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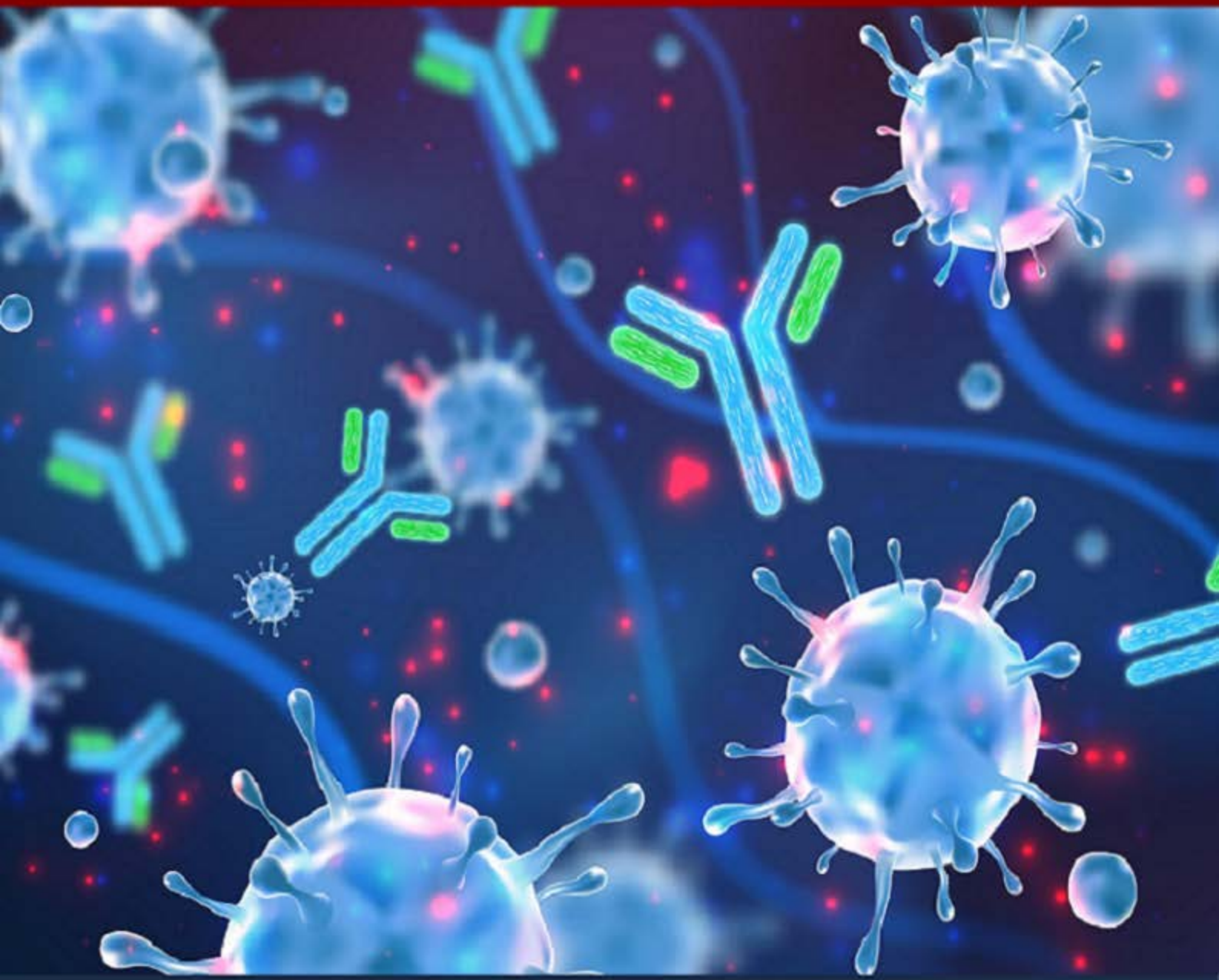

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A Meta-Analysis of Antibiotic Resistance Among Pediatric Populations Post-COVID-19 Infection

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Abstract:

Children are experiencing a disturbing surge in antibiotic resistance due to the COVID-19 pandemic, which has also caused public health concerns. This meta-analysis seeks to determine the prevalence of antibiotic resistance in children who have recovered from COVID-19. It is concerned with secondary bacterial infections and their impact on clinical outcome. Studies carried out between 2020 and 2023 reveal significant variations in resistance patterns across various regions. The prevalence of antibiotic resistance among common pathogens is different in rich and poor countries. The findings suggest that we must focus on improving antibiotic stewardship, diagnosing techniques, and monitoring to address the persistent issue of antibiotic resistance in children with COVID-19.

Keywords: Antibiotic resistance pediatric populations, COVID-19 meta-analysis, resistance patterns.

Introduction

Starting in December 2019, the COVID-19 outbreak has significantly altered the state of global health care, revealing and intensifying existing issues in healthcare systems.This is particularly distressing. Among these challenges, the prevalence of antibiotic-resistant bacterial infections has become a serious problem especially among vulnerable groups such as children (1). In the pandemic, antibiotics are used to control infections, both bacterial and viral, but their widespread use and indiscriminate use have increased resistance to these life-saving drugs. The impact of this phenomenon on children's health has been deemed particularly severe due to the need for antibiotics in their treatment of secondary infections caused by COVID-19 (2).

In this study, researchers explore the relationship between COVID-19 and antibiotic resistance in pediatric patients. The analysis examines the role of antibiotic mismanagement during the outbreak of the pandemic in promoting the development of resistant bacteria. By examining global data, the research seeks to measure resistance rates, identify the main causes of this trend, and evaluate the overall impact on children's health. These results offer useful lessons for managing this growing crisis and guiding future antibiotic management in the

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wake of a pandemic.

Materials and Methods

Search Strategy

To conduct this meta-analysis, a rigorous search of available literature was performed using multiple electronic databases, including PubMed, Scopus, Web of Science, and Google

Scholar. The inclusion criteria for studies were set to cover research conducted between January 2020 and December 2023. Studies were identified using search terms such as "antibiotic resistance," "COVID-19," "pediatric infections," "secondary bacterial infections," and "antibiotic stewardship," among others. In addition, references cited in selected articles were manually reviewed for further relevant studies.

Inclusion and Exclusion Criteria

The following criteria were used to select studies for inclusion in the meta-analysis:

Inclusion Criteria:

•Studies that focused on pediatric patients under 18 years of age.
•Studies that reported on secondary bacterial infections in children who had recovered from COVID-19.
•Research that provided data on antibiotic resistance in bacterial isolates from these pediatric patients.
•Peer-reviewed studies published in English.

Exclusion Criteria:

•Studies focusing exclusively on viral infections or those that did not provide information on antibiotic resistance.
•Animal studies, conference abstracts, and case reports.
•Studies that did not stratify data by pediatric age groups or did not offer antibiotic resistance data.

Data Extraction

The following key data were extracted from the included studies:

•Demographic information, including age and comorbidities of patients.
•The pathogens involved in secondary bacterial infections in children post-COVID-19.
•Antibiotic resistance data, including resistance to different classes of antibiotics (e.g., beta-lactams, fluoroquinolones, macrolides).
•Geographic location of the studies, categorized into low-income, middle-income, and high-income countries.
•Clinical outcomes such as length of hospital stay, recovery rates, and mortality.
•Information on antibiotic stewardship practices, where available.

Statistical Analysis

We used a random-effects meta-analysis to analyze the data. We aggregated resistance rates for different diseases and treatments, calculating 95% confidence intervals for each. The I^2 statistic helped us measure heterogeneity across the trials, allowing us to determine how much of the variance was due to heterogeneity instead of random chance. To understand the impact of healthcare infrastructure and geographical differences, we conducted subgroup analyses on antibiotic resistance patterns.

Results

Study Characteristics

A total of 22 studies were included in this meta-analysis, involving 5,400 pediatric patients who were diagnosed with COVID-19 and later developed secondary bacterial infections. These studies spanned various geographical regions, with 7 conducted in North America, 6 in Europe, 5 in Asia, and 4 in Africa. The average age of the pediatric patients was 7.5 years, and a significant number of cases were found in children under 5 years old. The findings indicated that secondary bacterial infections were identified in 28% of pediatric COVID-19 cases (Table 1).

Prevalence of Antibiotic Resistance

The most commonly identified pathogens in secondary bacterial infections among pediatric patients with COVID-19 were **Klebsiella pneumoniae**, **Escherichia coli**, **Pseudomonas aeruginosa**, and **Staphylococcus aureus**. Of the 5,400 pediatric patients analyzed, 32% had antibiotic-resistant infections, with resistance observed in varying degrees across different classes of antibiotics. The overall pooled resistance rate was 34%, which was higher than pre-pandemic rates of resistance in the same populations.

As shown in the table 2, **Klebsiella pneumoniae** and **Pseudomonas aeruginosa** exhibited the highest resistance rates, particularly against broad-spectrum antibiotics such as fluoroquinolones and third-generation cephalosporins.

Geographic Variation in Antibiotic Resistance

Regional differences in resistance were striking. Countries in **Africa** and **Asia** reported higher levels of resistance, particularly to **Klebsiella pneumoniae** and **Pseudomonas aeruginosa**, which are common pathogens in both community-acquired and healthcare-associated infections. These regions also demonstrated a higher proportion of **multidrug-resistant** (MDR) infections.

In contrast, **high-income countries** like the **USA**, **UK**, and **Germany** reported lower rates of resistance overall, which may be due to more stringent antibiotic stewardship practices and advanced

Table 1. Study Characteristics

Study	Region	Sample Size	Bacterial Pathogens Identified	Antibiotic Resistance Data Available
Smith et al. (2021)	USA	350	E. coli, Pseudomonas, Staphylococcus aureus	Yes
Chen et al. (2022)	China	450	Klebsiella pneumoniae, E. coli, Streptococcus pneumoniae	Yes
Patel et al. (2023)	India	300	Pseudomonas aeruginosa, Klebsiella, Streptococcus	Yes
Taylor et al. (2022)	UK	500	S. aureus, Pseudomonas aeruginosa, E. coli	Yes
Jackson et al. (2023)	South Africa	200	Klebsiella pneumoniae, Staphylococcus aureus	Yes

Table 2. Antibiotic Resistance Rates in Pediatric Populations Post-COVID-19

Pathogen	Resistance to Ampicillin (%)	Resistance to Trimethoprim-Sulfamethoxazole (%)	Resistance to Fluoroquinolones (%)	Resistance to Carbapenems (%)	Resistance to Third-Generation Cephalosporins (%)
Escherichia coli	28	22	17	7	21
Pseudomonas aeruginosa	40	15	35	13	25
Klebsiella pneumoniae	45	30	28	9	55
Staphylococcus aureus	12	10	6	0	2
Streptococcus pneumoniae	6	5	4	1	3

diagnostic capabilities. However, even in these countries, certain pathogens, such as **Pseudomonas aeruginosa**, showed concerning levels of resistance.

Impact on Clinical Outcomes

The presence of antibiotic resistance in pediatric patients with secondary bacterial infections following COVID-19 was associated with poorer clinical outcomes. The average length of hospital stay for children with resistant infections was 18 days compared to 11 days for children with non-resistant infections. Additionally, the mortality rate in the resistant group was 6.5%, compared to 2.1% in the non-resistant group.

Discussion

The results of this meta-analysis highlight several critical factors contributing to the rise in antibiotic resistance among pediatric populations following COVID-19 infections (3). The overuse and misuse of antibiotics during the pandemic are major drivers of this issue, which has been compounded by the uncertainty and challenges in distinguishing between viral and bacterial infections in pediatric COVID-19 cases (4). Throughout the pandemic, many clinicians opted to prescribe antibiotics empirically in cases of pediatric COVID-19, given the difficulty in promptly identifying bacterial co-infections (5). This practice, although aimed at preventing potential bacterial complications, has led to an increase in antibiotic resistance, as antibiotics are often unnecessary for viral infections (6). The frequent and sometimes unwarranted use of broad-spectrum antibiotics, particularly in hospitalized children, has created an environment conducive to the emergence of resistant pathogens (7).

The increased risk of secondary bacterial infections in children who have recovered from COVID-19 is another important aspect of this study. The most commonly identified pathogens in pediatric COVID-19 cases with secondary bacterial infections were **Klebsiella pneumoniae**, **Escherichia coli**, **Pseudomonas aeruginosa**, and **Staphylococcus aureus**, all of which are known to exhibit varying degrees of resistance (8). **Klebsiella pneumoniae** and **Pseudomonas aeruginosa**, in particular, demonstrated higher resistance rates, with resistance to commonly used antibiotics such as fluoroquinolones, carbapenems, and third-generation cephalosporins (9). These resistance patterns raise significant concerns about the effectiveness of current treatment regimens, as infections caused by these resistant strains may require alternative, more expensive, and often less effective therapies (10).

Geographic variation in antibiotic resistance is a critical finding from this meta-analysis. Studies conducted in low- and middle-income countries showed higher rates of resistance compared to high-income nations (11). This discrepancy likely reflects differences in healthcare infrastructure, diagnostic capabilities, and antibiotic stewardship programs. In many low-resource settings, the lack of access to rapid diagnostic tools means that antibiotics are often prescribed empirically, leading to overuse and the potential for resistance development (12). Additionally, limited access to healthcare resources and essential antibiotics may result in suboptimal treatment regimens, which further contribute to the emergence of resistant strains (13).

In contrast, high-income countries, where healthcare systems are generally better equipped and diagnostic capabilities are more advanced, tend to report lower rates of resistance. However, even in these settings, there are concerns about the overuse of antibiotics, especially in cases where bacterial co-infections are not confirmed but antibiotics are prescribed as a precautionary measure (14). While the prevalence of resistance is generally lower in high-income countries, this analysis highlights that the problem is not unique to resource-limited areas but is a global issue that demands attention (15).

One of the more striking findings of this study is the significant impact of antibiotic resistance on clinical outcomes in pediatric patients (16). The presence of resistant infections was associated with longer hospital stays, higher mortality rates, and poorer overall outcomes. Children with antibiotic-resistant infections spent an average of 18 days in the hospital, compared to 11 days for those with non-resistant infections (17). The increased duration of hospitalization not only places a strain on healthcare resources but also exposes patients to additional risks, such as hospital-acquired infections, further complicating their recovery (18). Mortality rates were also higher in the group with antibiotic-resistant infections, with a mortality rate of 6.5%, compared to 2.1% in those with non-resistant infections. These findings underscore the significant burden that antibiotic resistance places on both the healthcare system and patient health (19).

Given these findings, antibiotic stewardship emerges as a key strategy in combatting antibiotic resistance. Antibiotic stewardship programs, which promote the appropriate use of antibiotics, are essential in reducing unnecessary prescriptions and improving patient outcomes (20). These programs focus on ensuring that antibiotics are prescribed only when necessary and that the right antibiotics are used at the right doses and for the appropriate duration. In high-income countries, antibiotic stewardship programs have been successful in curbing the rise of resistance and improving clinical outcomes (21). However, in low- and middle-income countries, the implementation of such programs is often hampered by limited resources, lack of infrastructure, and inadequate training of healthcare providers (22).

The global nature of antibiotic resistance means that it is a problem that transcends national borders (23). While high-income countries have made significant strides in controlling antibiotic resistance, the rise of resistant strains in pediatric patients in low- and middle-income countries highlights the need for a coordinated global response (2). Effective surveillance systems, global collaboration in research, and the sharing of data on resistance patterns can help track the evolution of resistance and inform treatment strategies (24). In addition, there is a growing need for investments in diagnostic tools, which can help clinicians rapidly differentiate between bacterial and viral infections, reducing the unnecessary use of antibiotics (25).

Furthermore, the overprescription of antibiotics in pediatric COVID-19 cases is compounded by gaps in knowledge and awareness among both healthcare providers and the general public. In many cases, parents may pressure healthcare providers to prescribe antibiotics, believing that antibiotics are necessary for the treatment of viral infections (26). This, coupled with the uncertainty that healthcare providers face in diagnosing bacterial infections during the acute phase of COVID-19, contributes to the overuse of antibiotics. Education campaigns aimed at both healthcare providers and the general public are essential to address these misconceptions and encourage more responsible antibiotic use (27).

The rising prevalence of antibiotic resistance in pediatric populations post-COVID-19 is not just a clinical issue; it is a public health crisis that requires urgent attention (28). The long-term consequences of antibiotic resistance are profound, as infections

caused by resistant pathogens become more difficult to treat, leading to higher healthcare costs, prolonged illness, and increased mortality (29). It is essential to take a comprehensive approach to addressing this issue, which includes the judicious use of antibiotics, the promotion of antibiotic stewardship, and the development of new antibiotics and alternative therapies.

In addition to combating antibiotic resistance, there is a need for further research to understand the mechanisms behind resistance in pediatric populations and to identify novel approaches to prevention and treatmen (30). The ongoing surveillance of antibiotic resistance patterns, along with the development of more effective diagnostic tools and vaccines, will be key to managing the evolving landscape of infectious diseases (31). Given the increasing burden of antibiotic resistance, there is a pressing need for concerted global efforts to tackle this problem and mitigate its impact on pediatric health, particularly in the wake of the COVID-19 pandemic.

Conclusion

This meta-analysis has highlighted a concerning increase in antibiotic resistance among pediatric populations following COVID-19 infection. The misuse of antibiotics, particularly in the absence of bacterial co-infections, has contributed to the rise in resistance. The regional variations observed underscore the need for tailored antibiotic stewardship programs, better diagnostic tools, and improved healthcare infrastructure, particularly in low- and middle-income countries. Ultimately, addressing antibiotic resistance requires a global, multi-faceted approach that includes reducing unnecessary antibiotic use, improving diagnostic capabilities, enhancing surveillance, and investing in new treatment options. As the world continues to navigate the aftermath of the COVID-19 pandemic, the need for effective strategies to combat antibiotic resistance has never been more urgent.

Authors' Contribution

The authors read and confirmed the final manuscript.

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All data are obtainable after an appeal from the corresponding author.

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The Role of Hormonal Therapy in the Management of Hormone Receptor-Positive Breast Cancer: Current Trends and Future Directions

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Abstract:
Breast cancer is responsible for more than 2.3 million newly diagnosed cases each year, according to the statistics. A hormonal imbalance, which is defined by unregulated activity of estrogen and progesterone, is often the cause of this type of cancer. It has become easier to handle patients who have HR+ breast cancer, particularly in women who have both advanced and early-stage disease, as a result of the deployment of estrogen-blocking hormone treatment. The permissiveness of tamoxifen, which was the first selective estrogen receptor modulator (SERM) to be commercialized, made it possible for more hormonal therapies to be developed. The cornerstone of breast cancer treatment is comprised of aromatase inhibitors (AIs), selective estrogen receptor degraders (SERDs), and cyclin-dependent kinase inhibitors (CDK) 4/6. These three types of drugs ultimately lead to improved patient outcomes. On the other hand, the inherent or acquired resistance of cancers to hormone therapy continues to be a serious cause for concern. Alterations in the genetic makeup of the tumor, as well as the activation of alternate pathways, make this situation even worse. The increasing development of molecular biology, precision medicine, and targeted therapies, on the other hand, is pointing to a new strategy for dealing with these problems. The purpose of this study is to investigate prospective treatment options and to shed light on the significant role that hormone therapy plays in the management of HR-positive breast cancer.

Keywords: Breast Cancer, Selective Estrogen Receptors Modulator (SERM), Aromatase Inhibitors (AIs), Cyclin-Dependent Kinase Inhibitors (CDK)

Introduction

More than two million new cases of breast cancer are discovered every year, making it the disease that affects most people all over the world. The prevalence of breast cancer is a major concern for the public's health. An overwhelming majority of these cases are classified as hormone receptor-positive breast cancer, sometimes known as HR+ breast cancer (1). An increase in the number of estrogen and/or progesterone receptors is one of the characteristics of HR+ breast cancer patients. Cellular hyperplasia is induced by these receptors when they are triggered

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by hormonal signals, which ultimately leads to an increase in the growth of tumors (2). Given that estrogen and progesterone signaling may contribute to the genesis and progression of HR+ breast cancer, there is rising interest in the development of new hormonal treatments that precisely target this important signaling pathway. Patients who do not have HI have demonstrated an improvement in their prognosis as a result of these treatments; on the other hand, patients who have early and metastatic disease have reported a better prognosis for HI (3).

Tamoxifen, which is a selective estrogen receptor modulator (SERM) and the first medicine to precisely target estrogen receptors (4), is the drug that marks the beginning of hormonal therapy for breast cancer (5). Tamoxifen can achieve this by affixing itself to the estrogen receptors that are known to be present in breast cancer cells. Because of this, estrogen is unable to activate the cells, which results in a reduction in the proliferation of tumors. Because of the multiple benefits that are associated with their application, other medications that target the endocrine system have been very successful. A few examples of these benefits are increased rates of survival and decreased rates of recurrence of HR+ breast cancer when the patient is receiving treatment (6).

Over the course of several decades, tamoxifen has been the primary treatment for HR+ breast cancer. It is a substantial therapeutic strategy that improves the prognosis and reduces the risk of recurrence in both early-stage and metastatic circumstances (7).

We have made significant progress in our understanding of hormone receptor signaling, which has resulted in the revolutionary introduction of aromatase inhibitors (8) into the treatment paradigm, particularly for women who have gone through menopause. To accomplish their purpose, aromatase inhibitors, which include anastrozole, letrozole, and exemestane, work by inhibiting the enzyme aromatase (9). Aromatase is responsible for catalyzing the conversion of androgens into estrogen within the body. Aromatase inhibitors can successfully deprive HR+ tumors of the hormone that can boost their growth. This is accomplished by lowering estrogen levels (10). The results of clinical trials indicate that artificial insemination (6) is superior to tamoxifen in terms of reducing the rates of recurrence and improving disease-free survival (DFS) in postmenopausal women, particularly in the early stages of the illness. This advancement, in conjunction with the subsequent development of SERDs and cyclin-dependent kinase 4/6 (CDK4/6) inhibitors, has significantly expanded the variety of treatments available for HR+ breast cancer (11).

As a consequence, clinical results have been improved, and patients have managed to live for longer periods. A different approach is provided by SERDs, which include fulvestrant. These SERDs not only inhibit estrogen receptors but also make it easier for them to degrade, which further hinders the process of estrogen-mediated signaling (4). SERDs have shown to be successful in the treatment of early-stage as well as metastatic HR+ breast cancer, particularly in patients who have already developed resistance to tamoxifen or aromatase inhibitors. CDK4/6 inhibitors are a class of targeted medicines that work by inhibiting cyclin-dependent kinases that are related to the advancement of the cell cycle. Examples of these drugs include palbociclib, ribociclib, and abemaciclib. CDK4/6 inhibitors, when used in concert with AIs, have demonstrated significant improvements in progression-free survival (PFS) and overall survival (10) in patients with metastatic HR+ breast cancer. This is particularly true for individuals whose tumors have demonstrated resistance to endocrine therapy alone.

