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Artificial Intelligence in Personalized Breast Cancer Medicine: Current Trends and Future Directions

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Abstract:

Breast cancer is one of the most common types of cancer among women. This disease poses serious clinical challenges due to its variable response to treatment and biological heterogeneity. Therefore, conventional therapies fail to complement the characteristics of specific tumors, limiting the effectiveness of treatment even in personalized therapies. In recent years, powerful tools such as artificial intelligence(AI), have emerged to advance personalized medicine, especially in the field of breast cancer, with the help of which complex biomedical data can be better analyzed.

In this article, we aim to provide a comprehensive review of the impact of AI on early detection, prognosis and recurrence assessment, response prediction, biomarker discovery, and clinical decision-making in breast cancer. We will also explore how AI-based imaging analysis can help improve diagnostic accuracy, while integrated multi-omics models can enhance treatment decision-making and risk stratification. Emerging approaches such as explainable AI, radiogenomics, and AI-based multi-omics integration are also highlighted as key drivers in this field.

Despite encouraging results, significant challenges remain, including data heterogeneity, limited external and prospective validation, algorithmic bias, interpretability concerns, and ethical and regulatory barriers. Addressing these limitations through standardized data protocols, transparent and explainable models, and multi-center validation studies is essential for safe and equitable implementation. Overall, AI holds substantial potential to transform breast cancer management toward a more predictive, preventive, and patient-centered paradigm, provided that technological innovation is aligned with robust clinical validation and interdisciplinary collaboration.

Keywords: Artificial Intelligence, Breast Cancer, Personalized Medicine, Machine Learning, Multi-omics Integration.

INTRODUCTION

It is perhaps safe to say that breast cancer is one of the most common cancers in women, and it has emerged as one of the leading causes of death in the world (8, 61). Despite extensive advances in treatment, early detection, and clinical pathways, the biological heterogeneity of breast tumors has made traditional clinical pathways unable to provide an appropriate therapeutic response for individuals (3, 23). Precision medicine seeks to overcome these limitations by tailoring medical care based on patient-specific clinical, imaging, and

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molecular profiles (2, 62). However, the analysis of high-dimensional biomedical datasets poses significant challenges for conventional statistical methods due to scale, complexity, and non-linear relationships (16, 21). Artificial intelligence (AI), driven by advancements in machine learning and deep learning, has recently become a pivotal tool in oncology for deciphering complex data patterns and aiding clinical decision-making (1, 5). Comparative studies demonstrate that AI models surpass conventional methods in medical imaging by enhancing diagnostic sensitivity and specificity, refining risk stratification, and offering superior accuracy in predicting therapeutic outcomes (4, 58). Nevertheless, the seamless integration of AI into daily clinical workflows is hindered by challenges such as model opacity (lack of interpretability), the need for standardized data, and the necessity for rigorous external validation (10, 29). Consequently, a thorough examination of AI's role in precision breast cancer medicine is crucial to understanding how these innovations can be effectively translated into patient care. In the realm of breast cancer management, AI has shown immense potential in improving early detection, prognostic assessment, and tailored treatment planning (22, 66). Specifically, AI-driven algorithms have demonstrated superior performance over standard interpretative techniques in mammography and MRI, facilitating more consistent and precise tumor identification (32, 67). Beyond imaging, the application of AI in synthesizing multi-omics data has facilitated the identification of novel biomarkers and significantly improved predictive models for treatment efficacy and disease recurrence (13, 35). For instance, systematic reviews report that AI-based recurrence prediction models, trained on clinical and imaging cohorts, can stratify patients by risk with higher precision than conventional approaches. Furthermore, explainable AI methods are gaining attention for improving model transparency and clinical trust, which are crucial for adoption in precision oncology (18, 60). Despite the potential benefits, the broad adoption of AI-driven personalized medicine faces significant hurdles, including ethical dilemmas, data heterogeneity, and the requirement for extensive, annotated datasets (53, 29).

Epidemiology and Clinical Challenges of Breast Cancer

Globally, breast cancer continues to be the leading cause of cancer diagnosis among women, and its prevalence is increasing in many areas despite sustained public health initiatives (61). According to recent global estimates, breast cancer accounts for millions of new cases annually, with substantial geographic variation in rates due to differences in screening practices, lifestyle factors, and healthcare access (8). High-income countries often report higher age-standardized incidence rates, reflecting broad screening and early detection, whereas low- and middle-income regions bear disproportionately high mortality relative to incidence, highlighting disparities in care (8, 61). Risk factors for breast cancer encompass both non-modifiable elements such as age and genetic predisposition, and modifiable influences including obesity, alcohol consumption, and physical inactivity (3, 12). Moreover, epidemiological trends show an increasing burden of breast cancer among younger women and diverse populations, complicating traditional risk stratification and screening frameworks (66). The clinical phenotype is inherently linked to the underlying molecular subtype of the tumor. Notably, aggressive variants such as triple-negative breast cancer impose significant therapeutic difficulties, primarily due to the lack of viable targeted treatment strategies. These trends underscore the dynamic epidemiology of the disease and the need for adaptive clinical strategies that address both prevention and personalized treatment access. Consequently, understanding population-level patterns and underlying determinants of breast cancer remains foundational for advancing precision oncology. Despite improvements in early detection and therapeutic advances over recent decades, breast cancer continues to present significant clinical challenges that hinder optimal outcomes (11, 23). A pivotal challenge lies in the substantial heterogeneity of breast cancer, both molecularly and clinically. The disease encompasses distinct molecular entities, notably hormone receptor-positive (HR+), HER2-positive, and triple-negative subtypes, which display diverse growth dynamics, varying responses to therapeutic interventions, and differing prognostic outcomes (12, 31). This biological diversity complicates one-size-fits-all treatment paradigms and underscores the need for tailored approaches that integrate molecular profiling into clinical decision-making. Furthermore, disparities in healthcare infrastructure and resource availability contribute to delayed diagnoses and limited treatment options in many regions, exacerbating mortality rates and inequities in survival (11, 61). Even within high-resource settings, clinical challenges persist, including the management of advanced and metastatic disease, treatment resistance, and the balance between therapeutic efficacy and quality of life. Psychological, social, and economic burdens also add layers of complexity to patient care, requiring multidisciplinary support beyond conventional medical interventions. Addressing these multifaceted challenges necessitates an integrated approach that combines better epidemiologic insights, personalized treatment frameworks, and equitable healthcare delivery systems worldwide.

Principles of Personalized Medicine in Oncology Precision oncology marks a significant departure from conventional 'one-size-fits-all' strategies, adopting a framework that customizes diagnostic and therapeutic interventions according to the unique genetic, molecular, and environmental profile of each patient (62, 28). At its foundation, this approach synthesizes genomic profiling, biomarker discovery, and targeted treatments to align specific tumor characteristics with the most appropriate clinical management plans (12, 35). The advent of advanced technologies, such as next-generation sequencing (NGS) and liquid biopsies, has revolutionized the analysis of tumor DNA by offering rapid, economical, and clinically actionable data (31, 13). These tools empower clinicians to detect driver mutations, stratify patients based on risk levels, and prescribe therapies with a higher probability of success for specific molecular subtypes. Consequently, personalized oncology enhances therapeutic efficacy while minimizing unnecessary toxicity by avoiding treatments that are unlikely to benefit individual patients (28, 17). By prioritizing the molecular drivers of tumor behavior, this paradigm facilitates more informed decision-making and allows for dynamic adjustments in treatment protocols over time. Although traditional oncology has historically depended on histopathological classification, personalized medicine increasingly relies on multi-omics integration including genomics, proteomics, and transcriptomics to elucidate the complex biology of the disease (42, 21). Ultimately, the goal of this personalized approach is to improve patient survival and quality of life by tailoring therapeutic interventions to the specific biological characteristics of the disease (65, 62). Although personalized oncology holds significant promise, its clinical implementation is hindered by various practical and operational barriers that require resolution to maximize its therapeutic benefits (53, 29). Tumor heterogeneity, both within an individual tumor and between patients, complicates the identification of reliable biomarkers and predictive signatures, often leading to variable responses to targeted therapies (16, 36). Furthermore, ethical dilemmas concerning genetic testing, data security, and the fair distribution of advanced diagnostic and therapeutic tools significantly shape the adoption of these technologies in varied clinical environment (53, 29). In addition, high costs associated with comprehensive genomic profiling and targeted agents can limit availability, particularly in low-resource environments, reinforcing disparities in cancer care outcomes (11, 61). Another core challenge lies in integrating multi-disciplinary expertise and complex bioinformatic analyses into routine clinical workflows, requiring specialized infrastructure and trained personnel (9, 27). Furthermore, while molecular tumor boards and decision-support systems can help interpret genomic data, standardized guidelines for translating molecular insights into actionable treatment recommendations are still evolving (15, 54). Addressing these obstacles will be critical to extending the benefits of personalized medicine beyond specialized centers and harmonizing precision oncology with broader healthcare delivery. Continued research efforts, collaborative networks, and policy frameworks are essential to support equitable, evidence-based implementation of personalized cancer care (54, 63).

Overview of Artificial Intelligence in Healthcare

The evolution of artificial intelligence (AI) in healthcare has swiftly progressed from theoretical exploration to a pivotal, transformative paradigm, fundamentally altering clinical operations and patient care outcomes (17). Central to this advancement is the synthesis of machine learning (ML), deep learning (DL), and natural language processing (NLP) techniques, which enable the extraction of subtle, non-obvious patterns from complex biomedical datasets that exceed human analytical capabilities (6). These computational approaches have demonstrated considerable success in diagnostic imaging, including the detection of pathological features in radiographs, MRI, and CT scans, leading to enhanced accuracy and reduced time to diagnosis compared to traditional methods (22). Beyond imaging, AI systems contribute to predictive analytics for disease progression, automated triage, and real-time monitoring of patient health, thereby facilitating proactive care strategies. Crucially, AI-driven decision support tools are capable of synthesizing diverse clinical inputs including electronic health records (EHRs), genomic profiles, and real-time biometric metrics to generate precision therapeutic strategies tailored to individual patient vulnerabilities (15). Although the capacity of intelligent systems to accelerate clinical operations and optimize resource distribution is well-documented, significant hurdles regarding information confidentiality, system interoperability, and modeling inequities continue to impede widespread adoption (27). Nevertheless, as ongoing research aims to enhance algorithmic robustness and mitigate normative constraints, the widespread adoption of intelligent tools in medical practice holds the promise of substantially elevating both operational efficiency and the standard of patient care (34). A summary of key studies regarding AI applications in healthcare between 2020 and 2025 is presented in (Table 1).

The widespread adoption of AI-driven solutions in healthcare mandates a balanced approach that harmonizes technological innovation with stringent ethical oversight and clinical validation (53). A pivotal strength of artificial intelligence in modern

Table1. Key AI in Healthcare Studies (2020–2025) – Updated with Real References.

Study Title	AI Method	Data Type	Key Findings/ Achievements	Advantages	Limitations	Reference
Use of Artificial Intelligence in Healthcare: A Comprehensive Review	ML & DL	Clinical, Imaging, Genomics	Broad-spectrum AI utility: from detection to therapy	Broad scope, identifies trends	General review, not disease-specific	Rob,M.(2025)
AI Applications in Healthcare: Advances in Cancer, Diabetes, and Epidemiology	2025	ML, DL	Multi-modal (clinical, imaging, lab)	Highlights AI use in cancer prediction and patient monitoring	Multi-disease applicability	Limited experimental validation
Use of Artificial Intelligence in Healthcare: A Review	2025	ML, DL	Clinical, Health Records	Discusses adoption, challenges, and future trends	Insight into real-world implementation	Broad focus, lacks quantitative metrics
Role of AI in Healthcare Settings: A Systematic Review	2025	ML Ensemble	Clinical, Lab, Imaging	Improves decision-making and workflow efficiency	Evidence-based insights	Small sample sizes in included studies
AI in the Health Sector: Skills for Future Professionals	2025	ML, NLP	Clinical, Training	Identifies key skills for healthcare professionals to work with AI	Focus on workforce readiness	Not focused on clinical outcomes
AI for Early Cancer Detection	2024	CNN + ML	Imaging (Mammography, MRI)	Enhanced diagnostic accuracy for breast and lung cancer	Early detection, improves survival rates	Requires large labeled datasets
Predictive Analytics for Patient Outcomes Using AI	2024	DL + Ensemble ML	Clinical, Lab, Multi-omics	Predicts treatment response and risk of adverse events	Supports personalized medicine	Computationally intensive

medicine is its capacity to synthesize massive, multi-source clinical datasets. This proficiency facilitates advanced prognostic analytics, which empower clinicians to forecast patient trajectories with high precision, customize therapeutic interventions based on individual profiles, and optimize prophylactic health strategies (5). For instance, AI-based tools can identify subtle trends in longitudinal health records that are associated with disease onset or progression, helping clinicians tailor interventions before symptoms escalate (19). Moreover, AI accelerates research workflows by facilitating drug discovery, optimizing clinical trial design, and enhancing molecular profiling, which collectively support the advancement of precision medicine (37). The convergence of AI with telehealth infrastructures and remote monitoring ecosystems allows intelligent care models to transcend physical hospital boundaries, thereby extending high-quality medical support into community and home-based environments (39). However, achieving widespread clinical utility requires overcoming substantial barriers, such as ensuring algorithmic transparency, addressing inequities in data representation, and establishing standardized validation frameworks for AI applications (18). Sustained collaborative inquiry and stringent regulatory frameworks are paramount for translating the theoretical promise of artificial intelligence into tangible clinical benefits on a global scale (30).

AI Algorithms and Techniques in Breast Cancer

Artificial intelligence (AI) frameworks employ sophisticated computational methodologies to decode complex biomedical patterns, significantly enhancing breast cancer diagnostic precision and management protocols. Traditional machine learning classifiers specifically support vector machines (SVM), random forests, and neural networks have been extensively deployed to differentiate benign from malignant tumors using clinical and imaging records, frequently surpassing conventional statistical approaches in both accuracy and computational speed (31). Within this landscape, deep learning (DL), which mimics the hierarchical information processing of the human cerebral cortex, has emerged as a dominant paradigm due to its capacity for autonomous feature extraction from raw data, thereby obviating the need for manual engineering. Notably, convolutional neural networks (CNNs) have demonstrated superior efficacy in analyzing mammographic, sonographic, and magnetic resonance imaging (MRI) scans, yielding high sensitivity and specificity in lesion detection (32). Furthermore, emerging transformer-based architectures are being investigated to synthesize multi-view and multi-scale data, improving diagnostic robustness. Hybrid models that integrate classical machine learning with deep learning, or leverage transfer learning, have also shown promise, particularly in data-scarce environments. To address the 'black-box' opacity of these models and foster clinical trust, explainable AI (XAI) frameworks, such as SHAP (Shapley Additive Explanations), are increasingly adopted to enhance model interpretability. However, concerns regarding generalizability across diverse patient populations and clinical settings persist, emphasizing the necessity for rigorous validation and standardized benchmarking of AI algorithms in oncology (15).

Recent advancements in breast cancer AI research have shifted focus from mere predictive accuracy toward clinical utility and interpretability. Transfer learning, which leverages pre-trained models for specific imaging tasks, has proven effective in mitigating the reliance on extensive labeled datasets while sustaining high diagnostic performance, particularly within convolutional frameworks like ResNet and DenseNet (45). Similarly, ensemble methods that aggregate predictions from multiple algorithms have demonstrated enhanced robustness and reduced overfitting across heterogeneous data sources. To bridge the gap between algorithmic output and clinical decision-making, explainability tools such as model-agnostic interpreters like LIME and SHAP are being adopted to clarify algorithmic logic, a prerequisite for establishing clinical trust and ethical compliance (18). Furthermore, sophisticated recurrent and transformer-based architectures are currently under investigation for processing sequential and multimodal inputs, such as longitudinal time-series data from electronic health records, thereby extending AI capabilities beyond static image analysis. The incorporation of federated learning paradigms also facilitates collaborative model development across institutions while preserving patient privacy, thus addressing critical data governance issues (27). Despite these technical strides, rigorous validation across large, diverse clinical cohorts remains indispensable to guarantee equitable performance and mitigate bias before these systems can be safely integrated into routine clinical workflows (6).

AI in Early Detection of Breast Cancer

Improving clinical outcomes and mitigating mortality rates in breast cancer are inextricably linked to early detection, a domain where artificial intelligence has emerged as a transformative catalyst (2). Within this context, machine learning and deep learning frameworks, particularly convolutional neural networks (CNNs), have become instrumental in the analysis of mammography, ultrasound, and MRI scans, demonstrating enhanced sensitivity for early-stage malignant lesion identification compared to conventional radiological standards (38). The deployment of AI-driven triage protocols facilitates the automated flagging of suspicious regions, thereby alleviating cognitive burden on radiologists while minimizing inter-observer variability across diverse healthcare settings. Furthermore, the capacity of these systems to harmonize heterogeneous data sources such as patient histories, genetic markers, and prior imaging records enables more precise risk stratification and personalized screening intervals (66). At the population level, the integration of AI into mammography workflows has been correlated with increased detection rates of early-stage tumors and a concomitant reduction in false-positive findings, underscoring its dual contribution to both patient care quality and systemic efficiency (58). However, the generalizability of these models remains contingent upon the availability of high-quality, diverse, and large-scale training datasets, presenting a significant hurdle for widespread implementation. Overcoming challenges related to class imbalance, image artifacts, and protocol heterogeneity is essential for robust clinical deployment. Ultimately, AI is envisioned not as a replacement for radiologists, but as an adjunctive tool that augments human judgment, paving the way for a more efficient and accurate early detection paradigm (26).

Optimizing surveillance protocols and selecting supplementary imaging modalities for susceptible individuals necessitates a more precise identification of high-risk groups, a task where artificial intelligence plays a pivotal role. The field of radiomics, by leveraging quantitative data mined from medical imagery and integrating it with intelligent algorithms, can unveil subtle textural and morphological precursors of malignancy that often precede clinically palpable lesions (38). The precision of screening recommendations is significantly augmented by synthesizing individual risk variables such as age, familial history, and hormonal status with AI-driven imaging predictions. Evidence derived from multi-institutional studies indicates that AI-based risk stratification strategies exhibit superior efficacy compared to traditional tools like the Gail or Tyrer-Cuzick calculators, particularly when applied to heterogeneous and diverse populations (19). Moreover, AI can assist in reducing disparities in breast cancer screening by standardizing image interpretation and decision-making, minimizing observer variability that often affects early detection in community clinics and low-resource settings (66). Despite these advancements, regulatory approval, ethical considerations, and clinician trust remain key factors influencing adoption in routine clinical practice (53). Continuous retraining with diverse datasets, performance monitoring, and transparent reporting of algorithmic limitations are essential for safe deployment (56).

The diagnostic scope of artificial intelligence has expanded well beyond traditional radiological modalities to encompass the analysis of genetic profiles and molecular signatures. Innovative approaches utilizing non-invasive blood-based assays, particularly those analyzing circulating tumor DNA (ctDNA) alongside other biomarkers, have demonstrated significant potential in detecting microscopic disease remnants and predicting early tumor relapse (31). Concurrently, sophisticated algorithms harnessing the convergence of genomics, transcriptomics, and proteomics are capable of unveiling hidden molecular alterations that manifest months or even years before any radiological findings become apparent. This capability lays a robust foundation for truly personalized early detection paradigms (16). Such approaches allow for longitudinal monitoring and rapid intervention, potentially improving survival outcomes. Additionally, AI models can be incorporated into mobile health platforms and telemedicine frameworks to expand screening access, particularly in underserved areas (39). Challenges remain in data harmonization, algorithm interpretability, and establishing standardized clinical thresholds, but ongoing research and prospective trials are addressing these limitations. Collectively, AI-based early detection represents a convergence of imaging, molecular diagnostics, and predictive analytics, offering a transformative potential for breast cancer management (22).

