administration of oncolytic VV. Additional techniques for VV coating must be developed (47).

Conclusions

Presently, the majority of research on oncolytic viruses focuses on adenovirus and lentiviral vectors as prototypes. Nevertheless, the distinctive benefits of the vaccinia virus render it a good vehicle for gene therapy, offering potential for patients with many forms of cancer. For the treatment of various cancer patients, it is recommended to use thorough and personalized techniques. Hence, it is essential to alter the vaccinia virus to decrease its toxicity and enhance its stability for expression. pathogen vector. It has been determined that when foreign genes are incorporated into the TK region of the VV, they have a beneficial impact on cancer cells. Choosing the most suitable gene is likewise a difficult task. Hence, more research on gene therapy, using the oncolytic vaccinia virus as a carrier, may aid in halting the development and decline of cancer. Furthermore, this research is crucial for resolving the current issues and establishing the foundation for future therapeutic applications.

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Authors' Contribution

Akram Sadat Ahmadi and Yasaman Vojgani were involved in the conceptualization, design and writing of the manuscript draft. All authors read and confirmed the final manuscript.

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Advantages, Disadvantages and Risks of CRISPR/ Cas9 Technique for Gene Therapy

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Abstract

The CRISPR/Cas9 system has gained significant attention as a gene editing method in recent years because of its simple design, cost-effectiveness, high efficiency, and ease of use. Additionally, it allows for the simultaneous editing of many locations. Additionally, it may be performed without the use of plasmids, so avoiding the many complications associated with plasmids. CRISPR/Cas9 has shown significant promise in the investigation of genes and genomic activities in microbes, plants, animals, and humans. Transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD) are genetic disorders characterized by severe and possibly life-threatening symptoms. In this article, we discuss the use of CRISPR/Cas9 technology in the treatment of these two diseases and FDA-approved drugs based on CRISPR/Cas9. In addition, we address the most important challenges of gene therapy using this technology.

Keywords: CRISPR/Cas9, Gene therapy, β-thalassemia, Sickle cell disease.

Introduction

CRISPR/Cas9, also known as Clustered regularly interspaced short palindromic repeats, is a rapidly expanding tool that has a wide range of applications in therapeutics. It is particularly useful for making modifications to genes, to repair or eliminate any defective genes that cause diseases such as cancer and AIDS (Acquired immunodeficiency syndrome). Additionally, it involves the identification of the target sequence using sgRNA and then replacing a defective gene with a functional one. It provides a high level of efficiency, specificity, and effectiveness after gene editing. However, it also has some unintended effects on non-target areas and may trigger immune responses (1). The efficacy of CRISPR/Cas9 has been predominantly shown in vitro utilizing animal germ cell lines. However, its use in in vivo models is currently limited owing to ethical constraints. Recent developments include research and clinical trials that specifically target the treatment of diverse genetic illnesses. An example of the use of the CRISPR gene knock-in technology is its use in treating Leber Congenital Amaurosis 10 in vivo. CRISPR/Cas is often used to develop disease models for conditions such as Alzheimer's disease, osteogenesis imperfecta, X-linked adrenoleukodystrophy, and

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aniridia-related keratopathy. Furthermore, CRISPR/Cas has shown its role in the treatment of monogenic human genetic diseases, which is a captivating and very hopeful advancement in this technology. The main emphasis of this topic is on the intersection of scientific advances and ethical debates, which are crucial for the continued development and acceptance of CRISPR-based drugs (2, 3).

Function of the CRISPR/Cas9 technology

The mechanism by which bacteria resist the invasion of foreign nucleic acids primarily involves three stages: adaption, expression, and interference. Upon the first invasion of a virus into a bacterium, the bacterium will enzymatically break down the viral DNA into spacer sequences of appropriate lengths. This process occurs by identifying certain PAM sequences and integrating them into the bacterium's CRISPR spacer region. As a result, the bacterium gains the ability to remember and recognize the invading virus in the future. Upon subsequent invasion by the same kind of virus, the bacteria possess the ability to identify and convert the spacer sequences into pre-crRN (4). The pre-crRNA molecule will undergo pairing with tracrRNA and subsequent processing by CnsI and RNase III enzymes to produce mature crRNA. The crRNA identifies and attaches to the foreign DNA using complementary sequences, which is why it is also referred to as guide RNA (gRNA). Cas nuclease cannot independently break foreign DNA. However, when it is joined with a mature tracrRNA and crRNA to create the ribonucleoprotein complex, the Cas protein may be directed by the crRNA to cleave the invading DNA. This is accomplished by detecting the PAM site on the DNA, destroying the foreign DNA and the achievement of self-defence (5).

