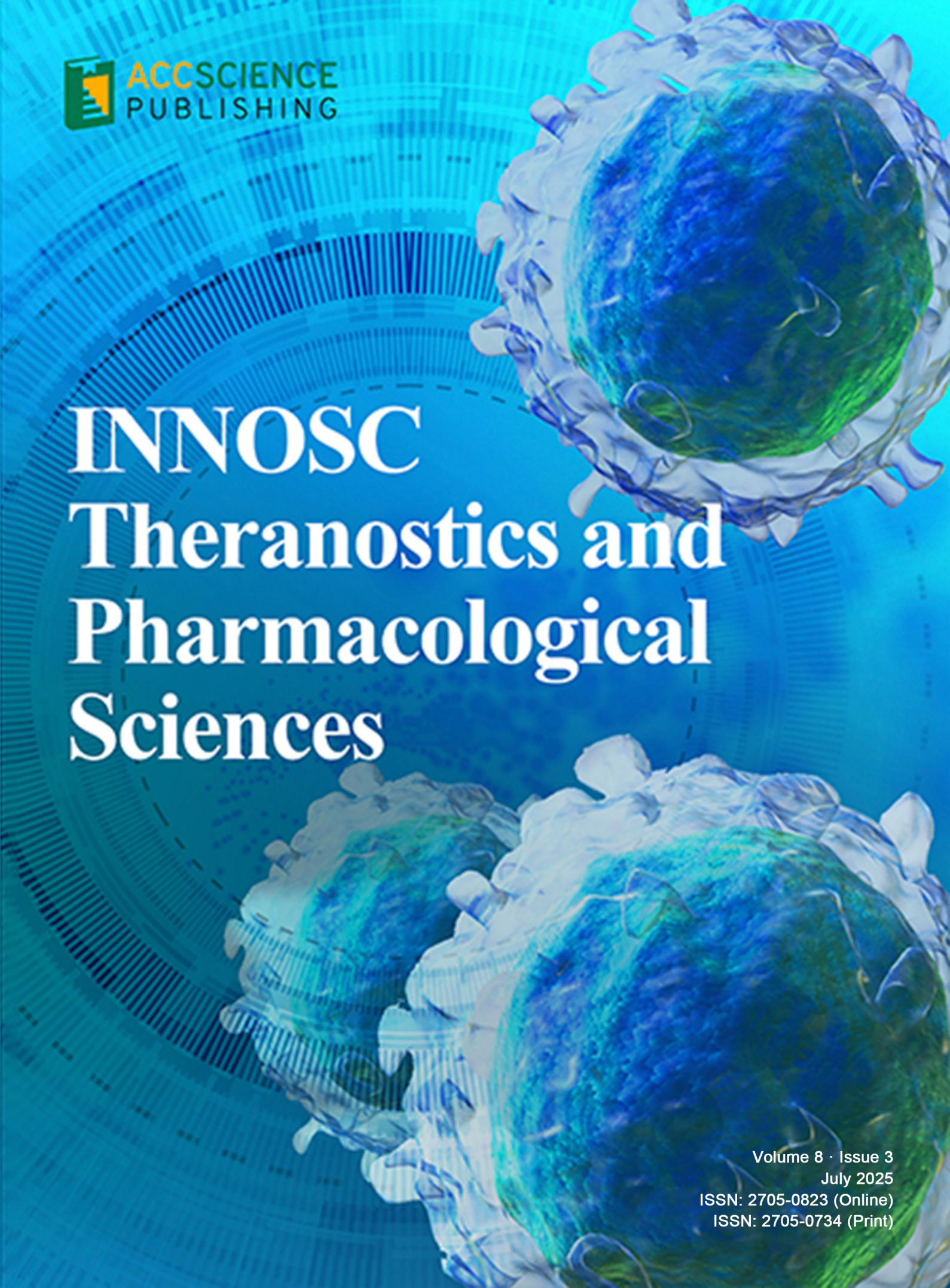

Volume 8 · Issue 3
July 2025
ISSN: 2705-0823 (Online)
ISSN: 2705-0734 (Print)

ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

Print ISSN: 2705-0734

Online ISSN: 2705-0823

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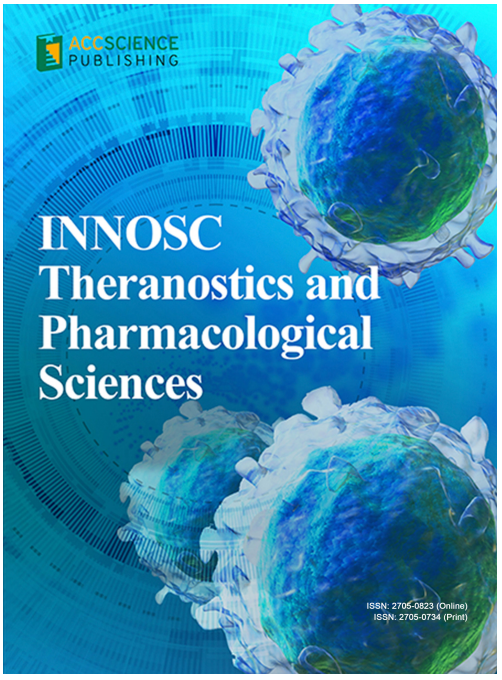

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Volume 8 • Issue 3 • July 2025
ISSN 2705-0734 (print) ISSN 2705-0823 (online)

INNOSC Theranostics and Pharmacological Sciences

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INNOSC THERANOSTICS AND PHARMACOLOGICAL SCIENCES

ISSN: 2705-0734 (print)

ISSN: 2705-0823 (online)

Editorial and Production Credits

Publisher: AccScience Publishing
Managing Editor: Zhao Jinling
Production Editor: Sharmila Velapasamy
Article Layout and Typeset: Sinjore Technologies (India)
Cover Design: ProPub (China)

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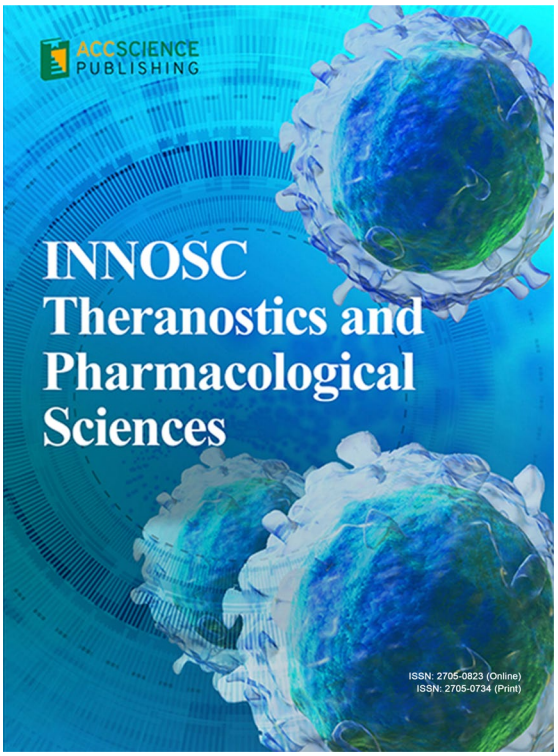

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REVIEW ARTICLE

Leveraging artificial intelligence to revolutionize medical device safety

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(This article belongs to the *Special Issue: Advancing Medicine and Healthcare through Federal Learning*)