AI in Predicting Treatment Response

Precise forecasting of treatment response serves as the pivotal element in personalized breast cancer management, enabling the alignment of therapeutic protocols with the unique biological and therapeutic profile of each patient's tumor. Recent advancements in artificial intelligence (AI) have demonstrated substantial efficacy in this domain by harnessing multi-modal data streams encompassing histopathology, clinical imaging, and molecular profiles to forecast individualized outcomes with greater reliability than conventional statistical models (47). For instance, interpretable deep learning models applied to digital whole-slide histopathological images have exhibited strong discriminative power in predicting neoadjuvant therapy responses. These models correlate predictive scores with tumor-infiltrating lymphocyte patterns and microenvironmental features, providing biologically relevant insights into treatment efficacy (10). The clinical utility of such AI-driven frameworks lies in their capacity to stratify patients based on their potential therapeutic response, thereby enabling healthcare providers to mitigate futile toxicity in unlikely responders and facilitating a more precise pivot to alternative treatment strategies. Furthermore, integrating radiomic signatures from magnetic resonance imaging (MRI) or mammography with clinical data enhances model granularity, leading to improved stratification of responders versus non-responders (48). Notwithstanding these technological strides, widespread clinical adoption of AI is still constrained by formidable obstacles, particularly regarding model interpretability, generalizability across independent cohorts, and the provision of actionable insights for medical practitioners. Overcoming these limitations is a prerequisite for transitioning predictive algorithms from research environments into integrated diagnostic workflows. Such a transition is critical to enable the precise guidance of therapeutic strategies and, consequently, the optimization of patient clinical trajectories (20).

Although artificial intelligence offers substantial promise in anticipating how breast cancer patients will respond to therapy, rigorous evaluations underscore ongoing methodological and translational barriers. Resolving these issues is a prerequisite for realizing the technology's true clinical value. A broad systematic review of existing models highlighted issues such as limited external validation, data and code unavailability, and under-reporting of demographic variables, which collectively contribute to a high risk of bias in many predictive frameworks (48). Furthermore, although deep learning and machine learning algorithms have achieved remarkable precision in retrospective studies, it is crucial to conduct prospective validations across heterogeneous patient populations. This step is necessary to verify the models' robustness and applicability in varied clinical environments.

Current research efforts also seek to optimize predictive models by incorporating tumor genomics, proteomics, and dynamic changes in longitudinal datasets, which can capture treatment response trajectories with greater fidelity (13). Explainable AI (XAI) methods are increasingly integrated into predictive systems to enhance clinician trust and facilitate interpretation of model decisions, making them more amenable to clinical adoption (18). Subsequent developments in this domain are anticipated to arise from concerted efforts aimed at harmonizing reporting standards, enhancing data interoperability, and embedding AI-generated prognoses into multidisciplinary clinical workflows. Such integration is pivotal for advancing precision medicine and delivering tailored therapeutic regimens to women diagnosed with breast cancer (28).

AI in Prognosis and Recurrence Prediction

Precision in prognostic assessment and recurrence forecasting constitutes the cornerstone of optimal breast cancer care, directly guiding the selection of adjuvant treatments and surveillance protocols. While conventional predictive frameworks predominantly utilize standard clinicopathological parameters specifically tumor dimensions, histological grading, and nodal involvement these traditional metrics frequently fall short of encapsulating the intricate biological heterogeneity that drives disease evolution (8). The integration of multi-modal data including clinical histories, radiomic features, and molecular signatures has positioned Artificial Intelligence (AI), specifically Machine Learning (ML) and Deep Learning (DL) architectures, as a pivotal tool for refining prognostic precision in breast cancer. Empirical evidence from systematic reviews indicates that algorithms such as Support Vector Machines (SVM), Random Forests (RF), and Neural Networks outperform traditional statistical methods in forecasting overall survival and recurrence risk. This superiority stems from their capacity to extract subtle, non-linear patterns from high-dimensional datasets (43). Consequently, these AI-driven frameworks facilitate the stratification of patients into distinct risk tiers, thereby empowering clinicians to tailor follow-up schedules and therapeutic aggressiveness to individual risk profiles. Nevertheless, the clinical translation of these models is currently hindered by a lack of robust external validation across heterogeneous populations, alongside variability in dataset quality and scale, which may compromise model generalizability (50). Despite these limitations, the incorporation of AI into prognostic frameworks remains a promising avenue for advancing precision oncology and enhancing outcome prediction (65).

By deciphering intricate morphological patterns in hematoxylin and eosin (H&E) stained slides that escape human detection, deep learning algorithms have significantly broadened the horizon of AI-driven prognostics. These models can extract rich predictive insights from histopathological images, correlating subtle morphological cues with recurrence risks, thereby surpassing the limitations of conventional grading systems (19). Furthermore, the synergy of radiomic signatures derived from medical imaging with genomic and clinical data has elevated predictive accuracy, enabling a more granular mapping of disease progression and the identification of high-risk individuals (12). To mitigate the risks of overfitting inherent in heterogeneous datasets, ensemble techniques that aggregate outputs from multiple machine learning models have proven effective in stabilizing risk assessments. To address the 'black box' nature of these complex models, Explainable AI (XAI) frameworks like SHAP (Shapley Additive Explanations) are increasingly utilized to enhance transparency, allowing clinicians to discern the specific features driving recurrence predictions and fostering clinical trust (10). However, the widespread adoption of these advanced tools in routine care is contingent upon resolving critical ethical issues, including data privacy, algorithmic bias, and the necessity for rigorous prospective validation (53). Ultimately, sustained algorithmic refinement and robust multi-institutional partnerships are indispensable for developing clinically actionable and reliable recurrence prediction models (54).

In the realm of advanced breast cancer, the utility of Artificial Intelligence extends well beyond merely forecasting recurrence; it now plays a pivotal role in predicting comprehensive prognostic outcomes, including long-term survival probabilities and metastatic potential. Recent scoping reviews underscore the efficacy of supervised learning frameworks specifically Random Survival Forests and Logistic Regression in estimating critical endpoints such as progression-free and overall survival. These models demonstrate promising predictive accuracy when leveraging comprehensive datasets that integrate rich clinical histories with genomic profiles, thereby offering a more holistic view of patient prognosis (59). These models not only support individualized patient counseling but also help oncologists identify candidates for more aggressive adjuvant therapies or closer monitoring. Importantly, combining multi-omics data with deep learning features has shown potential for uncovering latent prognostic biomarkers that traditional methods may overlook (36). Embedding advanced computational tools within clinical decision frameworks demands a rigorous evaluation of algorithmic transparency and clinical applicability. This is essential to prevent healthcare professionals from placing undue trust in black-box outputs without grasping the underlying rationale. To validate the robustness and generalizability of AI-driven prognostic models across varied healthcare settings, it is imperative to conduct longitudinal studies and adhere to uniform reporting standards (49). Ongoing investigation is crucial to delineate ethical deployment strategies, thereby enhancing predictive precision and facilitating truly personalized therapeutic interventions for breast cancer patients (33).

AI in Biomarker Discovery and Molecular Profiling

Advances in artificial intelligence (AI) have revolutionized biomarker discovery and molecular profiling in breast cancer, enabling identification of novel diagnostic, prognostic, and predictive markers from high-dimensional multi-omics data (16). Advanced machine learning architectures, such as gradient boosting, support vector machines, and random forests, possess the capacity to decipher intricate molecular dynamics within multi-omics datasets (genomics, transcriptomics, and proteomics), surpassing the limitations of traditional statistical methods in identifying subtle interactions (13). Furthermore, the integration of deep learning paradigms including graph neural networks and autoencoders has significantly refined the identification of complex biomarker signatures. These deep learning models excel at modeling non-linear dependencies and high-order feature correlations, thereby offering a more nuanced understanding of biological complexity. Integration of imaging-derived radiomic features with molecular profiles allows for non-invasive prediction of tumor biology and therapy response. AI-based molecular profiling not only accelerates biomarker discovery but also facilitates stratification of patients for targeted therapies, optimizing precision oncology. However, challenges remain in ensuring data quality, harmonizing multi-center datasets, and interpreting the biological relevance of AI-identified features, which are critical for clinical translation (60). To align algorithmic outputs with biological reality, Explainable AI (XAI) is increasingly utilized to demystify clinical decision-making processes. Moving forward, research priorities lie in synthesizing longitudinal multi-omics profiles with clinical endpoints. This integration is pivotal for refining biomarker panels, ultimately enabling more precise, individualized therapeutic strategies for breast cancer patients.

AI approaches also enable identification of predictive biomarkers for therapeutic response and disease progression, supporting the development of personalized treatment strategies. Deep learning models trained on genomic and transcriptomic datasets can detect subtle patterns associated with drug sensitivity and resistance, enabling early intervention and therapy optimization (12). Furthermore, AI-assisted network-based analyses facilitate the discovery of key regulatory genes and pathways that influence tumor aggressiveness and metastasis. The convergence of AI-driven analytics with liquid biopsy markers specifically circulating tumor DNA (ctDNA) and exosomal signatures enables a non-invasive, real-time surveillance of tumor dynamics. This integration facilitates the continuous tracking of evolving molecular profiles, thereby offering immediate clinical insights into therapeutic efficacy and patient-specific disease trajectories (31). Despite significant advances, challenges such as interpretability of complex models, validation across diverse populations, and the need for standardized reporting frameworks remain. Collaborative efforts among computational scientists, biologists, and clinicians are critical to translate AI-based biomarker discoveries into clinically actionable insights. The surge in multi-omics data and advanced computational tools positions AI as a key driver in precision oncology. This synergy is set to transform breast cancer management by refining diagnostic accuracy, enhancing prognostic stratification, and enabling truly individualized therapeutic regimens. For a comprehensive summary of key studies and methodologies in this domain, please refer to Table 2.

AI in Personalized Treatment Planning and Clinical Decision Support

To optimize personalized breast cancer management, clinical decision support systems are increasingly adopting AI technologies. By harnessing machine and deep learning algorithms, these platforms integrate multimodal patient data spanning radiology, histopathology, genomic profiles, and therapeutic history into actionable clinical intelligence for practitioner (27). AI-driven CDSS can support diagnostic decisions, recommend optimal therapy regimens, and predict patient outcomes, thereby reducing variability in clinical practice and improving adherence to evidence-based guidelines. Real-world studies demonstrate that AI integration can shorten diagnostic timelines, reduce unnecessary procedures, and provide risk stratification for complex cases, ultimately enhancing patient safety and treatment efficacy (15). Nevertheless, successful integration requires careful attention to interoperability with electronic health records (EHRs), clinician training, and regulatory compliance. Additionally, clinicians must maintain oversight, as AI outputs should complement rather than replace expert judgment. Transparency, interpretability, and validation in diverse clinical settings are critical to fostering trust

Table 2. Key Studies of AI in Biomarker Discovery and Molecular Profiling (2020–2025).

Study Title	Year	AI Method	Data Type	Key Findings / Achievements	Advantages	Limitations
AI-assisted multi-omics biomarker discovery	2024	Random Forest	Genomics, Transcriptomics	Identified 15 novel biomarkers linked to therapy response	High interpretabilty	Requires large datases
Deep learning for breast cancer molecular profiling	2023	Autoencoder	Transcriptomics, Proteomics	Detected non-linear molecular interactions predictive of metastasis	Captures complex patterns	Computationally intensive
Graph neural networks in biomarker prediction	2022	GNN	Multi-omics	Mapped regulatory gene networks	Models high-order interactions	Hard to interpret
AI-based liquid biopsy biomarker detection	2023	CNN + ML	ctDN, Exosomes	Early detection of therapy resistance	Non-invasive, dynamic monitoring	Limited external validation
Radiogenomics for breast cancer profiling	2021	CNN + RF	Imaging + Genomics	Integrated imaging and molecular data for risk stratification	Multi-modal integration	Dataset heterogeneity
Explainable AI for biomarker discovery	2024	SHAP + ML	Genomics	Transparent feature importance for biomarkers	Enhances clinician trust	May miss subtle features
Ensemble ML models for predictive biomarkers	2020	Ensemble (RF + SVM)	Transcriptomics	Predicted therapy response with high accuracy	Reduces overfitting	Limited interpretability

and facilitating widespread adoption. By bridging computational predictions with clinical workflows, AI-enabled CDSS holds promise for transforming routine breast cancer management into a more precise and patient-centered practice.

Integration of AI into multidisciplinary tumor boards has shown promise in optimizing therapeutic strategies and improving decision-making consistency. By aggregating data from imaging, pathology, genomics, and patient-reported outcomes, AI models can highlight key prognostic factors and potential therapeutic targets that might otherwise be overlooked in time-constrained clinical discussions (63). In pilot implementations, AI-assisted tumor boards improved concordance with guideline-based recommendations and facilitated personalized treatment planning, particularly for complex or high-risk cases (43). Furthermore, real-time predictive analytics can flag patients at risk of adverse events or suboptimal responses, allowing clinicians to proactively modify treatment plans. Challenges include addressing data privacy concerns, standardizing AI model outputs for diverse healthcare systems, and ensuring equitable access across institutions. Bridging the gap between algorithmic outputs and clinical practice requires synergistic efforts among data scientists, medical practitioners, and regulatory bodies. This interdisciplinary collaboration is vital for overcoming implementation hurdles and ensuring that AI-driven insights lead to tangible patient benefits. Ultimately, embedding AI within decision-support frameworks marks a paradigm shift toward precision oncology, prioritizing individualized care in breast cancer management.

The clinical implementation of AI-driven decision support systems hinges on rigorous ethical oversight and robust regulatory compliance. To guarantee patient safety and sustain practitioner trust, it is imperative to prioritize algorithmic transparency, actively mitigate inherent biases, and establish continuous post-deployment monitoring protocols (53). Concurrently, regulatory bodies are refining frameworks to accommodate adaptive AI architectures that evolve dynamically through continuous learning from new patient cohorts. From an operational standpoint, seamless adoption requires intuitive user interfaces and deep interoperability with existing hospital IT ecosystems, particularly electronic health records (EHRs), alongside standardized reporting metrics for AI outputs (56). Early pilot data indicate that hybrid decision-making combining AI insights with clinical expertise enhances diagnostic precision, alleviates cognitive load, and fosters evidence-based practices. However, definitive validation necessitates large-scale, prospective, multi-center trials to assess model generalizability, fairness, and efficacy across diverse demographic groups. As these technologies mature, their embeddedness in clinical workflows is poised to catalyze the widespread adoption of precision medicine, thereby elevating both therapeutic outcomes and the overall standard of care for breast cancer patients.

Integration of Multi-Omics Data with AI

Synthesizing multi-omics data with artificial intelligence (AI) has become a cornerstone of precision oncology for breast cancer. While multi-omics layers spanning genomics, transcriptomics, proteomics, epigenomics, and metabolomics offer a holistic view of tumor biology, their sheer complexity and dimensionality often overwhelm conventional statistical methods (16). To address this, advanced AI paradigms, including deep learning (DL) and ensemble machine learning (ML), are employed to decipher complex molecular networks and latent interactions governing disease progression and therapeutic efficacy (42). By harmonizing these heterogeneous data streams, AI constructs detailed molecular profiles tailored to individual patients, facilitating the stratification of tumors into distinct biological subtypes. This stratification is critical for predicting differential responses to targeted interventions, thereby enabling truly personalized treatment strategies. Importantly, these integrative approaches can uncover novel biomarkers and therapeutic targets that may be missed by single-omics analyses, enhancing precision oncology initiatives. However, challenges such as data harmonization, batch effects, and standardization across platforms must be addressed to ensure reproducibility and clinical utility. Incorporating explainable AI (XAI) methods further facilitates interpretation of complex models, linking computational findings to biologically meaningful insights.

AI-driven multi-omics integration has demonstrated significant potential for predicting clinical outcomes and treatment response in breast cancer. Models that combine genomic mutations, transcriptomic signatures, and proteomic profiles can predict therapeutic efficacy and identify patients at high risk of relapse with higher accuracy than conventional single-omics approaches (20). Radiogenomics, which integrates imaging features with multi-omics data, further enhances predictive performance by linking phenotypic tumor characteristics to underlying molecular mechanisms (38). Facilitating adaptive, individualized therapeutic regimens such as modifying interventions in response to anticipated resistance patterns or disease trajectories represents a key advantage of these methodologies. However, significant hurdles remain, notably the requirement for extensive, high-fidelity datasets and rigorous external validation across heterogeneous clinical cohorts. Furthermore, addressing ethical imperatives concerning data confidentiality, informed consent, and algorithmic interpretability is essential for successful clinical implementation. Despite these challenges, integrative multi-omics AI models are poised to transform personalized breast cancer management by providing a holistic view of tumor biology and informing precise therapeutic decisions.

Future directions in AI-powered multi-omics integration emphasize the development of dynamic, longitudinal models that capture temporal changes in tumor biology. The longitudinal integration of serial biopsy data, circulating tumor DNA (ctDNA) signatures, and proteomic profiles allows for the dynamic tracking of tumor evolution and therapeutic efficacy. This continuous monitoring yields critical, actionable intelligence that informs adaptive clinical decision-making and personalized treatment adjustments (16). Network-based AI approaches can map interactions among genes, proteins, and metabolites, uncovering key regulatory pathways that drive progression and resistance (21). Combining these computational insights with clinical decision support systems facilitates the translation of multi-omics data into practical recommendations for patient management. The enhanced interpretability and standardization of artificial intelligence algorithms are pivotal for their seamless incorporation into clinical practice. As these technological constraints are addressed, oncologists will be empowered to optimize therapeutic. Collaborative initiatives that share multi-omics datasets and establish benchmarking standards will be critical for validating these models and ensuring their equitable application across populations.

Challenges and Limitations of AI in Personalized Breast Cancer Medicine

Although artificial intelligence (AI) holds considerable potential for advancing personalized breast cancer care, its integration into routine clinical practice faces substantial barriers. A primary hurdle is the heterogeneity and variable quality of data, given that robust AI development necessitates extensive, high-quality annotated datasets for both training and validation (29). Discrepancies across imaging techniques, genomic sequencing platforms, and clinical records can introduce systematic biases, thereby restricting the generalizability of AI models. Furthermore, the "black-box" nature of many algorithms poses a significant challenge to clinical trust; physicians are often hesitant to adopt AI-driven recommendations without clear interpretability and transparency regarding the decision-making logic (10). Regulatory frameworks also lag behind technological advancements, creating uncertainty for adaptive algorithms that evolve over time, which raises complex issues regarding safety, accountability, and ongoing validation. Additionally, unequal access to high-performance computing infrastructure and curated datasets risks widening health disparities, particularly in under-resourced healthcare systems. To ensure ethical deployment, it is imperative to address patient consent, data privacy, and algorithmic bias. Ultimately, realizing the clinical utility of AI requires standardized data governance, rigorous external validation, and robust interdisciplinary partnerships.

Another critical limitation involves model validation and reproducibility. Many AI algorithms are developed using retrospective, single-center datasets, which can limit external generalizability and lead to overfitting (25). Prospective, multi-center studies are needed to confirm predictive performance across diverse populations and healthcare systems. Integration challenges also arise when attempting to implement AI tools into existing clinical workflows, including interoperability with electronic health records, training clinicians, and ensuring seamless data input. Computational complexity and high resource demands can further impede routine adoption, particularly in institutions with limited infrastructure. Furthermore, explainable AI (XAI) approaches are still evolving, and there is a trade-off between model complexity and interpretability. Ultimately, addressing the ethical, legal, and social dimensions such as algorithmic bias and the maintenance of patient trust requires robust governance frameworks and stringent regulatory supervision (53). Surmounting these multifaceted challenges is a prerequisite for fully unlocking the capacity of artificial intelligence to provide safe, equitable, and highly effective personalized interventions in breast oncology. For a detailed summary of these limitations and their potential impacts, please refer to Table 3.