The CRISPR/Cas system has the potential to edit genes because of the particular target sequence identification capacity of crRNA, the DNA cleavage activity of Cas nuclease, and the DNA repair processes of cells. Cells will initiate their intrinsic repair systems to mend DNA damage and prevent cellular demise when double-strand breaks (DSBs) occur. There are two ways of repairing: non-homologous end joining (NHEJ) and homology-directed repair (HDR) (6). If there are homologous sequences present, cells often use the HDR method to repair DSBs by homologous recombination, which involves incorporating homologous segments into the DNA. By using this repair process, we may deliberately create a repair template (donor) DNA fragment with matching arms and introduce it along with a gene-editing vector into the cell. This enables the integration of the donor DNA into the genomic DNA of the cell, resulting in the successful insertion of the desired gene. In the absence of homologous DNA, cells often activate the NHEJ process, which directly connects the damaged DNA. This repair method is susceptible to base pair insertions or deletions (indels) that may cause gene frameshift alterations, leading to gene knockout (7, 8).

For gene editing purposes, Cas9 and gRNA may be combined into a single vector or separated into two independent vectors. The artificially engineered gRNA is a hybrid RNA molecule that incorporates all the necessary crRNA and tracrRNA elements (9). The anterior segment of the gRNA is referred to as single guide RNA (sgRNA), and its primary function is to identify and bind to the specific target site. This section serves as a framework for attaching to the Cas9 protein. Modification of sgRNA is necessary for each identification of distinct targets. The optimal length of the single guide RNA (sgRNA) is 20 nucleotides, which facilitates the construction of the CRISPR/Cas9 vector. Given that, on average, there is a possible target present in every 8 bp DNA sequence, there exists a considerable number of potential targets that are suited for CRISPR/Cas9. The CRISPR/Cas9 system's popularity in gene editing is due to these features. Upon transformation of CRISPR/Cas9 vectors into target cells, the cells will produce Cas9 and gRNA, enabling them to carry out gene editing.

FDA-approved diagnostics and clinical interventions

Sickle cell disease and β-thalassemia

SCD and TDT are prevalent monogenic illnesses that result from mutations in the hemoglobin β component gene, making them two of the most common genetic disorders globally. SCD is defined by a disruption in the hemoglobin chain and the occurrence of hemolytic anemia. Typically, this condition is managed with blood transfusions and iron-chelation treatment (10). Individuals diagnosed with TDT have sickle-shaped red blood cells that have reduced oxygen-carrying capacity, leading to frequent discomfort. Consequently, these patients often need treatment in a clinical setting, which includes the administration of hydroxyurea, pain medications, and blood transfusions. Both disorders have also been treated via bone marrow transplantation, however finding a suitable match is challenging. Upon genetic analysis of the pathogenesis of these two hematological diseases, the transcription factor BCL11A was discovered to inhibit the production of fetal hemoglobin and γ-bead protein. Sustaining elevated levels of these proteins proved to alleviate the symptoms of SCD and TDT (11).

In 2019, Wu et al. used CRISPR/Cas9 to precisely cut the BCL11A enhancer region in hematopoietic stem cells (HSCs) and effectively reduced its expression without causing notable adverse consequences. In December 2020, clinical data was

disclosed for CTX001, a gene therapy developed by CRISPR Therapeutics in collaboration with Vertex Pharmaceuticals. CTX001 is a single-dose medication designed for the treatment of SCD and TDT. In this clinical experiment, Cas9 was used to precisely cut the BCL11A enhancer region in hematopoietic stem and progenitor cells (HSPCs), resulting in the loss of enhancer function in these cells. This method decreased the level of BCL11A and reinstated the synthesis of γ-hemoglobin and fetal hemoglobin. Afterwards, the researchers performed a transplantation of the altered Hematopoietic Stem and Progenitor Cells (HSPCs) into two patients who were diagnosed with SCD and TDT. The subsequent investigation revealed a significant increase in fetal hemoglobin levels in both individuals 12 months after the treatment. During the last follow-up visits at 18 and 15 months, both patients successfully attained normal levels of fetal hemoglobin. The subsequent treatment of eight patients had comparable outcomes to the first two patients, demonstrating the overall suitability and effectiveness of this approach. Nevertheless, this approach is not entirely innocuous, since both of the first patients encountered different levels of detrimental consequences, which were not life-threatening and subsided after receiving therapy. In a separate clinical trial aimed at treating SCD a method using RNA interference (RNAi) was used to suppress the expression of BCL11A. A lentiviral vector containing short hairpin RNA (shRNA) was created and used to introduce the lentivirus into CD34+cells derived from individuals with SCD. This approach resulted in successful therapeutic outcomes (12, 13).