Nonetheless, despite these therapeutic advancements, the clinical landscape of HR+ breast cancer is confounded by endocrine resistance, which may be intrinsic (existing from the initiation of therapy) or acquired (growing over time) (12). Endocrine resistance is a significant obstacle in the treatment of HR+ breast cancer, as tumors that initially respond to hormonal therapy may ultimately recur due to the activation of alternative signaling pathways or genetic changes that circumvent estrogen receptor reliance (13). The principal causes contributing to endocrine resistance encompass tumor heterogeneity, mutations in the estrogen receptor (notably ESR1 mutations), activation of the PI3K/AKT/mTOR pathway, and modifications in other cell cycle regulators, including cyclin D1 and CDK4/6. The tumor microenvironment and the presence of inflammatory mediators can exacerbate resistance to treatment (14).

Recent advancements in molecular biology, genomics, and precision medicine have yielded profound insights into the mechanisms underlying hormone resistance. These developments are facilitating the creation of innovative therapeutic ways to surmount resistance and enhance patient outcomes (15). Targeted medicines focused on specific molecular modifications, such as PI3K inhibitors or combination therapy addressing several signaling pathways, demonstrate potential in surmounting resistance. Moreover, the incorporation of liquid biopsy and sophisticated genetic profiling in clinical practice facilitates more accurate, personalized therapy approaches tailored to the distinct genomic attributes of a patient's tumor (16).

This review seeks to elucidate the present role of hormonal therapy in the management of HR+ breast cancer, analyzing the diverse therapeutic choices, their clinical effectiveness, and the obstacles

presented by endocrine resistance. Furthermore, it will examine novel tools in precision medicine and molecular targeting that possess the potential to enhance the efficacy of hormone therapy and tackle the intricate problem of resistance. The integration of tailored treatment strategies, innovative targeted medicines, and enhanced comprehension of the molecular biology of HR+ breast cancer will be essential for improving outcomes and alleviating the worldwide burden of this illness.

Current Approaches to Hormonal Therapy

Aromatase Inhibitors: The Backbone of Therapy in Postmenopausal Women

Aromatase inhibitors, also known as AIs, are the principal treatment for hormone receptor-positive breast cancer in postmenopausal women who have passed through menopause. Adipose tissue, liver, and muscle are all examples of peripheral tissues that are affected by these drugs because they inhibit the aromatase enzyme, which is responsible for facilitating the conversion of androgens, which are precursors to estrogen, into estrogen (17). In women who have gone through menopause, the ovaries stop producing a significant amount of estrogen, and the majority of the estrogen that is found in the body comes from several peripheral conversion mechanisms (18). Aromatase inhibitors reduce the amount of estrogen that is circulating in the body, preventing estrogen receptor-positive (ER+) tumors from receiving the hormone that is necessary for their growth and proliferation (19).

The efficacy of artificial intelligence as a therapeutic agents has been undeniably demonstrated, particularly in the context of adjuvant treatment for early-stage HR+ breast cancer. In postmenopausal women, aromatase inhibitors are superior to tamoxifen in terms of reducing the risk of breast cancer recurrence and improving disease-free survival (DFS) (20). This has been demonstrated by a large number of substantial clinical trials that were conducted using randomised methods. Two studies, the ATAC (Arimidex, Tamoxifen, Alone or in Combination) experiment and the BIG 1-98 study, demonstrated that aromatase inhibitors, such as anastrozole, letrozole, and exemestane, significantly reduce the rates of recurrence and improve survival outcomes when administered as adjuvant therapy after surgery. This is in comparison to the use of tamoxifen (21).

In comparison to tamoxifen, artificial insemination can reduce estrogen levels in a more effective manner, which is one of the primary advantages of AIs. Tamoxifen is a SERM that blocks estrogen receptors in breast tissue while demonstrating partial estrogenic activities in other tissues, such as the bones and the uterus (22). Tamoxifen is used to treat breast cancer. On the other hand, artificial intelligence solutions offer a more comprehensive estrogen blockade by systematically lowering the production of estrogen. Artificial insemination treatment is particularly advantageous for postmenopausal women since it targets the primary source of estrogen in the body while simultaneously lowering the likelihood of uterine side effects that are linked with tamoxifen (6).

Although AIs have significantly improved outcomes for postmenopausal women who have HR+ breast cancer, the deployment of these technologies raises several challenges. AIs have been related to particular side effects, including decreased bone density, arthralgia, vasomotor symptoms, and an increased probability of fractures. These bad effects have been linked to prolonged administration of such medications (23). As a consequence of the fact that estrogen plays a crucial role in maintaining bone health, AIs have the potential to lower estrogen levels, which in turn can lead to a drop in bone mineral density (BMD). As a consequence of this, individuals who are receiving AI therapy typically require constant monitoring of their bone health. Certain bone-modifying medications, such as bisphosphonates or denosumab, may be utilized to mitigate the risk of bone fractures (24).

Furthermore, musculoskeletal disorders, which include joint stiffness, pain, and edema, are common side effects that can have a significant impact on the quality of life of a patient. These symptoms, which are sometimes referred to as “AI arthralgia,” have the potential to render certain individuals unable to function normally and perhaps lead to the termination of treatment (25). Consequently, it is crucial to regulate these adverse effects in the care of women who are receiving AI therapy. Controlling these symptoms involves utilizing a variety of strategies, including analgesics, physical therapy, and adaptations to the patient’s lifestyle, to improve patient comfort and adherence to treatment (26).

There are several cases in which artificial intelligence is applied in the management of advanced or metastatic HR+ breast cancer. It has been established that AIs can improve PFS and overall survival in patients who have metastatic sickness when they are taken in conjunction with other targeted therapies, such as cyclin-dependent kinase 4/6 (CDK4/6) inhibitors (27). Certain CDK4/6 inhibitors, such as palbociclib, ribociclib, and abemaciclib, perform their role by impeding the advancement of the cell cycle, which in turn inhibits the proliferation of cancer cells. The combination of artificial intelligence with CDK4/6 inhibitors has resulted in the establishment of a standard treatment protocol for metastatic HR+ breast cancer (28). This approach offers patients extended disease management and improved outcomes in comparison

to monotherapy.

Even though they are effective, a significant obstacle continues to exist in the form of endocrine resistance, which can develop either naturally or as a result of prolonged treatment (29). Alterations in the estrogen receptor or the activation of alternative signaling pathways can lead to the development of resistance to aromatase inhibitors in some malignancies over the course of treatment. This resistance represents a considerable impediment to the efficacy of treatment interventions over the long term, particularly in the case of metastatic sickness. New chances to overcome resistance and improve patient outcomes have arisen as a result of the development of innovative drugs. These therapies include SERDs and combination treatments that target alternative biochemical pathways (30).

Clinical Efficacy and Applications

Early-Stage Breast Cancer

While conventional treatments like tamoxifen are effective in reducing the risk of recurrence and improving DFS in patients with early-stage HR+ breast cancer, AIs are more effective in clinical settings (31). Anastrozole, letrozole, and exemestane are examples of artificial insemination drugs that work by lowering estrogen levels in the body, which in turn inhibits the growth of tumors that are mediated by estrogen. Because estrogen is primarily produced by the aromatase enzyme rather than the ovaries, this method is particularly useful for women who have gone through menopause (32).

The function of aromatase inhibitors in the adjuvant setting has been elucidated by significant clinical trials. In this context, aromatase inhibitors are supplied after surgery to reduce the likelihood of recurrence taking place (33). Through the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial, which was a significant study, it was determined that anastrozole, which is an aromatase inhibitor, was more effective than tamoxifen in reducing the recurrence rates of HR+ breast cancer in postmenopausal women (34). Following the administration of letrozole as adjuvant therapy, the BIG 1-98 study indicated that letrozole was more effective than tamoxifen in improving DFS and overall survival respectively. Artificial intelligence is an effective alternative to tamoxifen for the treatment of early-stage HR+ breast cancer, particularly in postmenopausal women. These studies have established the use of AIs as a standard treatment for breast cancer (35).

Extended Adjuvant Therapy

There have been several important studies that have demonstrated the benefits of extending adjuvant AI therapy beyond the traditional timeframe of five years. Women who had completed their original five years of treatment with tamoxifen were included in the MA.17 trial, which proved the significant advantages of extending the duration of letrozole medication for an additional five years of treatment (36). Notably among high-risk patients, such as those with lymph node-positive sickness or those diagnosed at a younger age, the prolonged therapy led to a considerable drop in the incidence of late recurrences. This was notably true for individuals who had advanced stages of the disease (37). It has become apparent that increasing the use of AI therapy for these patients is a potential strategy for further reducing the rates of recurrence and improving the outcomes of long-term survival. Nevertheless, the benefits of prolonged adjuvant therapy need to be weighed against the possibility of long-term detrimental repercussions, such as decreased bone density, cardiovascular events, and musculoskeletal difficulties, which calls for careful monitoring and administration of care services (8).

Metastatic Breast Cancer

In the context of metastatic breast cancer, aromatase inhibitors are crucial, especially for patients with hormone receptor-positive tumors. In advanced disease, the objective is to manage the cancer, extend survival, and preserve quality of life. Artificial intelligences are frequently utilized with other therapies to augment their effectiveness (38). A promising strategy in metastatic HR+ breast cancer is the utilization of AIs alongside targeted medicines, including cyclin-dependent kinase 4/6 (CDK4/6) inhibitors. Pharmaceuticals such as palbociclib, ribociclib, and abemaciclib, which inhibit the CDK4/6 pathway, function by obstructing cell cycle advancement, hence averting cancer cell multiplication. In conjunction with AIs, CDK4/6 inhibitors have demonstrated a substantial enhancement in PFS and overall survival for patients with metastatic HR+ breast cancer (39).

The integration of AIs with CDK4/6 inhibitors has emerged as a fundamental treatment for metastatic breast cancer, yielding enhanced clinical results relative to AIs alone. Additional targeted medicines, including PI3K inhibitors or mTOR inhibitors, may be utilized in conjunction with AIs for specific individuals, contingent upon the tumor's molecular features (40). This comprehensive therapeutic strategy has transformed the management of metastatic breast cancer, providing patients with enhanced options for disease control and prolonging survival with a comparatively advantageous side effect profile compared to chemotherapy.

Limitations and Side Effects

The use of aromatase inhibitors for an extended

period in the treatment of HR+ breast cancer has been associated with several adverse effects, particularly on bone health (41). To function, artificial intelligence inhibits the enzyme aromatase, which is responsible for catalyzing the conversion of androgens to estrogen. This results in a decrease in the amount of estrogen that is present in the body (42). Even though these treatments are effective in reducing the proliferation of tumors that are caused by estrogen, they may also cause a significant reduction in bone density. As a consequence, the risk of fractures and other musculoskeletal consequences, such as joint pain, stiffness, and edema, is increased. Estrogen deficiency, which is essential for maintaining bone density, is thought to be the cause of these adverse consequences (43).

When these risks are taken into consideration, it is necessary to carefully monitor the bone health of patients who are undergoing AI therapy. The degree of bone loss can be evaluated with the help of bone density examinations, such as DEXA scans, which are frequently recommended (44). To add insult to injury, medical professionals may recommend bone-modifying medications, such as bisphosphonates (for example, zoledronic acid) or denosumab (a monoclonal antibody), which reduce the likelihood of fractures by increasing bone density and reducing bone resorption (45). It is the goal of these therapies to reduce the bone-debilitating effects of AIs, which will allow patients to continue receiving treatment for their cancer while simultaneously minimizing the number of skeletal difficulties they have. Furthermore, managing musculoskeletal disorders is an essential component of patient care because these symptoms can harm a patient's quality of life as well as their ability to comply with treatment (46).

Selective Estrogen Receptor Modulators (SERMs): A Historical Perspective

In the treatment of ER+ breast cancer, selective estrogen receptor modulators, also known as SERMs, are an important component of hormonal therapy (47). The therapeutic approach for HR+ breast cancer was revolutionized by tamoxifen, which was the first SERM to be approved for clinical application when it was first introduced. Specifically, tamoxifen accomplishes its effect by attaching itself to estrogen receptors in breast cancer cells (48). This prevents estrogen from connecting to the receptors, which in turn promotes the proliferation of cancer cells. Tamoxifen acts as an estrogen antagonist in breast tissue, which means that it inhibits the proliferative effects of estrogen on tumor cells. As a result, it reduces the likelihood of recurrence in women who have HR+ breast cancer (49).

In addition to its effect on the breast, tamoxifen also demonstrates estrogenic activity in other tissues, such as the uterus and the bones. Because tamoxifen has the potential to protect bone density while simultaneously increasing the risk of endometrial cancer, the risk-benefit profile has become more complicated as a result of the dual action (50). Tamoxifen has been the cornerstone of breast cancer treatment for decades, despite the concerns that have been raised about its effectiveness. There is strong clinical evidence that supports its effectiveness in reducing the risk of breast cancer recurrence and improving overall survival rates in women who have ER+ tumors (51).

Recently, innovative selective estrogen receptor modulators SERMs with tissue-specific effects have been developed as our understanding of breast cancer biology has progressed. Along the same lines as tamoxifen, raloxifene is a drug that targets estrogen receptors. It is primarily used for the treatment of osteoporosis and the prevention of breast cancer in women who are at a high risk of developing the disease after menopause (52). The development of SERMs has been a significant technological accomplishment in the field of hormonal therapy for breast cancer. Furthermore, continuing research continues to investigate novel options that have improved efficacy and safety profiles (53).

Clinical Impact

Tamoxifen remains an essential treatment for premenopausal women with HR+ breast cancer and in certain postmenopausal contexts. Tamoxifen, a SERM, functions by attaching to estrogen receptors on cancer cells, inhibiting the proliferative effects of estrogen in breast tissue (54). Although newer medicines like aromatase inhibitors are accessible for postmenopausal women, tamoxifen continues to be a fundamental treatment, especially for premenopausal women who still synthesize estrogen from their ovaries. The enduring therapeutic advantages of tamoxifen have been thoroughly recorded in significant clinical trials, such as the NSABP P-1 (National Surgical Adjuvant Breast and Bowel Project P-1) and ATLAS (Adjuvant Tamoxifen: Longer Against Shorter) trials (55). The trials have shown that tamoxifen substantially decreases the incidence of breast cancer recurrence and contralateral breast cancer in women with HR+ breast cancer. The ATLAS trial, featuring extensive long-term follow-up, validated that tamoxifen maintains its efficacy beyond 10 years of treatment, decreasing the probability of relapse and enhancing DFS and overall survival (10). The NSABP P-1 study, which primarily examined tamoxifen as a prophylactic intervention for high-risk women, showed a significant decrease in the occurrence of both invasive breast cancer and non-invasive (ductal carcinoma in situ) illness. Additionally, tamoxifen

has demonstrated efficacy in diminishing the risk of contralateral breast cancer, which is particularly significant for women previously diagnosed with cancer in one breast (56). The clinical advantages have established tamoxifen as a standard treatment, providing an effective approach to enhance long-term survival and quality of life in women with HR+ breast cancer.

Challenges

Although tamoxifen is a crucial element in breast cancer therapy, its administration is linked to various side effects and constraints that may affect patient compliance and overall results. A significant adverse effect of tamoxifen is the heightened risk of thromboembolic events, such as deep vein thrombosis (DVT) and pulmonary embolism (57). These concerns are especially pertinent for older women, those with additional thrombosis risk factors, or individuals with pre-existing cardiovascular diseases. The risk of these life-threatening consequences necessitates meticulous screening and management of patients, especially those with concurrent comorbidities (58).

Furthermore, tamoxifen has been linked to a heightened risk of endometrial cancer, especially in postmenopausal women. Due to tamoxifen's role as an estrogen agonist in specific tissues, including the endometrium, it can promote the proliferation of endometrial cells, potentially resulting in hyperplasia or cancer (59). The risk of endometrial cancer is dose-dependent and seems to be elevated in older women on prolonged tamoxifen treatment. This requires vigilant observation for signs of endometrial cancer (such as atypical uterine bleeding) and consistent gynecological assessments, particularly for women who have undergone prolonged tamoxifen therapy (60).

A notable difficulty with tamoxifen is its restricted effectiveness in cancers that are inherently resistant to estrogen inhibition. Although tamoxifen is efficacious in numerous HR+ breast cancers, certain tumors may possess genetic mutations or modifications in the estrogen receptor (e.g., ESR1 mutations) that diminish tamoxifen's capacity to bind efficiently and impede estrogen signaling (61). In certain instances, the tumors may persist in their growth despite tamoxifen treatment. Furthermore, cancers that initially respond to tamoxifen may ultimately acquire resistance, resulting in disease recurrence. This inherent or developed resistance to tamoxifen poses a significant therapeutic obstacle in the treatment of HR+ breast cancer, particularly in advanced or metastatic cases (62).

Significant advancements have been made in the development of alternative therapies to address these constraints. Aromatase inhibitors are now the standard treatment for postmenopausal women with HR+ breast cancer, providing comprehensive estrogen suppression by decreasing estrogen synthesis. Patients with tamoxifen-resistant cancers can benefit from novel targeted medicines, including SERDs and CDK4/6 inhibitors, which offer alternative therapy choices to enhance outcomes and combat resistance mechanisms (63). In conclusion, although tamoxifen is a crucial therapy for premenopausal women and specific postmenopausal scenarios, its adverse effects, such as thromboembolic events, heightened risk of endometrial cancer, and restricted effectiveness in tamoxifen-resistant malignancies, pose considerable obstacles. Continued research aimed at addressing resistance, enhancing side effect profiles, and formulating combination medicines is crucial for optimizing treatment results in women with HR+ breast cancer (64).

Advancements in Hormonal Therapy: CDK4/6 Inhibitors and Beyond

The introduction of CDK 4/6 inhibitors has substantially altered the therapeutic approach for HR+ breast cancer, particularly in advanced and metastatic phases (65). These medications signify a significant advancement in enhancing outcomes for patients with restricted alternatives following progression on standard hormonal therapy, including aromatase inhibitors or tamoxifen. CDK4/6 inhibitors have transformed the treatment of HR+ breast cancer by introducing a new mode of action that improves the effectiveness of endocrine therapy (66).

Mechanism of Action

CDKs are crucial regulators of the cell cycle, particularly governing the transition from the G1 phase (cell proliferation) to the S phase (DNA synthesis). CDK4 and CDK6 are essential in facilitating this transition by phosphorylating the retinoblastoma (Rb) protein, a principal regulator that typically inhibits cell cycle advancement (67). The phosphorylation of Rb by CDK4/6 facilitates the release of E2F transcription factors, which initiate the expression of genes essential for the S phase of the cell cycle, culminating in cell division (68).

CDK4/6 inhibitors, including palbociclib, ribociclib, and abemaciclib, selectively inhibit CDK4 and CDK6 activity, obstructing Rb phosphorylation and impeding cell cycle progression from G1 to S phase (69). This strategy significantly inhibits cell proliferation in HR+ breast cancer cells, which is essential for malignancies driven by estrogen receptor signaling. Combining CDK4/6 inhibitors with conventional hormonal treatments, such as aromatase inhibitors or fulvestrant, enhances therapeutic efficacy by more efficiently inhibiting tumor proliferation (39).

Clinical Evidence

First-Line Therapy

It has been established time and again in clinical studies that the incorporation of CDK4/6 inhibitors into endocrine therapy results in a significant improvement in PFS and overall outcomes for patients who have advanced HR+ breast cancer (70). To demonstrate the clinical efficacy of these combinations, studies such as PALOMA-2, MONALEESA-2, and MONARCH-3 have been extremely important. The PALOMA-2 trial demonstrated that the combination of palbociclib and letrozole, which is an aromatase inhibitor, resulted in a significant improvement in progression-free survival (PFS) when compared to letrozole alone (71). This resulted in a considerable reduction in the likelihood of the illness progressing. The MONALEESA-2 trial, which included both ribociclib and letrozole, produced comparable results. These results indicated a considerable improvement in progression-free survival (PFS) in addition to a positive safety profile (72).

In the context of first-line treatment, the MONARCH-3 study evaluated the performance of abemaciclib in combination with letrozole, which resulted in the production of strong evidence for the benefits of combining CDK4/6 inhibitors with aromatase inhibitors. The results of these clinical trials make it abundantly evident that the incorporation of CDK4/6 inhibitors not only increases the efficacy of endocrine therapy but also provides patients with extended disease management and delays progression, hence improving both quality of life and overall survival rates (73).

Second-Line Therapy

The use of CDK4/6 inhibitors in second-line treatment regimens has been beneficial in patients whose illness has progressed after first-line hormone therapy. In patients with metastatic HR+ breast cancer who had previously had hormonal treatment, the MONARCH-2 study found that combining abemaciclib with fulvestrant, an estrogen receptor antagonist, significantly improved overall survival and PFS (74). For patients who have shown resistance to aromatase inhibitors or tamoxifen, the results of MONARCH-2 validated the use of abemaciclib as a second-line treatment. It has been shown that CDK4/6 inhibitors, when combined with other endocrine drugs, can overcome endocrine resistance and produce substantial therapeutic benefits, even at the late stages of the disease (75).

According to the results, CDK4/6 inhibitors are now more commonly used in combination therapy as a first-line and second-line treatment strategy for metastatic HR+ breast cancer. This has greatly improved survival rates and given patients fresh hope for controlling their disease in the long run (70).

Toxicities and Management

Even though CDK4/6 inhibitors are highly effective in the treatment of HR+ breast cancer, they are also associated with several undesirable side effects. Neutropenia, fatigue, diarrhea, and hepatic enzyme abnormalities are the most common adverse effects that have been associated with CDK4/6 inhibitors (76). Neutropenia is a significant cause for concern because it can contribute to an increased risk of infections and may require a reduction in the dosage of the medication or a temporary cessation of treatment. Consistent monitoring of blood counts is essential for the early discovery and management of neutropenia. Patients may require growth factor support or alterations to their treatment plan, depending on the severity of their condition (77).

One of the most common unpleasant effects, particularly one that is related to abemaciclib, is diarrhea, which can be quite severe in some individuals. It is common practice to treat this adverse effect with supportive treatment, which may involve the prescription of anti-diarrheal medications and the reduction of dosage as necessary. To prevent dehydration and maintain the patient's quality of life, it is essential to effectively manage diarrhea (78).

It has been observed that patients who have been treated with ribociclib and abemaciclib have displayed abnormalities in liver function, including elevated levels of liver enzymes (AST and ALT). It is recommended that liver function be evaluated regularly, particularly in the beginning stages of treatment. In cases where there is a significant amount of liver toxicity, patients may be forced to make adjustments to their dosage or perhaps stop receiving treatment altogether (79).

Even though fatigue is a common adverse effect, it is often manageable via the use of supportive care procedures and symptomatic therapy. Even though some individuals may experience symptoms such as nausea, headaches, or baldness, it is important to note that these symptoms are typically less severe than the toxicities that are associated with conventional chemotherapy (80).

Additionally, even though the introduction of CDK4/6 inhibitors has revolutionized the treatment of HR+ breast cancer, particularly in the later and metastatic stages of the disease, it is vital to address adverse effects to achieve the best possible outcomes for patients. The alleviation of these toxicities can be accomplished by the implementation of individualized treatment protocols, consistent observation, and supportive interventions (81). This will enable patients to continue receiving therapy and benefit from significant gains in disease management and survival rates. There is reason to be optimistic about

the possibility of improved long-term management of HR+ breast cancer, as the development of novel medications and combination therapies continues to advance (82).