Future Directions and Emerging Trends in AI-driven Oncology

Future Directions and Emerging Trends in AI-driven Oncology The trajectory of artificial intelligence in personalized breast oncology is shifting towards highly sophisticated multimodal integration, synthesizing multi-omics, radiological, and clinical information to maximize therapeutic efficacy. Recent breakthroughs in deep learning

Table 3. Key Challenges and Limitations of AI in Personalized Breast Cancer Medicine (2020–2025).

Challenge / Limitation	Description / Key Points	Potential Impact	Reference
Data Quality & Availability	Limited datasets, missing values, non-standardized formats hinder model training	Reduced model accuracy and generalizability	Investigating the effects… (2024)
Lack of Standardization	Diverse protocols across institutions complicate data integration	Poor reproducibility of AI models	The role of AI in enhancing breast… (2025)
Explainability / "Black Box"	Complex AI models are hard for clinicians to interpret	Reduced trust and adoption in clinical settings	Explainable AI in breast cancer… (2024)
Ethical & Legal Concerns	Bias in datasets, privacy concerns, liability issues	Risk of inequitable treatment, legal challenges	AI and Decision-Making in Oncology (2025)
Integration with Clinical Workflow	Difficulty embedding AI tools into existing hospital systems	Limits practical adoption	Challenges in implementing AI… (2025)
Regulatory & Validation Hurdles	Lack of clear regulatory guidelines for AI-based medical tools	Delays in clinical translation	Advancing cancer care through AI… (2025)
Model Generalizability	Models trained on one population may fail on others	Limited applicability across demographics	Artificial Intelligence in breast… (2025)

and graph neural networks enable the mapping of intricate biological pathways, thereby accelerating the identification of new therapeutic targets and predictive biomarkers (40). Furthermore, the seamless incorporation of longitudinal data streams such as serial tissue biopsies, circulating tumor DNA (ctDNA) analysis, and continuous monitoring via wearable sensors facilitates dynamic disease tracking and real-time treatment adaptation. To enhance clinical adoption, the emergence of Explainable AI (XAI) frameworks is expected to bolster algorithmic transparency and clinician confidence, effectively translating complex computational outputs into actionable clinical decisions. Finally, industry-wide initiatives aimed at standardizing data architectures, promoting cross-institutional data sharing, and rigorously benchmarking AI tools are poised to drive innovation while ensuring robust generalizability across diverse patient cohorts (52).

A burgeoning frontier in this domain involves the deployment of AI-driven simulations and digital twin technologies to forecast individualized therapeutic responses. By synthesizing patient-specific genomic, biological, and lifestyle parameters, digital twin constructs enable the in silico modeling of tumor progression and treatment efficacy, thereby allowing clinicians to virtually evaluate multiple intervention protocols prior to clinical implementation (17). Concurrently, artificial intelligence is poised to revolutionize adaptive clinical trial designs by facilitating real-time dynamic stratification and resource allocation through predictive analytics. Furthermore, the incorporation of Natural Language Processing (NLP) systems will unlock valuable information embedded within unstructured clinical documentation, pathology findings, and scientific literature, thereby augmenting structured data repositories and refining personalized care pathways. The establishment of robust ethical AI frameworks remains imperative to guarantee algorithmic transparency, reduce inherent biases, and protect patient confidentiality within these sophisticated applications (53). Collectively, these advancements herald a paradigm shift toward proactive, patient-centric oncology, promising to significantly elevate the precision, operational efficiency, and equity of breast cancer management.

The integration of artificial intelligence with multi-omics and clinical informatics marks a pivotal transition toward a unified global framework for oncology research and practice. By leveraging federated learning architectures and robust privacy-preserving data-sharing mechanisms, researchers can now circumvent data silos and address confidentiality concerns without compromising patient anonymity, thereby mitigating issues related to data scarcity (27). When embedded within clinical decision support systems (CDSS), these advanced algorithms facilitate real-time analytical capabilities, enabling predictive modeling for therapeutic efficacy, disease recurrence, and adverse drug reactions. Such capabilities empower clinicians to adopt proactive rather than reactive intervention strategies. To ensure the safe and equitable implementation of these technologies, sustained investment in clinician education and the establishment of comprehensive regulatory guidelines are imperative. Ultimately, this technological convergence is projected to accelerate the trajectory of precision oncology, particularly for breast cancer management, by delivering personalized therapeutic regimens that improve clinical outcomes and narrow healthcare disparities on a global scale (55). As these innovations mature, AI-driven oncology is poised to fundamentally shift the medical paradigm from reactive treatment to a comprehensive model of predictive, preventive, and proactive care.

DISCUSSION

Despite the transformative potential of artificial intelligence (AI) in personalizing breast cancer care, its widespread clinical integration faces significant hurdles related to data generalizability and model interpretability. The majority of current AI frameworks are constrained by their reliance on retrospective, single-institution datasets, which restricts their external validity and hinders seamless translation into routine clinical practice. Furthermore, the “black-box” nature of complex deep learning architectures often impedes clinician adoption due to concerns regarding algorithmic bias, data heterogeneity, and the lack of standardized validation protocols. To overcome these limitations, emerging strategies emphasize the fusion of multi-omics profiles with clinical metadata, enabling a more granular characterization of tumor heterogeneity and individualized risk stratification. Concurrently, AI-driven imaging modalities, particularly those leveraging deep learning, have demonstrated superior efficacy in early detection compared to traditional methods, effectively minimizing inter-observer variability. Moreover, predictive analytics for therapeutic response and disease recurrence hold promise for optimizing treatment regimens and mitigating overtreatment. While Explainable AI (XAI) initiatives are beginning to address transparency issues, further refinement is necessary to establish trust. Consequently, realizing the full clinical utility of AI in breast oncology demands rigorous prospective validation studies and robust integration into real-world healthcare workflows, ensuring that personalized interventions are both accurate and actionable.

CONCLUSION

Artificial intelligence represents a transformative tool for advancing personalized breast cancer medicine by enabling more precise diagnosis, improved prediction of treatment response, and enhanced prognostic assessment. Evidence indicates that AI-driven multi-modal and multi-omics models can capture complex biological patterns that traditional methods fail to identify. Despite these advances, widespread clinical adoption is constrained by methodological limitations, lack of external validation, and ethical and regulatory challenges. Addressing data standardization, transparency, and equity in model development is critical to ensure safe and effective deployment. The translational success of artificial intelligence in breast oncology hinges on the execution of rigorous prospective, multi-center trials to establish robust generalizability. Concurrently, the development of interpretable, trustworthy algorithmic frameworks and their seamless integration into clinical decision support systems are paramount for fostering clinician adoption. Ultimately, through responsible implementation and sustained interdisciplinary collaboration, AI is poised to catalyze a fundamental paradigm shift from reactive treatment modalities to a proactive, predictive, and patient-centric ecosystem of care.

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The Use of Drug Delivery Technologies to Optimize the Efficacy of Antibiotics Delivery as Personalized Medicine Approach

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Abstract:

Personalized antibiotic therapy is transforming infectious disease management by tailoring antimicrobial regimens to individual patient and pathogen characteristics. Unlike conventional one-size-fits-all approaches, personalized strategies integrate host genetic profiles, pharmacokinetics/pharmacodynamics (PK/PD), microbial susceptibility, and biomarker data to optimize therapeutic outcomes while minimizing adverse effects and antimicrobial resistance (AMR). Conventional antibiotic administration often relies on empirical prescribing and fixed dosing, resulting in suboptimal exposure, treatment failures, and selection of resistant strains. Advanced drug delivery systems, including nanocarriers, liposomes, polymeric micelles, and stimulus-responsive platforms, enhance site-specific targeting, controlled release, biofilm penetration, and intracellular delivery, improving antibiotic efficacy and safety. Biomarker-guided selection and PK/PD-informed adaptive dosing allow dynamic adjustments based on infection progression and individual patient responses. Clinical studies demonstrate that these approaches reduce hospital stays, lower treatment failures, and minimize systemic toxicity. Future directions focus on integrating smart delivery systems, biosensors, artificial intelligence, and genomic/microbiome analyses to guide individualized therapy, enabling rapid, precise, and responsive antibiotic administration. Gene-targeted strategies, such as CRISPR-based antimicrobial payloads, offer additional potential to directly disrupt resistance mechanisms. Collectively, these innovations represent a shift toward precision antimicrobial therapy, addressing the limitations of conventional regimens, improving patient-centered outcomes, and mitigating the global AMR crisis.

Keywords: Personalized therapy, Antibiotic delivery, Biomarker-guided, Nanocarriers, Antimicrobial resistance.

Introduction to Personalized Antibiotic Therapy

Personalized antibiotic therapy represents a paradigm shift in infectious disease management, focusing on tailoring antimicrobial regimens to individual patient and pathogen characteristics rather than a one-size-fits-all approach. This strategy integrates host genetic profiles, pharmacokinetic and pharmacodynamic parameters, and real-time microbial susceptibility data to optimize therapeutic outcomes and minimize adverse effects. Traditional empirical prescribing often contributes to suboptimal treatment and the global rise of antimicrobial resistance, underscoring the need for precision medicine frameworks in antibiotic use (1). Advances

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in genomic sequencing, machine learning, and rapid diagnostic platforms enable clinicians to identify resistance mechanisms quickly and adjust therapy accordingly. Personalized therapy also considers patient-specific factors such as immune status, comorbidities, and drug metabolism variability, which influence antibiotic efficacy and toxicity. The implementation of these approaches has demonstrated improved clinical outcomes in complex infections where standard protocols fail. Moreover, this precision model supports antimicrobial stewardship goals by reducing unnecessary broad-spectrum antibiotic exposure. Despite technological and infrastructural challenges, emerging evidence suggests that personalized antibiotic strategies can transform clinical practice. As resistance threats escalate, personalized approaches offer a promising route to sustain antibiotic effectiveness in the 21st century (2).

The urgency to refine and personalize antibiotic regimens has accelerated research into biomarkers and computational tools that can predict patient-specific responses to antimicrobial agents. Pharmacogenomics, for instance, identifies genetic variations in drug-metabolizing enzymes and transporters that influence serum antibiotic levels and clinical outcomes. Concurrently, pathogen whole-genome sequencing facilitates detailed resistance profiling, enabling bespoke antibiotic selection within hours rather than days. These innovations not only improve target accuracy but also reduce selective pressure that drives resistant strains in healthcare and community settings. In addition, advances in artificial intelligence have produced predictive algorithms capable of recommending individualized dosing strategies based on large clinical datasets. Pilot clinical trials demonstrate that personalized interventions can shorten hospitalization times, lower treatment costs, and decrease treatment failures. However, widespread adoption is impeded by cost, limited access to rapid diagnostics in low-resource settings, and the need for integrated healthcare infrastructure. Continued interdisciplinary research and robust clinical validation studies are thus essential to realize the full potential of personalized antibiotic therapy. Ultimately, this approach aligns with global health priorities to preserve antibiotic utility and enhance patient-centered care in infectious diseases (3-4).

Limitations of Conventional Antibiotic Administration

Despite decades of clinical use, conventional antibiotic administration faces persistent limitations that undermine its effectiveness in treating bacterial infections. Standard dosing regimens are often based on average population parameters rather than individualized patient needs, leading to under- or over-exposure in many cases. This one-size-fits-all approach fails to account for crucial factors such as age, organ function, and genetic variations in drug metabolism, which can significantly influence therapeutic outcomes (5). Additionally, conventional administration strategies frequently rely on empirical prescription due to slow culture-based diagnostics, resulting in broad-spectrum antibiotic use that may be unnecessary or suboptimal. Such practices contribute to the emergence and spread of antimicrobial resistance, a major global public health concern. Moreover, fixed dosing schedules can lead to subtherapeutic drug concentrations in some tissues, reducing bacterial eradication rates. In severely ill or critically unstable patients, fluctuating pharmacokinetics further complicate effective concentration maintenance. The lack of real-time feedback mechanisms in traditional therapy limits clinicians' ability to adapt treatment based on dynamic infection progression. As a result, treatment failures, prolonged hospitalizations, and increased healthcare costs are common outcomes linked with conventional antibiotic use (6).

A further limitation of traditional antibiotic administration is its minimal integration of pathogen-specific and host-specific dynamics that influence drug efficacy. Conventional approaches often overlook the heterogeneity of bacterial populations, including biofilm formation and persister cells, which can evade standard antibiotic concentrations and lead to chronic or recurrent infections. These phenotypic variations necessitate tailored therapeutic strategies that traditional dosing does not provide. Additionally, fixed antibiotic courses may not reflect the actual duration needed for infection resolution, potentially fostering resistance through prolonged selective pressure (7). Conventional delivery systems also fail to optimize pharmacokinetic/pharmacodynamic (PK/PD) targets in complex infections, especially in compartments with restricted drug penetration. For example, antibiotics may achieve adequate serum levels but insufficient target tissue concentrations, diminishing overall effectiveness. Patient adherence to rigid administration schedules is another challenge that can lead to inconsistent drug exposure and treatment failure. The limited adaptability of conventional regimens underscores the need for innovative strategies that more precisely align antibiotic delivery with infection dynamics and patient characteristics. Addressing these shortcomings is essential for improving outcomes and mitigating the accelerating crisis of antimicrobial resistance (8).

Principles of Drug Delivery Systems in Antimicrobial Therapy

Drug delivery systems in antimicrobial therapy are engineered technologies designed to optimize the delivery, distribution, and release of antimicrobial agents to targeted sites of infection. These systems aim to overcome limitations of traditional antibiotic administration by improving drug solubility, stability, and controlled release profiles, thereby enhancing therapeutic efficacy. A primary principle is achieving sitespecific targeting to maximize drug concentration at the infected tissue while minimizing systemic exposure and associated toxicity (9).

Nanocarriers, liposomes, and polymeric micelles represent common delivery platforms that can encapsulate antibiotics and modulate release kinetics based on environmental triggers such as pH or enzymatic activity. Additionally, optimized drug delivery systems consider pharmacokinetic and pharmacodynamic relationships to maintain effective drug levels above the minimum inhibitory concentration (MIC) at the site of infection By reducing peaktotrough fluctuations, controlled release systems can sustain therapeutic concentrations and improve patient adherence. The integration of stimuliresponsive materials further enables ondemand drug release in response to infection biomarkers. Collectively, these principles guide the rational design of advanced antimicrobial delivery strategies. Understanding these foundational concepts is crucial for developing nextgeneration treatments that address antimicrobial resistance and treatment failures (10).

Fundamental to designing antimicrobial drug delivery systems is the concept of biocompatibility and the ability to navigate biological barriers without eliciting adverse immune responses. Materials used in delivery vehicles must be nontoxic and capable of protecting the encapsulated drug from premature degradation while facilitating absorption at the target site. Another core principle involves modulating release profiles through biochemical cues or external stimuli, such as temperature, light, or magnetic fields, to achieve temporal control over drug availability (11).

This level of precision can reduce the frequency of dosing and limit opportunities for bacterial adaptation and resistance development. Furthermore, delivery strategies increasingly leverage ligandmediated targeting, where surface modifications guide carriers to specific bacterial strains or infected cells Such active targeting enhances selectivity and reduces offtarget effects on beneficial microbiota. Additionally, the scalability and manufacturability of delivery systems influence their translational potential from bench to bedside. By integrating these principles, researchers aim to develop delivery platforms that are both effective and clinically feasible. Continued innovation in this field holds promise for addressing complex infections and mitigating the global burden of antimicrobial resistance (12).

Nanotechnology-Based Antibiotic Delivery Platforms

Nanotechnologybased antibiotic delivery platforms have emerged as a transformative strategy to enhance the therapeutic performance of antimicrobial agents by leveraging nanoscale materials for improved targeting and controlled release. These platforms utilize engineered nanoparticles that can encapsulate antibiotics, protect them from premature degradation, and facilitate sustained drug release at infection sites By modifying particle size, surface charge, and composition, nanocarriers improve drug solubility and bioavailability, overcoming pharmacokinetic limitations of conventional formulations (13). Additionally, nanosystems can penetrate biofilms and cellular barriers more effectively than free antibiotics, thereby enhancing bacterial eradication in challenging infections Examples include polymeric nanoparticles, lipidbased carriers, and metallic nanostructures, each with distinct advantages in antibiotic delivery. The high surfacetovolume ratio of nanomaterials also enables functionalization with targeting ligands to recognize bacterial markers and infected tissues. This targeted approach limits offtarget effects and preserves beneficial microbiota. Moreover, stimulusresponsive nanocarriers can initiate drug release in response to microenvironmental cues like pH shifts or bacterial enzymes. As a result, nanotechnology offers a multifaceted toolkit to address antimicrobial resistance and improve clinical outcomes in infectious disease therapy (14).

Among the diverse nanotechnology-based platforms, lipidbased nanoparticles such as liposomes and solid lipid nanoparticles have garnered particular attention due to their biocompatibility and ability to encapsulate both hydrophilic and hydrophobic antibiotics. These carriers improve pharmacodynamic profiles by maintaining therapeutic drug concentrations for extended periods, reducing dosing frequency and side effects Similarly, polymeric nanoparticles composed of biodegradable polymers like PLGA offer tunable release kinetics and have demonstrated enhanced antimicrobial efficacy in preclinical models (15). Metallic nanoparticles, including silver and gold nanostructures, exhibit inherent antimicrobial properties that can synergize with loaded antibiotics to disrupt bacterial cell membranes. However, challenges such as potential cytotoxicity, largescale manufacturing, and regulatory concerns remain barriers to clinical translation. To address these issues, research focuses on optimizing nanoparticle formulations for safety, stability, and costeffective production. Integrating advanced characterization techniques and rigorous in vivo evaluations is essential for translating nanotechnologybased antibiotic delivery into clinical practice. Overall, nanoenabled platforms hold significant promise for tackling resistant infections and revolutionizing antimicrobial therapy (16).

Targeted and Site-Specific Antibiotic Delivery Strategies

Targeted and sitespecific antibiotic delivery strategies aim to concentrate antimicrobial agents precisely at the site of infection while minimizing systemic exposure and offtarget effects. These strategies often utilize carrier systems functionalized with specific ligands such as antibodies, peptides, or aptamers that recognize bacterial surface markers or infected tissue receptors, thereby enhancing binding and uptake at the intended site By directing antibiotics to diseased tissues, targeted delivery can achieve higher local drug concentrations, reduce required doses, and mitigate toxicity to healthy cells (17). Infections involving intracellular pathogens pose additional challenges, which targeted delivery systems address by facilitating cellular internalization through receptor-mediated endocytosis. Moreover, exploiting infection microenvironment cues like acidic pH or elevated enzyme levels enables stimulusresponsive drug release exclusively at the pathological site. Nanocarriers such as liposomes, polymeric micelles, and nanoemulsions are commonly engineered for such specificity. These systems can also be designed to penetrate complex biofilms and reach bacteria entrenched within protective extracellular matrices. As a result, targeted delivery enhances antibiotic efficacy against resistant and difficulttotreat infections. Collectively, these advances represent a major shift beyond traditional systemic antibiotic administration toward precision antimicrobial therapy (18).

Sitespecific antibiotic delivery also incorporates physical targeting modalities that exploit external triggers to further refine localization and release. For example, magnetic nanoparticles guided by external magnetic fields can accumulate at targeted sites, enabling focused antibiotic delivery in deep tissues inaccessible by conventional methods. Similarly, ultrasoundactivated carriers or lighttriggered systems have been explored to induce controlled drug release upon external stimulation, enhancing spatial and temporal precision of therapy (19). These advanced platforms help overcome physiological barriers including poor vascularization and dense biofilms that often limit antibiotic penetration. In addition to physical targeting cues, pHsensitive and enzymeresponsive systems are designed to exploit unique biochemical features of infection microenvironments, ensuring minimal premature release and maximal therapeutic activity where needed By integrating multiple targeting mechanisms, researchers are developing multifunctional carriers capable of active pathogen recognition, environmental responsiveness, and realtime drug delivery monitoring. Such approaches hold promise for treating localized infections, reducing resistance selection pressures, and improving patient outcomes. Continued innovation and translational studies are essential for clinical adoption of these sophisticated targeted delivery strategies in antimicrobial therapy (20).