Transthyretin amyloidosis

Transthyretin (TTR) amyloidosis is a genetic ailment that is primarily caused by the accumulation of amyloid fibrils surrounding cells. This condition mostly affects the human nervous system and heart. TTR monomers are produced in the liver to form tetrameric complexes in their normal condition, which play a role in the transportation of thyroid hormones. Mutant TTRs fail to maintain the tetrameric structure and disassemble before reassembling into amyloid fibrils (14). Nevertheless, individuals suffering from TTR amyloidosis do not exhibit notable symptoms of thyroid hormone insufficiency, indicating that TTR could not play a large role in transporting thyroid hormones. Consequently, lowering TTR expression might be a potential strategy for treating this condition. The primary therapeutic choices are liver transplantation and the use of the tiny chemical tafamidis to stabilize tetramers. However, the latter strategy is not stable and successful.31 Patisiran, a siRNA medication, has been authorized by the FDA to treat this condition. Patisiran inhibits the translation of TTR to decelerate the progression of the illness, but, several injections are necessary on an ongoing basis for the duration of the patient's life. Given the absence of notable adverse consequences from reduced TTR expression, it may be possible to eradicate TTR amyloidosis by using a method that permanently destroys the mutant TTR gene. Gillmore et al. published the findings of clinical studies conducted in June 2021, which examined the in vivo administration of a CRISPR-based gene-editing medication called NTLA-2001. NTLA-2001 is composed of a liver-targeted lipid nanoparticle (LNP) that contains sgRNA targeting the TTR gene and messenger for SpCas9. The LNP has been used several times as a carrier for gene medicines to be delivered to the liver (12, 15).

During initial trials conducted on animal models, NTLA-2001 demonstrated very effective and long-lasting suppression. A total of six individuals were chosen to participate in this experiment, and each patient had the medication injection without experiencing any negative effects during the treatment period. By the seventh day of medicine administration, the patient's blood indicators and liver function indicators had returned to normal levels. Three patients were administered a dosage of 0.1 mg per kg, while the other three patients were given a dosage of 0.3 mg per kg to assess the effectiveness of NTLA-2001. On the 28th day, the blood TTR concentrations of the three patients who got the low dosage were reduced by 47%, 52%, and 56%. The three patients who received the high dose had decreases of 80%, 84%, and 96% in their blood TTR concentrations. This discovery suggests that the effectiveness of NTLA-2001 is directly influenced by the dosage and is very effective. Several months later, the FDA awarded orphan drug status to the approach, acknowledging both NTLA-2001 and the in vivo administration of CRISPR-based gene therapy (16, 17).

Limitations and challenges

Precisely delivering CRISPR/Cas gene medicines to the body has promise for treating illnesses in both laboratory and clinical settings. The great specificity, efficacy, and simplicity of handling this technology make it very desirable for future applications. Nevertheless, scientists have unearthed unforeseen circumstances when using CRISPR technology for gene editing.

Genomic integrity, off-target risk, on-target unwanted editing

Although CRISPR is an effective gene editing technique, it has several drawbacks, including off-target effects, off-target binding, and editing that can lead to dangerous problems. Human cells are

more susceptible to off-target mutations than mice or zebrafish. Gene editing with CRISPR assistance may result in massive deletions, ineffective DEAD spot repair, and inadvertent complicated rearrangements that have a deleterious effect and cause cell death. In recent years, several techniques such as IDLVs (integrase-defective lentiviral vectors), DISCOVER-seq (discovery of in-situ Cas off-targets and verification by sequencing), CIRCLE-seq (Circularization for in vitro reporting of cleavage effects by sequencing), digenome-seq etc., have been employed to identify off-target genome modifications. Several techniques can be employed to achieve this, such as the manipulation of Cas9 nucleases, the use of dimeric Cas9 nuclease, the combination of Cas9 with artificial inhibitory domains, the design of optimized sgRNA, the "hit-and-run" approach involving the transfer of Cas9 protein instead of the Cas9 gene, and the utilization of non-viral delivery methods to reduce off-target effects. dCas9, or dead Cas9, is more effective in therapeutic applications because it can alter the expression of target genes without causing double-stranded breaks (DSBs). On the other hand, Cas9n exclusively induces single-stranded breaks (SSBs) (18).