Abstract

Materiovigilance is a crucial component of health-care policy designed to ensure patient safety by monitoring and addressing safety issues associated with medical devices. However, traditional systems encounter challenges related to timely reporting, standardization, and the detection of adverse events. Artificial intelligence (AI) has the potential to transform materiovigilance by improving data processing, real-time monitoring, and predictive analytics. This review explores the potential of AI in strengthening medical device safety, highlighting its benefits in enhancing patient safety, personalizing medical devices, and streamlining regulatory reporting. AI-powered systems can detect adverse events, predict patient deterioration, and provide personalized treatment plans, ultimately improving patient outcomes. Furthermore, AI enables the analysis of large and complex datasets, facilitating proactive decision-making and the early identification of emerging risks associated with medical devices. By automating routine tasks and improving accuracy, AI can significantly reduce the administrative burden on health-care professionals. In addition, AI can enhance post-market surveillance by identifying trends and anomalies in real time, thereby accelerating corrective actions. However, ethical and regulatory considerations, such as algorithmic biases, data privacy, and accountability, must be addressed to ensure the responsible development and implementation of AI in materiovigilance. Establishing robust regulatory frameworks, fostering transparency, and promoting interdisciplinary collaboration are essential to overcoming these challenges and fully realizing AI's potential in health care.

Keywords: Materiovigilance; Artificial intelligence; Medical device safety; Patient safety; Medical devices

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Citation: Mishra HP, Loriya K, Shah N, Grover S, Mishra SS. Leveraging artificial intelligence to revolutionize medical device safety. *INNOSC Theranostics and Pharmacological Sciences*. 2025;8(3):1-11.
doi: 10.36922/itps.6204

Received: November 18, 2024

Revised: January 4, 2025

Accepted: January 10, 2025

Published online: January 22, 2025

1. Introduction

The term "materiovigilance" refers to a coordinated system for detecting, gathering, reporting, and analyzing adverse events associated with the use of medical devices.[1,2]

The primary goal of materiovigilance is to safeguard public health by monitoring and addressing potential safety issues related to medical equipment.[3] It plays a vital role in improving medical device efficiency and design, reducing complications from devices, and alerting patients and health-care providers to counterfeit or substandard devices.[4] As a crucial component of health policy in both public and private health-care settings, materiovigilance helps minimize the likelihood of incidents caused by medical equipment.[5] With the global increase in medical device use, materiovigilance has become increasingly important for ensuring patient safety and promoting the responsible use of life-saving devices.[6]

Different countries and regions, such as the United States, the European Union, Japan, China, and India, have distinct systems for implementing their materiovigilance programs.[3] For instance, the Materiovigilance Program of India, established on July 6, 2015, aims to generate safety data and track adverse events related to medical devices.[1,2] Interestingly, although many nations have established materiovigilance initiatives, these programs are often less developed and refined compared to the systems in place for medications.[7] This limitation emphasizes the ongoing need for efforts to improve post-market surveillance of medical devices. As health-care technology advances, robust materiovigilance procedures are becoming increasingly crucial, particularly with the integration of artificial intelligence (AI). AI has revolutionized post-market surveillance by enabling more effective signal detection, risk assessment, and regulatory compliance.[8] AI is transforming health-care monitoring by providing previously unattainable capabilities in patient care, disease detection, and health management. Machine learning (ML) algorithms and sophisticated data analysis enable the processing of large volumes of medical data, including electronic health records, medical imaging, and real-time patient data from medical devices.[9,10]

However, the use of AI in health-care monitoring raises concerns related to interpretability, algorithm bias, and data privacy, emphasizing the need for transparent and ethically sound AI implementation within materiovigilance frameworks.[8] In addition, the emergence of AI/ML-enabled medical devices presents new regulatory challenges, making it essential to incorporate sustainability principles into the materiovigilance ecosystem.[11]

Despite these challenges, AI holds immense potential for health-care monitoring. By integrating big data analytics, ML, and blockchain technology, AI can transform patient care models, streamline health-care delivery, and ultimately improve patient outcomes while reducing health-care costs.[12,13] The review aims to explore how the incorporation of AI into materiovigilance systems can address current challenges in medical device monitoring and ultimately enhance patient outcomes.

2. Challenges in the traditional materiovigilance system

Despite the implementation of materiovigilance programs in numerous countries, post-marketing surveillance of medical devices remains less advanced and reliable compared to that of medicines.[1,7] This circumstance suggests that traditional methods may be inadequate in monitoring and managing risks associated with medical devices once they are on the market.

There are significant variations in the materiovigilance regulatory systems of different nations, and there is insufficient empirical evidence to establish the overall superiority of any one system.[3] This lack of standardization can lead to inconsistencies in how adverse events are reported and addressed globally.