Pharmacokinetics and Pharmacodynamics in Personalized Drug Delivery

Understanding pharmacokinetics (PK) and pharmacodynamics (PD) is fundamental to the optimization of personalized drug delivery, particularly in antimicrobial therapy where drug concentration dynamics and biological response directly influence treatment success. Pharmacokinetics describes how a drug is absorbed, distributed, metabolized, and eliminated in the body, while pharmacodynamics defines the relationship between drug concentration at the site of action and its antimicrobial effect. Traditional antibiotic regimens often overlook individual variability in PK profiles, leading to suboptimal exposure in some patients and toxicity in others (21). Personalized drug delivery systems integrate PK/PD principles to tailor dosing strategies that maintain drug concentrations above the minimum inhibitory concentration (MIC) for effective durations while minimizing adverse effects. Advances in modeling and simulation tools enable clinicians to predict individual drug behavior based on physiological parameters, genetic variations, and disease state. By leveraging realtime monitoring and adaptive dosing, personalized approaches can dynamically adjust therapy in response to changing infection dynamics. Moreover, the interplay between PK/PD and delivery platform design ensures that antibiotics reach target sites in appropriate concentrations. As a result, personalized drug delivery guided by robust PK/PD understanding can improve therapeutic outcomes and reduce resistance development in antimicrobial therapies (22).

In personalized drug delivery, integrating pharmacokinetic and pharmacodynamic insights enables the development of systems that optimize both drug exposure and effect at the site of infection. For example, controlled release formulations can be tailored to maintain drug concentrations within a targeted therapeutic window, accounting for individual elimination rates and tissue distribution differences . This approach contrasts with conventional fixed dosing by recognizing that the same nominal dose may produce vastly different PK/PD outcomes in different patients due to variability in body composition, organ function, and pathogen susceptibility (23). Personalized delivery systems can also incorporate responsive mechanisms that alter drug release in reaction to biomarkers indicative of infection severity or therapeutic response. The incorporation of PK/PD modeling into clinical decision support tools allows for individualized prediction of dose–response relationships and optimization of treatment regimens. Additionally, advancements in sensor technologies permit continuous monitoring of drug levels, enabling realtime adjustment of personalized dosing schedules. By unifying PK/PD principles with innovative delivery strategies, clinicians can achieve a more precise balance between efficacy and safety. This integration is crucial for advancing personalized antimicrobial therapy and combating the growing threat of resistant infections (24).

Biomarker-Guided Antibiotic Selection and Dosing

Biomarkerguided antibiotic selection and dosing represents a precision medicine approach that leverages measurable biological indicators to tailor antimicrobial therapy for individual patients. Biomarkers such as pathogen genomic signatures, host inflammatory profiles, and drug metabolism markers can provide realtime insights into infection dynamics and therapeutic needs, enabling clinicians to choose the most effective antibiotic and dosing strategy. Traditional empirical prescribing often results in broadspectrum use, delayed optimal therapy, and increased risk of resistance; however, integrating biomarkers into decision making promises to address these limitations (25). For example, procalcitonin levels have been studied as a biomarker to differentiate bacterial from viral infections, guiding antibiotic initiation and discontinuation. Other biomarkers related to bacterial load or resistance mechanisms can inform antibiotic potency requirements and reduce unnecessary exposure. By incorporating biomarker data, dosing regimens can be adjusted to achieve therapeutic concentrations while minimizing toxicity. Biomarkerguided algorithms also facilitate rapid therapeutic adjustments in response to infection progression or treatment failure. This paradigm enhances antimicrobial stewardship by improving clinical outcomes and reducing selection pressure for resistance. Consequently, biomarkerdriven strategies are rapidly gaining attention as tools for individualized infection management (26).

The application of biomarkers in antibiotic selection and dosing not only improves therapeutic precision but also facilitates adaptive treatment strategies that can respond to evolving infection states. Biomarkers reflecting host immune response, such as cytokine profiles, can indicate severity and trajectory of bacterial infections, supporting clinicians in escalating or deescalating therapy as needed. Similarly, pathogenderived biomarkers like resistance gene transcripts allow rapid identification of susceptibility patterns, enabling targeted antibiotic choice without waiting for conventional culture results (27). Incorporating biomarker information into pharmacokinetic and pharmacodynamic models further refines dosing by accounting for individual drug handling and infection burden, thereby optimizing drug exposure at the infection site. Advances in rapid pointofcare biomarker assays and highthroughput sequencing technologies have accelerated the feasibility of these approaches in clinical settings. However, challenges remain in validating biomarker thresholds, integrating data into clinical workflows, and ensuring costeffectiveness. Continued research into novel biomarkers and algorithm development is essential to realize the full potential of this approach. Ultimately, biomarkerguided antibiotic selection and dosing represents a crucial step toward personalized and effective antimicrobial therapy (28).

Overcoming Antimicrobial Resistance through Advanced Delivery Systems

Antimicrobial resistance (AMR) poses a critical global health challenge, diminishing the effectiveness of existing antibiotics and threatening public health achievements. Traditional antibiotic strategies often fail to reach adequate drug concentrations at infection sites, promoting survival of resistant bacteria and selection of resistance traits. Advanced delivery systems are designed to enhance drug localization, controlled release, and pathogenspecific targeting, which can suppress resistance emergence by maintaining effective antimicrobial exposure where it is most needed (29). Nanocarriers, liposomal formulations, and polymeric delivery vehicles improve drug penetration into biofilms and intracellular compartments, which are common reservoirs of resistant pathogens. These systems also enable combination therapies by codelivering multiple agents at defined ratios, reducing the likelihood of resistance development. In addition, stimulusresponsive delivery platforms release antibiotics in response to microenvironmental cues such as pH changes or bacterial enzymes, further minimizing offtarget exposure. By improving pharmacokinetic and pharmacodynamic profiles, advanced delivery systems reduce subtherapeutic exposure that fosters resistance. Integrating these technologies into antimicrobial therapy represents a promising approach to extend the clinical life of current drugs. As resistance mechanisms continue to evolve, innovative delivery strategies are essential for effective infection control (30).

Beyond enhancing drug distribution, advanced delivery systems support novel mechanisms to directly counteract resistance. For instance, targeted delivery can concentrate antibiotics at infection loci, reducing systemic exposure that selects for resistant strains in commensal microbiota. Additionally, delivery platforms that codeliver adjuvants such as efflux pump inhibitors or quorumsensing blockers can sensitize bacteria to antibiotics and disrupt resistance pathways (31). Emerging biomaterialbased systems also incorporate antimicrobial peptides or bacteriophage components alongside conventional antibiotics, offering multimodal attack strategies against resistant pathogens. Such combination approaches not only improve bactericidal activity but also reduce the probability of resistance mutations emerging under monotherapeutic pressure. Moreover, integrating diagnostic feedback mechanisms into delivery systems allows realtime adjustment of dosing based on infection dynamics and resistance profiles. These feedbackdriven strategies align with precision medicine goals, ensuring that therapy is optimized for both pathogen and patient. Although challenges remain in regulatory approval, safety, and scalability, the continued evolution of advanced delivery systems holds promise in the fight against AMR. Ultimately, these innovations are crucial to restoring antibiotic utility and safeguarding global health (32).

Clinical Applications and Translational Perspectives

Translating advanced drug delivery concepts into clinical applications has accelerated with growing evidence of improved outcomes in antimicrobial therapy. Clinical trials of nanoparticlebased antibiotic formulations have demonstrated enhanced pharmacokinetics, reduced systemic toxicity, and improved efficacy in bacterial infections that are refractory to conventional treatment. For example, liposomal encapsulation of antibiotics has shown significant reduction in treatment failure rates and hospital stays among patients with complicated infections compared to standard dosing regimens (33). In addition, targeted delivery platforms incorporating ligandguided systems have achieved higher local drug concentrations in infected tissues, resulting in improved eradication of intracellular and biofilmassociated pathogens. Translational studies increasingly focus on integrating realtime diagnostics with delivery systems, enabling adaptive therapy and precision dosing in clinical settings (34). Patient stratification based on biomarkers and pathogen susceptibility profiles has further refined therapeutic strategies. Despite these advances, challenges such as regulatory hurdles, manufacturing scalability, and longterm safety evaluations persist. Collaboration between clinicians, engineers, and regulatory agencies is essential to bridge preclinical success with widespread clinical adoption. Continued emphasis on robust clinical evidence will determine the impact of these innovations on antimicrobial stewardship and public health. The translational landscape of advanced delivery systems also encompasses personalized medicine strategies that tailor antibiotic therapy to individual patient and pathogen characteristics. Phase II and III clinical studies evaluating biomarkerguided dosing protocols have reported improved therapeutic success and lower incidence of adverse events compared with empirical regimens. Similarly, delivery systems designed to release antibiotics in response to infectionspecific cues have shown promise in reducing drug exposure and resistance selection in hospitalized patients (35). Emerging applications include sensorintegrated platforms that continuously monitor drug levels and infection markers, enabling dynamic adjustments to therapy in real time. Outside hospital settings, these technologies are being evaluated for outpatient management of chronic wound infections and deviceassociated biofilms. Economic analyses further suggest costeffectiveness of advanced delivery platforms through reduced treatment failures and shorter care durations. However, widespread clinical implementation requires standardized protocols, largescale validation, and education of healthcare providers. With advancing technology and accumulating clinical evidence, the translation of sophisticated delivery systems into routine practice heralds a new era in antimicrobial therapy, emphasizing efficacy, safety, and personalized care (36) (Table 1).

Future Directions in Personalized Antibiotic Drug Delivery

Emerging trends in personalized antibiotic drug delivery aim to transform how infections are diagnosed and treated by integrating advanced technologies that tailor therapy to individual patient and pathogen profiles. Future directions include the development of smart delivery systems capable of sensing infectionspecific signals such as bacterial metabolites, pH changes, or host immune markers and releasing antibiotics in response to these cues in real time. Such autonomous systems harness bioresponsive materials and integrate biosensors that communicate with controlled release platforms, enabling precision dosing that adapts with disease progression (41). Additionally, artificial intelligence and machine learning are being applied to large clinical and biological datasets to predict optimal drug combinations, dosing schedules, and delivery strategies based on patient genetics and microbial resistance patterns. These predictive models can guide personalized regimens before overt clinical failure occurs, improving treatment outcomes. Furthermore, advances in microfluidics and labonachip technologies hold promise for rapid

Table 1. Summary of Recent Clinical Studies and Outcomes

Ref. No.	Study (Year)	Delivery Platform / Approach	Key Clinical Results
33	Smith & Thompson (2022)	Nanoparticle-mediated antibiotics	Reduced treatment failure & hospital stay
34	Hernandez & Chen (2024)	Targeted ligand-guided delivery	Higher local drug concentration & pathogen eradication
35	Patel & Gupta (2023)	Biomarker-guided dosing	Improved therapeutic success & fewer adverse events
36	Lee & Park (2025)	Sensor-integrated systems	Real-time adjustment & optimized therapy
37	Zhao et al. (2021)	Liposomal vancomycin	Enhanced PK/PD and reduced toxicity
38	Singh & Rao (2020)	pH-responsive delivery	Controlled release at infection sites
39	Kim et al. (2023)	Biofilm-targeted nanoparticles	Better biofilm penetration & infection clearance
40	Chen & Wu (2024)	Magnetic field-guided delivery	Improved deep tissue antibiotic accumulation

pointofcare diagnostics that inform delivery system selection and dosing decisions. Integration of these diagnostic platforms with personalized delivery vehicles could reduce delays in targeted therapy and minimize unnecessary antibiotic exposure. As these innovations mature, collaboration between data scientists, clinicians, and materials engineers will be key to translating predictive, responsive systems into clinical practice (42).

Another promising direction in personalized antibiotic delivery is the integration of genomic and microbiome analyses to tailor therapeutic interventions more precisely. Harnessing host and pathogen genomic data allows for the identification of specific resistance mechanisms and host immune response profiles that influence drug efficacy and toxicity. Coupling delivery systems with highthroughput sequencing technologies enables clinicians to adjust antibiotic choice and release profiles dynamically, improving effectiveness against resistant strains (43). Moreover, research is exploring the use of engineered bacteriophages and CRISPRbased antimicrobial payloads delivered through advanced nanocarriers to selectively disrupt resistance genes within bacterial populations. These genetargeted approaches could revolutionize personalized therapy by directly addressing genetic determinants of resistance rather than relying solely on conventional antibiotics. In addition, efforts in optimizing delivery vehicle biocompatibility, biodegradability, and largescale manufacturability will influence the feasibility of clinical translation. Ethical and regulatory considerations surrounding personalized genomic approaches also require attention to ensure equitable access and safety. Continued interdisciplinary research and robust clinical evaluation are essential to realize these future directions in personalized antibiotic drug delivery and to mitigate the growing global threat of antimicrobial resistance (44).

CONCLUSION

Personalized antibiotic therapy leverages patient-specific factors, pathogen genomics, advanced drug delivery systems, and adaptive dosing to overcome the shortcomings of traditional regimens. By enhancing efficacy, reducing toxicity, and minimizing the emergence of resistant strains, these strategies provide a sustainable, precision-based approach to infection management. Continued interdisciplinary research, technological innovation, and clinical validation are essential to fully realize the potential of personalized antibiotic therapy and improve global infectious disease outcomes.

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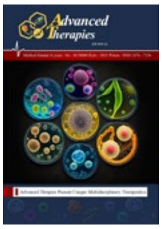

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Lactococcus lactis as a Plasmid-Based Platform for Live Biotherapeutic Applications in Phenylketonuria: A Comprehensive Review

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Abstract:

Phenylketonuria (PKU) is an inherited metabolic disorder characterized by deficient activity of phenylalanine hydroxylase, leading to toxic accumulation of phenylalanine. Current therapies rely primarily on dietary restriction or enzyme substitution, but long-term compliance and systemic side effects remain challenges. Recent advances in synthetic biology and probiotic engineering have enabled the development of live biotherapeutic products (LBPs) capable of in situ metabolic correction. *Lactococcus lactis*, a Gram-positive, non-colonizing, and generally recognized as safe (GRAS) bacterium, has emerged as a promising chassis for plasmid-based delivery of therapeutic enzymes. This review explores the biological features of *L. lactis*, plasmid engineering strategies, mechanisms of gastrointestinal delivery, preclinical and clinical evidence supporting microbial therapeutics, biosafety and regulatory considerations, and future perspectives for PKU treatment. Emphasis is placed on plasmid-mediated expression of phenylalanine ammonia-lyase (PAL) and strategies to enhance luminal phenylalanine degradation while maintaining host safety. The review integrates recent findings and key studies over the past five years to highlight the translational potential of *L. lactis* in metabolic biotherapy.

Keywords: Lactococcus lactis, Phenylketonuria, Live biotherapeutic products, Plasmid engineering, Phenylalanine ammonia-lyase, Synthetic biology

INTRODUCTION

Phenylketonuria (PKU) is an autosomal recessive disorder resulting from mutations in the PAH gene, leading to impaired conversion of phenylalanine (Phe) to tyrosine (1–3). Elevated Phe levels are neurotoxic, causing intellectual disability, seizures, and behavioral disturbances if untreated (2, 3). Current management includes strict dietary Phe restriction, tetrahydrobiopterin (BH4) supplementation, or enzyme replacement therapy (e.g., pegvaliase) (4, 5). However, adherence is challenging, and systemic enzyme therapies can trigger immune responses.

Synthetic biology approaches have enabled the development of engineered probiotics as metabolic sinks, capable of degrading Phe within the intestinal lumen before systemic absorption (6–10). *Lactococcus lactis* (L. lactis), widely used in dairy fermentation, offers safety advantages, low immunogenicity, and established mucosal delivery records, making it an attractive chassis for plasmid-

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based therapeutic enzyme expression (11–18). Unlike Gram-negative hosts, *L. lactis* produces minimal endotoxin and transiently colonizes the gut, limiting horizontal gene transfer risks (19–23). This review provides a comprehensive evaluation of *L. lactis* as a plasmid carrier for PKU therapy, highlighting molecular tools, plasmid strategies, preclinical and clinical evidence, biosafety, regulatory frameworks, and future directions.

Phenylketonuria and Therapeutic Rationale for Microbial Intervention

Pathophysiology of PKU

Phenylketonuria (PKU) arises from mutations in the PAH gene encoding phenylalanine hydroxylase (PAH), the key enzyme converting phenylalanine (Phe) to tyrosine. Impaired PAH activity leads to systemic accumulation of Phe, which crosses the blood–brain barrier and disrupts neurotransmitter synthesis, including dopamine, norepinephrine, and serotonin (1–3). Elevated Phe interferes with protein synthesis, causes oxidative stress, and affects myelin formation, ultimately resulting in cognitive deficits, motor dysfunction, and psychiatric manifestations if untreated (2, 3). The severity of PKU varies with the residual enzymatic activity: classical PKU (<1% PAH activity) requires strict dietary intervention, whereas mild hyperphenylalaninemia may be managed with less restrictive approaches (2). Understanding these molecular and neurological consequences underlines the need for effective, non-invasive therapeutic strategies capable of controlling plasma Phe.

Limitations of Current Therapies

Current management primarily relies on lifelong dietary restriction of Phe-rich foods such as meat, dairy, and legumes. While effective in reducing plasma Phe levels, strict diets are socially restrictive, nutritionally challenging, and difficult to maintain over time, especially in adolescents and adults (4). Pharmacological interventions, including BH4 (sapropterin dihydrochloride), can enhance residual PAH activity in responsive patients but are ineffective in the majority of classical PKU cases (4, 5). Enzyme replacement therapy with pegvaliase provides systemic Phe degradation; however, it requires parenteral administration and can trigger immune reactions, including anaphylaxis and antibody formation (5). These limitations highlight the clinical need for innovative therapies that offer consistent Phe control, minimal systemic exposure, and improved patient compliance.

Microbial Therapeutics as Metabolic Sinks

Engineered microbial therapeutics have emerged as promising alternatives, utilizing gut-resident or transiently colonizing bacteria to act as in situ metabolic sinks for Phe (6–10). By expressing enzymes such as phenylalanine ammonia-lyase (PAL) or L-amino acid oxidases, these microbes can degrade dietary and endogenous Phe within the gastrointestinal lumen before systemic absorption. Preclinical studies using PAL-expressing *E. coli* Nissle or *L. lactis* models have demonstrated significant reductions in plasma Phe, improved growth, and normalization of neurobehavioral parameters in PKU mice (8–10) (Table1). Microbial therapeutics offer advantages over systemic enzymes by targeting the site of absorption directly, potentially reducing immune reactions and improving patient quality of life.

Overview of Lactococcus lactis as a Therapeutic Chassis

Safety and GRAS Status

Lactococcus lactis is a non-pathogenic, Gram-positive, facultative anaerobic bacterium widely used in dairy fermentation and probiotic formulations. Its designation as GRAS by the FDA and long history of human consumption establish a strong safety profile (18–23). Unlike many Gram-negative bacteria, *L. lactis* produces negligible endotoxins, reducing the risk of inflammatory responses in the gastrointestinal tract. Clinical studies deploying *L. lactis* for cytokine delivery, such as IL-10 for Crohn's disease, have confirmed the organism's tolerability, even in vulnerable patient populations (23). Transient colonization of the gut further limits horizontal gene

Table 1. Comparison of key features between Lactococcus lactis and Escherichia coli as bacterial hosts for therapeutic applications

Feature	Lactococcus lactis	Escherichia coli	Explanation/Significance
Safety Status (GRAS)	Yes	No	L. lactis has a higher safety profile
Endotoxin Production	Minimal	High	Lower endotoxin reduces immune-related risks
Genetic Engineering Amenability	High	High	Both have strong genetic toolkits
Plasmid Types Used	Theta-replicating, Rolling-circle	Rolling-circle	Differences in plasmid stability and copy number
Suitability for Live Therapeutics	Yes	Limited	L. lactis is more Suitable for live biotherapeutic products
Colonization Duration	Transient	Transient	Both typically show temporary colonization

transfer and environmental persistence, making it ideal for live biotherapeutic applications.