Challenges in Hormonal Therapy: Mechanisms of Resistance

Resistance to hormonal therapy constitutes a major obstacle to the efficient management of (HR+) breast cancer, especially in late or metastatic phases. Notwithstanding the considerable clinical efficacy of therapies such as SERMs, AIs, and other endocrine-based treatments, a significant proportion of patients encounter either intrinsic or acquired resistance, resulting in disease progression (13). Comprehending the mechanisms underlying this resistance is essential for formulating innovative therapeutic techniques that can overcome these obstacles and enhance patient outcomes (83).

Intrinsic vs. Acquired Resistance

Intrinsic Resistance

Intrinsic resistance denotes cancers that are unresponsive to hormone therapy from the beginning. This form of resistance frequently arises from certain genetic mutations or modifications in essential signaling pathways that inhibit the efficacy of the therapy (84). For instance, certain HR+ cancers may include mutations that impair estrogen receptor signaling, or they may have anomalies in alternative growth-promoting pathways that reduce their dependence on estrogen for proliferation. These tumors exhibit a diminished likelihood of responding to standard hormonal therapy, like tamoxifen or aromatase inhibitors, from the onset of treatment (85).

Acquired Resistance

Acquired resistance occurs when cancers first react to hormonal therapy but subsequently establish methods to circumvent estrogen reliance. Over time, these tumor cells acclimate to the selective pressures of hormone therapy, resulting in disease progression despite ongoing treatment. This condition is particularly prevalent in metastatic contexts, where tumor cells undergo prolonged endocrine treatments (86). As the tumor progresses, it may activate alternate signaling pathways or acquire mutations that enable it to circumvent the requirement for estrogen, so rendering the therapy ineffective. Acquired resistance poses a significant difficulty in the management of advanced HR+ breast cancer, as it restricts the efficacy duration of previously effective medicines (87).

Key Mechanisms of Resistance

Several mechanisms have been identified that contribute to both intrinsic and acquired resistance to hormonal therapy in HR+ breast cancer. These include genetic mutations, activation of alternative signaling pathways, changes in hormone receptor expression, and epigenetic modifications (88). Below are some of the most prominent mechanisms that drive resistance:

1.**ESR1 Mutations**

A prominent cause of acquired resistance is the development of mutations in the ESR1. These mutations frequently occur in cancers that initially responded to tamoxifen or aromatase inhibitors but subsequently acquired resistance. ESR1 mutations result in ligand-independent activation of the estrogen receptor, indicating that the receptor remains active without the presence of estrogen or other activating ligands (89). This enables tumor cells to persist in proliferation independent of estrogen stimulation. Aromatase inhibitors, which function by diminishing estrogen synthesis, thus lose their efficacy. ESR1 mutations correlate with unfavorable prognosis and underscore the necessity for alternative treatments, such as SERDs, capable of targeting and degrading the mutated receptor (90).

2.**Alternative Pathway Activation**

A significant form of resistance involves the development of alternative signaling pathways that completely circumvent the estrogen receptor pathway. In HR+ breast cancer, tumors may enhance signaling pathways such as the PI3K/AKT/mTOR pathway, HER2 signaling, or fibroblast growth factor receptor (FGFR) signaling, all of which can facilitate tumor growth and survival independently of estrogen (91). Activation of the PI3K/AKT/mTOR pathway can promote cell proliferation and survival, even when medications that inhibit estrogen signaling are present. Likewise, HER2 overexpression or amplification may activate alternate growth signals, causing resistance to endocrine treatment. These alternative pathways present novel therapeutic targets, and combination therapies aimed at both estrogen signaling and these pathways are being explored to address resistance (92).

3.**Loss of ER Expression**

Certain malignancies may experience a significant alteration, completely losing estrogen receptor expression. The phenomenon termed “ER downregulation” frequently transpires with the advancement of breast cancer to a more aggressive and therapy-resistant phenotype (93). Tumors that exhibit a loss of ER expression become independent of estrogen for proliferation and are consequently resistant to hormonal treatments, like AIs or tamoxifen. The absence of ER expression is generally linked to an unfavorable prognosis and is indicative

of more aggressive, poorly differentiated neoplasms. This resistance mechanism is a considerable difficulty, as these cancers necessitate an entirely distinct treatment strategy, frequently incorporating chemotherapy or targeted medicines instead of hormone therapy (94).

4.**Epigenetic Modifications**

Epigenetic modifications, such as changes in DNA methylation and histone abnormalities, may also play a role in the resistance to hormone treatments in HR+ breast cancer. These alterations can influence the expression and functionality of the estrogen receptor without modifying the fundamental genetic sequence (95). Hypermethylation of the ESR1 gene promoter can result in decreased expression of the estrogen receptor, thereby reducing the efficacy of therapy aimed at the ER. Likewise, modifications in histone acetylation or methylation can influence chromatin architecture and gene expression, encompassing genes pertinent to cell cycle regulation and apoptosis. Epigenetic alterations enable tumor cells to circumvent the growth-inhibitory effects of hormone therapy, hence increasing their resistance to treatment (96).

Biomarker-Driven Personalized Medicine

Progress in genomic profiling has markedly improved the capacity to customize breast cancer therapies according to the specific molecular traits of the tumor. Personalized medicine, informed by biomarkers, is progressively establishing itself as a fundamental aspect of breast cancer care. By identifying particular genetic mutations, modifications in signaling pathways, and other molecular characteristics, clinicians can determine the best suitable medicine for each patient, so ensuring a more tailored and effective treatment approach (97).

Multi-Gene Assays

Multi-gene assays, including Oncotype DX and MammaPrint, have transformed the management of HR+ breast cancer by delivering essential insights regarding recurrence risk and the possible advantages of adjuvant therapies. These assays evaluate the expression of several genes in tumor specimens to forecast the probability of recurrence and inform treatment strategies. Oncotype DX evaluates the expression of 21 genes and generates a recurrence score that aids in determining the need for chemotherapy alongside hormonal therapy (98). MammaPrint, a prevalent genomic assay, assesses the expression of 70 genes and offers insights about the patient's recurrence risk and potential advantages of adjuvant chemotherapy. Utilizing these assays, oncologists can categorize patients into distinct risk groups, facilitating more tailored treatment strategies that prevent both overtreatment and undertreatment (99).

The integration of biomarker-driven medicines, shown by alpelisib for PIK3CA-mutated tumors, illustrates the influence of precision medicine on the future of breast cancer treatment. Advancements in biomarker research are expected to yield better-tailored medicines, enhancing patient outcomes and minimizing superfluous side effects (100).

Emerging Therapies

Several promising therapies are currently being investigated in clinical trials with the potential to complement or enhance existing treatments for HR+ breast cancer. These emerging therapies are focused on disrupting estrogen receptor signaling at multiple levels and overcoming the challenges associated with resistance to current therapies (101).

Selective ER Coregulator Modulators (SECRMs)

Selective estrogen receptor coregulator modulators (SECRMs) represent an innovative category of pharmaceuticals aimed at precisely and specifically modulating estrogen receptor function. In contrast to conventional SERMs, which either inhibit or stimulate ER signaling based on the tissue type, SECRMs function by modifying the recruitment of coregulatory proteins to the ER (102). This may result in the suppression of ER-mediated transcription in tumors while preserving normal estrogenic function in other tissues, including bone. SECRMs offer a promising treatment strategy for HR+ breast cancer by specifically inhibiting ER signaling in cancer cells while maintaining its function in normal tissues, especially for patients resistant to standard hormonal therapy (103).

Epigenetic Modulators

Epigenetic modulators represent a promising field of investigation focused on undoing or altering the epigenetic modifications that lead to resistance in HR+ breast cancer. Alterations in DNA methylation, histone modification, and chromatin remodeling can modify gene expression patterns in cancers, particularly those associated with estrogen receptor signaling, resulting in resistance to hormonal treatments (104). Epigenetic modulators aim to target and counteract these alterations, perhaps reinstating sensitivity to endocrine therapy. Agents that inhibit DNA methyltransferases or histone deacetylases are being evaluated in conjunction with hormone therapy to ascertain their potential to surmount resistance and enhance therapeutic efficacy (105).

Immune Checkpoint Inhibitors:

While immune checkpoint medications, including

PD-1 and PD-L1 inhibitors, have demonstrated significant potential across various malignancies, their effectiveness in HR+ breast cancer has been comparatively limited. HR+ breast cancer generally exhibits a reduced mutational burden and diminished immune cell infiltration, resulting in decreased responsiveness to immune checkpoint inhibitors. Researchers are investigating the integration of immune checkpoint inhibitors with hormone treatments to augment anticancer immunity (106). The objective of integrating immune checkpoint inhibition with endocrine therapy is to surmount the immunosuppressive tumor milieu and elicit a more robust immune response. Current early-phase clinical trials are in progress, and although the results to date have been inconsistent, this strategy possesses the potential to enhance outcomes for HR+ breast cancer patients, particularly those with metastatic illness (107).

Conclusion

Hormonal therapy has revolutionized the treatment of HR+ breast cancer, markedly enhancing survival rates and quality of life. Nonetheless, obstacles like as resistance, tumor heterogeneity, and treatment-associated toxicity highlight the necessity for further innovation. The future of HR+ breast cancer management is rooted in personalized medicine, with novel drugs such as oral SERDs, combination therapies, and biomarker-driven strategies providing renewed optimism. By overcoming the constraints of existing medicines and utilizing advancements in molecular biology, the forthcoming phase of hormonal therapy is poised to enhance results for patients with HR+ breast cancer.

Authors's Contribution

Kosar Helmi: Conceptualization and review, Farnoosh Honarmand: Writing and editing the draft. The authors read and confirmed the final manuscript.

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The Emerging Role of RET Alteration in Solid Tumors: From Pathogenesis to Targeted Therapies

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Abstract:

The study investigates the emerging role of RET (rearranged during transfection) alterations in solid tumors, focusing on their pathogenesis and implications for targeted therapies. Ret alterations have been proven to be critical oncogenic factors in various types of solid tumors, most notably PTC and NSCLC. These alterations cause continuous activity of RET signaling pathways, contributing to tumor proliferation and progression. A comprehensive review of recent literature was conducted to elucidate the mechanisms by which RET alterations promote oncogenesis across different tumor types. The efficacy of targeted therapies, specifically RET- selective TKIs such as selpercatinib and pralsetinib, was also evaluated through clinical trial data. In conclusion, RET alterations represent a promising target for precision oncology in solid tumors. Continued research is essential to fully understand the genomic landscape of RET-positive cancers and to optimize therapeutic approaches. The findings support the integration of RET testing in clinical practice to facilitate the development of personalized treatment plans for affected patients.

Keywords: RET alterations, Solid tumors, Targeted therapy, Tyrosine kinase inhibitors, Precision medicine.

Introduction

RET is a receptor tyrosine kinase that is essential for the growth, differentiation, and survival of cells and is involved in embryonic development. The RET signaling pathway is constitutively activated by RET changes, which include gene fusions, point mutations, and amplifications (three functional domains). This promotes carcinogenesis and aids in the development of the neuroendocrine, sympathetic nervous, and genitourinary systems. Numerous malignancies and developmental problems are associated with RET mutations (1-3).

The growth of the sympathetic nervous, GU and neuroendocrine systems during embryonic life is influenced by RET. RET's transforming potential was originally reported in 1985. One of the

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earliest oncogenes found in solid tumors. This was particularly true for papillary thyroid carcinomas (PTCs) including RET fusions. In 30%-70% of invasive breast malignancies (particularly those with ER+ and HER2+ fractions) and 50%-65% of pancreatic ductal adenocarcinomas, the RET protein is overexpressed (17, 18).

The efficacy of RET mutation detection techniques varies. For germline mutations, Sanger sequencing is the gold standard; for somatic mutations, qPCR and digital PCR are preferable. The analysis provided by next-generation sequencing (NGS) is more thorough (4-6).

Point mutations are widespread in medullary thyroid cancer and other solid tumors, while RET fusions, such as KIF5B-RET and CCDC6-RET, are common in papillary thyroid cancer and non-small cell lung cancer (7-9).

RET mutations are variations in the RET gene's DNA sequence. These changes may be: Mutations in Germlines are inherited and found in every human cell. In MEN2, germline mutations are pathognomonic and are categorized as familial medullary thyroid cancer (FMTC), MEN2A, and MEN2B. The M918T mutation and classical cysteine mutations are associated with multiple endocrine neoplasia types 2A (MEN2A) and 2B (MEN2B) (10).

Somatic mutations are acquired changes that occur in specific cells during a lifetime. These are unique to tumor cells and are not inherited. In sporadic MTC, somatic RET mutations are linked to more aggressive tumor characteristics and a poorer prognosis (11, 12).

RET rearrangements are chromosomal structural modifications that align the RET gene with another gene to form a fusion gene. This may result in the atypical protein that promotes cancer growth. Recurrent epithelial-mesenchymal transitions (RETs) are frequently observed in a subset of thyroid malignancies (ten percent to twenty percent of PTCs; RETs are less common in anaplastic, follicular, and medullary thyroid carcinomas) and lung cancers (non-small cell lung cancer). Patients with RET-rearranged NSCLC are younger (≤60 years old), and have poorly differentiated tumors, minimal to no smoking history, low tumor mutational burden, low PD-L1 expression, and poor response to immunotherapies. Also present in several cancers, such as esophageal cancer, cholangiocarcinoma, head and neck cancer, bladder carcinoma, chronic myeloproliferative neoplasms, mesothelioma, atypical lung carcinoid tumor, low-grade glioma KRAS wild-type pancreatic ductal adenocarcinoma, it and gastric adenocarcinoma (13, 14).

The term "RET amplification" describes a rise in the RET gene's copy quantity inside the cell. The RET protein may be overproduced as a result, which may encourage the growth and survival of cancer cells. Numerous tumors have been shown to have RET amplifications, including non-small cell lung cancer (NSCLC) and thyroid cancers such as medullary thyroid carcinoma (MTC), papillary thyroid carcinoma (PTC), and anaplastic thyroid cancer. RET copy number amplifications and gains are more frequent in NSCLC than RET rearrangements. Glioblastoma, colorectal adenocarcinoma, gastric cancer, prostate cancer, breast cancer, urothelial carcinoma and hepatobiliary cancers are among other conditions that have RET amplifications (15, 16).

Overexpression of RET is connected to prostate cancer that is moderately to poorly differentiated, as well as node metastases and nerve invasion in pancreatic cancer. Shorter median progression-free survival (mPFS) and overall survival (OS) are correlated with high RET expression in renal clear cell carcinoma (19- 21).

The identification of genetic changes responsible for carcinogenesis has transformed the management of cancer by facilitating the creation of tailored treatments. Rearranged during transfection (RET) gene modifications are one type of these changes that have become important in the pathophysiology of many types of solid tumors. In this overview, we explore the growing significance of RET modification in solid tumors, covering everything from its molecular underpinnings to the creation of tailored treatments (5, 22).

Molecular Mechanisms & Pathogenic Role of RET Alteration in Solid Tumors:

Chromosome 10q11.2 contains the RET oncogene, which genes for a receptor tyrosine kinase with three domains: intracellular, transmembrane, and extracellular. Activation necessitates a complex involving glial cell-derived neurotropic factor and a co-receptor (23-25). RET mutations activate carcinogenic signaling pathways, such as the MAPK and PI3K/AKT pathways, resulting in uncontrolled cell proliferation, survival, and metastasis. In addition to their function in tumor launch and development, RET mutations have been associated with resistance to conventional therapies, underscoring the significance of targeting the RET pathway in cancer treatment (Fig.1) (26, 27, 191).

There are two ways that RET mutations might cause oncogenesis: constitutive kinase activation from tyrosine kinase domain mutations or constitutive dimerization and activation from extracellular domain mutations. Chromosome rearrangements and germline or spontaneous mutations can potentially activate RET, resulting in ligand-independent phosphorylation and tumor development (28–30).

The two most prevalent RET gene fusions in cancer are 3' kinase fusions and 5' kinase fusions,

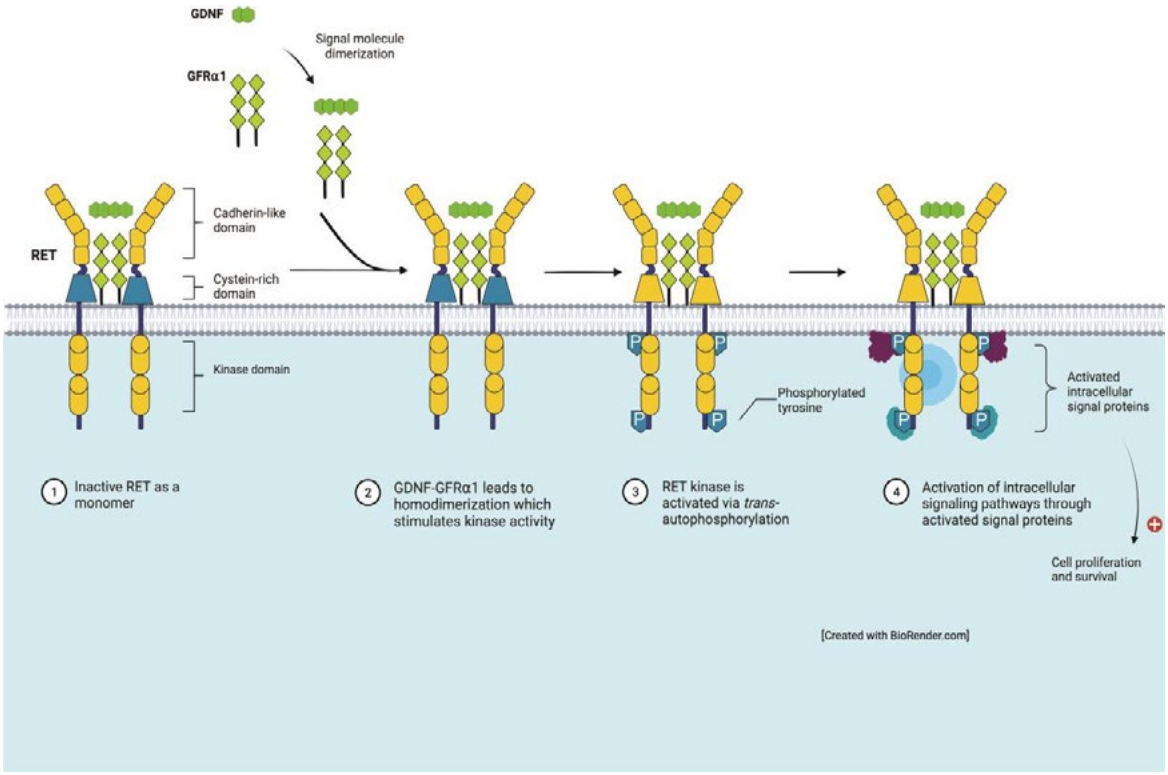

Fig 1. Subtitle: GDNF: glial cell-line-derived neurotrophic factor, GFRα1: GDNF family receptor alpha1, RET: Rearranged during Transfection.

both of which preserve the kinase domain. These fusions increase carcinogenic activity by activating kinases and triggering signal transduction via pathways like PI3K/AKT and MAPK. Increased kinase expression, ligand-independent activation, and modified intracellular localization represent a number of the mechanisms. KIF5B-RET fusion is caused by a pericentric inversion of chromosome 10, which is one of the structural abnormalities that drive RET fusions, along with translocations and inversions (31-33).

A hybrid gene that generates a fusion protein is produced when the RET gene fuses with another gene, a process known as RET fusion. This fusion protein may exhibit aberrant behavior that encourages the growth of cancer. it appears in 1-2% of lung carcinomas and 5-10% of spontaneous PTCs, mainly in NSCLC with adenocarcinoma histology. KIF5B and CCDC6 in NSCLC and CCDC6, PRKAR1A, NCOA4, GOLGA5, TRIM24, TRIM33, KTN1, and RFG9 in PTC are examples of common fusion partners (31, 34).

RET fusions trigger many receptor tyrosine kinases (RTKs) and downstream signaling cascades, as well as ligand-independent dimerization of fusion proteins. Because RET fusions can be targeted by particular treatments, patients with these genetic abnormalities may have a new avenue for treatment. Poorly differentiated tumors and a poorer prognosis for non-small cell lung cancer (NSCLC) are associated with RET fusion genes (27, 32, 35).

By activating the MAPK and PI3K pathways, RET fusions show transformative potential in preclinical models and are linked to poorer overall survival (OS) in several malignancies. MTC and NSCLC, two RET-driven malignancies, exhibit reduced PD-L1 expression and a low tumor-mutational load. In NSCLC, RET fusion is an independent risk factor for brain metastases (22, 32, 36).

Chromosome rearrangements that result in fusion genes with the RET kinase domain and gain-of-function mutations in its extracellular and cytoplasmic domains are the primary mechanism by which RET is activated in cancer. Cancer is also associated with increased expression of wild-type RET. Although introns 7 and 10 can also be involved, RET rearrangements usually involve intron 11, which results in fusions with the cytoplasmic part of RET. More than 35 genes can fuse with RET to produce dimerization domains that improve RET activation and expression. These rearrangements are thought to be driven by the repair of DNA double-strand breaks, which are frequently brought on by ionizing radiation and genotoxic substances (5, 22, 27, 32).

Standard Methods for Detection:

Common Techniques for Identification When available, universal screening for RET alterations

should be taken into consideration. Patients with thyroid carcinoma, NSCLC, and tumors identified with RET fusions histology are included in this category. The US FDA has approved selective-RET treatments, or they are being tested in countries without approval (37).

The ESMO guidelines state that FISH or RT-PCR may utilized in malignancies in which RET mutations or fusions are quite prevalent. It is recommended to use NGS in malignancies like NSCLC where RET fusions are uncommon. This allows for histocyte-agnostic screening (4).

Due to comparatively large percentages of false positives (about 62%) and false negatives (approximately 46%) in specimens that were previously evaluated using RT-PCR, IHC is not a safe technique to detect RET rearrangement. When combined, FISH and PCR are a useful toolkit. PCR is not enough, for finding new fusions. On the other hand, NGS, particularly those utilizing hybrid DNA/RNA-based systems, offers the benefit of being able to identify new fusions, identify the gene partner, and effectively detect somatic mutations in addition to gene fusions (38).

Since IHC could be utilized for measuring RET protein overexpression, scientists have looked into the diagnostic method of RET IHC as a possible detecting tool for oncogenic RET changes, especially RET fusions. RET IHC is rarely used in clinical practice, nonetheless, because immunostaining methods and antibodies are still not well standardized (4, 39).

The specificity and sensitivity of IHC are 82% and 87% respectively, with cytoplasmic staining involved by ≥1% of tumor cells. The RET IHC sensitivity varies depending on the fusion partner; for example, KIF5B has a 100% sensitivity, while RET rearrangements involving the NCOA4 fusion partner only have a 50% sensitivity (39). The tracheal epithelium (40), adrenal glands (41), parafollicular C cells (4), and colorectal tissue are among the normal cells in which wild-type RET expression may be found by IHC (42).

Additionally, a study showed that even without evidence of RET fusions, RET expression can increase in NSCLC (43). Thus, as a stand-alone test, RET IHC may be deceptive, and as a screening tool for RET changes, alternative diagnostic tests typically perform better than IHC. To clinically screen for carcinogenic RET mutations, RET IHC is not advised due to these difficulties (4, 44).