One of the primary issues that the world encounters is the underreporting of adverse events. Healthcare workers often struggle to translate their knowledge and positive attitudes into effective reporting of medical device adverse events.[2] This situation indicates that conventional systems may not be adequately encouraging reporting from those most likely to encounter these events.

Challenges including the absence of global standards and poor reporting protocols underscore the need for continuous strengthening and enhancement of materiovigilance programs to improve patient safety and medical device monitoring.

Over the past decade, AI has revolutionized materiovigilance by automating adverse event detection, data analysis, and pattern recognition. Traditional materiovigilance relied on manual data entry, static databases, and reactive approaches, often resulting in delayed detection of safety signals. In contrast, modern AI-driven systems leverage real-time monitoring, natural language processing (NLP), and predictive analytics to proactively identify risks from vast datasets, including unstructured sources such as social media and medical records. AI enhances accuracy, reduces reporting biases, and facilitates faster regulatory compliance. However, issues such as data privacy and algorithm transparency remain pivotal in ensuring the efficacy and reliability of AI in materiovigilance.[8,9]

Timely reporting of any event occurrence is important, and the reporting period is outlined in Table 1. Table 2 discusses the differences in medical device vigilance programs in India, the United States, and the United Kingdom.

Table 1. Reporting period for an event or occurrence in India[14]

Reporter	What to report	Timeline	Recipient of report
Device manufacturer	The initial report of an event on the "MDAE" reporting form, accompanied by corrective actions to protect the public from undue risk. This action serves as the first notification of a fatality or significant public threat caused by an adverse event or incident	After learning of an occurrence; within 5 working days	MvPI
Device manufacturer	The "MDAE" reporting form, which includes a report on the causation assessment and any remedial or preventative measures implemented within a certain timeframe	After learning of an occurrence; within 30 calendar days	MvPI
Health-care providers	Using the causality assessment report and "MDAE" reporting form	The "MDAE" report must be submitted within 5 working days of becoming aware of the event, and root cause analysis must be completed within the following 30 calendar days	MvPI

Abbreviations: MDAE: Medical device adverse events; MvPI: Materiovigilance program of India.

Table 2. Differences in medical device vigilance in India, the United States, and the United Kingdom[14-16]

Specifications	CDSCO (India)	FDA (US)	MHRA (UK)
Definition of medical devices	These include mechanical devices, contraceptives, disinfectants, insecticides, *in vitro* diagnostic materials, surgical dressings, surgical bandages, and devices intended for internal or external use in the diagnosis, treatment, mitigation, or prevention of disease or disorder in humans or animals	All tools, equipment, supplies, machinery, implants, software, accessories, and disinfectants used in *in vitro* testing or diagnosis fall under this category	Excludes substances used to clean medical equipment
Medical device classification	Four classes: I, II, III, and IV	Three classes: I, II, and III	Four classes: I, IIa, IIb, and III
Classification basis	Based on risk	Control level and marketing specifications	Based on risk
Medical device post-marketing surveillance	Started in 2015 under the Materiovigilance Program of India	Established in 1990 under the Safe Medical Device Act	PSURs apply to class IIa, IIb, and III medical devices under the MDR
Individuals who hold the authority to report adverse events	Manufacturers, health-care professionals, pharmacists, nurses, hospital technology managers, biomedical engineers	Manufacturers, importers, device user facilities, patients, health-care professionals, consumers	Manufacturers, users, health professionals, authorized representatives, and MHRA
Reporting requirements	Device malfunction, serious injury, death	Death, serious injury, device malfunction	Event has occurred in association with the medical device, which may have led or could potentially lead to death or serious injury
Report types	• First reporting • Trend reporting • Last reporting	• 30-day report • 5-day report • Reports of individual adverse events • Initial report • Additional reports: Semiannual reports and annual summary reports	• Early notification of adverse events • Final reports • Regular summary reporting • Trend reporting
Applicable forms	• Medical device adverse event reporting (MDAER) form • Field safety corrective action (FSCA) form	• FDA 3500 • FDA 3500A • FDA 3419 • FDA 3381 • FDA 3417	• The incident report form provided by the manufacturer • Online reporting by manufacturers through MORE

Abbreviations: CDSCO: Central drugs standard control organization; FDA: Food and drug administration; MDR: Medical devices regulation; MHRA: Medicines and health-care products regulatory agency; MORE: Manufacturer's online reporting environment; PSUR: Periodic safety update report; UK: United Kingdom; US: United States.