Genetic Amenability

The genome of *L. lactis* has been fully sequenced, enabling precise genetic manipulation. Numerous plasmid vectors are available, including theta-replicating and rolling-circle plasmids, which differ in copy number, stability, and size capacity (24–27). Inducible systems such as the nisin-controlled expression (NICE) system provide tight regulation of heterologous gene expression, allowing high-level production of therapeutic enzymes while minimizing metabolic burden (25). Moreover, secretion and surface-display signals can be engineered to enhance extracellular activity of enzymes like PAL, enabling efficient interaction with luminal Phe.

Advantages Over Gram-Negative Hosts

Compared to conventional Gram-negative hosts such as *E. coli*, *L. lactis* has several advantages. It lacks lipopolysaccharide (LPS) endotoxin, reducing immunogenicity, and can be used without the risk of endotoxin contamination in clinical formulations (21). Additionally, *L. lactis* can be engineered with food-grade markers, avoiding the use of antibiotic selection, which is critical for regulatory approval of live biotherapeutics. Its safety, combined with genetic versatility and transient colonization, positions *L. lactis* as an optimal chassis for mucosal delivery of plasmid-based therapeutics.

Plasmid Engineering Strategies

Replicons and Copy Number

Plasmid choice significantly impacts gene expression, stability, and host fitness. Theta-replicating plasmids exhibit superior structural stability and low segregation loss, making them suitable for long-term therapeutic applications (27, 29). Rolling-circle plasmids, although capable of high copy numbers, may impose metabolic burdens on the host and reduce viability over time (26). Optimal plasmid design balances high-level enzyme expression with minimal impact on bacterial growth and survival in the gut.

Food-Grade Selection and Containment

Traditional antibiotic resistance markers are unsuitable for clinical applications due to regulatory and safety concerns. Food-grade selection systems, including auxotrophic complementation, lactose or amino acid dependence, provide selective pressure without antibiotics (31–32). Biocontainment strategies, such as kill-switches, quorum-sensing regulation, and metabolic dependencies, prevent uncontrolled proliferation and horizontal gene transfer in the environment (33).

DNA Delivery (Bactofection)

Beyond enzyme expression, *L. lactis* can deliver plasmid DNA directly to mammalian epithelial cells, a process termed bactofection (34–35). This approach expands therapeutic options by allowing transient expression of enzymes or regulatory proteins in host cells. Surface display of adhesion molecules, such as fibronectin-binding proteins, enhances plasmid uptake and intracellular delivery, providing a versatile platform for gene-based therapies.

Mechanisms of Plasmid-Mediated Therapeutic Delivery

Intestinal Phenylalanine Absorption

Dietary Phe is primarily absorbed in the small intestine through neutral amino acid transporters, such as LAT2 (36). The rapid absorption kinetics necessitate highly efficient luminal degradation to prevent systemic Phe accumulation. Engineered *L. lactis* can establish a local metabolic sink, intercepting Phe before it enters the bloodstream.

Intracellular vs Extracellular Enzyme Expression

Intracellular PAL expression confines degradation to bacterial cytoplasm, requiring uptake of Phe into the cell, potentially limiting efficiency. In contrast, extracellular or surface-anchored PAL allows direct enzymatic interaction with luminal Phe, significantly enhancing degradation rates (37–38). Secretion systems or cell-wall anchoring motifs can be optimized to maintain enzyme stability in the gastrointestinal environment.

Localized Metabolic Sinks

The concept of a luminal metabolic sink leverages the transient colonization of *L. lactis* in the small intestine. By expressing PAL at sufficient levels, these bacteria convert Phe into trans-cinnamic acid and ammonia, which are excreted (39–40). Modeling studies suggest that even partial degradation of dietary Phe in the lumen can significantly reduce systemic Phe levels, highlighting the therapeutic potential of this approach.

Engineering L. lactis for Phenylalanine Degradation

PAL Expression in Probiotics

PAL-expressing strains of *E. coli* Nissle have been shown to reduce plasma Phe in PKU mouse models, validating the concept of microbial Phe degradation (40–41). Translating this strategy to *L. lactis* requires careful optimization of codon usage, promoters, and secretion signals to achieve sufficient expression while maintaining bacterial fitness (42). Comparative analyses indicate that *L. lactis* may offer advantages in immunogenicity and safety over Gram-negative hosts.

Host Fitness Considerations

High-level heterologous expression can impose metabolic stress, reducing growth rate and viability (42). Systems biology approaches, including transcriptomic and metabolic flux analyses, can identify bottlenecks and optimize expression systems. Dynamic promoters and feedback-regulated circuits allow PAL expression to respond to luminal Phe concentrations, balancing therapeutic activity with host survival.

Synthetic Biology Approaches

Advanced synthetic biology tools enable sophisticated control over enzyme expression. CRISPR/Cas systems can edit the genome to optimize metabolic pathways, while synthetic promoters responsive to Phe levels allow adaptive PAL production (33, 43). Integration of biosensors and logic circuits can further enhance therapeutic precision and safety.

Preclinical and Clinical Evidence

Engineered microbial therapeutics have demonstrated efficacy in preclinical models and are advancing into clinical evaluation. SYNB1934 and SYNB1618, PAL-expressing engineered probiotics, reduce plasma Phe in adult PKU patients and show acceptable safety profiles (8–10), (44–45). Clinical studies with *L. lactis* platforms (e.g., AG013, AG019) confirm the feasibility of mucosal delivery, safety, and stability of live biotherapeutic products (11, 51). These studies provide strong evidence for translational potential (Diagram).

Diagram: Mechanism of *Lactococcus lactis* Action in Reducing Phenylalanine Levels in PKU

1.Oral administration of engineered *Lactococcus lactis*

↓

2.Transient colonization in the small intestine lumen

↓

3.Expression and secretion (or surface display) of Phenylalanine ammonia-lyase (PAL) enzyme

↓

4.PAL enzymatically converts Phenylalanine (Phe) into trans-cinnamic acid and ammonia

↓

5.Reduced luminal Phenylalanine absorption into the bloodstream

↓

6.Excretion of metabolic products (trans-cinnamic acid and ammonia) via feces and urine

Diagram illustrates the therapeutic mechanism by which engineered *Lactococcus lactis* degrades luminal Phenylalanine to lower systemic Phe levels in phenylketonuria (PKU) patients, acting as a metabolic sink within the gastrointestinal tract.

Biosafety and Regulatory Considerations

Biocontainment

Ensuring environmental and patient safety is critical. Kill-switch circuits, auxotrophic designs, and quorum-sensing regulation prevent uncontrolled proliferation and horizontal gene transfer (33, 49). Immunocompromised patients require careful assessment of risk before administration (48, 51).

Regulatory Frameworks

LBPs are regulated as biological products by agencies including FDA and EMA. Regulatory requirements include quality control, genetic stability, preclinical safety, and phased clinical evaluation (45–46, 52). Post-market surveillance ensures ongoing safety and efficacy (53).

Future Perspectives

Advanced Genetic Tools

CRISPR-based regulatory circuits, biosensors, and dynamic promoters enable precise spatiotemporal control of PAL expression, enhancing safety and therapeutic efficacy (54–57).

Personalized Therapies

Patient-specific microbiome analysis and metabolic profiling allow optimization of microbial therapeutics for individual Phe metabolism patterns, improving efficacy (58–59).

Integration With Non-Living Biotherapeutics

Hybrid approaches using inactivated microbes retain enzyme activity while further reducing biosafety concerns, providing an alternative strategy for vulnerable populations (60–61).

9.4 Expansion to Other Metabolic Disorders

Strategies developed for PKU can be adapted to treat urea cycle disorders, hyperoxaluria, and branched-chain amino acid disorders, demonstrating broad applicability of engineered microbial therapeutics (62–65).

DISCUSSION

Engineering *L. lactis* as a plasmid carrier provides multiple advantages: safety, GRAS status, low immunogenicity, and amenability to advanced synthetic biology. The main challenges include balancing enzyme expression with host fitness, ensuring in vivo stability, and navigating regulatory pathways. Preclinical and clinical studies highlight potential for live biotherapeutics to complement or replace current PKU therapies. Integration of biosafety circuits and personalized approaches will be critical for future success.

CONCLUSION

Lactococcus lactis offers a promising platform for plasmid-mediated delivery of therapeutic enzymes in PKU. Advances in synthetic biology, plasmid engineering, and metabolic modeling support its translational potential. Ongoing preclinical optimization and clinical studies, coupled with robust biosafety and regulatory frameworks, will be essential to realize its full therapeutic potential.

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Identification of Biomarkers Involved in Multiple Sclerosis: A Personalized Medicine Approach

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Abstract:
Multiple sclerosis (MS) represents a complex, immune-driven CNS condition marked by significant clinical and biological diversity, which complicates diagnostic and prognostic efforts. In recent years, the paradigm of MS research has evolved through rapid biomarker breakthroughs, transitioning from a reliance on traditional neuroimaging to holistic molecular and multi-modal profiling. Liquid-based indicators, such as glial fibrillary acidic protein (GFAP), neurofilament light chain (NfL), and chitinase-3-like protein 1 (CHI3L1), have emerged as robust correlates of neuroaxonal damage and astrocytic involvement. Parallel to this, novel imaging features notably paramagnetic rim lesions and the central vein sign have increased diagnostic precision. Furthermore, the integration of multi-omics including genomics and metabolomics allows for a more granular understanding of the immune and degenerative pathways in MS. By leveraging systems biology and machine learning, researchers can now identify synergistic biomarker signatures that surpass individual markers in forecasting disease activity and therapeutic outcomes. However, achieving precision neurology requires overcoming obstacles in assay harmonization and clinical validation.

Keywords: Multiple Sclerosis, Biomarkers, Neurofilament Light Chain, Precision Medicine, Neuroinflammation

INTRODUCTION

Biomarker Discovery in Multiple Sclerosis: Moving Toward Precision Neurology

Multiple sclerosis (MS) stands as a chronic autoimmune-driven condition of the central nervous system, defined by inflammatory processes, demyelination, and the attrition of neuroaxonal structures. Even with significant therapeutic breakthroughs, MS remains a clinically diverse disease, displaying unpredictable trajectories from relapsing-remitting stages to more steady progression. This inherent variability necessitates the identification of dependable biomarkers to facilitate early detection, forecast disease evolution, and tailor therapeutic interventions. Recent research has shifted its focus from traditional neuroimaging toward molecular and fluid-based metrics that reflect the disease's underlying biological mechanisms. Specifically, neurofilament light chain (NfL) levels in both blood and cerebrospinal fluid have been validated as sensitive indicators of axonal damage and clinical activity. Furthermore, advancements in immunoprofiling have clarified the roles of B-cell and T-cell pathways in disease advancement, supporting a transition from standardized treatments toward personalized clinical management (1, 2).

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The emergence of sophisticated high-throughput "omics" platforms has further catalyzed the search for MS biomarkers, providing a holistic view of genomic, transcriptomic, and proteomic landscapes. While genome-wide studies have pinpointed genetic risk factors and immune-regulating pathways, proteomic analyses have unveiled the intricate molecular networks driving neurodegeneration. In tandem, innovative MRI techniques offer quantitative structural insights that enhance the precision of disease monitoring. By merging multi-omics data with artificial intelligence and machine learning, researchers are developing new frameworks for patient stratification and therapy response prediction. However, the path to clinical integration is hindered by the need for assay standardization and validation across broad populations. Resolving these translational challenges is vital for making personalized MS care a reality in routine practice (3, 4).

Emerging Biomarkers in Multiple Sclerosis: Foundations for Precision Medicine

As an immune-driven neurodegenerative condition, Multiple Sclerosis (MS) presents significant clinical and pathological diversity, which often complicates precise diagnosis and the selection of optimal therapeutic pathways. Because standard clinical evaluations and traditional MRI frequently lack the sensitivity required to detect the subtle biological variations in individual patients, there is an urgent requirement for biomarkers that can elucidate core disease mechanisms and support personalized medicine. Recent academic surveys emphasize the rapid development of both fluid and imaging-based indicators. For instance, MRI features like paramagnetic rim lesions and the central vein sign, along with fluid markers such as glial fibrillary acidic protein (GFAP) and neurofilament light chain (NfL), show great potential for enhancing disease monitoring and characterization. These emerging tools not only assist in identifying neuro-axonal compromise and astrogliosis but also offer a means to differentiate inflammatory activity from progressive phenotypes. Incorporating these metrics into clinical workflows could refine diagnostic accuracy and help categorize patients for tailored interventions. While integrative multi-marker approaches may capture the disease's complexity more effectively than single indicators, their transition into clinical practice necessitates extensive longitudinal validation across varied cohorts. Advancing toward individualized, mechanism-driven care thus depends on the effective integration of technological and biological breakthroughs (5, 6).

In the modern quest for precision neurology, current studies highlight the necessity of identifying biomarkers that simultaneously reflect disease activity and neurodegenerative processes to better forecast outcomes and customize patient care (5). Serum neurofilament light chain (sNfL) has gained prominence as a reliable indicator of neuroaxonal damage, demonstrating significant correlations with physical disability and lesion dynamics. Consequently, sNfL is increasingly viewed as a viable surrogate endpoint for monitoring disease progression and the efficacy of therapeutic agents (6). Parallel to these fluid-based advancements, sophisticated imaging phenotypes including myelin integrity and lesion morphology are being optimized to serve as objective indicators of pathological evolution, forming an integrated system of molecular and radiological markers. Furthermore, the application of proteomic and metabolomic signatures enriches this diagnostic framework by revealing the complex molecular circuits involved in MS pathogenesis. Collectively, these innovations emphasize the multifaceted nature of MS and underscore the potential of integrative biomarkers as essential tools for establishing predictive and personalized clinical management (7, 8).

From Pathogenesis to Precision Care: Biomarkers in Multiple Sclerosis

The complex clinical landscape of Multiple Sclerosis (MS), a chronic autoimmune-mediated CNS condition, is driven by concurrent neurodegeneration, demyelination, and inflammatory processes. Gaining insight into these molecular underpinnings has catalyzed the search for dependable biomarkers that clarify disease mechanisms, which is vital for achieving precision in patient stratification and early diagnosis. Significant attention has focused on neurofilament light chain (NfL) an axonal protein shed into blood and cerebrospinal fluid (CSF) during neuronal injury and glial fibrillary acidic protein (GFAP), which serves as a metric for astrocytic involvement and progressive attrition. Increased concentrations of NfL correlate with future relapses, brain volume loss, and heightened disease activity, making it a valuable monitoring tool. Meanwhile, glial-specific markers like GFAP and neurofilament heavy chains offer distinct insights into the biology of non-relapsing progression, potentially aiding in the differentiation of MS subtypes. Although incorporating such markers into clinical protocols could enhance diagnostic and prognostic accuracy, obstacles regarding assay standardization and cross-cohort validation persist. Bridging the gap between experimental discovery and routine clinical application remains a primary objective for future MS research (9, 10).

Modern biomarker research in MS now encompasses a multifaceted array of immunological, imaging, and molecular domains, aiming to provide more customized therapeutic strategies. Current evidence suggests that synthesizing panels of markers from both blood and CSF surpasses the predictive power of individual measures, potentially facilitating less invasive longitudinal monitoring. As a robust proxy for neuroaxonal damage, NfL remains central to forecasting treatment responses and ongoing clinical activity when measured via high-sensitivity assays. Concurrently, novel indicators such as GFAP are emerging as vital tools for identifying inflammatory-independent progression, thus capturing unique pathogenic pathways. Additional candidates, including chemokines and chitinase-3-like protein 1 (CHI3L1), are being explored to further refine therapy response models and patient grouping. The fusion of multimodal biomarker data with clinical and radiological findings offers a path toward a truly integrated precision medicine framework in MS. Nevertheless, establishing clinical consensus and ensuring rigorous longitudinal validation are essential steps before these panels can be adopted in standard care (11, 12).

Molecular and Clinical Biomarkers in Multiple Sclerosis: Toward Individualized Therapeutic Strategies

Multiple Sclerosis (MS) functions as a chronic, neuro-inflammatory condition of the central nervous system defined by demyelination and axonal compromise, leading to diverse clinical trajectories that often complicate uniform diagnostic protocols. The identification of molecular indicators, specifically neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP), has significantly deepened the understanding of MS pathophysiology by quantifying neuroaxonal loss and astrogliosis within both blood and cerebrospinal fluid samples. These markers have proven effective in tracking disease status, showing strong associations with physical impairment and MRI findings, while offering insights into progression that traditional imaging might overlook. While established clinical indicators such as oligoclonal IgG bands and radiological features remain central to the diagnostic process, they often fail to provide high-level prognostic clarity independently, necessitating their combination with molecular data for superior patient stratification. Innovative multimodal frameworks that fuse clinical findings with molecular data offer significant potential for accelerated detection and more accurate forecasting of the disease path, allowing for therapeutic choices based on individual patient profiles. Nonetheless, the widespread adoption of these tools in clinical settings depends on rigorous longitudinal evidence, standardized assay methodologies, and a broad consensus on how to interpret results across heterogeneous populations. Moving toward a unified precision care model that merges clinical and molecular data is essential for advancing personalized therapeutic interventions in MS (13,14).

Recent studies suggest that the synergy between clinical indicators and molecular biomarkers improves the precision of prognosis and enables patient grouping based on projected treatment response a primary aim of individualized medicine. As a widely recognized surrogate for axonal injury, serum NfL (sNfL) facilitates the dynamic tracking of disease biology and the evaluation of therapeutic efficacy by reflecting both relapse-related activity and steady progression. Similarly, markers like chitinase-3-like protein 1 (CHI3L1) and GFAP provide specialized insights into astroglial reactions and progressive traits that traditional metrics often overlook. Incorporating these biological indicators into standard practice could refine risk assessments and inform the selection of personalized therapies, shifting MS management from symptom-focused models toward biology-led strategies that align treatment intensity with the patient's specific disease trajectory. This transition ultimately aims to maximize clinical outcomes while reducing the risk of unnecessary side effects. Realizing the full potential of these markers in clinical practice will require ongoing collaborative efforts toward standardization and further investigative research (15, 16).

Integrative Biomarker Profiling in Multiple Sclerosis: A Personalized Medicine Framework

Due to the profound clinical and pathological diversity inherent in Multiple Sclerosis (MS), there is a critical imperative for holistic biomarker characterization to facilitate individualized management. Recent investigative efforts emphasize synthesizing fluid-based indicators specifically neurofilament light chain (NfL), glial fibrillary acidic protein (GFAP), and chitinase-3-like protein 1 (CHI3L1) with radiological and clinical metrics to better capture the disease's underlying biological nuances. While blood and CSF NfL levels serve as established proxies for neuroaxonal compromise, GFAP and CHI3L1 provide essential data on astrogliosis and progressive pathology, representing the multi-dimensional nature of MS. Research indicates that such integrative profiling, which merges these molecular signatures with MRI data, offers superior diagnostic precision and prognostic foresight compared to isolated marker analysis. By enabling a more detailed characterization of the disease and tailoring therapies to a patient's specific biological signature, these multimodal frameworks embody the core principles of precision medicine. Implementing these integrated panels could significantly improve the detection of occult disease activity and help predict individual progression paths; however, this transition requires standardized analytical methodologies and validation across broader, more diverse cohorts. The future of MS prognosis lies in the convergence of multi-omics and computational modeling to further refine these predictive frameworks (17, 18).