Chromosomal disorganization

Researchers have expressed significant worry regarding the safety of CRISPR-based gene-editing technologies. Double-stranded DNA is typically cleaved by Cas9, leading to the activation of NHEJ repair. As a consequence, the resulting repaired DNA strands often exhibit a little deficiency or excess of base pairs, which aligns with the anticipated outcome. Nevertheless, throughout the process of validating editing efficiency, researchers discovered instances of significant base losses and chromosomal structural translocations. These errors can result in conditions such as malignant tumors and are unacceptable in clinical settings, notwithstanding their low likelihood of occurring. CRISPR/Cas9's repetitive cleavage of target genes is a significant factor in the occurrence of chromosomal translocations and deletions. Yin et al. integrated an exonuclease structural domain with Cas9 to mitigate the frequency of these alterations. This structure executes post-processing shortly after cleavage, hence minimizing the probability of generating intact ends. This method efficiently inhibits the complete restoration of the DNA strand, hence preventing the replication of the genome by Cas9. The researchers combined spCas9 with improved three-prime repair exonuclease 2 (TREX2) to create a modified version of Cas9 called Cas9TX. Through research involving modified T cells and other cells, it was evident that Cas9TX effectively inhibited chromosomal translocations in comparison to the high-precision SpCas9 version (19).

Stimulation of the immune system

The required treatment area or cell count varies considerably based on the specific target organ or tissue, the kind of disease, and its severity. For instance, when aiming at a small organ with a restricted number of cells, it is generally quite simple to reach a therapeutic threshold with a single injection. Nevertheless, tissues and organs of considerable size, INCLUDING skin, liver OR lung contain a significant number of cells (20). Hence, the capacity for recurrent administration is a crucial characteristic for effectively treating illnesses that impact extensive tissues or organs (20). The delivery technique must provide sufficient safety and be capable of evading the host immune system to facilitate multiple administrations. As mentioned before, AAV is rendered ineffective by an anti-capsid antibody, which is why the second injection does not work. The number of potential administrations significantly affects the extent of the treatable region for genome editing therapy. Repeated dose for local injection enables gradual extension of the treated area over a while. Systemic infusion can lead to the accumulation and enhancement of both the treated region and the efficacy through multiple treatments. It is crucial to understand that the idea of administering many doses is only relevant when the effects of genome editing build up over a significant period (21).

The potential worry of immunogenicity to CRISPR-Cas9 protein has been explored for therapeutic applications due to the presence of preexisting anti-Cas9 antibodies in over 50% of the human population. These antibodies are specifically targeted against the routinely utilized SaCas9 and SpCas9 bacterial orthologs. To reduce the problem of immunogenicity, the Cas9 protein can be modified to eliminate immunogenic epitopes. Transient immunosuppression can be used as a countermeasure to reduce immunity to both Cas9 and the delivery payload. When Cas9 RNP is directly delivered with CPP, the presence of the anti-Cas9 antibody can greatly impact the efficacy of delivery. This is because the antibody's binding may conceal the CPP portion, hindering its function. However, it is worth noting that the majority of alternative delivery systems do not externally expose the Cas9 protein. Therefore, the impact of the anti-Cas9 antibody in serum should be negligible, particularly throughout the delivery process. Utilizing a nanocarrier technology to encapsulate the Cas9 enzyme could potentially offer a temporary safeguard against neutralizing antibodies and degradation caused by nuclease/protease activity (22).

Limitations of targeted delivery

Deviation from the desired position

Viral and nonviral vectors are typically

administered systemically to animals, effectively protecting CRISPR gene medicines from degradation in the blood and tissues. Nevertheless, unaltered vectors are vulnerable to being taken up by metabolic organs within the body. The CRISPR/Cas system retains its functionality while entering nontarget cells but genetically alters healthy cells, perhaps resulting in unforeseeable ramifications. Enhanced vehicles are required to minimize the infiltration of gene medications into non-targeted cells (23). Methods to enhance delivery functionality involve applying a biofilm to the carriers or including peptides that are recognized by receptors on the target cells. The design of nanoparticles that are responsive to the specific microenvironment of the target organ can boost the concentration of gene drugs. This can be achieved by considering factors such as pH fluctuations, reactive oxygen species (ROS), and adenosine triphosphate (ATP) levels. The nanomaterial shell undergoes disintegration in a particular environment, so revealing the core, which subsequently penetrates the cell via endocytosis. Nevertheless, if the microenvironment in specific diseased tissues is not substantially distinct from that of other tissues, it is possible to create a nanoparticle that can be triggered by various situations to release its contents, so enabling targeted treatment for the disease. Furthermore, there have been advancements in the development of CRISPR/Cas9 delivery systems that are responsive to light, magnetic fields, and ultrasound. These systems are designed to ensure accurate and targeted delivery (23).