3. Transformation of materiovigilance through AI

AI is significantly transforming medical device surveillance by enhancing the processes of monitoring, diagnosis, and health management. Core AI technologies, including computer vision (CV), ML, artificial neural networks, and data fusion techniques, are central to these advancements.[17,18] The vast amounts of data generated by real-time sensor measurements, which are essential to medical devices, have made it possible to extract valuable insights using ML and neural networks.[19] These tools are crucial for accurate diagnosis, effective supervision of medical devices, and real-time monitoring.

In addition to these technologies, NLP plays a key role in analyzing patient data and medical records, whereas CV is essential for interpreting visual data, including images and videos.[20,21] In addition, the integration of blockchain technology with AI is enhancing data security, addressing issues of data integrity and authenticity, particularly within health-care settings.[5] This combination ensures the authenticity of acquired data, thereby strengthening the reliability of medical device surveillance.

The integration of blockchain, NLP, ML, and CV is driving significant progress in medical device surveillance. Together, these technologies improve the precision of device monitoring, enhance diagnostic capabilities, and ensure the confidentiality and integrity of medical data. As AI technology continues to evolve, its applications are expected to expand into areas such as global pandemic forecasting, personalized medicine, and predictive health care.[22,23]

AI enhances data processing, model training, and software implementation in the context of medical devices.[24] These applications improve patient outcomes, reduce the burden on health-care providers, and increase diagnostic accuracy.[25] AI has also demonstrated promise in fields such as ophthalmology, where it aids in disease detection, diagnosis, treatment planning, and tracking disease progression.[26] Figure 1 illustrates a flowchart outlining the AI workflow in materiovigilance. This workflow involves a structured process aimed at enhancing medical device monitoring and safety. It begins with data collection from diverse sources such as medical devices, clinical records, and patient feedback. The raw data undergoes preprocessing, which involves standardization, cleaning, organizing, and labeling to prepare it for AI analysis. Subsequently, AI model development utilizes advanced techniques such as anomaly detection, NLP for extracting insights from clinical text, and predictive modeling to forecast potential device failures. These AI models enable real-time monitoring, incorporating Internet of Things tracking for continuous performance assessment and generating automated alerts for anomalies. In the risk assessment stage, AI tools classify devices by risk levels and prioritize safety signals to address critical issues. Incident analysis and reporting follow, with root cause analysis identifying adverse event triggers and producing automated, standardized reports for regulatory purposes. The process concludes with decision support, where safety alerts recommend recalls or advisories, and feedback loops contribute to iterative improvements in device safety and functionality. This workflow ensures a proactive, dynamic approach to materiovigilance, leveraging AI's ability to process and analyze vast datasets for timely interventions.