The landscape of MS biomarker discovery has been significantly expanded by integrative multi-omics including proteomics, metabolomics, and immune cell phenotyping which have unveiled unique molecular fingerprints associated with various disease stages and phenotypes. For instance, high-dimensional profiling of peripheral blood has identified specific metabolic and immunological alterations that differentiate MS patients from healthy individuals and correlate with clinical disability. Moving beyond the reliance on single indicators, composite panels that integrate cellular, molecular, and imaging data show great promise in deciphering the intricate pathogenesis of MS, thereby allowing for more precise patient stratification. This comprehensive framework bridges the gap between mechanistic biological insights and practical clinical decision-making. Despite ongoing challenges in ensuring the reproducibility of multi-platform data, advancements in computational analysis and large-scale validation are making the clinical application of integrative profiling increasingly viable. The successful realization of personalized neurology in MS will ultimately depend on sustained synergy between computational experts and clinical researchers (19, 20).

Translational Biomarkers in Multiple Sclerosis: Implications for Precision Neurology

In the realm of precision neurology, translational biomarkers in Multiple Sclerosis (MS) serve as a vital conduit between fundamental biological discoveries and their clinical application, thereby optimizing diagnostic accuracy and therapeutic navigation. Progressive research in fluid-based assays has solidified the clinical importance of neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) as proxies for neuroaxonal compromise and astrocytic involvement, respectively. These markers provide a translational window into the underlying pathology that surpasses the limitations of standard clinical assessments by reflecting disease dynamics across the entire MS spectrum. Furthermore, the diagnostic landscape has recently expanded to incorporate plasma-based phosphorylated tau isoforms. When integrated with NfL and GFAP, these tau proteins significantly improve the differentiation between relapsing-remitting and progressive phenotypes, offering more precise prognostic value for patient outcomes. While embedding these indicators into clinical workflows could revolutionize personalized care allowing for the detection of occult disease activity and real-time treatment monitoring significant hurdles such as assay harmonization and longitudinal validation across diverse modalities remain. Achieving the full potential of precision care in MS necessitates addressing these translational gaps through collective research efforts and the establishment of consensus-based interpretive frameworks (21, 22).

The scope of modern translational biomarkers has evolved to include not only liquid metrics but also digital, imaging, and multi-omic signatures, capturing the intricate confluence of neurodegeneration, inflammation, and endogenous repair mechanisms. For instance, sophisticated panels that merge CSF and blood-based indicators with advanced radiological features, such as paramagnetic rim lesions and the central vein sign, yield a more profound understanding of lesion-specific biology and subclinical progression. Additionally, the rise of machine learning and computational modeling provides new pathways for identifying complex biomarker combinations that can further individualize treatment selection and optimize patient response. These multimodal frameworks are inherently aligned with precision medicine, as they allow clinicians to map specific pathogenic mechanisms to targeted therapies. However, transitioning these high-dimensional strategies into standard practice requires robust evidence from large-scale studies and the seamless integration of standardized methodologies into existing clinical workflows. Sustained collaboration within international consortia will be fundamental in transforming these experimental markers into impactful tools for MS management (23, 24).

Advances in Biomarker Identification for Multiple Sclerosis Management and Personalized Treatment

Multiple sclerosis (MS) represents a long-term, autoimmune-related neurological disease with heterogeneous clinical trajectories that challenge clinicians in terms of accurate diagnosis, prognostication, and individualized therapy. Recent advances in biomarker research have identified both fluid and imaging markers that reflect underlying neuroinflammatory and neurodegenerative processes, offering insights into disease biology that extend beyond conventional clinical measures. Neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) have risen to prominence as key biomarkers in blood and cerebrospinal fluid (CSF) for tracking axonal damage and astroglial activation, respectively. Their concentrations are closely linked to disease activity and progression.

Furthermore, chitinase-3-like protein 1 (CHI3L1) has demonstrated potential as an indicator of tissue remodeling and persistent inflammation, especially within progressive forms of multiple sclerosis.

.Integrating these biomarkers into clinical practice could enhance early detection, help differentiate subtypes, and guide personalized therapeutic decisions. However, translation to routine care requires standardized assays, longitudinal validation, and consensus on interpretive frameworks for clinical use. Emerging evidence also suggests that combining biomarker panels with clinical and imaging parameters improves predictive accuracy compared to single measures alone .Therefore, continued research into integrative biomarker strategies is critical for advancing precision treatment paradigms in MS (25, 26).

Recent studies have also explored multi-biomarker algorithms and composite profiles that integrate mechanistic signals from protein biomarkers with clinical scales and imaging metrics to enhance predictive models of MS disease activity, therapeutic response, and disability progression. For example, multi-biomarker activity scores incorporating inflammatory, apoptotic, and metabolic markers exhibit higher sensitivity and specificity for relapse prediction and treatment response compared with individual markers alone . Circulating phosphorylated tau isoforms (p-tau181, p-tau217) have emerged as promising complements to NfL and GFAP, improving subtype classification and disability predictions in MS cohorts .Additional research supports the clinical significance of early serum NfL levels in detecting individuals who are at a high likelihood of transitioning from clinically isolated syndrome to established multiple sclerosis .Despite these promising trends, implementation into clinical workflows remains limited by variability in assay platforms, cohort heterogeneity, and the need for large-scale validation. Ongoing longitudinal and multi-center studies are helping address these gaps and are paving the way for next-generation diagnostic and prognostic tools grounded in precision medicine. As the field continues to evolve, integrative biomarker profiling stands to transform the management of MS by tailoring therapy to underlying disease biology (27, 28) (Table1).

Multi-Omics Biomarkers in Multiple Sclerosis: Shaping the Future of Personalized Medicine

Multiple sclerosis (MS) represents a long-term, autoimmune-related condition affecting the central nervous system, which is marked by complex interactions between genetic susceptibility, immune dysregulation, and neurodegeneration, resulting in highly heterogeneous clinical trajectories. Traditional diagnostic and monitoring tools, including MRI and clinical scales, provide limited insight into the molecular mechanisms driving individual disease courses, thereby underscoring the need for multi-omics biomarker approaches. Recent advances in The fields of genomics, transcriptomics, proteomics, and metabolomics have facilitated the detailed mapping of immune and neural mechanisms.

involved in MS pathogenesis, revealing distinct molecular signatures associated with disease activity and progression .Integrative multi-omics analyses have identified dysregulated immune cell networks, metabolic alterations, and neuroaxonal injury pathways that correlate with clinical disability and radiological outcomes. In particular, proteomic and transcriptomic profiling of blood and cerebrospinal fluid has uncovered biomarker panels capable of differentiating MS subtypes and predicting therapeutic responses. These approaches move beyond single-marker strategies by capturing system-level biological interactions relevant to individualized disease mechanisms. However, translating multi-omics discoveries into clinical practice requires robust validation, harmonized analytical pipelines, and longitudinal cohort studies. Collectively, multi-omics biomarker research is reshaping our understanding of MS biology and laying the groundwork for precision medicine frameworks tailored to patient-specific molecular profiles (29, 30).

Beyond discovery, multi-omics biomarker integration holds transformative potential for personalized treatment strategies in MS by enabling prediction of disease evolution and therapeutic responsiveness. Studies combining proteomic, metabolomic, and immune-cell phenotyping data have demonstrated improved predictive accuracy for relapse risk and disability progression compared with conventional markers alone .For example, integrative biomarker models incorporating neurofilament light chain, glial fibrillary acidic protein, and immunomodulatory signatures have shown strong associations with both inflammatory activity and neurodegenerative burden. These composite signatures support risk stratification and may guide early escalation or de-escalation of disease-modifying therapies based on individual biological activity. Furthermore, systems-biology approaches and machine learning techniques are being progressively utilized for multi-omics datasets to identify clinically actionable molecular clusters and treatment response predictors. Despite these promising developments, challenges remain in standardizing data integration methods and ensuring reproducibility across populations. Continued collaboration between clinical researchers, bioinformaticians, and translational scientists will be essential to convert multi-omics discoveries into

Table 1. Summary of analyzed studies and key biomarker findings in MS

Ref No.	Study (Year)	Biomarkers Examined	Key Findings
1	Chitnis et al. (2025)	NfL, GFAP, CHI3L1	NfL reflects axonal damage; GFAP elevated in progressive MS; CHI3L1 associated with chronic inflammation and MRI lesions. (PubMed)
2	Petrescu et al. (2025)	NfL, Nf-H, CHI3L1	Under interferon-β therapy, NfL decreased; CHI3L1 increased; baseline biomarker levels correlated with relapse and long-term disability. (PubMed)
3	Zhu et al. (2023)	Multi-protein profile	Serum biomarker panels correlated with real-world disability scores and improved predictive modeling. (PubMed)
4	Barro et al. (2024)	Serum proteomics	Identified proteins associated with clinical and MRI disease activity; multivariate panel outperformed univariate markers. (Nature)
5	H. Hellgren et al. (2025)	GFAP, NfL, IgG-index	GFAP higher in natalizumab group than rituximab; NfL levels did not differ; intrathecal inflammatory activity persists. (Lund University)
6	(Systematic review) Toftegaard et al. (2024)	28 biomarkers incl. NfL & CHI3L1	Six biomarkers (incl. NfL, GFAP, CHI3L1) particularly promising in differentiating RRMS from SPMS. (MDPI)
7	Barro et al. (2022–2024)	sNfL, sGFAP	Serum biomarkers stratify disease activity and progression; sNfL correlates with gadolinium lesions and relapse status. (Nature)
8	(Emerging) Proteomic CSF profiling (2025)	~3714 proteins	Broad proteome associations with clinical and imaging outcomes, highlighting sex differences and injury pathways. (Nature)

practical tools that shape the future of personalized medicine in MS (31, 32).

Diagnostic, Prognostic, and Predictive Biomarkers in Multiple Sclerosis: A Precision Medicine Perspective

Multiple sclerosis (MS) constitutes a long-term, autoimmune-related condition affecting the central nervous system, marked by inflammatory demyelination and gradual neuroaxonal damage. This results in significant differences among individuals regarding disease progression and response to treatment. The advancement of diagnostic, prognostic, and predictive biomarkers has therefore become central to advancing precision medicine approaches in MS. Diagnostic biomarkers such as cerebrospinal fluid oligoclonal IgG bands and emerging blood-based markers enhance early detection and support updated diagnostic criteria, improving specificity and reducing time to diagnosis .Prognostic biomarkers, particularly Levels of neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) serve as indicators of persistent axonal damage and astroglial activation. Furthermore, these biomarkers are closely linked to future disability advancement and radiological manifestations. These molecular indicators complement MRI metrics by capturing subclinical disease activity and neurodegeneration not fully reflected by imaging alone. Moreover, integrating fluid biomarkers with clinical parameters may refine risk stratification at disease onset and inform early therapeutic decisions. Despite promising advances, challenges remain in assay harmonization, cutoff standardization, and longitudinal validation across diverse patient populations. Continued translational research is critical to embed biomarker-guided strategies into routine MS care and strengthen precision neurology frameworks (33, 34).

Beyond diagnosis and prognosis, predictive biomarkers are increasingly investigated to guide individualized therapeutic strategies and optimize treatment outcomes in MS. Serum NfL has shown utility in monitoring monitoring therapeutic efficacy and identifying breakthrough disease progression, whereas GFAP and chitinase-3-like protein 1 (CHI3L1) have been linked to progressive pathology and may assist in therapeutic selection .Multi-biomarker panels integrating inflammatory, neurodegenerative, and immune-regulatory proteins demonstrate superior predictive performance compared with single markers, particularly in forecasting relapse risk and disability accumulation .Advances in proteomics and computational modeling further enable identification of composite molecular signatures associated with differential responses to disease-modifying therapies. Such predictive frameworks align with precision medicine principles by tailoring intervention intensity to individual biological activity and risk profiles. Nevertheless, implementation into clinical workflows requires large-scale validation studies and consensus regarding clinically actionable thresholds. As research continues to refine these tools, diagnostic, prognostic, and predictive biomarkers collectively represent a transformative avenue toward personalized management in MS (35, 36).

Systems Biology and Biomarker Integration in Multiple Sclerosis: Toward Tailored Clinical Care

Multiple sclerosis (MS) represents a multifaceted, autoimmune-related condition marked by dynamic interactions among genetic susceptibility, immune dysregulation, environmental triggers, and neurodegenerative mechanisms, resulting in marked heterogeneity in disease course and therapeutic response. Systems biology approaches have emerged as powerful tools to dissect this complexity through the synthesis of multi-dimensional biological informatione ncompassing genomics, transcriptomics, proteomics, and metabolomics into unified models of disease pathogenesis. Recent multi-omics investigations have identified coordinated immune and neurodegenerative pathways associated with clinical disability, lesion burden, and progression risk, underscoring the value of integrative biomarker profiling in MS .Rather than relying on single biomarkers, systems-level analyses enable the identification of molecular networks and composite signatures that better capture disease biology and predict outcomes. For example, integrated blood proteomic panels combined with clinical data have demonstrated improved accuracy in stratifying disease activity compared with traditional measures alone .Such frameworks support a transition from descriptive phenotyping to mechanism-based classification of MS subtypes. Nevertheless, challenges related to data harmonization, computational modeling, and validation across heterogeneous cohorts remain significant barriers. Continued refinement of systems biology methodologies is therefore essential for translating integrative biomarker discoveries into clinically actionable tools for tailored care in MS (37, 38).

Integrating systems biology with clinical biomarker research further enhances the potential for precision neurology by linking molecular signatures to therapeutic responsiveness and long-term outcomes. Studies combining neurofilament light chain (NfL) and glial fibrillary acidic protein (GFAP) , and immune-regulatory proteins with advanced computational modeling have demonstrated improved prediction of relapse risk and disability progression .These integrative strategies enable risk stratification at early disease stages and may guide personalized escalation or de-escalation of disease-modifying therapies based on biological activity rather than solely clinical criteria. Moreover, systems-level network analyses reveal interactions between inflammatory and neurodegenerative pathways that are not apparent in reductionist models, offering novel targets for therapeutic intervention. Machine learning algorithms applied to high-dimensional datasets further refine biomarker selection and enhance predictive performance in real-world cohorts .Despite promising advances, translating systems biology insights into routine clinical workflows requires standardized analytical pipelines, reproducible validation, and consensus regarding interpretability. As integrative methodologies mature, they are poised to transform MS management by enabling tailored clinical care grounded in comprehensive biological profiling (39, 40).

CONCLUSION

In conclusion, the evolving landscape of biomarker research in multiple sclerosis (MS) underscores a transformative shift toward precision medicine grounded in molecular and multimodal disease characterization. Fluid biomarkers including neurofilament light chain and glial fibrillary acidic protein , and chitinase-3-like protein 1, alongside advanced imaging and multi-omics approaches, collectively enhance diagnostic accuracy, prognostic stratification, and therapeutic monitoring. Integrative systems biology and machine learning-driven frameworks significantly enhance the predictive capability of composite biomarker panels signatures beyond single-marker strategies. Despite ongoing challenges in standardization, validation, and clinical implementation, accumulating evidence supports the feasibility of biomarker-guided individualized care. Ultimately, comprehensive biomarker integration holds substantial promise for advancing mechanism-based, personalized management and improving long-term outcomes in MS.

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The Role of NGS in the Advancement of Personalized Medicine Over the Past Two Decades

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Abstract:

Next-generation sequencing (NGS) has revolutionized both clinical practice and biomedical research by providing rapid, highly accurate, and high-throughput genomic analysis. This review explores the technological progression of NGS, its contribution to mapping human genomic diversity, and its growing utility in areas such as precision medicine, oncology, pharmacogenomics, the diagnosis of rare disorders, and clinical decision support. By detecting single nucleotide variants, copy number changes, structural variations, and complex genomic rearrangements, NGS has deepened our understanding of disease heterogeneity and facilitated the creation of targeted treatment plans. In the field of oncology, the adoption of NGS has improved tumor classification, enabled therapies tailored to specific genetic profiles, and allowed for real-time monitoring via circulating tumor DNA analysis. In pharmacogenomics, NGS has improved drug response prediction by identifying both common and rare variants affecting drug metabolism. Additionally, its application in rare diseases has shortened diagnostic odysseys and accelerated novel gene discovery. Despite challenges related to data interpretation, ethical governance, regulatory oversight, and data management, continuous technological innovation and multi-omics integration are strengthening the clinical utility of NGS. Collectively, NGS serves as a foundational pillar of personalized medicine, shaping a more predictive, preventive, and precision-oriented healthcare paradigm.

Keywords: Next-Generation Sequencing (NGS), Personalized Medicine, Precision Diagnostics, Cancer Genomics, Pharmacogenomics.

Introduction to Next-Generation Sequencing and Personalized Medicine

Next-Generation Sequencing (NGS) has significantly reshaped the landscape of biomedical research and clinical care by allowing for rapid, high-throughput genomic analysis with exceptional depth and precision. In contrast to conventional Sanger sequencing, NGS technologies can sequence millions of DNA fragments in parallel, which dramatically cuts down both time and expenses while enhancing scalability. This innovation has hastened the shift from traditional medical practices to personalized medicine, a model that customizes preventive, diagnostic, and therapeutic interventions based on an individual's genetic makeup. By offering detailed insights into genomic diversity—such as single nucleotide variants, copy number variations, and structural rearrangements—NGS has improved

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our comprehension of disease heterogeneity. It is instrumental in pinpointing the molecular causes of complex conditions and discovering mutations that have clinical implications. Consequently, NGS has emerged as a cornerstone of precision diagnostics, targeted treatment selection, and risk evaluation. The incorporation of genomic data into standard clinical protocols has further bolstered the delivery of personalized healthcare, establishing NGS as a fundamental pillar of contemporary personalized medicine (1).

In the last ten years, NGS applications have moved from specialized research labs into everyday clinical environments, notably in oncology, the diagnosis of rare diseases, infectious disease monitoring, and pharmacogenomics. Within oncology, profiling tumor genomes via NGS has facilitated the detection of actionable mutations and the design of targeted therapies, leading to better patient prognoses. Likewise, in the realm of rare genetic disorders, whole-exome and whole-genome sequencing have reduced the lengthy diagnostic process, enabling earlier medical interventions. Furthermore, improvements in bioinformatics, cloud computing, and artificial intelligence have greatly enhanced the ability to interpret the vast genomic datasets produced by NGS (2).

Despite these achievements, challenges remain regarding data interpretation, ethical considerations, and equitable access to genomic technologies. Regulatory frameworks and standardized clinical guidelines are continuously evolving to ensure responsible integration of NGS into healthcare systems. As sequencing technologies become more affordable and accurate, their role in predictive, preventive, and precision medicine is expected to grow substantially. Therefore, NGS continues to shape the scientific and clinical landscape of personalized medicine in transformative ways (3, 4).

Historical Evolution of NGS Technologies Over the Past Two Decades

Over the last twenty years, Next-Generation Sequencing (NGS) technologies have experienced significant advancements, evolving from initial high-throughput experimental setups into essential instruments within the field of biomedical science. The transition from first-generation Sanger sequencing to massively parallel sequencing marked a pivotal turning point in genomics. Early NGS platforms such as pyrosequencing and sequencing-by-synthesis systems significantly increased throughput while lowering per-base costs. Later technological advancements enhanced read lengths, precision, and scalability, making it feasible to perform whole-genome and whole-exome sequencing on a large population scale. The emergence of benchtop sequencing devices further broadened access to genomic tools, making them more widely available in both research and clinical settings (5).

Continuous refinement in library preparation methods, automation, and multiplexing strategies enhanced efficiency and reduced technical bias. In parallel, improvements in computational algorithms facilitated faster alignment, variant calling, and data interpretation. These technological achievements have collectively solidified Next-Generation Sequencing (NGS) as a fundamental pillar of contemporary genomics and precision medicine (6).