Before the widespread availability of DNA-based NGS, FISH was a more popular alternative technique for RET rearrangement detection. When analyzing 100 non-overlapping nuclei, break apart FISH is generally regarded as positive if at least 10% to 15% of tumor cells show a rearrangement pattern with two signals surrounding the RET gene separated (4, 39).

FISH has significant drawbacks despite its benefits, which include fast turnaround times and single-cell resolution. Firstly, information about unusual the inclusion of the RET kinase domain or breakpoints in the RET fusion cannot be obtained by FISH analysis due to its lack of spatial resolution. To determine whether the RET rearrangement is harmful and, hence, warrants intervention, these datapoints are essential. Secondly, the fusion partner involved determines how accurate FISH is. Break-apart FISH has a 100% sensitivity for KIF5B and CCDC6 intrachromosomal rearrangements, but a 67% sensitivity for NCOA4 rearrangements (39, 45). These are most likely caused by the proximity of RET and NCOA4 on chromosome 10, which can produce a more modest splitting pattern, which is probably the cause of these discrepancies (46).

FISH is also relatively expensive, requires technical expertise for interpretation, and is typically only available in larger centers and reference laboratories (47). NSCLC has the highest sensitivity when taking tumor primary into account (100%), the second is thyroid cancer (88%), and then other tumors (75%). These variations are probably caused by the varying frequencies of fusions like NCOA4-RET (39).

Sanger sequencing based on DNA PCR Assays, provides an accurate way to identify recognized single-nucleotide alterations in cases where their relative allelic frequency exceeds 15%. It is only useful in MEN2A and FMTC, and it still serves as a confirming test because it cannot identify gene mutations with low VAFs, gene rearrangements or partial deletions. Hot-spot mutations at VAFs as low as 1% can be reliably detected using quantitative PCR on DNA. However, a major factor limiting the tests' capacity to detect fusions and less common oncogenic mutations that don't occur in hot regions is the limited number of primers that are utilized with this technique. Although in practical practice, NGS-based tests are better at identifying RET-activating mutations, quantitative PCR is still a good substitute in cases where NGS is not easily accessible (4).

Advances in DNA sequencing techniques have made it easier to discover RET mutations in a variety of tumor types using DNA-based NGS. RET mutations and rearrangements can be detected simultaneously using targeted DNA-based NGS if there is adequate Intron inclusion and exonic coverage of RET fusion. A variety of gene panels with varying RET mutation detection rates are clinically accessible to identify canonical RET changes. However, in certain instances of rearrangements of unknown significance (i.e., non-canonical RET rearrangements), only RNA sequencing was able

to confirm the presence or absence of oncogenic RET fusion transcripts. This was the case when comparing the performance of MSK IMPACT (hybrid capture DNA-based NGS panel) to RNA-based NGS as a reference standard (MSK-Fusion) in detecting RET fusions. DNA-based NGS is also capable of detecting somatic mutations at low VAFs because of its great sensitivity (39).

Regardless of the underlying DNA-level processes, functionally definite RET fusion transcripts can be identified using RNA-based NGS. RNA-based testing is frequently regarded as the gold standard technique for RET fusion identification and is a crucial confirmatory assay in many clinical contexts (38).

There are generally two methods for incorporating RNA sequencing into clinical workflow. For a more conclusive and useful evaluation, RNA testing can be carried out on a subset of individuals with RET rearrangements of uncertain importance in processes that include screening by DNA-based sequencing. The best way to detect RET fusion is a DNA-sequencing test, and in advance along DNA-sequencing, RNA-sequencing could be done. But its not always necessary to be done. Quality control of preanalytical settings is essential to guarantee the correctness of results since formalin fixation and tissue block preservation are linked to considerable RNA degradation (48).

As another way to assess monitoring, diagnosis, and prognosis in several cancers, cfDNA offers a viable substitute for invasive biopsies. A non-invasive method of detecting RET changes is plasma-based circulating free DNA (cfDNA), which is an alternative to tissue-based DNA sequencing. However, cfDNA testing's diagnostic yield depends on the DNA turnover rates and tumor load at the moment of liquid biopsy; progressive or metastatic diseases have higher sensitivity than those with localized or stable diseases (39, 49).

The poor specificity of DNA-based sequencing and varying sensitivity are still problems for cfDNA-based liquid biopsy testing. But with longitudinal testing, cfDNA can capture tumor heterogeneity and treatment response dynamics, offering a useful platform to identify how clonal and subclonal mutations impact tumor growth. Therefore, when assessing therapy resistance mechanisms and investigating logical combination therapies, prospective cfDNA testing can offer important insights (50).

Targeted Therapies for RET-Altered Solid Tumors

Lung cancer

In 2012, they were identified as non-small-cell lung cancer (NSCLC) (53, 54). They account for only 1% to 2% of all non-small cell lung cancers (NSCLCs), with an estimated 10,000 new cases occurring every year worldwide. In NSCLC, KIF5B and CCDC6 are the most frequently occurring partner genes of RET fusions (55–57).

RET fusion-positive NSCLC have the following clinical characteristics: adenocarcinoma histology, poorly differentiated tumors, equal incidence in both sexes, younger patient (approximately 60 y), and little to no tobacco exposure (58, 59). Furthermore, in terms of the dissemination to the CNS, The attitude of NSCLC with RET alteration is intermediate to that of patients with ALK-positive and ROS1 positive (60).

Chemotherapy

Although chemotherapy has a low effect, RET-positive NSCLC seems to be susceptible to regimens which include pemetrexed, according to findings from retrospective investigations (61-64).

Immunotherapy

Immunotherapy data in patients with RET fusion-positive NSCLC is derived from retrospective research rather than prospective investigations. It is debatable if this treatment is beneficial for this population (65).

Multi-targeted agent

Cabozantinib

The multi-TKI targets not just RET but also VEGFR2, AXL, c-KIT, FLT3 and MET (66). It's interesting to note that the number of lines treated before, previous VEGF inhibitors, and gene partners all had a detrimental impact on cabozantinib's effectiveness (tumors containing CCDC6-RET or ERC1-RET showed no responses). In NSCLC, KIF5B-RET fusions had shorter mPFS than those with CCDC6-RET fusions, which is an unusual consequence for cabozantinib (67).

Increased alanine aminotransferase (ALT), increased aspartate aminotransferase (AST), hypothyroidism, diarrhea, palmar-plantar erythrodysesthesia, and skin hypopigmentation were the most frequent treatment-related adverse events (AEs) of any grade (79). While not required by its approval, periodic evaluation of electrolytes, calcium, and TSH should be part of safety monitoring during treatment (e.g., monthly at the beginning) (100).

Vandetanib

is an EGFR, VEGFR-2/3 and RET inhibitor (68). It is taken 300 mg per day. The three most frequent adverse events were rash acneiform, diarrhea, and hypertension (101). Start with 200 mg per day for people with mild renal impairment (creatinine clearance 30 to 50

mL/min). When the creatinine clearance is less than 30 milliliters per minute, use is not advised. Electrocardiograms (ECGs) and blood levels of potassium, calcium, magnesium, and TSH should be taken at 2–4 weeks and 8–12 weeks after beginning medication, and then every three months after that, as required by the REMS program. More regular monitoring may be necessary for those who have diarrhea (102).

Lenvatinib

This is a multi-TKI of platelet-derived growth factor receptor alpha (PDGFRα), fibroblast growth factor receptor (FGFR1-4), VEGFR1-3, RET, and KIT (69). CCDC6-RET and KIF5B-RET fusions produced similar responses; headache, diarrhea, decreased appetite, nausea, vomiting, proteinuria and Hypertension were the most frequent adverse events (70).

Ponatinib

is a RET and BCR-ABL inhibitor (71). The most frequent adverse events were dry skin, nausea, diarrhea, constipation, abdominal pain, and skin rash (103). Trials of several multi-TKIs in RET-positive NSCLC have shown that these medications have moderate activity but substantial toxicity, which is probably related to the inhibition of non-RET targets, particularly VEGFR-2.

Other Multi-TKIs

alectinib, sunitinib, sorafenib, nindetanib and regorafenib have very low function and little evidence available. Clinical information on these multi-TKIs from retrospective research and clinical trials (62, 72-77). All VEGF-targeted antiangiogenic MKIs (aaMKI) have the same side effects, which include muscle wasting, delayed wound healing, myelosuppression, arterial thromboembolism, hypertension, kidney toxicity, bleeding, hepatotoxicity, cardiotoxicity and hand-foot skin reaction. The higher dosage needed for derivatives of vitamin D and thyroid hormone can also result from aaMKIs in patients with hypothyroidism following thyroidectomy or decreased parathyroid function (104).

Arterial thromboembolic events during the last 6 to 12 months, encasement of major arteries by tumor, untreated hemorrhagic brain metastases current bleeding and major surgery within 28 days are all considered relative contraindications to aaMKIs. Because tracheoesophageal fistulas have been reported after external beam radiation therapy (EBRT) to the neck, we also attempt to limit the use of strong antiangiogenic medicines in these patients (104).

In conclusion, MKI for RET-altered advanced NSCLC was linked to both significant TRAEs and low efficacy outcomes. Although the non-selective inhibition of the RET-RTK is most likely the cause of these outcomes, patient selection may also play a role, as nearly all of the patients in the preceding trials had received prior treatment (75, 76). Therefore, in this context, none of these medications were approved by the US Food and Drug Administration (FDA) (78).

In some situations, multitargeted RET inhibitors such as sunitinib, alectinib, vandetanib, and cabozantinib may be helpful substitutes for selpercatinib and pralsetinib. They are substantially less effective than selective RET inhibitors, although the FDA has approved them for various applications (79, 80).

Selective RET Inhibitors (RET-Is)

Praseltinib

(Original name: BLU-667) is a powerful and selective RET-TKI. It has been proven to inhibit RET in vitro with a potency that is at least ten times greater than that of other multi-TKIs that do not target VEGFR2. Additionally, pralsetinib showed the same strong action against the V804R/L and the CCDC6-RET fusion, which may provide resistance to the multi-TKIs (81). it has demonstrated activity against KIF5B–RETV804L Ba/F3 and KIF5B–RET Ba/F3 allograft tumors in preclinical models (82).

Pralsetinib was tested in patients with RET-positive NSCLCs and other solid tumors as part of the global phase I/II investigation ARROW (83). hyponatremia and hypertension had occurred at 600 mg daily dose during Phase I Bayesian. Safety and pharmacokinetics data indicated 400 mg once daily as the recommended dose.

ORR as determined by safety and BIRC were the main goals in phase II of the experiment. Particularly in the individuals who had already received treatment, pralsetinib showed strong activity: the mPFS was 17.1 months, the mDoR was not reached, and the ORR was 61%. Crucially, the reactions were independent of ICI, previous multi-TKIs, and/or the RET fusion partner. The mDoR was mPFS were 9 months, and ORR was 70% among patients who had never received treatment (84, 85).

Pralsetinib exhibits intracranial action in tumor driven by CCDC6-RET or KIF5B-RET fusions, according to preclinical study findings (84). The IC-ORR was 56% among patients with demonstrable CNS illness and 51% among individuals with CNS metastases (62). Treatment-naïve and pretreated patients did not significantly differ in terms of toxicity. Treatment discontinuation was 7% in all cases. There was one pneumonia-related treatment-related death (85).

The ARROW experiment was further updated at the of ESMO 2022, with a median 16-month follow-up. The efficacy population, which included

RET + NSCLC (1:1 previously pretreated and treatment naïve), was enrolled at the data cutoff. As with previous data cuts, the ORR was 77.6% for patients who had never received systemic treatment and 63.1% for patients who had previously received platinum treatment. Pre-treated patients had a median overall survival of 44.3 months, while treatment-naïve patients did not. Among grade ≥3 adverse events (AEs), anemia, hypertension, reduced neutrophil count, pneumonia, and neutropenia were the most frequent (≥10%). As a result of treatment-related adverse events, 10% of patients stopped using pralsetinib (86).

The starting dosage for adults is 400 mg once daily, without food. Due to adverse reactions or possible drug interactions, dose modifications can be required. It is recommended to assess liver biochemistry tests before starting pralsetinib and every two weeks after starting it. Monitor every month after the first three months if liver tests stay stable (105).

Pralsetinib was given accelerated clearance by the FDA on September 4, 2020, for RET NSCLCs, regardless of previous treatment . EMA authorized pralsetinib on December 13, 2020, RET altered NSCLCs who had not received any previous RET inhibitor treatment (87).

The IC-ORR was 83% among patients with CNS target lesions, according to real-world data from the EAP of pralsetinib. Incidence of AEs was 39% with a G > 3 and occurred in 83.6% of the patients. Thrombocytopenia, oral mucositis, and neutropenia were the most prevalent G3 or greater AES. 42% of adverse events resulted in a dose reduction, and 12% led to treatment discontinuation. There were two documented treatment-related deaths: one from typhlitis and one from sepsis (88).

AcceleRET Lung was a worldwide trial that compared standard chemotherapy +/- pembrolizumab to first-line pralsetinib in patients who had not yet received treatment for RET + advanced non-small cell lung cancers. Patients randomly assigned to the control group were allowed to switch to pralsetinib as their condition progressed. PFS was the main endpoint, and it was evaluated with BIRC (89).

Selpercatinib

(previously LOXO-292) is a selective RET-TKI. Selpercatinib inhibits several point mutations, RET V804M, and RET fusions according to preclinical research. selpercatinib is demonstrated invivo, to suppress the proliferation of patient-derived xenografts, such as a patient-derived RET fusion-positive xenograft implanted orthotopically into the brain, and RET-altered human cancer cell lines. Two patients have also been described as receiving the first evidence of selpercatinib's activity: one MTC with RET (M918T) mutation who was not responding to cabozantinib, vandetanib or sorafenib, and another RET-altered NSCLC was advancing on alectinib, erlotinib, chemotherapy and nivolumab (90–92).

Libretto-001 has been a worldwide experiment that tested selpercatinib in individuals with solid tumors that had an activating RET change. Patients received seven doses throughout the phase I portion; DLTs were not reported. 160 mg twice dose day was determined to be the recommended for phase II (91). ORR via BIRC was the phase II part's main endpoint. The ORR, mDoR, and mPFS of the patients who had previously received treatment were 64%, 17.5 months, and 16.5 months, respectively. The ORR was 85%, and the mDoR and mPFS for the treatment-naive patients could not be estimated (92).

Libretto-001 was analyzed subgroup-wise among baseline CNS metastasis patients. Of that patients, 56% had previously undergone brain radiation treatment. IC-ORR for patients with CNS target lesions has been reported at 82%, but IC-mDoR couldn't be estimated. IC-mPFS was 13.7 months overall (93). Additionally, one EMLA4-RET-altered NSCLC that developed leptomeningeal carcinomatosis and advanced to agerafenib was shown to benefit from selpercatinib, achieving a PR with an indeterminate duration of response following 10.8 months (94).

The Libretto-001 has been updated to include all patients who were enrolled before the cutoff point for data and had a six-month follow-up. About 40 months was the median follow-up period. Patients who pre-received treatments, had an ORR of 61.5%, mDoR of 31.6 months, mPFS of 26.2 months and OS of 47.6 months. In patients who had never received treatment, the mDoR was 20.3 months, the ORR was 82.6%, the mPFS was 22 months and the OS was not estimable. In CNS metastasis patients, an IC-ORR and IC-mDoR were 84.6% and 9.36 months, respectively (95-97).

Based on the efficacy, safety, and tolerability of selpercatinib in patients with metastatic RET fusion-positive NSCLC, the LIBRETTO-432 trial demonstrates the benefits of adjuvant selpercatinib in patients with earlier stages (IB-IIIA) of RET fusion-positive NSCLC following definitive radiotherapy or surgery. Targeting treatment during the surveillance period after completing curative therapies and applicable adjuvant chemotherapy is expected to improve outcomes for patients with stage IB-IIIA RET fusion-positive NSCLC (192).

The most frequent adverse events (AEs) in terms of safety were weariness, skin rash, elevated ALT, elevated AST, dry mouth, edema, and diarrhea. Increases in ALT, AST, and hypertension were the most frequent G-≥3 adverse events, and the rate was 42%. Drug cessation was caused by 11% of adverse events, while dose reductions were caused by 48.9% of them (95, 96).

The starting dose is 160 mg twice daily among patients ≥50 kg and 120 mg twice daily for individuals under 50 kg. It's crucial to refrain from using gastric acid-lowering drugs at the same time as selpercatinib because this can lower plasma levels. Selpercatinib should be taken with food if that is not feasible. It must be administered two hours before the acid-reducing drug, or two or ten hours following a H2 receptor antagonist or locally acting antiacid, if the patient is taking any of these medications. It may be essential to lower the dose to address unpleasant reactions. Tests for LFT biochemistry must be performed before starting selpercatinib and every two weeks after starting it. Monitor every month after the first three months if liver tests stay stable. Before starting and at regular intervals following, measure the QT interval, electrolytes, and TSH (106, 107).

Among treatment-naive patients with advanced RET fusion-positive NSCLC (including unresectable stage IIIB, IIIC, and IV), the Libretto 431 (NCT04194944) compared conventional therapies (chemotherapy +/- pembrolizumab at the investigator's discretion) to first-line selpercatinib. In the selpercatinib group, mDoRs were longer (11.5 vs 24.2 months), and the experimental arm had higher responses (ORR 84% vs. 65%). In comparison to the control treatment, selpercatinib increased the median PFS. The overall survival rate data were not yet mature. Selpercatinib also improved the TTP impacting the CNS. Increases in alanine aminotransferase, aspartate aminotransferase, and hypertension were the most common grade ≥3 events with selpercatinib (98).

Selpercatinib was approved by the FDA on September 21, 2022, for adult patients with RET fusion positive NSCLCs, regardless of their previous treatment regimens. The European Medicines Agency (EMA) authorized selpercatinib on September 29, 2022, for advanced non-small cell lung cancers in people who had not received RET inhibitor treatment before (98). Using the RET inhibitor selpercatinib instead of immunotherapy and/or chemotherapy in the front-line scenario is recommended, but pralsetinib is a suitable substitute (albeit one with less evidence than selpercatinib). On the other hand, using these agents in the subsequent-line setting is equally permissible. Patients with locally progressed RET-positive non-small cell lung cancer are also eligible to receive selpercatinib (98, 99).

Thyroid cancer

PTC commonly exhibits RET rearrangements

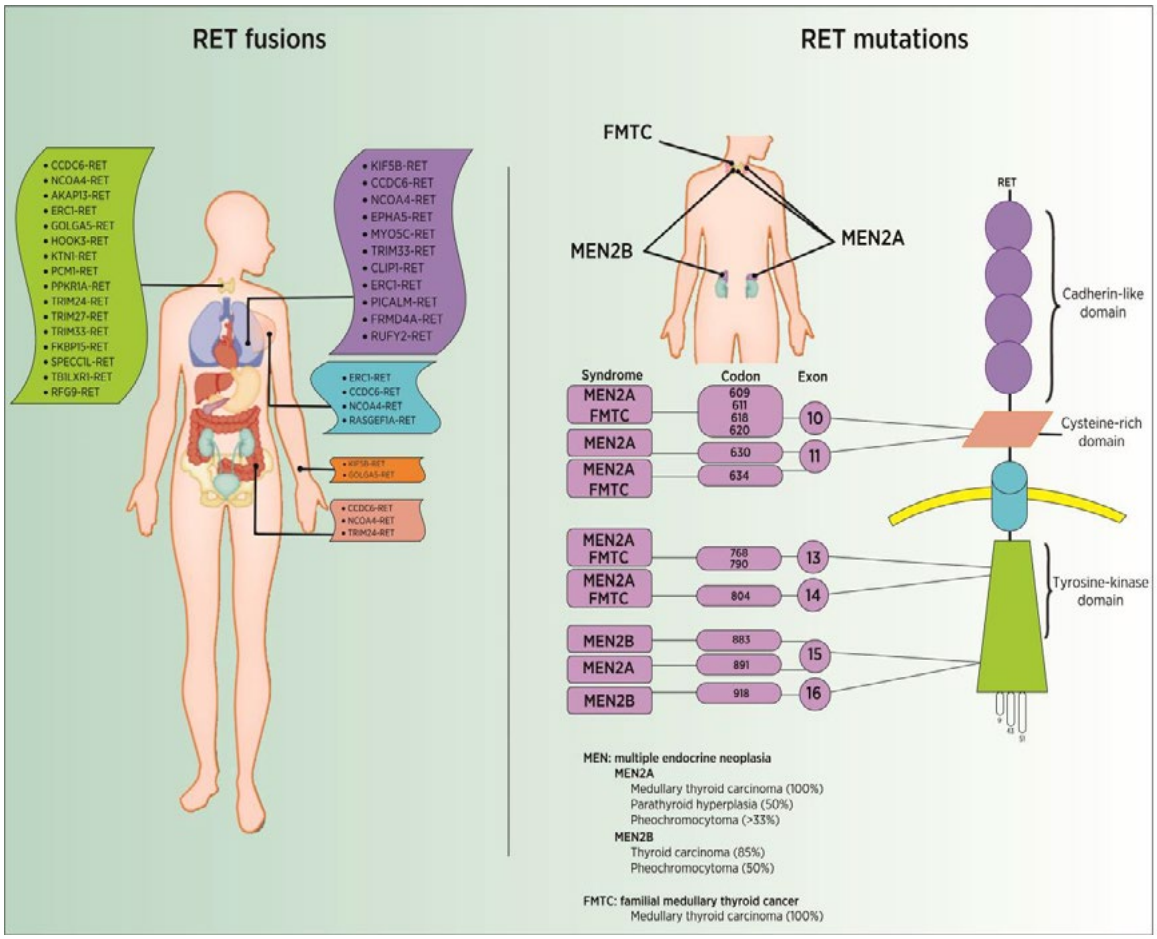

Fig 2. RET gene mutations and fusions in solid tumors

(Fig. 3), particularly in individuals who have previously been exposed to ionizing radiation. In post-Chernobyl PTC, 50% to 90% of children exhibit RET rearrangements because their follicular cells are prone to genetic alterations because of their high rate of proliferation. In a large PTC cohort, the Cancer Genome Atlas consortium recently discovered RET fusions for about 6.8% , however, other studies indicate that the incidence of rearrangements varies greatly (2.6%–70%) (108-111). The two most common RET fusion partners in PTC (>90% of cases) are CCDC6 and NCOA4, the latter of which is typically linked to a larger size of the tumor, higher stage at presentation, and aggressive behavior (112).

The autosomal dominant multi-tumor syndrome known as MEN2 is further classified as familial MTC, MEN2B, and MEN2A, which accounts for over 90% of cases (114). The bulk of the most prevalent variations are located in important residues in the extracellular and kinase domains, with extracellular domain mutations more commonly observed in MEN2A and FMTC (Fig. 2), even though about half of the 200 RET variants that have been identified in MEN2 are known to be harmful (115). Molecular testing can detect germline RET mutations, a pathognomonic feature of MEN2, in 98% to 100% of cases (113).

Missense mutations impact the cysteine-rich domain in the extracellular area by substituting alternative amino acids for the cysteine (C609, C611, C618, C620, C630, and C634). (116, 117)

The most prevalent substitution seen in MEN2A patients is an On exon 11 there may be a mutation that involves C634 which is the most prevalent substitution in MEN2A(about 85%). Each of the different cysteine residues (C611, C609, C620, C630, and C618) had an equal number of mutations in FMTC (118). MEN2B syndrome is pathognomonic for mutations in the kinase domain, 95% of them have M918T on exon 16 and the additional 2-3% have A883F on exon 15 (119). Compared to other RET mutations, the M918T mutation causes enhanced aggression by changing the RET receptor's catalytic core, which increases Receptor activation and ATP binding independent of receptor dimerization (120, 121).