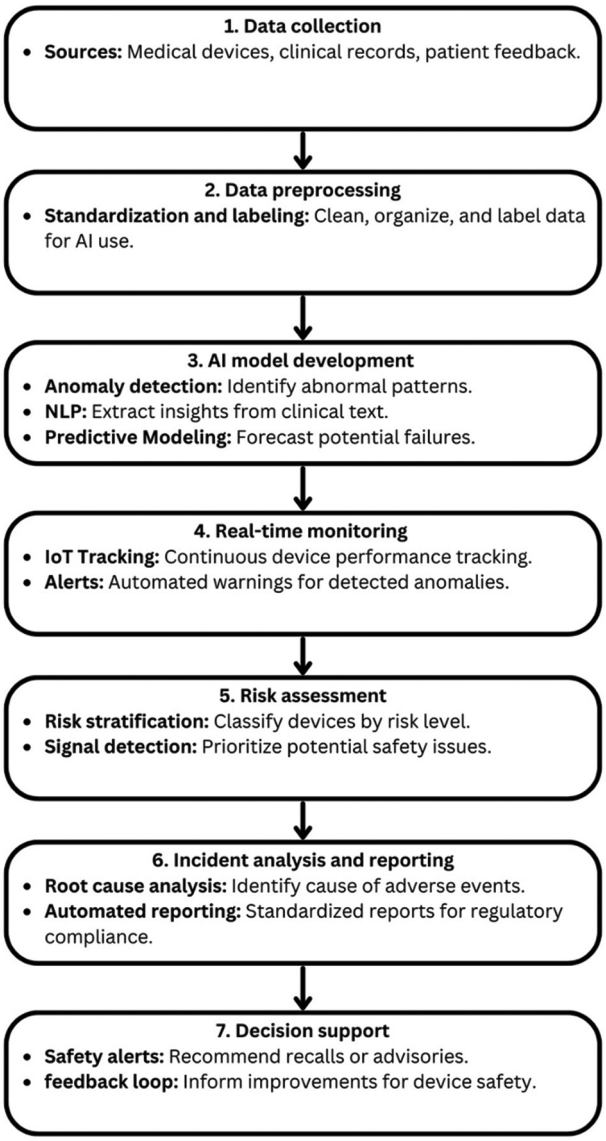

Figure 1. Flowchart of the "artificial intelligence in materiovigilance" workflow
Abbreviations: AI: Artificial intelligence; IoT: Internet of things; NLP: Natural language processing.

3.1. Real-time data monitoring and predictive analytics in patient safety

AI has proven to be a highly effective tool for improving patient safety through predictive analytics and continuous real-time data monitoring. AI-driven systems enable early identification of potential safety issues and prompt risk-reduction measures by automating the analysis of real-world data, such as patient outcomes, adverse event reports, and electronic health records.[8,9] These systems, leveraging big data analytics and ML algorithms, provide actionable insights into device safety profiles and emerging trends by identifying patterns and anomalies.

In both clinical and home settings, AI-driven systems have significantly improved the accuracy of real-time monitoring and predictive capabilities through the application of ML techniques.[27] These systems have the capacity to continuously collect and evaluate data from various sources, including physiological signals, enabling timely intervention and the early detection of potential health issues.[27,28]

AI technologies also facilitate the identification of patient decline in its pre-symptomatic stages by converting streaming clinical data into real-time visual risk assessments, thereby improving triage processes and patient care strategies.[29]

Moreover, AI systems use predictive models based on real-time patient data to generate automated alerts for health-care providers. These notifications are essential for preventing complications, ensuring timely responses, and improving patient outcomes. When abnormalities are detected, the constant flow of data processed by AI models enables real-time monitoring of clinical conditions, patient vitals, and device performance, thereby enabling prompt action.

AI-based predictive analytics also support medical professionals in complex clinical decision-making, particularly in high-stress situations such as the coronavirus disease 2019 (COVID-19) pandemic. During the pandemic, AI applications provided critical information for patient risk assessments, allowing health-care systems to efficiently manage patient loads. These systems continue to evolve, offering personalized treatment plans and enhancing diagnostic precision through predictive analytics.[28]

3.2. ML in detecting adverse events

ML models have shown significant promise in identifying and reporting safety concerns related to medical devices, particularly in the detection of adverse events. These models are trained on large datasets to detect patterns and anomalies that may indicate device malfunctions. ML algorithms have been applied in clinical trials to predict adverse outcomes, including fatalities and other critical events. For example, one study developed five ML models using data from 28,340 clinical trial reports. The best-performing model, logistic regression, achieved a receiver operating characteristic score of 0.7344.[30] This finding demonstrates how ML can assist researchers in estimating risks and predicting unfavorable events, thereby facilitating the development of more effective measures to protect participants.