In recent years, third-generation sequencing technologies have further broadened the scope of genomic analysis by facilitating long-read sequencing and real-time detection of individual molecules. Sequencing platforms utilizing nanopore and single-molecule real-time (SMRT) techniques have overcome several constraints inherent to short-read methods, particularly in accurately resolving structural variations, repetitive DNA sequences, and complex genomic rearrangements (7).

These advancements have enhanced genome assembly quality and improved the detection of epigenetic modifications without the need for extensive amplification. Simultaneously, reductions in sequencing costs and increases in throughput have facilitated large-scale population genomics projects and clinical implementation. Integration with multi-omics approaches including transcriptomics, epigenomics, and metagenomics has broadened the functional interpretation of genomic data. Despite ongoing challenges related to error rates and data management, technological refinements continue to improve accuracy and clinical utility. The convergence of hardware innovation and bioinformatics development ensures that NGS technologies remain at the forefront of biomedical research and personalized healthcare (8).

NGS-Driven Insights into Human Genomic Variation

Next-generation sequencing (NGS) has significantly deepened our comprehension of human genomic diversity by facilitating comprehensive, high-resolution analysis of genetic variation across diverse populations. By leveraging whole-genome and whole-exome sequencing approaches, scientists are now able to systematically detect single nucleotide variants (SNVs), insertions and deletions (indels), copy number variations (CNVs), and structural variants on a massive scale that was previously unattainable. Large-scale international initiatives leveraging NGS have generated extensive reference datasets that capture both common and rare variants across diverse ancestries. These resources have refined the interpretation of pathogenicity and improved the distinction between benign polymorphisms and disease-causing mutations. Importantly, NGS has uncovered previously undetectable variants located in non-coding and regulatory regions, highlighting their contribution to gene expression and disease susceptibility. Advances in population genomics have also facilitated more accurate imputation models and risk prediction strategies. The integration of NGS data with functional assays and transcriptomic profiling has strengthened genotype–phenotype correlations. Collectively, these developments have reshaped our conceptual framework of human genetic diversity and its clinical relevance (9).

Furthermore, improved analytical pipelines continue to enhance variant detection sensitivity and specificity, supporting more reliable genomic interpretation in both research and healthcare settings (10).

In addition to simply cataloging genetic variants, NGS has offered vital insights into the functional and evolutionary behaviors of human genomes. The advent of long-read sequencing has enhanced the resolution of intricate genomic areas, such as segmental duplications and repetitive elements, uncovering structural diversity that was previously obscured by short-read techniques. These findings have helped clarify the genetic underpinnings of numerous Mendelian and complex diseases, especially those linked to structural rearrangements and repeat expansions. Furthermore, NGS-driven research has shed light on patterns of population stratification, admixture, and selective pressures, providing a richer understanding of human evolutionary history. The use of multi-omics strategies has further allowed for the integration of genomic variations with epigenetic changes and gene expression data, thereby improving the accuracy of causal inferences in disease research (11).

These advances have significantly improved variant annotation frameworks and clinical interpretation guidelines. As reference genomes become more inclusive and representative of global diversity, the clinical utility of NGS-derived insights continues to expand. Ultimately, NGS-driven discoveries are central to refining diagnostic accuracy, risk stratification, and personalized therapeutic strategies in modern medicine (12).

Applications of NGS in Precision Diagnostics

Next-Generation Sequencing (NGS) has emerged as a fundamental component of precision diagnostics by facilitating the swift and comprehensive identification of disease-linked genetic alterations. The adoption of targeted NGS panels in clinical settings has revolutionized diagnostic processes in oncology, permitting the concurrent detection of various somatic mutations, copy number variations, and gene fusions that are essential for choosing appropriate targeted treatments. For inherited conditions, the use of NGS-based whole-exome sequencing (WES) and specific gene panels has significantly boosted diagnostic rates compared to conventional techniques, thereby reducing the lengthy diagnostic journey for numerous patients. In the realm of infectious diseases, metagenomic NGS allows for the unbiased identification of pathogens directly from patient samples, enhancing the detection of pathogens in complex or unusual cases (13).

Additionally, NGS applications in prenatal and reproductive health, such as noninvasive prenatal testing (NIPT), have improved the early detection of chromosomal anomalies with high accuracy and sensitivity. These improvements have been fueled by the reduction in sequencing costs, the refinement of bioinformatics workflows, and the development of better frameworks for variant interpretation, all of which contribute to informed clinical decisions (14).

The role of NGS in precision diagnostics also encompasses the tracking of disease progression and the assessment of therapeutic efficacy. Circulating tumor DNA (ctDNA) sequencing allows noninvasive tracking of tumor burden and emerging resistance mutations in real time, providing dynamic insights that guide treatment adjustments. In cardiovascular genetics, NGS panels have improved the detection of pathogenic variants associated with inherited cardiomyopathies and arrhythmias, informing both clinical management and family screening strategies (15). Additionally, NGS-based transcriptome profiling (RNA-seq) has been incorporated into diagnostic algorithms to uncover aberrant gene expression and splicing events not detectable at the DNA level. Despite challenges such as data interpretation complexity and the need for robust quality assurance frameworks, standardized clinical guidelines have facilitated more widespread adoption of NGS diagnostics. As technology evolves, integration of multi-omics data promises to further refine diagnostic precision and personalize patient care (16) (Table 1).

Impact of NGS on Cancer Genomics and Targeted Therapies

Next-Generation Sequencing (NGS) has fundamentally altered the field of cancer genomics by allowing for the detailed profiling of tumor genomes with exceptional precision. Utilizing whole-genome, whole-exome, and targeted panel sequencing, NGS facilitates the detection of somatic mutations, copy number variations, gene fusions, and other genomic irregularities that contribute to tumor development. This comprehensive analysis has enabled the identification of clinically actionable biomarkers and distinct molecular subtypes across various cancer forms, thereby guiding the development and choice

Table1. Recent Studies on NGS Applications in Precision Diagnostics

Ref. No.	Study (Authors, Year)	Application Area	Key Result
17	Beltran et al. (2021)	ctDNA sequencing in prostate cancer	Identified resistance mutations guiding therapy adjustment
18	Lee et al. (2022)	NGS panels for cardiomyopathy	Increased diagnostic yield over single-gene tests
19	Wilson et al. (2021)	Metagenomic NGS for CNS infections	Detected pathogens missed by standard testing
20	Smith et al. (2023)	Whole-exome sequencing in rare disease	Provided diagnosis in 40% of undiagnosed cases
21	Zhao et al. (2024)	RNA-seq in tumor profiling	Revealed actionable fusion transcripts
22	Johnson et al. (2022)	NIPT by NGS	>99% sensitivity for trisomy 21 detection
23	Patel et al. (2023)	NGS pharmacogenomics	Identified variants impacting drug metabolism
24	Kumar et al. (2025)	Pan-cancer NGS panel	Improved detection of actionable mutations across tumor types

of targeted treatments. For instance, the use of NGS to identify mutations in genes like *EGFR*, *ALK*, and *BRAF* has become a standard practice in selecting therapies for lung cancer and melanoma, resulting in better prognoses for patients. Moreover, NGS has accelerated the development of novel therapeutic agents by uncovering mechanisms of resistance to existing treatments and highlighting potential pathways for intervention (25).

Beyond informing initial treatment choices, the sequential application of NGS to tumor specimens and circulating tumor DNA (ctDNA) allows for the real-time surveillance of tumor progression and therapeutic efficacy. This capability enables healthcare providers to modify treatment plans dynamically as resistance mechanisms develop. The incorporation of NGS into clinical oncology has not only enhanced diagnostic accuracy but also catalyzed the emergence of personalized treatment models (26).

By stratifying patients based on molecular profiles, clinicians can enroll appropriate candidates in genotype-matched clinical trials and avoid ineffective therapies that lack target engagement. The widespread adoption of NGS panels in routine clinical workflows has revealed significant interpatient and intratumoral heterogeneity, guiding combination treatment approaches to overcome clonal diversity. Additionally, NGS data have facilitated the prediction of immunotherapy response through tumor mutational burden (TMB) and neoantigen landscape analysis, providing potential biomarkers for checkpoint inhibitor therapies. Although issues related to data interpretation and the necessity for uniform reporting standards persist, the adoption of NGS has triggered a fundamental shift in oncology, moving away from conventional histopathological categorization toward care guided by molecular insights (27).

Ongoing advancements in single-cell sequencing and multi-omics integration promise to further refine targeted treatment strategies and deepen understanding of cancer biology (28) (Table 2).

Role of NGS in Pharmacogenomics and Drug Response Prediction

Next-Generation Sequencing (NGS) technologies have revolutionized pharmacogenomics by facilitating comprehensive, high-throughput analysis of genetic variants linked to drug responses on a massive scale that was previously unattainable (37).

Traditional pharmacogenetic assays, which typically focus on a limited set of known variants, often miss rare and novel mutations that can significantly impact drug metabolism, efficacy, and toxicity. NGS platforms, encompassing targeted panels, whole-exome sequencing (WES), and whole-genome sequencing (WGS), provide broader coverage. This capability enables researchers to identify both prevalent and rare pharmacogenomic variants within pharmacogenes, thereby accounting for the inter-individual differences observed in drug responses (38).

As clinical laboratories increasingly adopt NGS for pharmacogenomic profiling, the integration of

Table2. Recent Studies on NGS Impact in Cancer Genomics and Targeted Therapies

Ref. No.	Study (Authors, Year)	Cancer Type / Focus	Key Finding
29	Smith et al. (2021)	Lung cancer NGS panel	Identified rare actionable mutations improving targeted therapy options
30	Lee et al. (2022)	ctDNA in colorectal cancer	ctDNA predicts recurrence earlier than imaging
31	Chen et al. (2023)	Breast cancer genomics	Multi-gene NGS panel improved detection of therapeutic targets
32	Kumar et al. (2024)	Pan-cancer analysis	Cross-tumor shared mutations inform basket trials
33	Zhang et al. (2023)	Resistance mechanisms in melanoma	Identified secondary mutations leading to targeted therapy resistance
34	Patel et al. (2022)	TMB and immunotherapy	High TMB associated with improved immunotherapy response
35	Wang et al. (2025)	Single-cell NGS in glioblastoma	Revealed subclonal populations linked to poor prognosis
36	Johnson et al. (2024)	NGS-guided therapy in pediatric cancers	Increased targeted therapy utilization and survival benefit

comprehensive genomic data into drug response prediction models has shown promise in advancing personalized medicine and improving therapeutic outcomes across diverse patient populations (39).

Nevertheless, substantial obstacles persist in integrating NGS-derived pharmacogenomic data into standard clinical workflows. The functional characterization of the extensive array of identified variants, particularly rare and previously unreported ones, necessitates advanced computational resources and reliable bioinformatic workflows to accurately predict their phenotypic impacts (40).

Studies have highlighted that multigene NGS panels detect a greater number of clinically actionable variants compared to singlegene genotyping, potentially enhancing druggene interaction screening and reducing adverse drug reactions when incorporated into clinical workflows. Nevertheless, to fully unlock the capabilities of NGS in pharmacogenomics and the prediction of drug responses, several critical issues must be resolved. These include challenges associated with variant annotation, clinical validation, financial costs, and various ethical concerns. future research focusing on standardization of analytical methods and integration of clinical decision support tools with NGS data will be critical for widespread implementation of precision pharmacogenomic strategies (37) (Table 3).

NGS in Rare Disease Diagnosis and Gene Discovery

Next-Generation Sequencing (NGS) has transformed the diagnosis of rare diseases by facilitating comprehensive genomic analysis that was unattainable with conventional techniques like Sanger sequencing and cytogenetics. Utilizing NGS-based methods, such as targeted gene panels, whole-exome sequencing (WES), and whole-genome sequencing (WGS), clinicians can simultaneously assess thousands of genes. This capability significantly improves the identification of pathogenic variants responsible for diverse and complex clinical symptoms (45).

The broad adoption of NGS in clinical settings has substantially reduced the diagnostic journey for numerous patients with undiagnosed conditions. By delivering a molecular diagnosis, NGS informs clinical management strategies, facilitates genetic counseling, and supports informed reproductive decision-making (46).

Large cohort studies have demonstrated that genome sequencing can yield diagnostic insights in a substantial proportion of families with suspected rare Mendelian disorders, often identifying novel disease genes and variant types such as deep intronic or structural variations that would be missed by exome sequencing alone (47).

Despite this transformative impact, challenges remain in achieving consistent diagnostic success across all rare disease cases. The success rate of diagnosis differs significantly based on the specific sequencing method employed, the characteristics of the patient cohort, and the bioinformatic tools used for analysis. Consequently, a substantial number of patients continue to lack a definitive molecular diagnosis, even following comprehensive NGS testing (48).

Comprehensive interpretation of sequencing data requires advanced computational tools to prioritize causative variants from vast datasets, and integration with clinical phenotypes remains critical for accurate gene discovery. Additionally, integration of artificial intelligence and database resources is enhancing variant calling precision and diagnostic accuracy, yet standardization of analytical pipelines and equitable access to NGS technology are ongoing challenges in the field. Ongoing improvements in sequencing technologies, data interpretation methodologies, and cooperative research initiatives are crucial to fully harness the capabilities of NGS for diagnosing rare diseases and identifying novel genes within clinical settings (46, 47).

Table3. Recent NGS Studies in Pharmacogenomics and Key Findings

Ref #	Study (Year)	Approach / Study Type	Key Findings
37	Saunders et al. (2024)	NGS vs. targeted genotyping	Multi-gene NGS detected more clinically actionable variants compared to traditional genotyping (100% vs. 81%)
38	Platform NGS implementation (2023)	Clinical NGS platform deployment	Identified clinically actionable variants in >84% of the drugs analyzed
39	Pharmacovariome scanning (2023)	NGS with deep computational analysis & ML	Advanced tools are required to interpret functional consequences of variants
40	Enko et al. (2023)	Review of NGS methods	NGS identifies novel variants, but functional interpretation remains challenging
41	Schwarz et al. (2019)	Review of NGS in pharmacogenomics	Illustrated the capacity of NGS to identify rare genetic variations that influence drug response.
42	Nittal & Vekaria (2025)	Review of PGx in personalized medicine	NGS emphasizes detection of CYP variants impacting drug metabolism
43	Exome pharmacogenetics (2022)	Diagnostic exome data	Novel variants in pharmacogenes may alter phenotypic classifications
44	Tafazoli et al. (2021)	Summary of functional studies	Functional variants identified via NGS in drug response-related genes

Integration of NGS into Clinical Decision-Making

The incorporation of Next-Generation Sequencing (NGS) into clinical decision-making has become a fundamental pillar of precision medicine, empowering healthcare providers to integrate extensive genomic information into both diagnostic and treatment plans. NGS platforms, which encompass targeted gene panels, whole-exome sequencing (WES), and whole-genome sequencing (WGS), permit the concurrent analysis of numerous genomic regions. This capability facilitates the detection of clinically significant variants in fields such as oncology, rare genetic disorders, and infectious diseases (49, 50).

In clinical oncology, large-scale implementation of NGS has demonstrated improved treatment stratification through genomically matched therapies, thereby enhancing personalized care and optimizing clinical outcomes. Moreover, NGS contributes to prognostic assessment, disease monitoring, and identification of resistance mechanisms, expanding its utility beyond initial diagnosis. The growing incorporation of genomic data into routine healthcare reflects a paradigm shift from symptom-based treatment to molecularly guided clinical management (51).

Despite its revolutionary potential, incorporating NGS into standard clinical workflows involves significant technical, logistical, and interpretative hurdles. The substantial volume and intricate nature of the genomic data produced by NGS necessitate standardized bioinformatics pipelines, validated frameworks for variant interpretation, and unified reporting systems to guarantee consistent clinical value. Furthermore, the smooth integration of genomic results into electronic health records (EHRs) and clinical decision support systems (CDSS) is crucial for delivering actionable insights directly at the point of patient care. Multidisciplinary collaboration among molecular pathologists, bioinformaticians, genetic counselors, and treating physicians is critical for accurate interpretation and translation of genomic results into therapeutic decisions. Addressing issues such as cost-effectiveness, reimbursement policies, clinician education, and regulatory standardization will be fundamental to ensuring equitable access and sustainable implementation of NGS-driven precision medicine strategies (52).

Ethical, Regulatory, and Data Management Challenges of NGS

Although the swift advancement of Next-Generation Sequencing (NGS) technologies has transformed genomic medicine, it has concurrently presented intricate ethical dilemmas that demand thoughtful attention. As NGS facilitates the comprehensive examination of entire genomes and exomes, critical concerns including informed consent, the handling of incidental findings, data privacy, and the fair distribution of genomic services have emerged as pivotal issues in both clinical and research environments (53).

The identification of secondary findings unrelated to the primary diagnostic question raises dilemmas regarding disclosure obligations and patient autonomy. Furthermore, the storage and potential reuse of genomic data for future research intensify concerns about confidentiality and long-term data governance. The inherently identifiable nature of genomic information complicates anonymization strategies and increases the risk of re-identification. Ethical frameworks must therefore balance innovation with respect for individual rights, cultural diversity, and social justice. Ensuring transparency in communication, strengthening genetic counseling services, and implementing robust consent models are essential components of responsible NGS integration into healthcare systems (54).

In addition to ethical concerns, regulatory supervision and data management systems continue to pose significant obstacles to the uniform adoption of NGS in clinical environments. While regulatory bodies have progressively established guidelines to assess the analytical, clinical validity, and clinical utility of genomic assays, achieving consistency in these frameworks across different regions remains a challenge (55).

The dynamic evolution of sequencing platforms and bioinformatic pipelines further complicates regulatory approval and quality assurance processes. In addition, NGS generates massive volumes of data requiring secure storage, interoperable formats, and standardized annotation systems to ensure reproducibility and clinical applicability. Data governance policies must address cross-border data transfer, cybersecurity threats, and long-term sustainability of genomic repositories. Incorporating NGS data into electronic health records requires adherence to data protection regulations, all while ensuring that the information remains readily accessible to support clinical decision-making. Developing globally aligned standards, accreditation systems, and scalable bioinformatic infrastructures will be essential to maximize the benefits of NGS while safeguarding patient rights and data integrity (56).

Future Perspectives of NGS in Personalized Medicine

Next-generation sequencing (NGS) is poised to further transform personalized medicine by enabling deeper molecular characterization of diseases and more precise therapeutic stratification. New advancements, including long-read sequencing, single-cell genomics, multi-omics integration, and real-time sequencing technologies, are anticipated to improve diagnostic precision and reveal genetic variations that were previously undetectable (57).

Furthermore, the synergy between NGS and artificial intelligence (AI) or machine learning algorithms is speeding up the interpretation of variants, the discovery of biomarkers, and the development of predictive models for treatment outcomes. In oncology, infectious diseases, and rare genetic disorders, future applications of NGS are anticipated to support earlier detection, dynamic disease monitoring, and adaptive treatment strategies. Additionally, decreasing sequencing costs and improved automation are likely to expand accessibility across diverse healthcare systems. Combining genomic data with transcriptomic, proteomic, and metabolomic information will foster a systems biology framework for personalized healthcare (58).

As technological advancements continue, NGS is expected to move beyond tertiary centers and become embedded in routine clinical workflows worldwide. In the future, the role of Next-Generation Sequencing (NGS) in personalized medicine will rely not just on technological advancements, but also on its successful translation into healthcare strategies designed for large populations. Large genomic initiatives and biobank-driven research are generating expansive datasets that enable population genomics, pharmacogenomics, and risk prediction models tailored to diverse ancestries (59).

The incorporation of NGS into preventive medicine frameworks may facilitate proactive disease risk assessment and targeted intervention before clinical manifestation. Furthermore, advancements in point-of-care sequencing technologies and decentralized testing platforms may democratize access to precision diagnostics, particularly in low-resource settings. Standardized data-sharing infrastructures and federated learning models are anticipated to enhance collaborative research while preserving data privacy. Ethical governance, clinician education, and reimbursement reform will remain pivotal in ensuring sustainable implementation. Ultimately, the evolving landscape of personalized medicine will be defined by the combined power of integration of genomic innovation, digital health technologies, and evidence-based clinical translation (60).