In MEN2A and FMTC, tyrosine kinase domain mutations have also been reported (V804M and V804L in exon 14, and L790F and E768D in exon 13). These mutations are typically linked to a later onset of illness or more indolent disease.FMTC is also considered a clinical variation of MEN2A with reduced penetrance due to the shared mutational signatures between these two disorders in the extracellular and kinase domains. Desmoplastic melanoma (20%), melanoma (6.6%), colorectal cancer (3.6%–6.9%), cutaneous SCC (10%), Anaplastic thyroid carcinoma (4.3%), ureter urothelial carcinoma, and breast cancer and paraganglioma, are among the various thyroid cancers that also include somatic RET mutations (122).

MTC, or medullary thyroid cancer has been more common during the past years, and it accounts for 1–5% of cases (25% inherited and 75% sporadic). MTC is responsible for over 14% of all thyroid cancer-related deaths, despite its modest frequency (123).

MTC can run in families and is derived from parafollicular C cells. It may occur sporadic or linked to one of the two forms of MEN2 syndromes. Roughly 50% of sporadic MTCs and over 95% of hereditary MTCs have RET mutations. Other Solid Tumors Affected by RET Of all differentiated thyroid cancers, 80–85% are papillary thyroid cancers. About 20–40% of sporadic cases of papillary thyroid cancer have been reported to feature RET fusions, in which RET fusions following radioiodine exposure have higher rates (124, 125).

The most common genetic changes in MTC are RET-activating mutations. Germline gain-of-function mutations in the RET proto-oncogene cause around 25% of MTCs to be inherited and are associated with types 2A and 2B (128). Almost all individuals with MEN2A have MTC, along with a variety of additional symptoms including primary hyperparathyroidism, pheochromocytoma, and in rare cases, Hirschsprung disease and cutaneous lichen amyloidosis. In terms of MTC's penetrance and aggressiveness, as well as the presence of other related disorders, the genotype influences the syndrome's phenotypic presentation (131).

However, MTC is quite aggressive and frequently manifests in infancy in these patients (131). M918T, in exon 16 which is a germline RET mutation and replaces a methionine with a threonine inside RET kinase domain is nearly the only cause of MEN2B (126, 133-137).

Double mutations which involve V804M and A883F mutations (on exon 15) are present in less than 5% of MEN2B syndrome individuals (128,132). Apart from MTC, MEN2B syndrome is characterized by skeletal abnormalities like a marfanoid body habitus, pheochromocytoma, widespread ganglioneuromas of the aerodigestive tract, and ophthalmologic abnormalities (128, 131).

Unlike MTC, PTC has occasionally been found to be caused by RET fusions rather than mutations, particularly in children and after radiation exposure. Five to ten percent of PTCs have RET fusions, which most frequently involve RET with NCOA4-RET or coiled-coil domain containing 6 (CCDC6-RET) after a DNA double-strand break defective repaired (127–129). There are two possible ways that RET fusions increase the activation of downstream signaling

pathways: either the widespread expression of the upstream partner gene that causes expression of RET in cells by aberrant transcription and where typically, it isn't, or The dimerization domain contributed by the partner gene causes ligand-independent dimerization and RET kinase activation(4, 33, 127).

Chemotherapy and immunotherapy

Chemotherapy for RET-positive thyroid cancer, particularly in MTC and other cancers of the thyroid with RET alterations, has evolved significantly with the introduction of targeted therapies. Traditional chemotherapy generally targets rapidly dividing cells indiscriminately, which can cause a broad range of adverse effects.

Immunotherapy in RET-positive medullary thyroid cancer (MTC) is an emerging area of research, particularly as advancements in understanding tumor biology and immune mechanisms continue to evolve.

Multi-TKIs

Advanced radioiodine-refractory differentiated thyroid carcinomas (RR-DTCs), particularly the ones that have RET mutations, can be treated with lentvatinib, cabozantinib, and sorafenib. While overall survival (OS; 21.1 vs. 26.6 months) did not significantly increase in the EXAM trial, cabozantinib compared to placebo have been improved mPFS (4.0 vs. 11.2 months) and ORR (0% vs 28%). According to a retrospective review of the data from this trial, the presence of M918T mutation was correlated with improvements in ORR (2%vs. 34%), PFS (5.8 vs. 14.2 months), and OS (20.2 vs. 44.3 months) when compared to others (138, 139).

Biochemical tests have demonstrated the effectiveness of both cabozantinib and vandetanib against M918T (121, 140). The ZETA trial showed that vandetanib improved ORR statistically significantly (45% vs. 13%; P < 0.001) and lengthened the mPFS when compared to a placebo (30.5 vs. 19.3 months; P ¼ 0.001). Because there was not enough biological data available for this study, several of the patients in this clinical trial had unknown RET mutational status. However, the M918T mutation was once more linked to a higher ORR in the subgroup analysis when compared to the group that did not have this change (54.5% vs. 30.9%) (141).

Phase II clinical trials in thyroid malignancies have also evaluated lenvatinib, sunitinib, sorafenib, motesanib, and dovitinib which also are MKIs that have an anti-RET effect (142–146). mPFS and ORR varied from 5.4 to 17.9 months and 2% to 36%, respectively, in the world's demographic, irrespective of RET modification. Although post hoc RET subgroup analyses were performed in some of these studies, There was no discernible association between these patient groups and the response of the tumor (142, 143, 146).

The ORR for 33 RET-positive tumors in the motesanib study, which had the greatest number of RET-positive patients, was 0%, but it was 8% for 13 other tumors that were RET wild-type tumors. Finally, vandetanib and cabozantinib appear to provide superior outcomes in certain cancers of the thyroid with RET-positive; nevertheless, prospective trials with RET-positive chosen patients are required to confirm this concept. Notably, these medications have side effects that are comparable to those shown in NSCLC trials and are not insignificant. Vandetanib and cabozantinib had treatment discontinuation rates of 12% and 16%, respectively. Adverse effects necessitated a dose decrease for 35% and 79% of patients, respectively (146).

Selective RET Inhibitors (RET-Is)

Selpercatinib and pralsetinib, two medications with strong and specific anti-RET action, have been developed to address some of the limitations of MKIs (Table 1) (147-150). patients receiving RET-selective inhibitor therapy have been known to develop mutations

Table 1. Clinical trials of selpercatinib and pralsetinib in lung and thyroid cancer.

Selective RET inhibitor	Study name	Number		Follow-up (monthes)	ORR (%)	mPFS (months)	mOS (months)
Selpercatinib	LIBRETTO-531	T=291	S=193	12	69.4	NE	-
	phase3(Thyroid)		C=98	12	38.8	16.8	-
	LIBERTTO-431	T=261	S=159	17.9	84	24.8	NE
	phase3(Lung)		C=102	12.7	63	11.2	NE
	LIBERTTO-001	T=316	P=247	40	61.5	26.2	47.6
			N=69	40	82.6	22	NE
Pralsetinib	ARROW	T=145	PM=61	25.4	55.7	25.8	-
	(Thyroid)		NM=62	25.4	77.4	NE	-
			PTh=22	25.4	0.4	25.4	-
	ARROW	T=233	P=158	16	63.1	16.4	44.3
	(Lung)		N=75	16	77.6	12.6	NE

in codon 810 that result in selpercatinib resistance (152). For advanced or metastatic MTC and other thyroid tumors with a change (mutation or fusion) in the RET gene, the FDA has licensed selpercatinib (153).

Comparing sepercatinib to vandetanib or cabozantinib, the former lowers the probability of disease progression. Additionally, it works well for patients who have already received vandetanib and/or cabozantinib treatment. For instance: Among 291 patients with advanced, metastatic or progressive MTC with RET-mutation who were never treated with a kinase inhibitor, LIBRETTO-531 was a randomized open-label trial compared each of vandetanib or cabozantinib with selpercatinib. The median progression-free survival in the active comparator group was 16.8 months (151).

Selpercatinib had a higher 12-month progression-free survival (PFS) rate (86.8%) than either cabozantinib or vandetanib (65.7%). 57.5 and 34.7 percent, respectively, had partial responses, whereas 11.9 and 4 percent, respectively, had complete responses. The selpercatinib group saw fewer grade 3 or higher adverse events (76.3 versus 52.8 %), and fewer patients stopped taking the medication because of side effects (26.8 versus 4.7 %). At 12 months, the selpercatinib group's treatment failure-free survival rate was 86.2%, while the control groups were 62.1 %. Overall survival seemed to benefit the selpercatinib as well, despite the modest number of mortality events that were documented.

The overall response rate (ORR) for selpercatinib in the open-label LIBRETTO-001 study was 69 and 73 percent, respectively, in 143 patients with advanced MTC that all of them were RET-mutant and had either received treatment with vandetanib or cabozantinib or not (154). CR was seen in 9% of patients who had previously undergone treatment with an aaMKI., while 11 percent of treatment-naive patients reported it; 60 and 61 percent, however, reported PR respectively. The 12-month rates of PFS were 82 and 92 percent, respectively, but mPFS have not yet been attained.

Selpercatinib can result in quick palliation for patients experiencing symptoms of the condition, such as diarrhea or ectopic Cushing syndrome (156). Another trial is assessing selpercatinib before thyroidectomy in patients with nodal metastases or locally advanced primary tumors because of the drug's ability to rapidly decrease tumors (151). pralsetinib is approved by the FDA for the treatment of RET fusion-positive thyroid tumors that have advanced disease. A failure to finish the trial required to meet postmarketing conditions led the firm to voluntarily withdraw the FDA-approved preliminary indication for RET-mutant MTC in July 2023. No new safety or efficacy information led to the indication's withdrawal (151).

The open-label ARROW trial used pralsetinib to treat 122 patients with RET-mutant MTC. Patients who had previously received cabozantinib and/or vandetanib had an overall response rate of 60%, whereas those who had never received treatment had a response rate of 71%. The complete and partial response rates were 1.8 and 58 percent, respectively, among the 55 patients who previously had been treated with cabozantinib or vandetanib (154, 155).

Overall response rates were comparable in an updated study with two further years of follow-up (77.4 % in treatment naïve patients and 55.7 % in previously treated patients with vandetanib or cabozantinib) (155).

RET in other solid tumors

Other cancers that are associated with RET fusions include pancreatic acinar cell carcinoma (PACC), in 7.5% of cases which frequently conflicts with BRAF fusions. Less than 1% of colorectal carcinomas (CRC) include RET Fusions; these tumors are more common in older individuals, right-sided, RAS/BRAF wild-type, and MSI-high tumors, and they are linked to a poor prognosis (51).

In some instances, NCOA4-RET fusion serves as a resistance mechanism in breast tumors that are HER2-negative. Salivary gland cancers are uncommon and diverse, with some subtypes, such as intraductal carcinoma and mammary analogue secretory carcinomas (MASCs), containing RET fusions, including TRIM27-RET, ETV6-RET, NCOA4-RET, and KIAA1217-RET. A sporadic condition that involves many gene partners and is not associated with MEN 2 syndrome is pheochromocytoma (52).

Resistance mechanisms of RETtargeted therapy

Growing applications of cell-free DNA analysis and next-generation sequencing during tumor growth have improved our knowledge of potential resistance pathways and guided potential solutions. To date, two primary mechanisms have been identified for resistance to selective RET inhibitors: activation of alternate signaling pathways that allow RET inhibition to be bypassed and on-target mutations that degrade drug binding (150, 155). Non-gatekeeper mutations and Solvent-front mutations are acquired RET kinase domain mutations, which both disrupt the binding of the drug and provide resistance to selective RET inhibitors (150, 155, 156).

Several TKIs have been licensed for the treatment of advanced MTC, as previously mentioned, and have demonstrated significant efficacy; nevertheless, resistance to these TKIs has also developed. acquired and intrinsic resistance to RET-targeted TKIs, both, have been found in clinical practice, while the underlying mechanisms are still mostly unclear.

Table 2. New second generation RET inhibitors against resistance mutations

Drugs / Mutations	TPX-0046	BOS-172738	TAS0953/HM06	LOXO-260	EP0031/A400
G810C	+	–	+	+	+
G810R	+	–	+	+	+
G810S	+	–	+	+	+
V804L	–	+	+	+	+
V804M	–	+	+	+	+

Insufficient oncogenic RET kinase inhibition is at least partially responsible for the poor ORR that hinders the effectiveness of MKIs like Cabozantinib and Vandetanib. Many MTC patients (35–79%) who were using Vandetanib or Cabozantinib needed to have their dosages reduced because to the incidence of off-target adverse events. Therefore, it is challenging to use these MKIs to achieve the right drug concentrations for RET inhibition (127).

Even though the introduction of Selpercatinib and Pralsetinib, has significantly enhanced the out come for these patients who were resistant to MKI, demonstrating improved efficacy, less toxicity profile and significantly improved ORR (96,107,149), more than 30% of patients do not experience a partial response (PR) to these medications, and some patients experience rapid tumor recurrence or progression following an initial response to TKIs, which may indicate the presence of acquired and primary resistance. According to these findings, the field of RET resistance is complicated, and to overcome it, it is essential to comprehend the physiopathology of the several processes involved (157).

Understanding how RET inhibitors attach to RET kinase is essential before looking at how RET inhibitors cause resistance. To prevent kinase activity, TKIs totally or partially bind to the RET kinase domain's nucleotide-binding pocket. Additionally, depending on the activation loop's spatial orientation, kinases can take on an inactive or active form. It is referred to as "DFG-out" indicates that the aspartate-phenylalanine-glycine (DFG) motif at the N-terminal is flipped-out, while "DFG-in" indicates that it is in the activation loop. TKIs are divided into three categories: type I, type II, and type III. Each kind has a different mode of action. Type II inhibitors, such as sunitinib, work by competing with ATP for binding to the ATP binding site, preventing the kinase from adopting its active conformation. Type I TKIs, such as sorafenib, is only accessible in the DFG-out conformation, and stabilize the inactive kinase by indirectly competing with ATP by taking up residence in the ATP-binding site's nearby hydrophobic pocket (158). Type III TKIs, such as Vandetanib, function by covalently attaching to cysteines at particular, variable kinase sites to stop them from activating (159).

Notably, selective RET inhibitors and MKIs have distinct RET binding mechanisms. To get to the back cleft without passing through the gate, sepercatinib attaches itself to the front cleft and wraps around it., whereas MKIs enter the drug-binding pockets through the gate and take up residence in the front and back clefts (147).

Intrinsic resistance mechanisms

Alterations in RET that interact One mechanism of main resistance to MKIs have been identified as coexisting RET change. M918T is the most common mutation in MTC, that impacts on kinase's C-lobe. Patients with RET mutations may require a larger dose of Vandetanib, Cabozantinib, and Lenvatinib, as their half maximum inhibitory concentrations (IC50s) for RET M918T kinase were found to be many times that of the wild-type RET kinase (160). Intrinsic mechanisms of resistance have occasionally been defined as additional abnormalities, like RET V804L/M intrinsic gatekeeper mutations or other changes that normally function as acquired resistance mechanisms (90, 150, 158, 161).

Bypass signaling

Driver oncogenes such as mutation in EGFR and RAS, and amplification of the MET have been shown to co-occur in RET-altered tumors in clinical settings (50, 125), and preclinical studies have also detected acquired mutations of these driver oncogenes (162). The above driver genes may co-occur and circumvent the RET proto-oncogene requirements, reducing the effectiveness of RET-targeted TKIs. However, it hasn't been found in MTC. Since in vitro research has demonstrated how AKT2 amplification contributes to carcinogenesis and because serine/threonine kinases encoded by the AKT gene family phosphorylate downstream protein effectors like mTOR, It has been believed that AKT2 amplification contributes to both acquired and de novo resistance to targeted therapies like Vandetanib for MKIs, which persistently activate RET. However, the NCT01582191 trial (phase 1) showed longer PFS and greater ORR when Everolimus was added to Vandetanib in RET-driven malignancies suggesting that the inclusion of Everolimus and other mTOR inhibitors might be able to break through this resistance(161, 163-165).

According to a prior trial, patients with RET fusion-positive and MET amplification-positive non-small cell lung cancer (NSCLC) reacted to the combination of MET/ALK/ROS1-targeted TKI Crizotinib and Selpercatinib, whereas those who were resistant to Selpercatinib (166).

Tumor immune infiltration and microenvironment

The various biological components that make up the tumor microenvironment (TME) include vasculature, fibroblasts, extracellular matrix, tumor cells, immune cells, and a range of associated chemokines and cytokines (168). First, TME stressors and autophagy can impact EGFR-TKI resistance, and EGFR-TKIs may be more effective if CD4+ and CD8+ T cells are present in the TME (169). Second, TME can influence the tumor's responsiveness to certain TKIs. However, it is unknown and has not yet been shown how TME affects the efficacy of RET inhibitors (170).

NK cells, tumor-associated macrophages, myeloid-derived suppressor cells, TIL, and other immune cell subsets infiltrate TME locally. The many cell types and distributions comprise the intricate immunological features of TME (171). Tregs that suppress the immune system, which is associated with a poor prognosis and are very prevalent in TME (172, 173), are distinguished by their CD4+ CD25+ FOXP3+. Tregs are now using a variety of techniques to minimize early resistance to RET inhibitors, including downregulating MHCI or inhibiting FOXP3 through the utilization of RET inhibitors to counteract this immunosuppressive impact. One such strategy is the use of CXC chemokine receptor 4 (CXCR4) inhibitors (174).

CXCR4, a G-protein-coupled receptor that is triggered by C-X-C pattern chemokine ligand 12 (CXCL12), is one of the primary regulators of Tregs. Encouraging tumor growth and drawing in stromal and immune cells, it contributes significantly to TME. Thyroid cells that express RET or RET-positive MTC exhibit significant levels of CXCR4 expression, as do endothelial cells and Tregs (175, 176, 179). As a result, preclinical research shows that RET-mutant cell lines treated with Vandetanib exhibit downregulated CXCR4 expression (175, 177) and plans to combine CXCR4 inhibitors with traditional treatments are being developed for solid and hematologic tumors (176). The only CXCR4 inhibitor currently authorized for use in patients with multiple myeloma or non-Hodgkin's lymphoma is AMD3100 (Plerixafor or Mozobil) (178).

Acquired resistance mechanism

Secondary RET alteration

Gatekeeper mutations' emergence MKIs's Acquired resistance to Lenvatinib, Cabozantinb, or Vandetanib is usually the result of RET V804L/M/E. Both sunitinib and ponatinib continue to exhibit limited efficacy against V804M. However, considerable non-RET kinase activity explains the clinical response observed in 75% of patients who were treated with Vandetanib and had V804 mutation (90, 150, 158, 161). Other areas of acquired RET mutations include solvent front G810A/S mutations, RET Y806C mutations, and RET S904F activation loop mutations. The three RER mutation types mentioned above provide resistance to Vandetanib, whereas the S904F mutation is susceptible to Nintedanib, and the G810A/S mutation is susceptible to Ponatinib and Lenvatinib. A different mutation, RET I788N, is responsive to Ponatinib but resistant to AD80, Vandetanib, and Cabozantinib (58, 149, 150, 161, 180). Almost none of these mutations have been identified as germline changes. Secondary RET mutation is another significant acquired resistance mechanism for selective RET inhibitors (90, 150, 158, 161).

Bypass signaling

An escape strategy that frequently occurs across oncogenic drivers is the activation of alternative pathways linked to cell proliferation. When driver oncogenes are found in RETpositive tumors, they may circumvent the RET proto-oncogene's requirements and result in both acquired and primary resistance to RET-targeted TKIs. MKI resistance in RET-rearranged cells has been linked to the co-activation of the MAPK/ERK pathway. Trametinib, a particular MEK inhibitor with strong RET-blocking

activity, must be added to AD80 in this case to eradicate the resistant cells (149, 150, 181).

According to preclinical research, this pathway was downregulated when EGFR inhibitors Gefitinib or Cetuximab were added to MKI therapy. This prevented the phosphorylation of AKT and ERK (170). MET or EGFR inhibitors can overcome other bypass mechanisms, such as MET amplification in colorectal cancer or the acquisition of MET D1228V in NSCLC (161). Within the phase III EXAM trial, 21% of patients had changes in the genes CDK4, CCND2, CDK6, CCND1, CDKN2C or CDKN2A/B. The cyclin D-dependent kinases CDK4 and CDK6 may be activated as a result of these changes. It is yet unknown how CDK4/6 inhibitors affect cancers with RET mutations (128).

A further resistance mechanism to selective RET inhibitors is an acquired NTRK3 fusion, which has been reported in RET fusion-positive lung cancer treated with sepercatinib. Larotrectinib or Entrectinib, two NTRK selective inhibitors, may be a good strategy in this situation (167). Additionally, there have been reports of KRAS amplification and BRAF and ROS1 mutations of uncertain significance (77, 83).

In addition to the secondary RET mutation, Rosen's study revealed that another significant mechanism of resistance to Selpercatinib was bypass signaling, as evidenced by emergent MAPK activating changes comparable to those causing primary resistance. According to a polyclonal resistance theory, a complicated polyclonal resistance pattern to sepercatinib treatment may develop, as evidenced by the variety of Mechanisms of primary or acquired resistance induced by MAPK that were also seen throughout specific patients (Fig.3) (183).

New drug era

RET inhibitors of the second generation: The development of many next-generation RET inhibitors is part of the ongoing search for new medication for the treatment of malignancies with RET alterations. There is a new and powerful RET inhibitor TPX-0046 that works against 18 altrations of RET, which include solvent-front alterations in G810R and G810S that cause acquired resistance to pralsetinib and selpercatinib (68).