Biocompatibility

It is necessary to create appropriate vectors for candidate cells to minimize the risk of negative reactions resulting from off-target CRISPR/Cas9 systems. For the vector to effectively perform its role, it must possess biocompatibility, a strong encapsulation capacity, and the ability to penetrate the cell membrane. Three hundred thirty-six When building the system, it is important to take into account the immune reaction that occurs when the material is delivered into the body. It has been observed that Cas9 proteins often obtained from *S. pyogenes* and *S. aureus* can cause an immunological response in humans. To address this difficulty, a modified version of Cas9 that does not contain exons that trigger a response was administered to juvenile and adult mice via AAV, resulting in the successful prevention of both humoral and cellular immune responses. Furthermore, the modified Cas9 must also be carried in a vector specifically engineered to prevent activation of the host immune response. Within living organisms, viruses, lipids, and exosomes can evade the immune system, while synthetic chemical nanoparticles need to be coated with a protective layer on their surface. This coating not only stabilizes the polymer in the bloodstream but also requires the presence of modified CD47 protein. Moreover, plant exosomes have a higher probability of evading immune system recognition due to their inherent source. Plant exosomes are considered a safer option for delivering CRISPR/dCas9 systems due to the significant distinctions between plant and mammalian diseases. Nevertheless, the study of administering gene medicines through plant exosomes is still in its early stages, particularly due to the variability in properties among exosomes produced by various plants (12, 24).

Potential risk of cancer

Recently, two separate study teams discovered that CRISPR-Cas-induced DSBs could trigger the p53 signaling pathway. Genetically modified cells have the potential to become cancer-initiating cells, meaning they could start the development of cancer. Therefore, using CRISPR-Cas systems for treatment could unintentionally raise the risk of cancer. While there is yet no conclusive evidence to establish a direct link between CRISPR-Cas-mediated genome editing and the development of cancer, this research serve as a cautionary reminder regarding the use of CRISPR-Cas systems in gene therapy. It serves as a reminder that there is still a considerable distance to cover before CRISPR-Cas technologies can be effectively utilized in humans (25).

Application and advantages of CRISPR-Cas systems in the study of human diseases

Utilizing CRISPR-Cas systems to create animal and cell models

Animal models play a vital role in comprehending gene function, investigating the development of human diseases, and advancing the creation of new medications. Nevertheless, conventional techniques for producing animal models are intricate, expensive, and time-consuming, significantly restricting the use of animal models in fundamental medical research and preclinical investigations. Following the identification of CRISPR-Cas systems, a sequence of genetically altered animal models has been created with remarkable efficiency. Mice are extensively utilized as model animals in scientific studies and are considered the primary model animals for research on human diseases. Researchers have successfully created numerous genetically engineered mice models. However, because of significant disparities in species characteristics between humans and rats, they are unable to offer reliable evaluation and sustained monitoring for the study and management of human diseases (26).

Research has shown that the effectiveness of CRISPR-Cas mediated genome editing is greater in

laboratory conditions (in vitro) compared to living organisms (in vivo). Therefore, the utilization of genetically modified cell models can significantly reduce the time required for medical research. Previously, scientists have utilized CRISPR-Cas systems to conduct genetic alterations on many types of cell lines, including tumor cells, adult cells, and stem cells, to replicate a range of human disorders (26).

Uses of CRISPR-Cas systems in the diagnosis of diseases

CRISPR-based molecular diagnostic technology is advancing at a rapid pace, and it has been distinguished as one of the world's top ten science and technology advancements in 2018 due to the discovery of novel Cas enzymes (Cas12, Cas13, etc.) and the growth of CRISPR-Cas platforms (27). The 'collateral cleavage' activity of Cas13 enzymes is distinct from that of Cas9. This activity can result in the cleavage of nearby non-target RNAs following the cleavage of the target sequence (28). Feng Zhang et al. created SHERLOCK (Specific High Sensitivity Enzymatic Reporter UnLOCKing), an in vitro nucleic acid detection platform that uses Cas13a and is based on the "collateral cleavage" activity of Cas13. It consists of fluorescent RNA reporters, sgRNA that targets specific RNA sequences, and Cas13a. When the Cas13a protein recognizes and cleaves the target RNA, it will cut the report RNA and release the detectable fluorescence signal to achieve the diagnostic objective. Researchers have employed this approach to identify tumor DNA mutations, genotype human DNA, distinguish pathogenic microbes, and detect viruses. Subsequently, Feng Zhang et al. enhanced the SHERLOCK system and rebranded it as SHERLOCKv2, which is capable of simultaneously detecting four viruses. Collateral cleavage activity is also observed in Cas12 enzymes, in addition to Cas13 CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy (29).