CONCLUSION

Next-Generation Sequencing (NGS) has arisen as a revolutionary driver in contemporary healthcare, fundamentally altering the approach to disease comprehension, diagnosis, and management. By facilitating detailed genomic profiling with greater resolution and reduced expenses, NGS has effectively connected the divide between molecular scientific research and practical clinical implementation.

Its impact spans oncology, rare disease diagnostics, pharmacogenomics, infectious disease surveillance, and clinical decision-making, where it has improved diagnostic accuracy, enabled targeted therapeutic selection, and supported real-time disease monitoring. The integration of multi-omics data, artificial intelligence, and advanced bioinformatic pipelines has further enhanced the interpretative power and clinical relevance of genomic information. Collectively, these advancements position NGS as a central pillar of precision medicine and a catalyst for individualized healthcare delivery.

Despite its remarkable progress, the widespread implementation of NGS requires continued efforts to address obstacles concerning the analysis of data, the establishment of standard protocols, the alignment of regulatory frameworks, and the oversight of ethical considerations , and equitable access. Sustainable integration into healthcare systems depends on robust clinical guidelines, interoperable data infrastructures, and multidisciplinary collaboration among clinicians, geneticists, and bioinformaticians. Future innovations including long-read sequencing, single-cell analysis, and decentralized sequencing platforms are expected to further expand clinical applications and improve patient outcomes. As genomic technologies continue to evolve, their responsible and evidence-based integration are crucial for completely unlocking the promise of predictive, preventive, and personalized medicine on a global scale.

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Harnessing AI for KRAS Molecular Pathway Detection in Breast Cancer

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Abstract:

In various cancers, the KRAS pathway is central to governing cellular proliferation, differentiation, and survival. Despite the comparative rarity of KRAS mutations in breast malignancies, aberrant pathway activity significantly influences tumor progression, immune modulation, and clinical resistance. In the present review, we synthesize current knowledge on KRAS signaling in breast cancer, focusing on its prevalence and the molecular drivers behind its activation. Our discussion extends to how dysregulated cascades associated with KRAS, such as PI3K-AKT-mTOR and RAF-MEK-ERK, impact the biological landscape of the tumor beyond mere mutational status.

Furthermore, the review explores the transformative impact of omics technologies and artificial intelligence (AI) in decoding KRAS-driven molecular networks. Recent progress in genomics, transcriptomics, proteomics, and especially multiomics data integration has enabled a more comprehensive understanding of KRAS pathway dynamics. At the same time, machine learning and deep learning approaches have significantly improved tumor classification, biomarker identification, and prediction of therapeutic outcomes. Emerging AIdriven multimodal frameworks that combine molecular profiles, histopathological features, and imaging data show great promise for enhancing prognostic assessment and developing personalized treatment strategies in breast cancer.

Nevertheless, ongoing challenges such as data heterogeneity, limited interpretability of models, insufficient external validation, and ethical considerations remain to be addressed. Future efforts are oriented toward explainable AI, federated learning, and clinically validated predictive systems to establish a robust foundation for AIenabled precision oncology in breast cancer. Ultimately, integrating KRAS pathway biology with advanced AI analytics could accelerate the evolution of individualized diagnostic and therapeutic approaches in breast cancer management.

Keywords: KRAS signaling, breast malignancies, Artificial intelligence, Multi-omics integration, Precision oncology

Introduction to KRAS Signaling in Breast Cancer

KRAS (Kirsten rat sarcoma viral oncogene homolog) is a crucial hub in intracellular signaling circuits controlling proliferation, survival, differentiation, and migration by relaying extracellular cues to major downstream routes particularly the RAF–MEK–ERK and PI3K–AKT pathways frequently altered during tumor development. While somatic KRAS mutations occur less often in breast cancer than in many other cancers, aberrant KRAS expression and associated

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pathway activation have been shown to correlate with tumor progression, immune microenvironment modulation, and clinical outcomes across molecular subtypes of breast cancer, indicating a broader role for KRAS-mediated signaling in disease pathophysiology beyond mutation status (1, 2).

Emerging evidence suggests that elevated KRAS expression and enriched KRAS signaling signatures influence prognosis and biological behavior in breast cancer, with studies linking higher KRAS levels to distinct immune infiltration patterns and survival differentials, particularly in triple-negative and hormone receptor-positive subgroups. These findings highlight the importance of dissecting KRAS pathway dynamics within the complex landscape of breast cancer molecular biology to identify potential prognostic biomarkers and therapeutic targets (1, 2).

Molecular Mechanisms of KRAS Pathway Activation

KRAS is a small GTPase that alternates between an inactive GDP-bound form and an active GTP-bound form, a transition controlled by guanine nucleotide exchange factors (GEFs) such as SOS1 and by GTPase-activating proteins (GAPs). When upstream receptor tyrosine kinases (RTKs) including EGFR and HER2 are stimulated, SOS1 promotes GDP displacement and GTP loading, resulting in structural rearrangements of KRAS that allow binding to downstream targets and the initiation of signaling programs linked to growth, survival, and differentiation. Once engaged, KRAS triggers prominent pathways such as RAF–MEK–ERK and PI3K–AKT–mTOR supporting oncogenic communication across multiple cancer types (3, 4).

In cancer, activating KRAS mutations or upstream overexpression of RTKs result in persistent GTP-bound KRAS, driving continuous downstream signaling independent of normal regulatory controls. This constitutive activation promotes uncontrolled cell growth, metabolic reprogramming and resistance to targeted therapies, in part through crosstalk among signaling axes and feedback loops that stabilize the active state. Understanding these molecular mechanisms of KRAS activation is essential for identifying therapeutic vulnerabilities and developing effective inhibitors that can interrupt aberrant signaling in tumor cells (3, 5).

Prevalence and Clinical Relevance of KRAS Changes in Breast Cancer

Alterations in the KRAS gene in breast cancer are relatively uncommon when compared with many other tumor types. Large-scale genomic studies report KRAS mutations in roughly 0.6–0.7% of invasive breast cancers. In contrast, copy-number alterations and increased KRAS mRNA expression are observed more frequently and may contribute to key aspects of tumor behavior. Although canonical activating KRAS mutations are uncommon, variation in KRAS expression and genetic status has been linked to specific molecular subgroups and clinical characteristics, highlighting the multifaceted nature of KRAS involvement in breast tumor development and the possible value of KRAS-associated features for prognostic stratification.

Importantly, the clinical impact of KRAS alterations cannot be explained by prevalence alone. Elevated KRAS mRNA levels have been associated with worse overall and disease-specific survival, including in defined breast cancer subgroups, supporting its potential role as a prognostic biomarker and indicating broader pathway dysregulation that may affect responsiveness to therapy as well as the emergence of resistance.

Continued research is needed to clarify how these alterations contribute to clinical outcomes and to establish their relevance for precision oncology in breast cancer management (6, 7).

Omics Technologies for KRAS Pathway Investigation

High-throughput omics approaches including genomics, transcriptomics, proteomics, and metabolomics have transformed the study of intricate signaling networks such as KRAS. By allowing multi-layer characterization of molecular changes, these technologies provide a more integrated view of how alterations at different biological levels shape pathway activity.These platforms allow for the detection of genomic variants, differential gene expression, protein abundance and modifications, and metabolic states that collectively define KRAS pathway activity and downstream effects in cancer cells and tissues (8, 9). Integration of multi-omics datasets through systems biology approaches enhances the resolution of KRAS-associated networks and uncovers biomarkers that single-layer analyses might overlook (8, 9).

Specifically, omics strategies have been used to elucidate how KRAS influences tumor behavior by correlating genomic mutations with transcriptome changes and proteome dynamics, thereby providing insights into pathway activation, feedback loops, and interactions with other oncogenic modules (10, 11). In breast cancer research, expanding omics approaches and analytic frameworks such as proteogenomics and interactomics contribute to understanding tumor heterogeneity and KRAS pathway implications, offering opportunities for improved molecular classification and therapeutic targeting. (9, 11)

Artificial Intelligence Approaches in Cancer Molecular Profiling

Artificial intelligence (AI) has become a powerful tool for cancer molecular characterization, as it can extract meaningful structure from complex, high-dimensional biological datasets patterns that conventional analyses may miss. Using machine learning and deep learning models, researchers have analyzed multi-omics, imaging, and clinical data to support tasks such as tumor subtype classification, prognostic biomarker discovery, and treatment-response prediction, often achieving improved performance relative to standard statistical methods. (12, 13). These AIdriven strategies facilitate the discovery of molecular signatures across genomics, transcriptomics, and proteomics data, thus supporting precision oncology initiatives targeting heterogeneous cancer profiles (12, 13).

In recent years, AI has been integrated into diagnostic and therapeutic pipelines to interpret molecular features from diverse data modalities, ranging from RNA expression profiles to histopathological images, improving subtype classification and personalized treatment planning (14, 15). Additionally, AI approaches are increasingly employed to integrate multiomics and radiomics data, enhancing the robustness of cancer profiling and enabling more comprehensive insights into tumor biology and potential therapeutic vulnerabilities (114, 15).

AI-Driven Multi-Omics Integration for KRAS Pathway Detection

AI-guided multi-omics integration has become an effective approach for disentangling complex signaling systems such as the KRAS pathway by jointly analyzing complementary molecular layers, including genomics, transcriptomics, epigenomics, and proteomics. Analyses restricted to a single data type frequently miss the layered regulatory processes that govern KRAS activation and its downstream consequences. In contrast, machine learning and deep learning models can capture high-dimensional relationships and cross-talk patterns across heterogeneous omics datasets, offering a more comprehensive view of pathway behavior and associated molecular traits (16, 17). By leveraging this strategy, researchers can derive pathway-associated signatures, subtype-relevant biomarkers, and candidate therapeutic targets that are difficult to uncover with conventional statistical approaches alone (16, 17).

In oncology research, AIbased integration of multiomics data has demonstrated enhanced performance in characterizing tumor subtypes, predicting clinical outcomes, and uncovering molecular mechanisms across various cancer types, suggesting its applicability in KRAS pathway investigation and precision medicine. For example, integrative models employing clustering, feature selection, and predictive algorithms have successfully stratified tumors by biological and clinical characteristics based on multiomics profiles, illustrating the potential of AIassisted frameworks to decode signaling networks such as KRAS and translate molecular complexity into actionable insights (16, 18).

Deep Learning Applications in Histopathology and Imaging Biomarkers

Deep learning, a branch of artificial intelligence, has accelerated histopathology research by supporting automated extraction of clinically meaningful features and imaging biomarkers from digitized whole-slide images. Compared with conventional manual assessment, this strategy can improve diagnostic accuracy, standardize interpretations, and increase throughput. DL approaches including convolutional neural networks (CNNs), ResNet-

Table 1. Recent Studies on AI Driven Multi Omics Integration (Continuing Ref Numbers)

Ref #	Study (First Author, Year)	Main Findings
19	Li al ,2024	AI-assisted multi-omics integration elucidates complex cancer molecular profiles and signaling network patterns across multiple omics layers. (PubMed)
20	Sahu al , 2025	Review highlighting AI and multi-omics integration in precision oncology, including signaling pathways like KRAS among others. (PubMed)
21	Zhao al , 2025	Multi-omics machine learning identifies melanoma molecular subtypes and prognostic signatures, demonstrating integrative model utility. (PubMed)
22	Chong al , 2022	Integrated multi-omics characterization reveals KRAS mutant cancer subtypes and distinct pathway activity clusters. (PubMed)
23	Wang al , 2025	Integrative multi-omics machine learning reveals proliferating cell functions linked with prognosis and therapy response. (PubMed)
24	Ou al , 2025	Integrative machine learning and multi-omics analysis uncovered key cell death patterns and immunotherapy targets in renal carcinoma. (PubMed)
25	Ma al, 2024	Prognostic model based on mitochondrial function developed using multi-omics and machine learning in gastric cancer. (PubMed)
26	Other multi-omics integration reviews	AI multi-omics frameworks enhance understanding of signaling and clinical predictions across tumors. (Lippincott Journals)

style architectures, and transformer-enhanced frameworks have been used for tasks such as tumor detection, molecular subtyping, and prognostic modeling across diverse cancer types, with especially extensive work in breast cancer. In some settings, their performance is comparable to and in certain cases exceeds that of expert pathologists. (19,20). These methods have also shown promise in predicting underlying molecular characteristics, including gene expression and mutational status, from histological images, indicating their potential as noninvasive imaging biomarkers that link tissue morphology to molecular pathology (19, 21).

The integration of deep learning with highresolution imaging workflows has facilitated the development of robust biomarkers that correlate with clinical outcomes, therapeutic response, and tumor microenvironment features, thereby contributing to personalized oncology care and precision medicine strategies. Furthermore, systematic reviews reveal a growing trend toward multimodal DL models that combine histopathology with genomic data to improve biomarker prediction and clinical stratification, underscoring the expanding role of DL in bridging imaging and molecular phenotyping for cancer research and clinical application (20, 21). Despite these progressions, several limitations still need to be addressed particularly issues related to data standardization, the interpretability of model outputs, and the level of clinical evidence required through rigorous validation before these methods can be broadly adopted in routine practice.

Predictive Modeling of Therapeutic Response via KRAS Pathway Analysis

Predictive modeling focused on the KRAS pathway has become a critical area of research in precision oncology, as KRAS mutations and associated signaling dynamics influence response to targeted therapies, immune checkpoint inhibitors, and conventional treatments. Computational models that integrate molecular profiles including gene expression, mutation status, and pathway signatures can stratify tumors by likely therapeutic sensitivity or resistance, enhancing treatment selection and reducing unnecessary toxicity. For example, integrated predictive models such as the *K20* classifier have shown high performance in predicting KRAS dependency and therapy responsiveness in cancer cell lines and clinical datasets, outperforming models based solely on mutation status (22). Additionally, emerging radiogenomics and multiomics predictive frameworks combine imaging and molecular data to further refine response predictions .

Beyond targeted inhibitors, evidence from clinical analyses indicates that KRAS mutation status also modulates response to other systemic therapies, such as immune checkpoint inhibitors in nonsmall cell lung cancer, where KRAS mutations have been associated with improved survival outcomes compared to wildtype in some studies (24). Such findings illustrate the broader utility of KRAScentered predictive models in forecasting therapeutic outcomes across diverse treatments and cancer types, underscoring the importance of leveraging highdimensional data and machine learning approaches to inform personalized treatment strategies (23, 24).

Current Challenges and Limitations of AI-Based Molecular Detection

Although artificial intelligence (AI) has shown substantial promise for improving molecular detection in oncology, several challenges limit its routine clinical adoption. A key obstacle is data quality and heterogeneity: most AI methods rely on large, diverse, and accurately annotated datasets, but many existing resources contain class imbalances, insufficient representation of different populations, and inconsistent labeling. These factors can skew predictions and weaken a model's ability to generalize across healthcare settings (25, 26). In addition, model interpretability is a major concern. Many contemporary approaches especially deep learning systems operate as "black boxes," so the basis of their decisions is not transparent, which can undermine clinician confidence and make regulatory evaluation more difficult (25, 26).

Beyond technical constraints, ethical, legal, and regulatory barriers also slow the practical adoption of AI-based molecular detection tools. Key concerns include patient privacy, algorithmic fairness, and the requirement to follow standardized validation procedures. The existing evidence suggests that many cancer-focused AI studies are built on retrospective or proof-of-concept datasets with restricted external testing. This highlights the need for prospective, multicenter clinical trials and for rigorous benchmarking against accepted clinical standards before these systems can be responsibly incorporated into routine diagnostics (25, 27). Ultimately, addressing these interconnected limitations is crucial to fully realize AI's value in precision oncology while maintaining reliability, transparency, and equity before these systems can be responsibly integrated into routine diagnostics in clinical implementation (25, 27).

Future directions in AIenabled precision medicine for breast cancer

The role of artificial intelligence (AI) in precision oncology for breast cancer is expected to grow substantially in the coming years, propelled by progress in machine learning, multi-modal data integration, and real-world clinical adoption. Recent reviews suggest that AI models capable of combining imaging, pathology, genomic, and clinical information may enable more refined patient stratification, improve treatment planning, and strengthen prognostic performance relative to conventional approaches (28). As AI-based decision-support tools advance, future work will likely emphasize enhancing model interpretability, conducting robust external validation across diverse populations, and embedding these methods into everyday clinical workflows to deliver personalized management aligned with individual tumor biology and patient-specific features (29).

Furthermore, recent research indicates that precision oncology powered by artificial intelligence has the potential to minimize inequities in breast cancer management. This is achieved by increasing the availability of personalized therapeutic strategies and assisting clinical judgment across diverse medical environments, ranging from advanced facilities to underserved regions (29). To guarantee the reliability and broad applicability of AI algorithms, it is crucial to prioritize prospective, multicenter clinical trials alongside initiatives that promote inclusive data collection. Looking ahead, the field is expected to evolve through the adoption of federated learning techniques to bolster data security and diversity, the implementation of explainable AI (XAI) to foster trust among healthcare professionals, and the deployment of AI-enhanced biosignatures for dynamic, real-time monitoring and adjustment of cancer therapies (30).

CONCLUSION

In conclusion, while KRAS mutations aree infrequent in breast malignancy, the dysregulation of this signaling axis significantly influences disease progression, molecular diversity, immunological crosstalk, and resistance to therapy. Progress in omics-based technologies has deepened our insights into the molecular networks driven by KRAS, uncovering diagnostic biomarkers that transcend simple mutational profiling. Furthermore, combining artificial intelligence with multi-omics, imaging, and histopathology has bolstered our ability to map pathway activity, categorize patient subgroups, and forecast therapeutic outcomes with higher accuracy. However, to facilitate secure and fair clinical implementation, critical issues regarding data integrity, interpretability, rigorous validation, and ethical standards must be systematically resolved. Ultimately, the maturation of AI-assisted precision oncology, underpinned by reliable validation protocols and transparent, explainable frameworks, offers a transformative potential for converting our understanding of KRAS dynamics into tailored diagnostic and clinical management strategies for breast cancer.

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Table 2. Recent Studies and Future Trends in AI-Guided Precision Oncology (Continuing Ref Numbers)

Ref #	Study (First Author, Year)	Main Findings
51	Lowry et al., 2026	Mammography-based AI models show promise for screening and risk stratification but require prospective validation and diverse population studies. (pubmed.ncbi.nlm.nih.gov)
52	Feng et al., 2025	AI enhances breast cancer management across screening, diagnosis, prognosis, and treatment, supporting precision oncology and identifying future research needs. (pubmed.ncbi.nlm.nih.gov)
53	Salazar-Garcés et al., 2026	AI improves treatment planning and guideline adherence but highlights challenges in LMIC settings and the need for external validation and equitable data. (pubmed.ncbi.nlm.nih.gov)
54	Shukla al, 2025	AI/ML frameworks for systematic variant annotation may accelerate personalized treatment and drug repurposing strategies. (pubmed.ncbi.nlm.nih.gov)
55	Ran al, 2025	Multi-modal AI integration facilitates subtype identification and therapy resistance prediction, supporting personalized treatment design. (frontiersin.org)
56	Narayanan al, 2025	AI supports predictive modeling, treatment response evaluation, and precision medicine but highlights gaps in dataset diversity and algorithm transparency. (link.springer.com)
57	Future Ai oncology review	Reviews AI advancements across oncology and proposes future innovations in clinical decision support and personalized care. ((turn0search8))
58	Explainable AI research	Highlights the role of explainable AI in improving interpretability and trustworthiness for clinical adoption. ((turn1academia22))

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Dynamic Range	9
Experiment Types	Quantitative PCR with dye, Quantitative PCR with probe, Allele Discrimination with HRM, Allele Discrimination with probe, Comparative Quantitation, User Defined
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