Its usage in patients with RET V804 gatekeeper mutations is restricted, because it does not block these mutations. In vitro, TPX-0046 exhibits strong inhibitory effect against SRC kinase while sparing VEGFR-2. On RET-driven xenograft tumor models, this medication demonstrated strong in vivo anti-tumor action (184). SWORD1trial (phase I/II) is presently under progress to verify its effectiveness and assess its safety in humans. Initial findings in 21 patients with advanced MTC or NSCLC and RET-alteration revealed that 4/5 of those who were

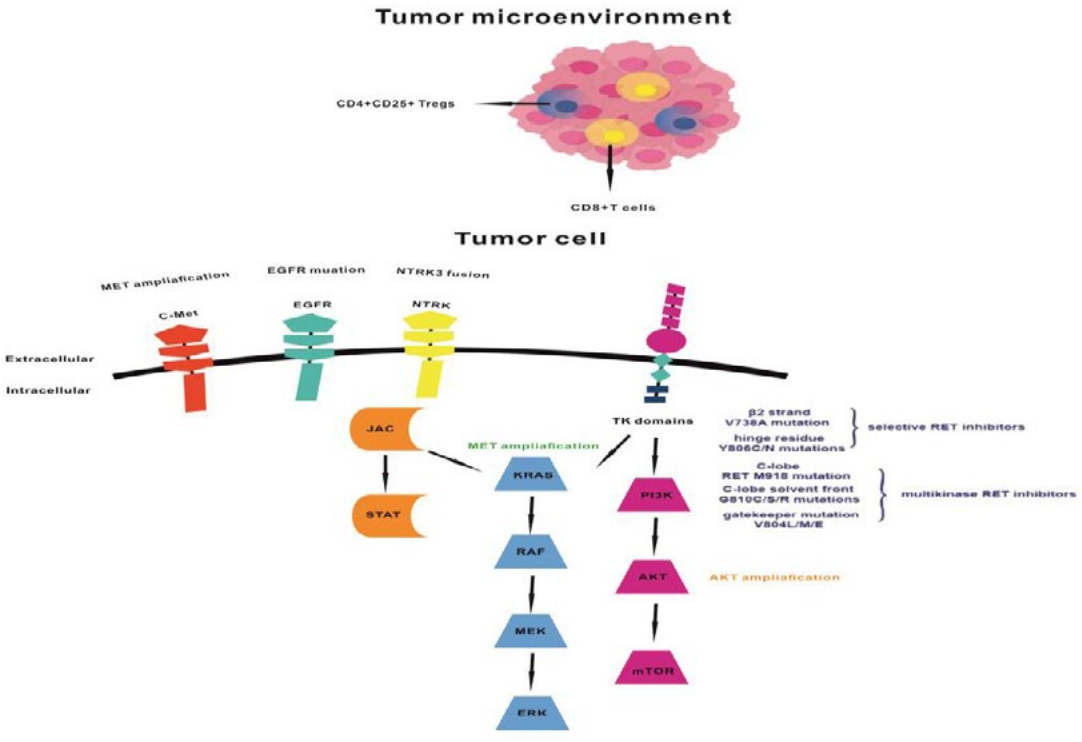

Fig3. Both primary and acquired resistance mechanisms to RET inhibitors.

TKI naïve (−3 to −42%) and 3/9 of those who were TKI pretreated (−17 to −44%) experienced tumor regression, with an acceptable toxicity profile (185).

The other second-generation RET inhibitor, BOS-172738, is presently being studied in a phase I trial. It was created with strong selectivity against VEGFR-2, nanomolar efficacy against RET, such as V804 M/L mutations, and Kd values ≤ 1 nM. Its strong anti-tumor efficacy and strong and specific RET inhibition were validated in vivo (186).

A new ATP-competitive and highly selective RET inhibitor, TAS0953/HM06 exhibits strong in vitro suppression of both G810R/S and RETV804M/L mutations in addition to RET-wildtype. It is insensitive to solvent front mutations because of its distinct binding mechanism to RET. In xenograft tumor models made from mice resistant to selpercatinib and pralsetinib, it showed substantial anticancer activity in vivo, indicating a good therapeutic promise (187). Additionally, data from animal models indicate that TAS0953/HM06 has higher suppression of brain xenograft tumors, increased survival in mice with intracranial metastases, and more effective CNS penetration than selpercatinib (188).

Highly selective RET inhibitor, LOXO-260 is made to be active for solvent-front and RET gatekeeper mutations while still exhibiting strong inhibition against other typical RET changes. In NSCLC or thyroid cancer patients that have RET altration or who have already taken a RET inhibitor, it is presently being studied in a phase I trial (NCT05241834) (189). Another powerful next-generation selective RET inhibitor, EP0031/A400, has improved effectiveness over selpercatinib against known resistance mutations, such as G810R/S/C and RET V804M/L, and has blood-brain barrier penetration. In 87 patients with RET-altered tumors, including 10 MTCs, the ongoing phase 1 basket trial's preliminary data (NCT05443126) indicate a 64% ORR, high tolerability, few adverse events, and no dose-limiting toxicities (190).

Other next-generation selective RET inhibitors are also being studied in advanced RET-altered tumors, including thyroid cancer. However, since bypass RAS mutations account for amount of acquired resistance to first-generation RET inhibitors and for which there is presently no specific targeted therapy, more research is necessary to address the shortcomings of the available treatments. Patients who have these acquired RAS mutations may have hope as several trials exploring the use of pan-RAF inhibitors in solid tumors are now in progress (Table 2) (191).

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CAR-T Cell Therapy in Systemic Lupus Erythematosus: Mechanisms, Toxicities, and Management Strategies

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Abstract:

Chimeric Antigen Receptor T-cell (CAR-T) therapy has revolutionized cancer treatment, particularly in hematologic malignancies, by genetically modifying a patient's T cells to specifically target and eliminate tumor cells. This groundbreaking approach has led to remarkable clinical outcomes, especially in patients with refractory or relapsed cancers. Over the past few years, CAR-T cell therapy has also been explored for the treatment of autoimmune diseases, including systemic lupus erythematosus (SLE), a complex and chronic autoimmune condition characterized by widespread inflammation and tissue damage. While the potential for CAR-T therapy in autoimmune disorders is significant, its application is accompanied by a range of toxicities that can pose substantial risks to patients, complicating its clinical use. These toxicities arise due to the powerful immune activation induced by CAR-T cells, which can affect various organ systems and result in serious side effects. This paper reviews the mechanisms behind CAR-T therapy-related toxicities, focusing on key adverse events such as Cytokine Release Syndrome (CRS), Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS), hematologic and cellular toxicities, as well as concerns regarding immunogenicity and oncogenic risks. Understanding these toxicities is critical to maximizing the therapeutic benefit of CAR-T therapy while minimizing potential harm to patients.

Keywords: Systemic lupus erythematosus (SLE), CAR-T cell therapy toxicities, Cytokine Release Syndrome (CRS), Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS).

Introduction

Systemic Lupus Erythematosus (SLE) is a complex and debilitating autoimmune disease that affects approximately 5 million people worldwide. Characterized by immune system dysregulation, SLE leads to the production of autoantibodies, causing widespread inflammation and damage to various organs (1-3). The disease course is unpredictable, with periods of flare-ups and remissions, and it is often associated with high morbidity and mortality. Traditional treatments for SLE, including corticosteroids, immunosuppressive drugs, and biologics such as rituximab and belimumab, aim to control immune hyperactivity but often come with significant side effects like increased infection risk and reduced patient quality of life (4). As a result,

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there is a growing need for novel therapies that can more precisely target immune dysregulation while minimizing adverse effects (5).

CAR-T cell therapy, a promising approach originally developed for cancer treatment, is emerging as a potential therapeutic strategy for autoimmune diseases like SLE. This therapy involves modifying a patient's T cells to express a chimeric antigen receptor (6), which allows the T cells to specifically recognize and target autoantibody-producing B cells, a hallmark of SLE (7). Although still in its early stages, CAR-T therapy has shown promise in preclinical and early clinical studies.

The application of CAR-T therapy in the treatment of autoimmune diseases, particularly in conditions like SLE, represents a paradigm shift in how autoimmune disorders could be managed in the future. In SLE, B cells and their resultant autoantibodies play a critical role in disease pathogenesis (8). By engineering CAR-T cells to target specific markers on autoreactive B cells, such as CD19 or CD20, researchers aim to directly eliminate these pathogenic B cells, potentially offering a more targeted approach compared to traditional immunosuppressive therapies (9).

However, CAR-T therapy is not without its challenges. The engineering process, which involves ex vivo manipulation of T cells, can result in a range of toxicities. These toxicities are particularly concerning in autoimmune disease contexts, where immune dysregulation is already at play (10). Thus, understanding the mechanisms behind these adverse events is crucial for advancing the clinical application of CAR-T therapy and mitigating the risks associated with its use in autoimmune disorders (11).

In this paper, we explore the complexities of CAR-T therapy in the treatment of autoimmune diseases, focusing on its potential application in SLE. Specifically, we examine the mechanisms of toxicities like CRS, Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS), and other adverse effects related to CAR-T therapy. We also delve into strategies to mitigate these risks, discuss recent clinical advancements, and explore ongoing research that holds promise for improving the safety and efficacy of CAR-T therapy in autoimmune diseases.

CAR-T Cell Therapy: Mechanisms and Development

The development of CAR-T therapy has undergone several phases, with key innovations improving the safety and efficacy of this treatment. Initially, the first generation of CARs utilized a simple extracellular single-chain variable fragment (scFv) to target tumor-associated antigens (12). Over time, more advanced designs known as second- and third-generation CARs have incorporated additional co-stimulatory domains, such as CD28, 4-1BB, and ICOS, which enhance T cell activation, persistence, and anti-tumor activity (13). The use of co-stimulatory molecules has been shown to increase CAR-T cell expansion and provide better long-term efficacy, although they can also contribute to toxicities like CRS.

The complexity of the immune system, particularly in autoimmune diseases like SLE, requires careful consideration when designing CAR-T therapies. In SLE, targeting B cells through CAR-T is one of the most promising strategies, as B cells contribute significantly to disease progression through the production of autoantibodies. By targeting surface proteins like CD19 or CD20 on these B cells, CAR-T therapy could offer a selective means to reduce autoreactive B cell populations and ameliorate the disease (14).

While the clinical efficacy of B cell-targeted CAR-T therapy in autoimmune diseases like SLE is still being evaluated, early preclinical and clinical studies have shown some promising results. This approach has the potential to reduce or even eliminate the need for systemic immunosuppressive drugs, which can cause long-term complications and increase the risk of infections and malignancies (15).

One of the challenges with CAR-T therapy in autoimmune diseases is the potential for off-target effects. Since many antigens expressed on B cells are also found on other immune cells or tissues, there is a risk that CAR-T cells could attack healthy tissues, leading to autoimmunity or exacerbation of existing disease. Researchers are working to identify more specific antigens or use a combination of CAR-T designs that can minimize off-target effects while maintaining therapeutic efficacy (16, 17).

Cytokine Release Syndrome (CRS)

Mechanism and Pathophysiology

Cytokine Release Syndrome (CRS) is a systemic inflammatory response triggered by the activation of CAR-T cells. Upon recognizing and binding to their target antigen, CAR-T cells become activated and release large quantities of cytokines—signaling molecules that mediate immune cell communication (18, 19). Key cytokines involved in CRS include**Interleukin-6 (IL-6)**, **Tumor Necrosis Factor-alpha (TNF-α)**, and **Interferon-gamma (IFN-γ)**. These cytokines initiate a cascade of immune responses, leading to inflammation, endothelial damage, and increased vascular permeability. The resulting vascular leakage can cause tissue edema, hypotension, and, in severe cases, multi-organ failure (20).

The pathophysiology of CRS is complex and multifactorial. In addition to direct CAR-T cell activation, antigen-presenting cells (APCs) such as dendritic cells are stimulated, leading to the release

of further inflammatory cytokines and amplifying the immune response. In autoimmune diseases like **SLE**, the baseline immune activation is already heightened, which can exacerbate CRS and make management more challenging (21).

The incidence and severity of CRS are influenced by several factors, including the CAR-T cell dose, their persistence in circulation, the specific target antigen, and the patient's underlying immune profile (11). Patients with autoimmune diseases, particularly those with dysregulated immune responses like SLE, may be more prone to CRS, either due to an increased likelihood of immune activation or because their immune systems may already be primed for exaggerated responses. Therefore, careful patient selection, vigilant monitoring, and early intervention are crucial when administering CAR-T therapy in autoimmune disease populations (22).

Insights from Highly Cited Studies on CRS

A study published in Blood, titled "Chimeric Antigen Receptor T Cells for Autoimmune Diseases

Understanding Cytokine Release Syndrome" provides a detailed analysis of the mechanisms and incidence of CRS in the context of CAR-T therapy. The study emphasizes that CRS is a dose-dependent phenomenon, with higher doses of CAR-T cells often correlating with more severe cytokine storms. The authors discuss how patients with pre-existing immune dysregulation, such as those with autoimmune diseases like SLE, are at increased risk for CRS due to their heightened baseline immune activity. The paper stresses the importance of early detection and intervention in these high-risk patients, recommending preemptive cytokine-blocking strategies and close monitoring of inflammatory markers to mitigate CRS severity (23).

The article, "The Immune System and Cytokine Release Syndrome

Understanding the Complex Interactions in CAR-T Therapy," published in *Nature Reviews Clinical Oncology*, explores the intricate relationship between immune dysregulation and CRS in autoimmune patients. This study highlights that individuals with autoimmune diseases, such as lupus and rheumatoid arthritis, are especially susceptible to CRS when undergoing CAR-T therapy. Given their already heightened inflammatory states, these patients are more likely to experience exaggerated immune responses. The authors advocate for the use of targeted therapies like **tocilizumab** (an IL-6 receptor antagonist) to manage CRS and recommend rigorous monitoring of immune markers, as well as the use of supportive care measures to address CRS-related complications (Nature Reviews Clinical Oncology Article) (23).

A seminal study published in The Journal of Clinical Investigation, titled "CRS in CAR-T Therapy

Pathogenesis, Risk Factors, and Clinical Management," delves into the risk factors and pathogenesis of CRS, particularly in patients with autoimmune diseases. The authors note that CRS is notably prevalent in individuals with pre-existing autoimmune conditions, such as SLE, where it can be exacerbated by pre-existing immune activation. The study identifies key biomarkers, such as **elevated IL-6 levels**, that serve as early indicators of severe CRS. The paper emphasizes the need for biomarker-driven approaches to predict CRS risk and suggests that personalized dosing regimens, along with targeted immunomodulatory therapies, can help manage CRS in autoimmune disease patients (The Journal of Clinical Investigation Article).

These highly cited studies collectively underscore the complex relationship between CRS and autoimmune diseases, revealing how pre-existing immune activation can complicate the effectiveness of CAR-T therapy. As research in this area progresses, it becomes increasingly clear that managing CRS in autoimmune disease patients will require not only timely interventions with cytokine blockers but also a comprehensive understanding of individual immune profiles. This approach may help mitigate the risks associated with CAR-T therapy and optimize treatment outcomes for patients with autoimmune diseases.

Grading and Management of CRS

As discussed earlier, CRS is graded based on the severity of symptoms. The grading system typically ranges from Grade 1 (mild symptoms such as fever) to Grade 5 (fatal complications). Management of CRS is tailored to the severity of the symptoms, with supportive care being essential for all patients. For Grade 1 or 2 CRS, symptomatic treatment such as antipyretics and intravenous fluids may suffice. For more severe forms (Grade 3 or 4), aggressive interventions are required (24).

The standard treatment for severe CRS includes the use of **tocilizumab**, an IL-6 receptor antagonist, which effectively blocks IL-6 signaling and curtails the cytokine storm. Other interventions may include corticosteroids, which suppress the immune response and reduce the inflammation caused by the release of cytokines. In cases where tocilizumab and corticosteroids are ineffective, additional therapies such as Janus kinase (JAK) inhibitors may be explored (25).

In autoimmune disease patients, the management strategy for CRS may need to be adjusted. For instance, SLE patients often have elevated baseline levels of immune activation, so their response to CRS-triggering CAR-T cells may be unpredictable.

Moreover, the use of immunosuppressive drugs in these patients could complicate CRS management, requiring a more careful balancing of treatment to avoid exacerbating the autoimmune response or inducing relapse (26).

Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS)

Mechanisms and Pathophysiology

The pathophysiology of ICANS involves neuro-inflammation, which is triggered by the release of pro-inflammatory cytokines and the infiltration of immune cells into the central nervous system (CNS). This phenomenon is particularly concerning in autoimmune diseases like SLE, as these patients already have a compromised blood-brain barrier (BBB), making it easier for immune cells to enter the CNS and cause damage. The endothelial cells that form the blood-brain barrier are often dysregulated in autoimmune diseases, potentially increasing the risk of ICANS (27).

Additionally, the activation of microglial cells—resident immune cells of the CNS—can further exacerbate neuroinflammation and contribute to neuronal injury. The exact mechanisms that lead to ICANS in the context of CAR-T therapy remain a subject of ongoing research, but it is clear that the systemic inflammatory response, combined with the immune dysregulation seen in autoimmune diseases like SLE, plays a significant role in triggering these adverse neurological effects (28).

ICANS can be further complicated by the presence of other comorbidities common in autoimmune diseases, such as vascular abnormalities, prior neurologic events, or the use of immunosuppressive drugs, which may predispose patients to neurological side effects (29). As CAR-T therapies continue to expand into autoimmune disease treatment, understanding the unique risks associated with ICANS in this patient population will be critical (6).

Diagnosis and Management of ICANS in CAR-T Therapy

Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) is a well-documented complication of CAR-T cell therapy, characterized by neurological symptoms ranging from mild cognitive dysfunction to severe manifestations such as delirium, seizures, and coma (30). The pathophysiology of ICANS is not fully understood, but it is believed to involve cytokine release, T-cell activity, and inflammation in the central nervous system. These mechanisms may be further exacerbated in patients with autoimmune diseases like **SLE**, as SLE itself is associated with dysregulation of the immune system, which could influence the neuroinflammatory response triggered by CAR-T therapy (31). Diagnosing and managing ICANS requires a comprehensive approach, with special attention to underlying autoimmune conditions like SLE, which may alter the clinical presentation and response to treatment (32).

Diagnosis of ICANS

The diagnosis of ICANS requires a thorough neurological evaluation, as symptoms can range from mild cognitive issues to life-threatening conditions like seizures and coma (33). A multidisciplinary approach is typically employed to rule out other potential causes of neurological symptoms, such as infection, stroke, or progression of underlying autoimmune disease. Common diagnostic methods include:

- **Magnetic Resonance Imaging (MRI)**

An MRI of the brain is commonly performed to assess any structural changes. In most cases of ICANS, MRI findings are often **normal.** However, in severe cases, subtle findings such as **edema** or **white matter lesions** may be seen. These findings may reflect areas of the brain affected by neuroinflammation, though they are not always diagnostic (34).

- **Electroencephalography (EEG)**

An EEG can be instrumental in detecting **subclinical seizures**, which may occur even in the absence of overt clinical seizures. Given that some ICANS patients may experience non-convulsive seizures, EEG is particularly useful for identifying neurological dysfunction that is not immediately obvious (35).

- **Cerebrospinal Fluid (CSF) Analysis**

While CSF analysis in ICANS is typically **unremarkable;** it can be used to rule out other potential causes of neurological symptoms, such as **infection** (e.g., meningitis or encephalitis) or **malignancy** (36)**.** CSF may show mildly elevated protein levels or white blood cells, but these findings are not specific to ICANS and may be more indicative of other conditions (37).

In patients with **SLE**, the diagnosis of ICANS may be particularly challenging because SLE is often associated with central nervous system (CNS) involvement, such as **lupus cerebritis**. Symptoms of SLE-related CNS involvement (e.g., cognitive dysfunction, seizures, psychosis) overlap significantly with those of ICANS (38). Therefore, distinguishing between ICANS and worsening SLE manifestations is crucial. The inclusion of **neurologic autoantibodies**, such as **anti-NMDA receptor antibodies** or **antiphospholipid antibodies**, may help assess the autoimmune contribution to neurological symptoms in SLE patients.

Management of ICANS

The management of ICANS is aimed at **reducing neuroinflammation** and **supporting neurological function.** As ICANS can range from mild to life-threatening, treatment strategies should be individualized based on the severity of symptoms. In patients with **SLE**, special attention is required to ensure that any immune-modulatory treatments used for ICANS do not exacerbate pre-existing autoimmune activity (39).

•Corticosteroids

The cornerstone of ICANS treatment is the use of **corticosteroids**, particularly **dexamethasone**. Dexamethasone is effective in reducing **neuroinflammation** and improving neurological symptoms. The dose and duration of corticosteroid therapy are typically adjusted based on the severity of symptoms. In cases where ICANS is severe or resistant to initial steroid treatment, corticosteroids may be escalated or given as high-dose therapy (40).

•Intravenous Immunoglobulin (IVIg)

In refractory cases of ICANS, **IVIg** is an option. IVIg has been shown to reduce inflammation and modulate immune responses. However, its role in ICANS remains investigational, and more research is needed to determine its effectiveness and optimal use in CAR-T-related neurotoxicity (41).

•Plasmapheresis

As another potential therapeutic option, **plasmapheresis** (also known as therapeutic apheresis) may be considered in severe cases of ICANS. This approach is aimed at removing circulating inflammatory mediators, autoantibodies, or other factors contributing to the neuroinflammatory response. Plasmapheresis is generally reserved for cases that do not respond to steroids or IVIg (42).

•Symptomatic Management

In patients with severe neurotoxicity, **symptomatic management** is critical. For patients experiencing seizures, **antiepileptic drugs** (AEDs) such as levetiracetam or phenytoin may be administered (43). Additionally, **sedation** may be necessary to ensure patient comfort and safety in cases of severe agitation or delirium. For patients with **respiratory failure**, **mechanical ventilation** may be required, particularly in those with compromised airway or breathing function (44).

•Ongoing Neurological Monitoring

Given that ICANS symptoms can evolve rapidly, continuous monitoring of neurological status is essential. **Frequent neurological exams**, including assessments of mental status, motor function, and seizure activity, should be conducted. The use of **EEG** may help monitor subclinical seizures, and periodic **MRI scans** can assess for worsening edema or structural changes (44).

For patients with **SLE**, it is essential to monitor for exacerbations of the underlying disease. Since SLE can also cause **neuropsychiatric manifestations**, distinguishing between **SLE-related neurotoxicity** and ICANS is crucial for tailoring therapy. If there is evidence of SLE-related CNS involvement (e.g., lupus cerebritis), additional treatments, such as **immunosuppressive agents** or **hydroxychloroquine**, may be considered, in conjunction with the management strategies for ICANS (45).

Prognosis

The prognosis of ICANS is largely dependent on the severity of symptoms and the timeliness of treatment. In mild cases, with prompt management, patients typically recover without long-term neurological deficits (46). However, in severe cases, if left untreated or poorly managed, ICANS can result in significant morbidity or even death. Patients with underlying autoimmune conditions, like **SLE,** may face a more complicated course, as the interaction between CAR-T therapy-induced immune activation and pre-existing immune dysregulation can lead to an unpredictable clinical course (47).

Early detection, appropriate management, and long-term follow-up are essential to improving outcomes for patients with ICANS, particularly those with complex autoimmune diseases such as SLE.

Expansion of Clinical Trials Testing CAR-T in Autoimmunity

Building on successes in lupus, clinical investigations into CAR-T therapies for autoimmune diseases have expanded significantly. As of November 2023, 29 ongoing trials were listed on ClinicalTrials.gov. Among these, 17 focus on lupus, while others explore vasculitis, neurological autoimmune diseases, and pemphigus vulgaris. The concentration on lupus reflects the challenges of treating the disease with existing therapies, unlike vasculitis, which often responds well to current anti-B cell strategies. This growing body of research underscores the widespread interest in CAR-T as a groundbreaking approach to autoimmune disease management (48).

Recent Data and Results from Clinical Trials

Among the most prominent studies is the investigation into **anti-CD19 CAR-T cell therapy** for lupus and other autoimmune diseases, which has shown promising results. Recent findings from a Phase 1/2 trial, published in *The Lancet*, demonstrated that autologous anti-CD19 CAR-T therapy led to

significant B cell depletion and clinical improvements in patients with refractory SLE. Over 70% of patients showed a reduction in disease activity, marked by a decrease in both the SLE Disease Activity Index (SLEDAI) and the need for corticosteroids. However, the study also highlighted that while CAR-T therapy showed promise, some adverse effects like Cytokine Release Syndrome (CRS) and Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) remained concerns (49).