Conclusions

CRISPR-Cas-mediated genome editing technologies are considered a significant milestone in the field of molecular biology in the 21st century due to their ability to provide a flexible and accessible method for modifying, regulating, and visualizing a genome. The extensive application of CRISPR-Cas systems in gene function analysis, human gene therapy, targeted medication development, animal model construction, and livestock breeding has thoroughly demonstrated their significant potential for further development. Nevertheless, there are still some constraints that must be surmounted in the practical applications of CRISPR-Cas systems, and significant efforts must be made to assess their long-term safety and efficacy.

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Authors's Contribution

Shabnam Radbakhsh: data curation; editing and review. Hoda Namdari Moghaddam: investigation and writing. All authors read and confirmed the final manuscript.

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Ethics approval and consent to participate

Not applicable

Conflict of Interest

The authors declared no conflict of interest.

Consent for publication

Not Applicable

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CANCER: A WORD, NOT A SENTENCE

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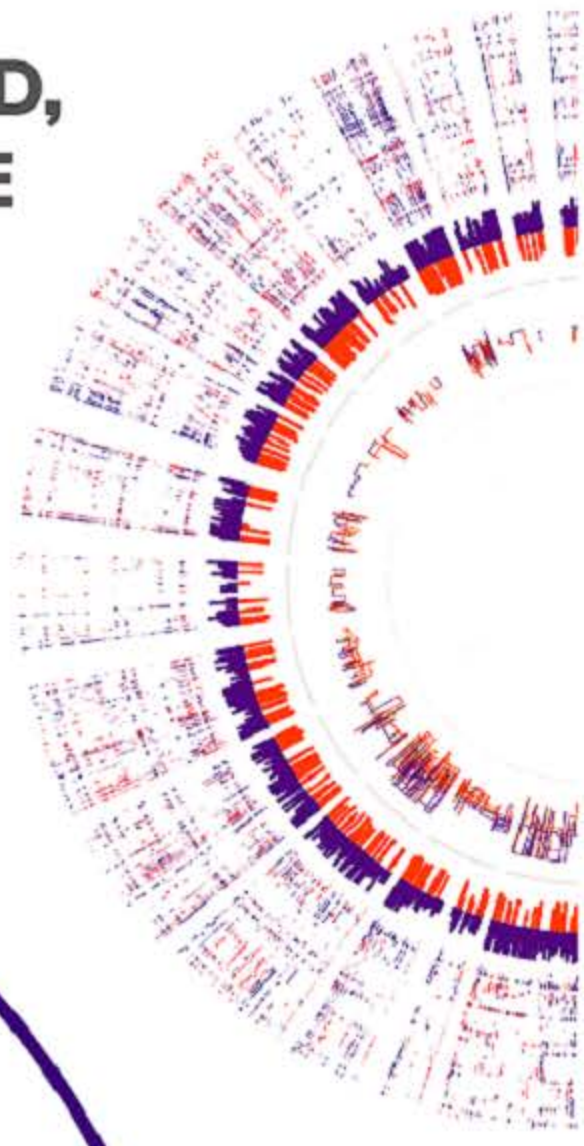