Adverse events are classified using various ML techniques, such as ensemble learning, unsupervised learning, and deep learning. Algorithms such as Random Forests, Support Vector Machines, and Neural Networks analyze complex health data to classify these occurrences. These classification models are essential in the field of medical devices by identifying irregularities and managing risks in interconnected medical systems.[31]

To ensure accurate event detection, ML models require extensive data validation during training. The training data is thoroughly examined and adjusted, frequently through cross-validation methods, to maximize model performance under real-world conditions. The reliability of these models in detecting and reporting safety issues in medical devices is enhanced by continuous updates and reassessments based on new data inputs.[31] As AI continues to evolve, further advancements in the efficiency and personalization of medical device safety monitoring are anticipated, particularly with the integration of new domains such as proteomics and genomics with ML.[32]

3.3. AI for post-market surveillance of medical devices

AI-powered systems are revolutionizing post-market surveillance by providing reliable methods for monitoring device performance and safety across patient populations after deployment. One example is the Data Extraction and Longitudinal Trend Analysis (DELTA) network study, which demonstrates how computerized safety surveillance systems can monitor cardiovascular devices. By continuously analyzing routinely collected data, this system enables the prompt identification of adverse event rates associated with specific device classes. It surpasses conventional retrospective analysis techniques by offering real-time analysis, significantly accelerating the identification of safety issues.[33]

In addition, the potential of AI in real-time health monitoring is demonstrated by AI-enabled devices, such as smartwatches with atrial fibrillation detection capabilities, which have been approved by the United States Food and

Drug Administration (FDA). These devices help prevent cardiovascular complications and provide early warnings to users.[34] However, the advent of these technologies raises concerns about security, ethical bias, accountability, and clinical effectiveness in practical settings. These concerns emphasize the need for regulatory frameworks that manage these risks while ensuring fairness and transparency.[35]

AI-driven post-market surveillance provides innovative approaches to identifying adverse events and device malfunctions at an early stage. For example, a framework for monitoring AI tools used in breast cancer screening across clinical centers highlights the importance of surveillance in detecting potential software malfunctions.[36] As AI continues to develop, establishing robust quality management systems and stringent post-market monitoring procedures will be crucial to ensuring the safety and efficacy of medical devices throughout their lifecycle.[37]

3.4. AI-driven automation in regulatory reporting

AI-driven automation in regulatory reporting holds significant potential for reducing human error and enhancing operational efficiency, particularly in the areas of materiovigilance and medical device safety. By streamlining data collection, analysis, and submission procedures, organizations can improve compliance and effectiveness in the medical device industry through the integration of AI technologies.[38]

AI-driven automation has already shown considerable promise in improving case reporting, data quality, and drug safety signal detection in the context of pharmacovigilance.[39] These advancements can also benefit in monitoring of medical device safety. AI systems significantly reduce the need for manual labor by processing thousands of adverse event reports each month, analyzing data, and interpreting results at impressive speeds.[39]

Despite the numerous benefits of AI, integrating these technologies into regulatory reporting systems presents several unique challenges. Two major obstacles are the lack of unified regulatory guidance and the availability of suitable training data for ML models.[39] In addition, while the goal of complete automation is appealing, it should be approached with caution, as stated in pharmacovigilance. A collaborative approach that combines technical expertise with intelligent technology must be prioritized, aiming to augment human capabilities rather than completely replace them.[40]

3.5. Enhancing decision-making with AI-powered insight

AI-powered insights are increasingly being employed to improve decision-making across various fields, including health care. Numerous studies highlight AI's groundbreaking potential in promoting data-driven decision-making. AI's ability to swiftly and accurately process vast amounts of data has made real-time, data-driven decision-making possible. Advanced language models, such as ChatGPT, are already being used in government sectors to improve operations, policy-making, and public services such as emergency response and public health management.[41] In materiovigilance, where timely decisions are critical for patient safety, this application can be expanded. AI-driven decision-making has been shown to improve organizational performance, with big data-powered AI playing a significant part in the development of AI capabilities within organizations.[42]

Despite its considerable potential to improve decision-making, AI presents several challenges that need to be addressed. Issues such as data privacy, ethical implications, and the risk of negative outcomes from poorly implemented AI remain prevalent.[43,44] These concerns are particularly significant in the context of materiovigilance, given the sensitive nature of medical records and the possible impact on patient safety.