Another promising study, published in *Nature Medicine*, explored **anti-B cell maturation antigen (BCMA) CAR-T cells** in autoimmune conditions, including lupus and myasthenia gravis. The study found that targeting BCMA in these patients was effective in depleting the pathogenic B cells that drive disease progression (50). Additionally, BCMA CAR-T therapy appeared to offer an advantage over traditional therapies in controlling disease activity, with patients experiencing fewer relapses and a reduced dependency on immunosuppressive drugs. However, researchers emphasized that these promising results require validation in larger clinical trials, particularly due to the potential for adverse events associated with CAR-T cell therapy (51).

Insights from Highly Cited Studies

Several highly cited studies have expanded our understanding of the role of CAR-T cell therapy in autoimmune diseases:

1. A review article titled **"CAR T Cells for Treating Autoimmune Diseases"** discusses the application of CAR-T cells in autoimmune disorders, focusing on both preclinical and clinical results. The review highlights the success of anti-CD19 CAR-T cells in targeting B cell populations in diseases like systemic lupus erythematosus (SLE), refractory antisynthetase syndrome, and myasthenia gravis. The study concludes that CAR-T therapy offers a highly targeted approach for B cell-driven autoimmune diseases, potentially reducing disease activity with fewer side effects compared to traditional treatments (52).
2. The article **"Dawn of CAR-T Cell Therapy in Autoimmune Diseases"** provides an overview of the progress made in this field, detailing both preclinical studies and early clinical trials. It highlights the promise of anti-CD19 CAR-T cells in animal models of lupus, where the therapy resulted in a marked reduction in disease symptoms. Clinical trials in humans have shown similar outcomes, with patients experiencing substantial improvements in disease activity. The article underscores the need for more comprehensive trials to fully understand the therapeutic potential of CAR-T cells in autoimmune diseases (53).
3. A key case series published in *The New England Journal of Medicine* titled **"CD19 CAR T-Cell Therapy in Autoimmune Disease"** evaluates the safety and efficacy of CD19-directed CAR-T cell therapy in patients with autoimmune diseases, including SLE. The results from this study demonstrated that the therapy was well-tolerated, leading to significant improvements in disease activity and reductions in disease flares. These findings suggest that CD19-directed CAR-T therapy could be a viable treatment option for patients with autoimmune diseases who are refractory to conventional therapies. However, as with other CAR-T approaches, careful monitoring for immune-related toxicities, including CRS and ICANS, is essential (54).

These studies collectively illustrate the growing optimism surrounding CAR-T cell therapy for autoimmune diseases. The ability to target specific immune cells involved in the disease process presents a transformative approach to treatment, offering potential advantages over conventional therapies. However, risks such as severe immune-related side effects like CRS and ICANS remain challenges that researchers are actively working to address (55).

Other Toxicities Associated with CAR-T Therapy

Beyond CRS and ICANS, several other toxicities are associated with CAR-T therapy, and these include hematologic, cardiac, and pulmonary complications, as well as long-term risks such as secondary malignancies and autoimmunity (56).

Hematologic Toxicity

Hematologic toxicities, including cytopenias (e.g., anemia, neutropenia, thrombocytopenia), are commonly observed after CAR-T cell therapy. These toxicities occur due to the exhaustion of the hematopoietic stem cell pool, as well as direct cytotoxicity induced by CAR-T cells on normal hematopoietic cells. The risk of severe cytopenias is higher in patients with autoimmune diseases due to the presence of altered immune dynamics. Management of hematologic toxicities generally involves supportive care, such as blood transfusions and growth factor support (e.g., granulocyte colony-stimulating factor [G-CSF]). In some cases, immune suppression may also be required to manage prolonged cytopenias (57).

Cardiac and Pulmonary Toxicity

Although less common, cardiac and pulmonary toxicities have been reported in patients undergoing CAR-T therapy. These include arrhythmias, myocardial infarction, acute pulmonary edema, and respiratory distress. In autoimmune disease patients, these risks may be further complicated by pre-

existing cardiovascular and pulmonary involvement, making careful monitoring essential (58).

Secondary Malignancies and Autoimmunity

Long-term use of CAR-T therapy may increase the risk of secondary malignancies, as prolonged immune suppression and T cell activation can alter normal cellular processes (59). Furthermore, there is concern that CAR-T therapy could inadvertently trigger autoimmune responses, leading to the development of new autoimmune diseases or exacerbation of pre-existing conditions like SLE. Long-term follow-up is crucial to monitor for the emergence of secondary cancers or the onset of autoimmune phenomena, which could have profound effects on patient health (60).

Conclusion

CAR-T cell therapy holds immense potential for the treatment of autoimmune diseases like SLE. However, the risk of toxicities associated with this innovative therapy remains a major concern. Understanding the underlying mechanisms of toxicities like CRS, ICANS, and hematologic complications, as well as the strategies for managing these toxicities, will be critical in making CAR-T therapy a viable treatment option for autoimmune diseases. With ongoing advancements in CAR-T design, the ability to minimize these risks and maximize therapeutic efficacy will likely improve, paving the way for more targeted and personalized treatments for patients with autoimmune diseases. The future of CAR-T therapy in autoimmune diseases depends on refining treatment protocols, developing better patient selection criteria, and advancing our understanding of the intricate relationship between the immune system and CAR-T therapy.

Authors' Contribution

…… were involved in the conceptualization, design and writing of the manuscript draft. The authors read and confirmed the final manuscript.

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The Impact of Long-Term Exposure to Air Pollution on Cancer Risk

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Abstract:

Air pollution is recognized as a critical global health challenge, contributing to approximately 7 million deaths annually and ranking among the most severe environmental crises confronting humanity. A substantial portion of the global population resides in regions where air pollution levels, driven by emissions from industrial activities, power generation, vehicular traffic, and residential combustion, significantly exceed the World Health Organization's recommended air quality guidelines. Ambient air pollution has emerged as a pressing global public health issue due to its widespread presence and severe adverse effects on human health, particularly its association with cancer.

Extensive evidence from epidemiological studies, experimental research in laboratory animals, and mechanistic investigations has established a strong link between air pollution particularly exposure to particulate matter (PM) and an increased risk of cancer incidence and mortality. Long-term exposure to PM and other air pollutants contributes to oxidative stress, inflammation, DNA damage, and epigenetic modifications, all of which are implicated in carcinogenesis.

In this study, we explore the intricate relationship between air pollution and the occurrence of specific cancers, including lung, bladder, and breast cancer, while also elucidating the underlying molecular mechanisms that drive pollution-induced carcinogenesis. By analyzing recent research findings and mechanistic data, we aim to enhance the understanding of how chronic exposure to air pollution contributes to cancer development. Addressing this issue requires global efforts to implement stringent air quality regulations, promote clean energy alternatives, and increase public awareness to mitigate the long-term health impacts of air pollution.

Keywords: Air pollution, Lung cancer, Particulate matter, Breast cancer

Introduction

Particulate matter (PM) is regarded as one of the most detrimental airborne pollutants, originating from both natural and human-caused sources or generated by atmospheric interactions (1). In the past few years, West Asia has seen desert dust storms, resulting in an increase in the frequency of dusty days and the daily average concentration of particulate matter with an aerodynamic diameter smaller than 10 μm (PM10) (2). Air pollution from the environment has been

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associated with a variety of health consequences, such as increased rates and death from heart disease, lung disease, and malignant illnesses (3). The respiratory and cardiovascular impacts of contaminants in air exposure have been thoroughly shown in both the industrial and general populations. Air pollution (AP) is a pervasive and intricate amalgamation of both solid and liquid particulates and gasses (4). PM and nitrogen oxides (NOx), particularly nitrogen dioxide (NO_2), are significant major constituents of pollution in the air. PM is often classified by size, differentiating particles with an aerodynamic diameter less than 10μm (PM10) from those with an aerodynamic diameter less than 2.5μm (PM2.5) (5). The former may be breathed, while the latter, classified within the PM10 fraction, can penetrate the lung alveoli and, in their tiniest form, enter the bloodstream. PM may originate from heating sources, vehicular traffic, industrial activities, and the agricultural sector, or it may be of natural origin (6).

AP is very detrimental, and even minimal concentrations pose a threat to human wellness. AP is currently regarded as a critical worldwide health concern and is accountable for an increasing array of health effects (7). Rapid development may result in increased exposure to harmful levels of environmental contaminants. Outdoors AP has long been a significant health issue, and the situation deteriorates daily (8). The primary contributor to AP in large urban areas is an inadequate transportation infrastructure. The level of AP in densely populated cities exceeds that of other regions, potentially causing significant adverse effects on human health by disrupting water and land environments (9). Tehran, Beijing, Sao Paulo, Shanghai, Cairo, Bangkok, Mexico City, and Jakarta are the most polluted regions globally. A significant sixteen percent of fatalities connected to illnesses that are not transmitted are attributable to AP (10). Ambient AP, recognized as a cancer-causing agent for humans, has been linked to the onset of several cancers. Numerous research examining industrial AP contact have shown a correlation between AP and an increased risk of lung cancer (11). The mechanisms behind this connection are thought to entail the onset and development of carcinogenesis via damage to the DNA, inflammatory processes, oxidative stress, and interruption of cell processes (12). Nonetheless, the correlation between air pollution and various cancer forms stays mostly ambiguous. Several observational investigations have attempted to investigate possible correlations between AP and cancers, including breast, bladder, and skin cancers; nevertheless, the results have been intricate and sometimes conflicting (13). The purpose of this study is to investigate the relationship between long-term exposure to AP and some cancers.

Carcinogenic mechanism of air pollution

Inflammatory substances associated with AP, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β), foster pro-inflammatory conditions that facilitate tumorigenesis (14). Inflammatory signaling networks, including nuclear factor-kappa B (NF-κB) and signal transducer and activator of transcription 3 (STAT3), are often active in cancer (15). These processes modulate the activity of genes associated with cell survival, growth, blood vessel development, and dissemination, hence creating a conducive environment for tumor formation. Inflammation fosters a tumor-promoting milieu by affecting several elements of the tumor's microenvironment, such as immune system cells, fibroblasts, endothelial cells, and the extracellular matrix (16). Additionally, pro-inflammatory and stromal cells have a role in the modification of the extracellular matrix, promoting tumor expansion and dissemination. Inflammation has a complex involvement in the emergence of cancer, affecting several facets of tumor origin, growth, and development (17). Long-term inflammation thus produces damage to DNA, establishes a tumor-promoting milieu, hinders immune surveillance, and enhances angiogenesis. A troubling element of air pollution is its capacity to produce mutagenesis, resulting in genetic modifications in living creatures (18). Laboratory rats situated close to contaminated manufacturing areas (1 km downwind from two integrated steel mills) exhibited a greater transmissible mutation rate at tandem-repeat DNA loci than those at a reference site 30 km distant, with this impairment mainly attributed to a rise in mutations inherited via the paternal germline (19). Mutations in essential regulatory genes may impair cellular processes, resulting in unregulated cell proliferation and the onset of cancer. The findings indicate that mitigating air pollution is essential to diminish its mutagenesis capacity on cells (20).

Breast cancer and air pollution

Present medical information indicates that breast cancer (BC) is the most often identified cancer among women internationally, resulting in significant morbidity and mortality, and is the main cause of cancer-related deaths in females globally (21). Based on the Global cancer prevalence statistics, the worldwide incidence of cancer is steadily growing, especially in developing nations, with an estimated 1.7 million new cancer cases and 521,900 deaths recorded globally in 2012. Globocan (2020) estimates 2261419 new BC cases (both sexes, all ages) in 2020, representing 11.7% of all new cases of cancer (22). BC killed an estimated 684996 people, accounting for 6.9% of all cancer fatalities. BC has a global prevalence rate of 47.8 and a death rate of

13.6 per one million individuals (23). Scientists at the National Institutes of Health discovered that residing in regions with elevated particle AP correlates with a higher prevalence of BC. The research, released in the Journal of the National Cancer Institute, is one of the biggest investigations currently underway examining the correlation between outside AP, particularly fine particulate matter, and the prevalence of BC (24). The study was conducted by researchers from the National Institute of Environmental Health Sciences (NIEHS) and the National Cancer Institute (NCI), both affiliated with NIH. Research in the Journal of the National Cancer Institute revealed that residing in regions with elevated particle AP correlates with an 8% rise in the prevalence of estrogen receptor-positive BC (25). Inconsistencies persist in the research about the correlation between AP and BC incidence. Crouse et al. identified a positive correlation between the amount of NO2 and BC risk with 95% confidence; however, other investigations reported positive relationships with limited, small, or null statistical significance (26). Hystad et al. identified a substantial correlation between levels of NO2 and premenopausal BC, whereas a marginal connection was seen between NO2 and postmenopausal BC in women (27). Datzman et al. identified substantial positive correlations between NO2, PM10, and BC occurrence via medical information from a local insurance provider in Germany, which encompasses about one million females and 9,577 cases (28). AP can raise the possibility of BC by engaging with estrogen receptors, inducing inflammatory and oxidative stress, and disturbing hormonal equilibrium. Certain research indicates that the impact of AP on BC risk is more pronounced in younger women (29). Garcia et al. examined the association between elevated BC possibility and various air pollutants, such as acrylamide, carbon tetrachloride, chloroprene, 4,4'-methylene bis (2-chloroaniline), propylene oxide, and vinyl chloride; however, the risk percentages were not significantly different (30). Furthermore, a review analysis synthesized the findings from 8 case-control investigations and 9 cohort investigations, indicating little evidence to substantiate a correlation between PM and BC incidence (25). Goldberg et al. indicated that exposure to atmospheric NO_2 and UFPs may elevate the likelihood of developing postmenopausal BC, particularly in women with positive estrogen and progesterone receptor expression (31). The varied results and inferences may be attributed to climate, cultural disparities, or population density; nevertheless, more investigations and analyses are required to establish a definitive association between AP and BC risk.

Lung cancer and air pollution

Lung cancer (LC) is the most prevalent malignancy and the main reason of death due to cancer globally (32). Tobacco use is the biggest recognized environmental factor, with 81.7% of LC occurrences attributed to it (33). Moreover, multiple investigations have shown that contact with outside pollutants, such as PM, NOx, ozone, and sulfur dioxide, could have an association with LC. LC is the predominant reason for worldwide cancer mortality and incidence, with around 2 million diagnoses and 1.8 million fatalities (34). Lung cancers are the second most prevalent diagnosis of cancer in both men and women, behind prostate and BC, respectively. Contaminated air comprises a minimum of two categories of carcinogens: polycyclic aromatic hydrocarbons (PAHs) and n-nitroso substances, including nitrosamines (35). Studies indicate that PM2.5 could encourage tumorigenesis in pulmonary tissues. PM2.5 exhibits elevated PAH concentrations, capable of inducing oxidative stress and activating aryl hydrocarbon receptors in human bronchial epithelial cells (BEAS-2B) (36). Stimulation of the aryl hydrocarbon receptor facilitates the spread and invasion of LC cells. Research revealed that death from LC attributable to PM2.5 is significantly greater in women than in males (37). Besides inducing local inflammation and oxidative stress, PM2.5 infiltrates the lungs and terminal bronchioles, subsequently entering the bloodstream and eliciting widespread inflammation. A recent study indicates that prolonged contact with PM10 in a human NSCLC cell line resulted in a reduction of the mitotic rate and the proportion of cells in the G2/M phase relative to a control group (38). Long-term contact to fine particulate AP may induce genotoxicity and mutagenicity. It can raise the likelihood of LC by proinflammatory destruction, the generation of reactive oxygen species, and DNA oxidation (39). Consequently, particularly in those with a significant genetic predisposition, extended exposure to AP may elevate the occurrence of lung tumors. Huang et al. established a synergistic impact of elevated genetic vulnerability (assessed using a polygenic risk rating derived from genome-wide correlation research) and increased PM2.5 exposure on lung cancer frequency (40). Outside air pollution has been associated with many epigenetic alterations, such as adjustments to post-translational histone modifications, 5-hydroxymethylation, and particularly DNA methylation (DNAm) (41). DNAm is a biochemical alteration that happens in cytosines, especially within the CpG context, and influences the expression of genes along with multiple other roles (42). As noted with TP53, hypermethylation facilitates gene silence, while DNA hypomethylation leads to chromosomal instability and the stimulation of retrotransposon sequences and repetitive motifs, including LINE-1 and Alu. DNA hypomethylation

additionally impacts essential chromosomal areas, including the subtelomeric and pericentromeric areas (43). Access to atmospheric air pollution, both short-term and long-term, correlates with aberrant DNA methylation. Additional research indicates that epithelial cells from humans exposed to PM2.5 have increased susceptibility to hypomethylation and transcriptional stimulation of multiple genes and microRNAs (miRNAs), hence altering cancer-related signaling networks (44). PM2.5 may generate alterations in long non-coding RNAs (lncRNA) like loc146880 through reactive oxygen species (ROS), facilitating autophagy and cancer in lungs (45).

Bladder cancer and air pollution

In 2018, bladder cancer ranked as the 10th most prevalent cancer globally. The rates of incidence were elevated in males, for whom it ranked as the sixth most prevalent malignancy (46). The greatest rates of incidence were seen in the advanced nations. The majority of bladder cancers are classified as urothelial carcinoma (UC), with the rest including squamous cell carcinoma, sarcoma, lymphoma, and adenocarcinoma (47). Around 75 percent of these cases are non-muscle-invasive bladder cancer (NMIBC). Non-muscle invasive bladder cancer (NMIBC) has a significant frequency owing to its sluggish natural history and elevated relapse rate (48). Environmental hazards and comprehensive exposome (the whole of exposure from both inside and outside elements) have a substantial role in the progression of BC. Consequently, comprehending these risk variables is essential for protection (49). Continuous exposure to AP, especially PM2.5, is linked to a heightened chance of bladder cancer. A comprehensive review and meta-analyses indicated that a 5 µg/m3 rise in PM2.5 exposure correlates with a 6% greater likelihood of bladder cancer. The research identified indicative evidence of a correlation between prolonged contact with PM2.5 and the prevalence of bladder cancer (8). Separate research indicated that the correlation between zinc and PM2.5 underscores the significance of pollution from factories. A comprehensive review and meta-analysis revealed that a 10 µg/m3 rise in NO2 correlates with a 4% elevated risk of bladder cancer (50). Nevertheless, several investigations have not shown a correlation between NO2 and bladder cancer. The existing information about the correlation between the prevalence and morbidity of bladder and kidney cancer and AP contact primarily originates from industrial settings including contact with gasoline vapors, chlorinated solvents, asbestos, pesticides, and polycyclic aromatic hydrocarbons (PAHs) (51). A study and meta-analysis revealed an elevated incidence of urinary bladder cancer among motor vehicle drivers who were professionally subjected to significant levels of traffic-related AP (52). Nonetheless, although the level of airborne contaminants in the general public is significantly lower than that experienced by drivers and workers in factories, it is plausible to infer that long-term contact in the general population may be linked to an elevated likelihood of bladder cancers (53). Multiple pathways could account for the association between AP contact and bladder cancer. Current research on animals indicates that contact with PM2.5 may disrupt the angiotensin/bradykinin pathway, leading to premature kidney injury, oxidative stress, and/or inflammation, ultimately resulting in cancer (54). These biological results indicate that prolonged contact to air pollution may cause abnormalities in the urinary tract, potentially resulting in urological malignancies (54).

Discussion and conclusion

The urban air comprises a complicated amalgamation of oxidizing gases and particulates of varying sizes and compositions. Contact with AP elements may harm the biological molecules of people and animals, potentially leading to illnesses like malignancy (55). The International Agency for Research on Cancer has classed contact with entire diesel engine exhaust as probably dangerous to people, according to little human data and significant evidence from animal studies (56). Several epidemiological research has demonstrated correlations between fine particulate matter in AP and deaths from LC. Emission origins can involve natural phenomena like wildfires, volcanic eruptions, and dust storms (57). Anthropogenic pollutants include emissions from combustion methods utilized in heating, energy generation, industrial activities, and vehicular traffic. The concentration of contaminants in urban air around us is influenced by both local emissions and extended transportation as well as meteorological factors (58). Irrespective of the extent of air pollution contact, it is standard procedure to classify its components as gases and particles. AP poses a considerable and extensive risk to public health, with 99% of the global population inhaling polluted air, as reported by the World Health Organization (WHO) (59). The hazards linked to AP parallel those induced by cigarette smoking. Contact with air pollution may result in cancer, stroke, respiratory disorders, heart problems, and other health complications. Air pollution jeopardizes advancements in alleviating the global cancer burden by exacerbating the annual increase in avoidable cancer cases (60). Mitigating air pollution directly decreases the risk of lung cancer; however, the measures implemented—such as expanding green spaces, utilizing cleaner energy sources, and promoting active transportation (walking and

cycling)—also yield numerous health advantages, including improved dietary habits, enhanced physical activity, and reduced prevalence of other non-communicable diseases and co-morbidities, thereby diminishing the probability of developing additional cancers (61). AP may adversely affect the quality of life for those with cancer by intensifying respiratory symptoms, elevating tiredness, diminishing physical activity, and aggravating treatment side effects. It may also hinder cancer treatments by diminishing the efficacy of chemotherapeutic agents, exacerbating surgical difficulties, and possibly interfering with targeted medicines and immunotherapies (62). AP become a substantial factor in its occurrence. This analysis examines the correlation between air pollution—particularly particulate matter (PM2.5), industrial pollutants such as vinyl chloride and benzene—and the heightened risk of cancer (63). Air pollution may mechanistically induce cell damage by oxidative stress, inflammation, and genetic alterations, hence leading to cancer formation. Epidemiological data from cohort and regional studies indicates a favorable association between prolonged contact with air pollution and higher rates and death of cancer (64). Moreover, air pollution has been demonstrated to deteriorate survival results in people with cancer, especially those detected during the initial stages (64). The analysis underscores the requirement for more stringent air quality laws and pertinent studies on the fundamental mechanisms affected by air pollution. Mitigating contact with air pollution is essential for decreasing the chance of cancer and enhancing overall health results.

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Synthetic Biology in Genomics: Redefining Genetic Engineering and Its Applications in Personalized Medicine

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Abstract:

Synthetic biology, an emerging interdisciplinary field combining biology, engineering, and genomics, has led to transformative advances in genetic engineering. By enabling the design of novel biological systems and organisms, synthetic biology is revolutionizing personalized medicine. This paper examines how synthetic biology intersects with genomics to create more precise and individualized medical treatments. The ability to manipulate genomes at a granular level, coupled with advanced techniques such as CRISPR-Cas9 gene editing and genome synthesis, has provided powerful tools for developing personalized therapies. This review explores the applications of synthetic biology in personalized medicine, focusing on the creation of tailored treatments, gene therapies, synthetic vaccines, and diagnostic systems. We also address the ethical, regulatory, and safety concerns surrounding these technologies, as well as their future potential.

Keywords: Synthetic Biology, Genetic Engineering, Personalized therapies, Synthetic vaccines.

Introduction

Personalized medicine is an evolving approach to healthcare that tailors medical decisions, practices, and treatments to the individual characteristics of each patient (1). Unlike the traditional "one-size-fits-all" model, which assumes a universal treatment plan for all individuals, personalized medicine takes into account a wide array of factors unique to each patient. These include genetic makeup, environmental exposures, lifestyle choices, and even cultural background, all of which can influence how a patient responds to different therapies (2). By integrating these individual characteristics into the treatment process, personalized medicine aims to provide more effective, precise, and safer healthcare, improving outcomes while minimizing the risk of adverse effects (3).