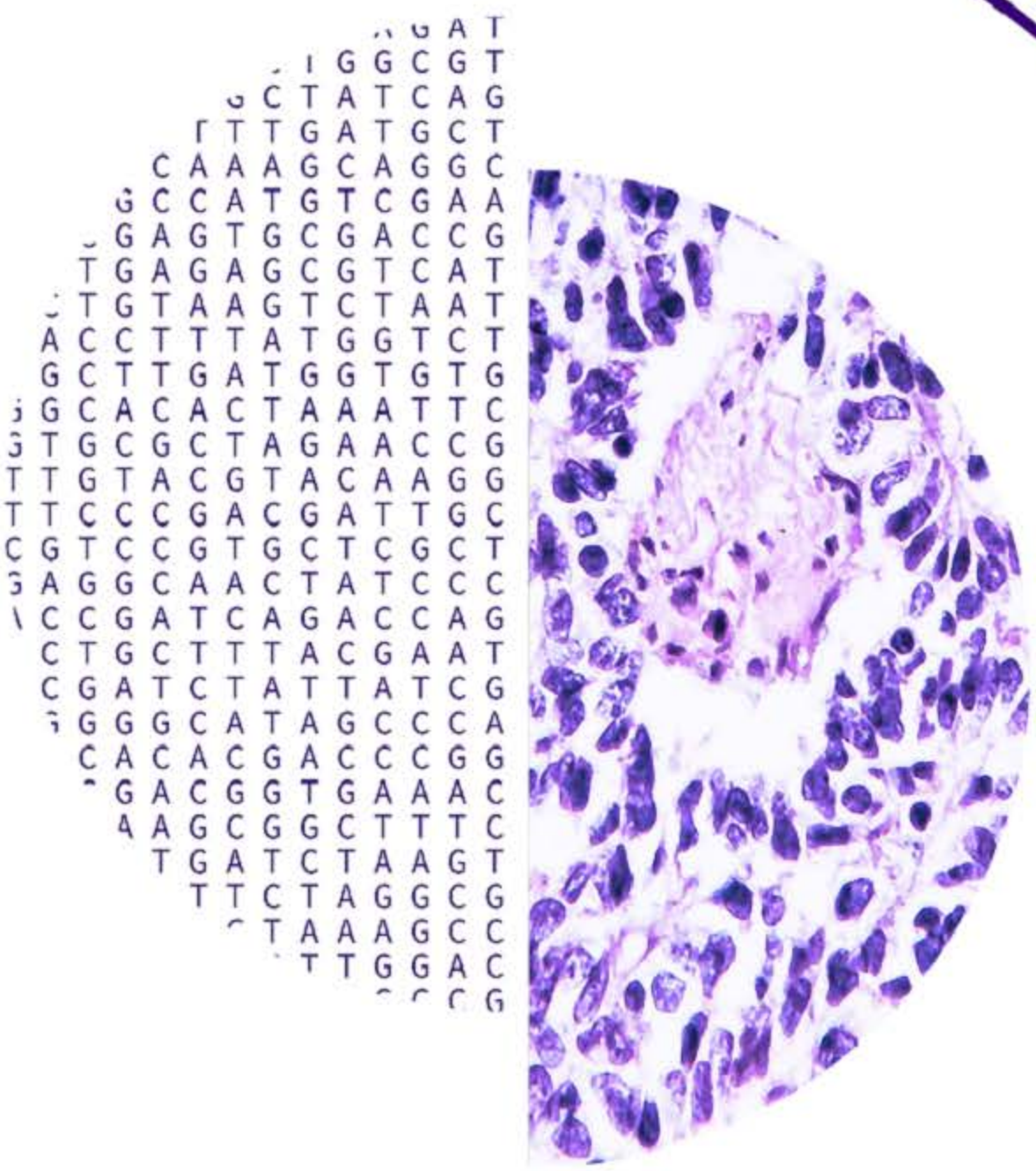

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SOLID TUMOR

ABOUT SOLID TUMORS

Cancer is a disease where cells grow out of control, and these cancer cells can spread to other parts of the body without proper treatment. Solid tumors are a type of cancer that can appear anywhere (e.g. lung cancer, breast cancer, liver cancer, etc.)

Generally, this problem is caused by various gene mutations in the cells. People with the same type of cancer may have different mutations. However, scientists have been able to design specialized treatments for some of these mutations, and they are known as clinically actionable mutations. Specifically targeting clinically actionable mutations is called precision cancer treatment.

What are the available precision cancer treatments?

Precision cancer treatments are designed to be more effective at killing cancer cells and less harmful to healthy cells.

Targeted Therapies: Able to specifically target cancer cells that have certain actionable mutations or abnormalities.

Immunotherapy: Helps immune cells to recognize and destroy cancer cells.

Why do I need genetic testing?

Genetic testing can help your doctor understand what's happening in your tumor. Some genetic mutations may make certain treatments more effective, while others may indicate resistance to certain therapies. Therefore, genetic testing can help your doctor create the optimal treatment plan designed for you.

WHAT WILL YOU KNOW FROM SmarTest'S REPORT?

What gene mutations are in your tumor?
Receive a full description of the mutations occurring in your tumor, including whether these mutations may also affect your children.

What treatment may work best for you?
Help your doctor to find the best treatments for you based on what mutations were found in your tumor to work alongside the latest professional guidelines and expert consensus.

Why does my current treatment not work well?
Understand why therapy resistance occurs and receive other personalized treatment options for your doctor to consider.

We are able to deliver the report within 2-3 weeks upon receiving the sample

Please be aware that our report should not replace professional advice from a doctor. Always consult your doctor about your condition and treatment options. Additionally, not all mutations have corresponding precision cancer treatments. Even in such cases, having a better understanding of your condition can be helpful for your current and future treatment plan.

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WHAT WE OFFER

Comprehensive solid tumor panels

Our all-in-one panels are designed for all solid tumors and characterize the tumor to better guide healthcare professionals in prescribing a precision treatment.