The emergence of synthetic biology and genomics has significantly advanced the potential of personalized medicine, marking the beginning

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of a new era in healthcare. Synthetic biology, which involves the design and construction of new biological parts, devices, and systems, is transforming the way we understand and treat disease (4). By enabling the manipulation of genetic material with unprecedented precision, synthetic biology offers innovative solutions to a range of medical challenges. It provides the tools to design tailored genetic systems that can address specific genetic mutations, which could lead to more effective treatments for genetic disorders. Additionally, synthetic biology has opened new possibilities in the development of personalized vaccines, which can be designed to target an individual's specific immune responses (5). Furthermore, advancements in diagnostic technologies, fueled by the integration of genomics and synthetic biology, promise to offer more accurate and rapid methods for detecting diseases at their earliest stages (6).

The combination of genomics and synthetic biology is particularly transformative in the field of personalized medicine, allowing for highly targeted therapies that are customized to the molecular characteristics of each patient's disease. For example, in oncology, genetic profiling of tumors can identify mutations and alterations specific to an individual's cancer, enabling the development of targeted therapies that attack cancer cells without harming healthy tissue. Similarly, personalized immunotherapies, such as CAR-T cell therapy, are being developed to harness and enhance a patient's immune system to specifically target and destroy cancer cells (7).

Despite the immense potential of personalized medicine, these advancements are not without their challenges. Ethical concerns surrounding privacy, genetic data sharing, and the accessibility of personalized treatments are critical issues that need to be addressed as these technologies continue to evolve (8). Additionally, the complexity of designing and implementing these advanced therapies in clinical settings presents logistical and regulatory hurdles that must be overcome to ensure that patients benefit from these innovations safely and equitably.

This paper explores how synthetic biology is revolutionizing personalized medicine by examining the technological advancements driving this transformation, the therapeutic applications that are already emerging, and the ethical considerations that accompany these groundbreaking developments. By delving into both the promise and the challenges of this new era in medicine, the paper aims to provide a comprehensive overview of how synthetic biology is reshaping the future of personalized healthcare.

Synthetic Biology and Personalized Medicine: A New Paradigm

The Role of Synthetic Biology in Personalized Medicine

Synthetic biology has introduced a transformative paradigm in medicine, often referred to as "programmable biology," where biological systems and genetic material are engineered and reprogrammed to serve specific therapeutic purposes. This capability represents a profound shift in the way diseases are understood and treated, as it enables the creation of highly customized therapies that are tailored to the individual genetic and molecular makeup of each patient. By manipulating biological systems at the genetic level, synthetic biology empowers clinicians and researchers to address the underlying causes of disease rather than merely treating symptoms.

In personalized medicine, the concept of tailoring treatment to an individual's unique genetic profile is becoming increasingly important. Traditional medical approaches often rely on generalized treatments that target broad mechanisms of disease, which may not be equally effective for all patients. In contrast, synthetic biology allows for the precise engineering of genetic circuits, biosensors, and gene-editing technologies to directly address the specific genetic mutations and molecular pathways driving a patient's disease. This results in therapies that are not only more effective but also carry fewer risks of adverse effects, as they can be fine-tuned to target the disease at its molecular source. The ability to design and deploy personalized treatments offers a profound advantage, particularly in fields like oncology, genetic disorders, and immunology, where individual patient responses can vary widely.

As a key enabler of personalized medicine, synthetic biology facilitates the creation of patient-specific therapies that provide more accurate, safer, and potentially curative options. The intersection of synthetic biology with genomics, bioinformatics, and advanced diagnostics is unlocking new avenues for treating diseases that were previously considered intractable or difficult to manage, further advancing the shift toward precision medicine.

Key Technologies Driving Personalized Medicine

Several groundbreaking advancements in synthetic biology are revolutionizing the way personalized medicine is conceptualized and delivered. These technologies allow for the precise manipulation of genetic material, enabling the development of highly personalized treatments that are tailored to the genetic and molecular profiles of individual patients. Key technologies driving this revolution include:

1. CRISPR-Cas9 and Genome Editing

The advent of CRISPR-Cas9 technology has marked a watershed moment in the field of genetics and personalized medicine. CRISPR-Cas9 allows for

precise, targeted editing of genes at specific locations in the genome, offering unprecedented accuracy and flexibility in correcting genetic mutations responsible for various diseases. This tool works by harnessing a naturally occurring bacterial defense mechanism to cut DNA at specific sites, which can then be modified or replaced to correct genetic defects.

For example, CRISPR has shown tremendous promise in treating genetic disorders like sickle cell anemia, where the underlying genetic mutation can be corrected in the patient's cells, offering the potential for a permanent cure. This capability extends to a wide range of diseases caused by single-gene mutations, such as cystic fibrosis and Duchenne muscular dystrophy, where personalized gene therapies could provide patients with individualized, life-changing treatment options. In the realm of cancer, CRISPR technology can be used to modify immune cells, enhancing their ability to target and destroy cancer cells more effectively, offering new prospects for personalized immunotherapies.

2. Gene Synthesis and Custom Pathways

Gene synthesis and the creation of custom metabolic pathways is another powerful tool enabled by synthetic biology. By synthesizing entire genes or creating new biochemical pathways from scratch, scientists can engineer biological systems that perform specific, therapeutic functions. These custom-designed pathways can be used to modify the metabolic processes within a patient's cells, offering potential treatments for a variety of conditions, including metabolic diseases, cancer, and neurodegenerative disorders.

For instance, synthetic biologists can design therapeutic enzymes that replace faulty ones in patients with genetic metabolic disorders, or they can reprogram cells to produce beneficial molecules such as proteins or hormones that the body cannot produce on its own. Additionally, the ability to construct artificial biosynthetic pathways enables the creation of personalized treatments for diseases such as cancer, where novel pathways can be engineered to specifically target cancerous cells, minimizing harm to healthy tissues.

The combination of gene synthesis with advanced techniques in genome editing allows for the construction of personalized therapies that are tailored to the individual biochemical environment of each patient, improving both efficacy and safety.

3. Cellular and Gene Therapy

Gene therapy, driven by synthetic biology, is poised to become a cornerstone of personalized medicine. This approach involves introducing modified genes into a patient's cells to correct genetic defects or to enable the body to produce therapeutic substances. The rise of gene therapy has been particularly transformative in the treatment of inherited genetic disorders, where traditional methods of treatment were either insufficient or non-existent.

Autologous stem cell therapy, for example, is being employed in personalized cancer treatment. In this method, a patient's stem cells are harvested, genetically modified, and reintroduced into their body to target specific diseases, such as cancer. In the case of personalized cancer immunotherapy, immune cells, such as T cells, are engineered to recognize and attack cancer cells more effectively. The result is a highly personalized treatment that harnesses the body's immune system to fight the disease, offering the potential for more effective and durable responses compared to traditional treatments like chemotherapy or radiation.

Gene therapies are also being developed for rare genetic diseases, such as spinal muscular atrophy (9), where the delivery of a functional copy of a missing or defective gene can dramatically improve patient outcomes. The use of viral vectors or CRISPR-based techniques to deliver gene therapies is rapidly advancing, enabling more targeted and efficient treatments with reduced side effects.

4. Synthetic Biology-Driven Diagnostics

Effective personalized medicine relies not only on precise treatments but also on equally precise diagnostics. The ability to accurately diagnose diseases based on an individual's unique genetic and molecular profile is essential for tailoring therapies that are most likely to be effective. Synthetic biology plays a crucial role in the development of highly sensitive diagnostic tools that can identify specific genetic markers, proteins, or other biomarkers associated with particular diseases (10).

Innovative biosensors, developed using synthetic biology techniques, can detect and measure minute levels of disease markers in a patient's body. These tools have the potential to identify diseases at their earliest stages, enabling early intervention and better outcomes. Additionally, synthetic biology-driven diagnostics can be used to monitor how a patient is responding to personalized therapy, allowing for real-time adjustments to the treatment plan based on the patient's response (11). This level of precision in monitoring and diagnosing disease is a key component of personalized medicine, ensuring that therapies remain tailored and optimized throughout the course of treatment (12).

Moreover, synthetic biology is enabling the development of portable and cost-effective diagnostic platforms, which could make personalized medicine more accessible to patients in low-resource settings. These advances may bring personalized healthcare to a broader population, making it more inclusive and

equitable (13).

These technological advancements in synthetic biology are ushering in a new era of personalized medicine, providing innovative tools to tailor treatments and diagnostics to the individual genetic and molecular profile of each patient. As these technologies continue to evolve, they hold the promise of revolutionizing the way diseases are treated, offering more effective, safer, and customized healthcare solutions that address the unique needs of every patient (14).

Applications of Synthetic Biology in Personalized Medicine

Gene Therapy and Editing

Gene therapy is a cornerstone of personalized medicine, and synthetic biology plays a pivotal role in its development. In traditional gene therapy, genes are introduced into a patient's cells to correct genetic defects or treat diseases (15). Synthetic biology enables the engineering of precise gene-editing tools, such as CRISPR, that can target specific genes and correct mutations with higher efficiency and accuracy. For instance, sickle cell disease is caused by a mutation in the hemoglobin gene (16). Using CRISPR-Cas9, scientists can edit the genome of hematopoietic stem cells, enabling the correction of the mutation in a patient's blood cells. This can potentially cure the patient by restoring normal hemoglobin function (17). Such gene-editing technologies are central to developing individualized treatments for genetic diseases, where the patient's specific genetic defects can be addressed. Another example is cystic fibrosis, which results from mutations in the CFTR gene (18). Synthetic biology techniques, including gene delivery systems, could be used to deliver a healthy version of the CFTR gene to lung cells, providing personalized treatments for affected individuals (19).

Personalized Cancer Therapy

Synthetic biology holds immense promise in the treatment of cancer. Personalized cancer therapies are designed based on the unique genetic makeup of a patient's tumor. Unlike traditional cancer treatments, which are based on general protocols, personalized therapies focus on the specific mutations and molecular markers present in the patient's cancer cells (20).

One approach involves CAR-T (Chimeric Antigen Receptor T-cell) therapy, where a patient's T-cells are genetically engineered to target cancer cells. Synthetic biology tools are used to design the receptors on T-cells that recognize and bind to tumor-specific antigens (21). This allows for a personalized approach, as the therapy is customized to the specific cancer type and tumor characteristics of each patient. Additionally, synthetic biology is used to engineer microbes that can target and destroy cancer cells (22). For instance, synthetic bacteria can be programmed to recognize cancerous cells and deliver therapeutic payloads, such as toxins or immune-stimulating compounds, directly to the tumor site. This precision medicine approach reduces the risk of damage to healthy tissues, a common side effect of traditional cancer treatments (23).

Personalized Vaccines

Vaccines are typically designed to elicit a broad immune response. However, personalized vaccines are tailored to an individual's genetic profile, enhancing their effectiveness (24). Synthetic biology can be employed to design synthetic vaccines based on the specific viral or bacterial strains that a person is most likely to encounter, taking into account their genetic makeup and immune system response (25).

Personalized cancer vaccines are another area of active research. These vaccines are tailored to target specific mutations or antigens expressed by an individual's cancer cells. Synthetic biology allows for the design of synthetic peptides or genetic materials that can stimulate an immune response against these tumor-specific markers. For instance, a synthetic vaccine could be designed for a patient with melanoma, targeting the specific mutations found in their cancer cells (26).

Personalized Diagnostic Systems

Accurate and early diagnosis is critical for the success of personalized medicine. Synthetic biology enables the development of highly sensitive and specific diagnostic tools, such as biosensors and genetic tests, that can detect disease markers at the molecular level (11). These tools can be tailored to an individual's unique genetic profile, providing more accurate diagnoses and guiding treatment decisions (27). For example, gene expression profiling can be used to identify biomarkers that are associated with specific diseases. These biomarkers can be used to develop diagnostic tests that can detect the presence of disease before symptoms arise, allowing for early intervention and personalized treatment (28, 29).

Synthetic Biology and Microbiome Engineering

The human microbiome— the trillions of microorganisms living in and on our bodies— plays a significant role in health and disease. Synthetic biology offers the potential to engineer the microbiome to benefit personalized medicine (30). By modifying the genetic makeup of microbial communities, researchers can develop personalized treatments that influence the microbiome in ways that improve health outcomes (31).

For example, the microbiome is known to affect drug metabolism, immune responses, and disease susceptibility. Synthetic biology can be used to design engineered microbes that produce therapeutic molecules or regulate the immune system. These engineered microbes could be delivered as probiotics to restore balance to the microbiome in individuals with specific health conditions, such as inflammatory bowel disease or metabolic disorders (32).

Challenges and Ethical Considerations in Personalized Medicine

While the integration of synthetic biology into personalized medicine holds tremendous promise, there are still several significant challenges that need to be addressed (33). These challenges encompass both technical and ethical issues, each of which plays a crucial role in the future development, accessibility, and societal acceptance of these advanced therapies (34). Overcoming these barriers is essential for ensuring that the full potential of personalized medicine can be realized in a safe, ethical, and equitable manner.

Technical Challenges

One of the primary technical hurdles is the complexity of designing and delivering gene therapies effectively. Gene therapies require precise modification of an individual's genetic material, and achieving this with a high degree of accuracy remains a challenging task (35). Technologies like CRISPR-Cas9 have shown remarkable potential in editing genes, but there are still concerns regarding off-target effects, where unintended parts of the genome may be altered, leading to unintended consequences (36). Moreover, ensuring the safe and efficient delivery of gene-editing tools to the right cells in the body is a critical challenge, as current delivery systems, such as viral vectors, can be inefficient and may trigger immune responses. Overcoming these technical limitations is crucial for advancing the field of gene therapy and ensuring its success in clinical applications (37).

Another challenge lies in the scalability and production of personalized therapies. As treatments are tailored to individual patients, the process of developing and manufacturing these therapies becomes highly individualized, which can make it difficult to produce them in large quantities (38). The high degree of customization required for each patient increases the time and cost involved in the development of treatments, raising concerns about the overall feasibility of delivering personalized medicine on a large scale (1).

Safety Concerns

Safety remains one of the most significant concerns in the use of synthetic biology in personalized medicine. Gene-editing technologies, such as CRISPR, present unique risks, particularly when it comes to editing the human genome (39). Unintended genetic changes or "off-target" effects could have serious health consequences, including the potential to activate cancer-causing genes or disrupt essential cellular functions. Although improvements in gene-editing technologies are reducing the risk of such unintended effects, further research and safeguards are required to ensure the long-term safety of these therapies (40).

Another safety concern is the use of synthetic microorganisms or engineered microbes in therapeutic applications. While genetically engineered bacteria or viruses may be used to deliver drugs or genes to specific areas of the body, there is the risk that these organisms may behave unpredictably, causing harm to the patient or triggering immune reactions (41). Rigorous testing and monitoring protocols must be established to ensure that these engineered organisms are safe and do not pose unforeseen risks (42).

Accessibility and Cost

The high cost of personalized therapies presents another challenge. Many of the cutting-edge treatments made possible by synthetic biology, such as gene therapies, are expensive to develop, produce, and administer (43). These costs are often passed on to patients, making access to personalized medicine a significant issue for many individuals, particularly those in low- and middle-income countries (44). The affordability of these therapies is a major concern, as the potential benefits of personalized treatments may be out of reach for large segments of the global population (45).

In addition to cost, there are also concerns about the availability of specialized infrastructure and expertise required to deliver these therapies. For example, gene therapies often require sophisticated diagnostic tools and facilities capable of monitoring patient responses to treatment (46). The lack of such resources in resource-poor settings could exacerbate existing healthcare disparities, leaving certain populations without access to potentially life-saving treatments.

Ethical Considerations

Alongside the technical and logistical challenges, several ethical issues must be addressed as synthetic biology is integrated into personalized medicine. These ethical concerns are pivotal in guiding the development, regulation, and public acceptance of new treatments (47). Some of the key ethical issues include:

Genetic Privacy and Data Security

The use of genetic data is at the core of personalized medicine, as treatments are increasingly based on an individual's genetic makeup. However, the collection, storage, and sharing of such sensitive information raise significant privacy concerns (48). Genetic data can reveal deeply personal information about an individual's health, ancestry, and predisposition to various diseases, making it a potential target for misuse. Ensuring that individuals' genetic data is protected from unauthorized access, hacking, or exploitation is critical to maintaining trust in personalized medicine (49). Strong data protection laws, as well as secure storage and encryption technologies, are essential to safeguard genetic information (50).

Additionally, there are concerns about how genetic data might be used by third parties, such as insurance companies or employers, which could potentially lead to discrimination based on genetic predispositions. Ethical guidelines and legal frameworks must be established to ensure that individuals' genetic data is used responsibly and for the benefit of patients, without infringing upon their privacy or rights (51).

Equity and Access

One of the most pressing ethical concerns in personalized medicine is ensuring that these advanced therapies are accessible to all, regardless of socioeconomic status or geographic location (52). The high cost of personalized treatments poses a significant barrier to access, and there is the risk that only wealthier individuals or those living in developed countries will benefit from these innovations (53). In a world where healthcare disparities already exist, the advent of personalized medicine could exacerbate inequities if not carefully managed.

There is a critical need for policies that ensure equal access to personalized treatments across all populations. This includes addressing the affordability of therapies, as well as improving healthcare infrastructure in underserved areas (54). Efforts must be made to ensure that personalized medicine is not a privilege for the few but a benefit for all, with equitable distribution of these therapies across diverse social, economic, and geographical groups (55).

Regulation of Gene Editing

The ability to edit the human genome using technologies like CRISPR-Cas9 presents both immense opportunities and significant risks. While gene editing holds the potential to cure genetic diseases and improve patient outcomes, it also raises concerns about unintended consequences and the ethical implications of altering the human germline (the genetic material passed on to future generations) (56). The modification of the human genome could have long-lasting effects, not only on the individual receiving treatment but also on their descendants (9).

As gene-editing technologies continue to evolve, regulatory frameworks must be established to ensure that they are used responsibly. These regulations must include guidelines for conducting research, clinical trials, and therapeutic applications, as well as oversight mechanisms to monitor the long-term effects of gene editing. International consensus on the ethical boundaries of gene editing will be crucial in preventing misuse or overly permissive policies that could lead to harmful genetic modifications (57).

Designer Babies

One of the most controversial ethical concerns related to gene editing is the potential for creating "designer babies." This term refers to the possibility of editing human embryos to select desirable traits, such as intelligence, physical appearance, or disease resistance. While the technology to edit embryos exists, the ethical questions surrounding this practice are profound (58). Many worry about the social and moral implications of allowing parents or clinicians to decide on genetic traits for their children, potentially leading to a form of genetic elitism or discrimination (59). The idea of creating "designer babies" raises concerns about the ethics of genetic enhancement, as well as the potential for societal pressures to influence genetic choices.

Conclusion

Synthetic biology is transforming personalized medicine, enabling highly targeted treatments tailored to individual genetic, molecular, and environmental factors. Technologies like CRISPR-Cas9 and gene synthesis offer the potential for more effective therapies with fewer side effects, revolutionizing the treatment of genetic disorders and cancers. However, challenges such as technical complexities, safety concerns, high costs, and ethical issues around genetic privacy, access, and enhancement remain. To fully realize its potential, ongoing research, regulatory frameworks, and a commitment to equity are essential. With these efforts, personalized medicine can become widely accessible, improving patient outcomes and advancing global health.

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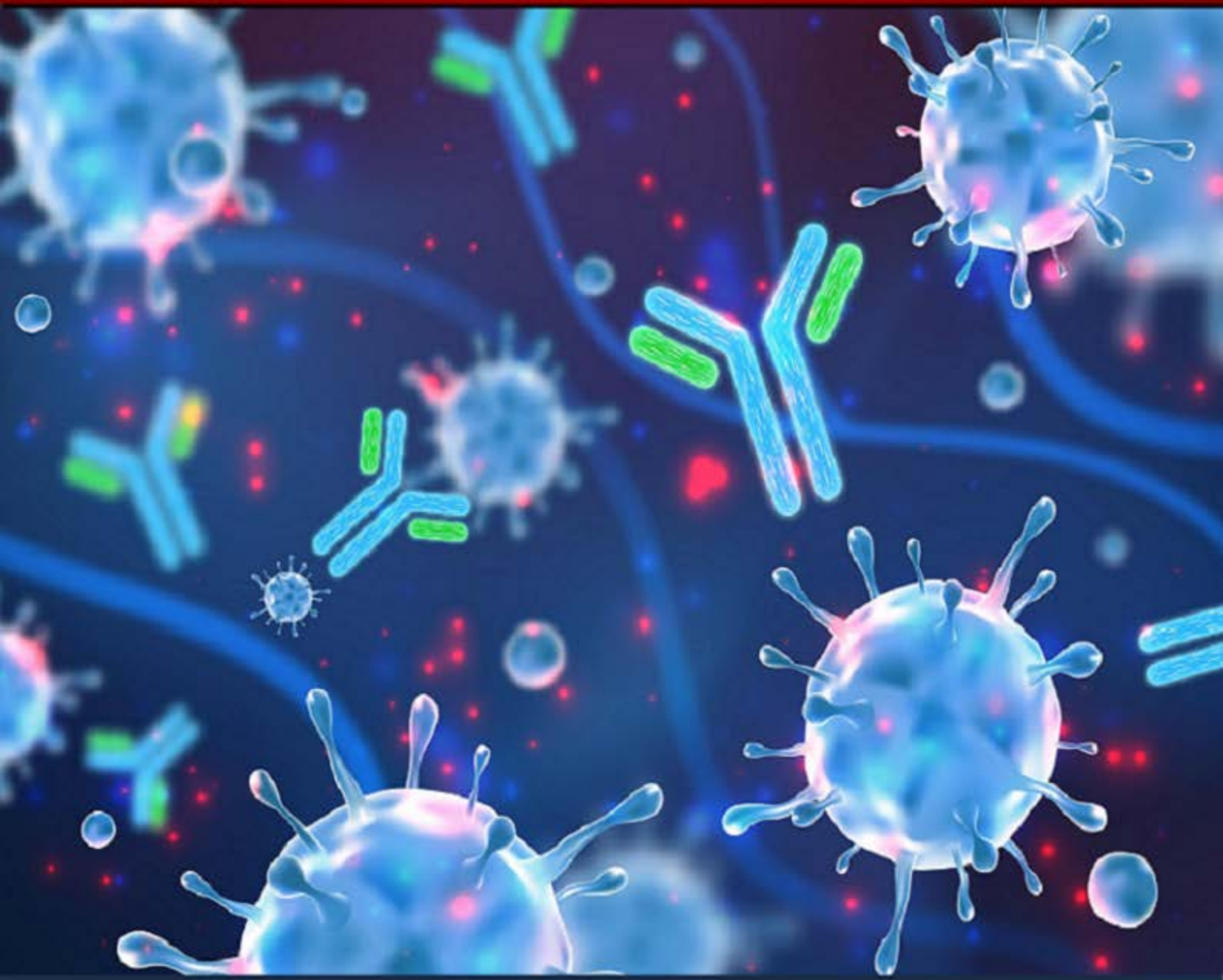

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