SmarTestPRIME™ CE

A 437-gene panel covering all solid tumors and additional key biomarkers. (TMB, MMR, MSI, HRR)

SmarTestPRIME™ HRD CE

A 437-gene panel + genome-wide homologous recombination deficiency (HRD) evaluation across all solid tumors. (TMB, MMR, MSI, HRR)

SmartTestRADIOTRON™

A 474-gene panel designed for personalized radiotherapy.

SmarTestLITE™

A 196-gene panel covering major actionable solid tumor genes and some key biomarkers (MMR and MSI).

SmarTestBRCASCAN™

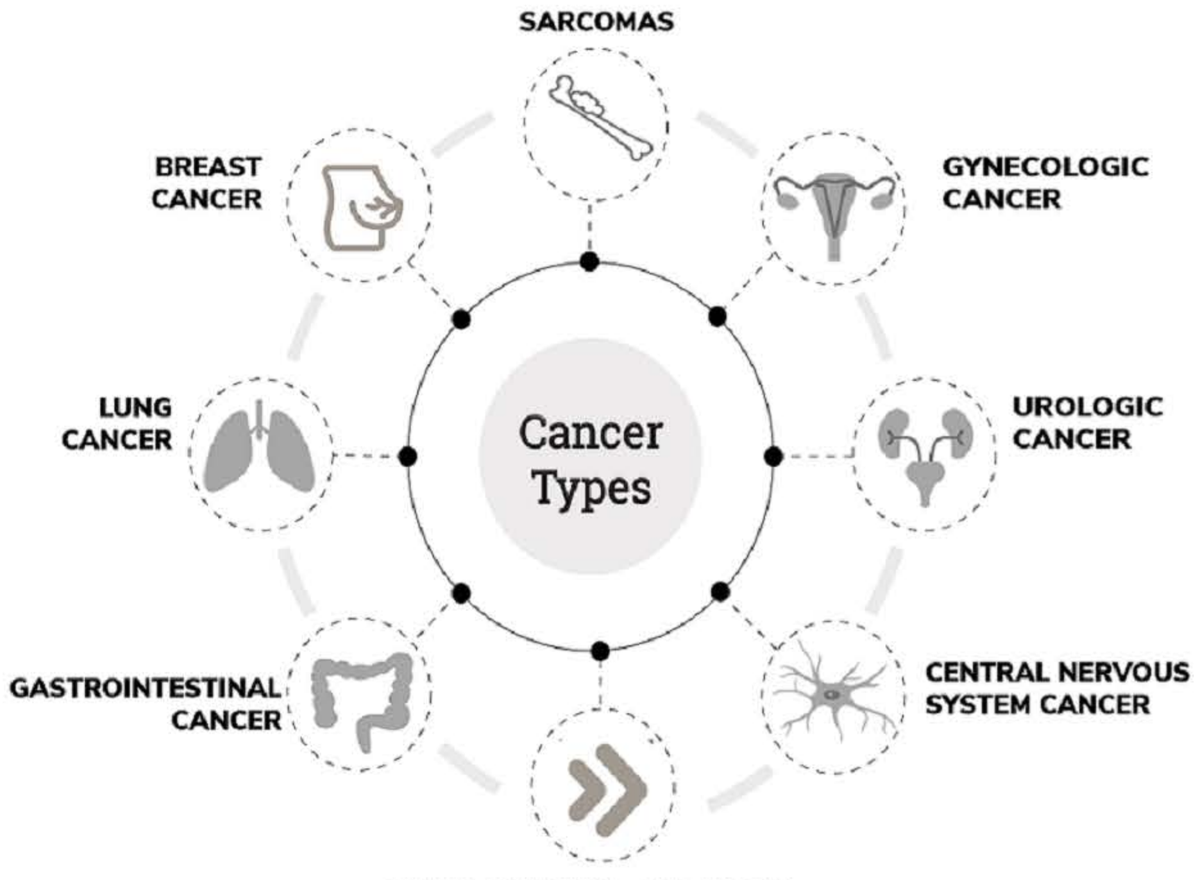
A 27-gene panel designed for cancers related to the HRR pathway (breast, ovarian, prostate, etc.) or for individuals with a family history of BRCA1 or BRCA2 mutations.

SmarTestPANCARNA™

A 117-gene panel designed for fusion genes, which are major drivers of tumorigenesis and tumor progression.

Cancer type-specific gene panels

If you prefer to test only a single cancer type, we also offer a cost-effective option that allows you to target specific types of cancer.



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