In conclusion, real-time, data-driven insights from AI have the potential to significantly improve decision-making in materiovigilance. However, for successful implementation, it is essential to carefully consider ethical concerns, data privacy, and the development of robust governance frameworks.[43]

4. Improving patient outcomes: Case studies of AI in materiovigilance

Technologies such as AI and ML have shown significant promise in improving patient outcomes and advancing materiovigilance processes. The utility of AI in enhancing patient safety and monitoring medical devices is demonstrated by numerous case studies.

AI has the potential to improve intraoperative patient care in anesthesiology by continuously monitoring vital signs and predicting complications, as exemplified by the Hypotension Prediction Index algorithm.[45] This AI application enables anesthesiologists to optimize medication dosages, reducing side effects and increasing effectiveness. Similarly, smartwatches with AI capabilities have proven effective in detecting cardiac arrhythmias by continuously tracking heart activity. One case study reported the use of a smartwatch to identify atrial fibrillation in a young patient, highlighting the potential of wearable AI technology for early diagnosis and intervention.[46] Furthermore, during the COVID-19 pandemic, CV and AI-driven predictive analytics were used to facilitate remote care, diagnosis, and screening.[22] These applications helped minimize physical

contact while enabling timely diagnosis and treatment, thus enhancing patient safety.

Despite the promise of AI to improve patient outcomes, several issues and inconsistencies remain. A systematic review of 53 studies on AI in patient safety revealed variability in AI reporting and the absence of standardized benchmarks.[47] This variability highlights the need for thorough validation of AI systems in real-world clinical settings to ensure their reliability in predicting safety outcomes.

To take everything into consideration, AI-powered decision support tools have demonstrated their ability to improve medication administration, patient stratification, and error detection, thereby bolstering patient safety.[47] Both in hospital and home settings, the integration of AI into patient monitoring systems has improved real-time monitoring, increased predictive accuracy, and accelerated response times.[27] However, addressing concerns related to data privacy, algorithm transparency, and integration into clinical workflows requires further investigation and validation.[48] As AI continues to evolve, it holds the potential to revolutionize materiovigilance and significantly improve patient outcomes.

AI is transforming materiovigilance, particularly in enhancing the safety and customization of medical devices. For individuals with physical disabilities, AI-optimized, 3D-printed assistive devices are improving satisfaction and mobility, fostering greater independence. A prime example is the FDA-approved Nevro HFX iQ system, an AI-powered spinal cord stimulator that personalizes pain treatment by continuously adjusting neurostimulation based on real-time patient feedback, thereby enhancing pain management. In addition, the system's adaptive algorithms detect early signs of infection or complications, allowing timely interventions. This AI-driven approach in materiovigilance exemplifies how advanced monitoring can significantly improve patient safety and outcomes.[49]

AI-powered bionic limbs by Össur further illustrate the impact of AI in materiovigilance, particularly for optimizing prosthetic limbs. These advanced prosthetics use ML to adapt in real time to each user's gait and movements, enhancing comfort, stability, and balance, even on challenging terrains. By continuously adjusting to the user's movements, they provide a more natural experience, improve mobility, and reduce the risk of falls, thereby significantly enhancing patient safety and satisfaction. This example underscores AI's role in personalizing device performance to meet individual needs, positively impacting patient outcomes.[50]

Another emerging approach to monitoring medical device safety is the concept of the digital twin. A digital twin is a virtual replica of a medical device or system, including its interactions with patients and the environment, which is powered by real-time data collection and AI-driven analysis. This virtual model mirrors the physical device's performance and the patient's response, enabling continuous monitoring of safety and performance. The digital twin can simulate various scenarios and predict potential risks or adverse events before they occur, providing an advanced layer of surveillance in materiovigilance systems. As AI enhances the digital twin, it becomes increasingly sophisticated, learning from ongoing patient interactions and device usage, thus enabling proactive safety measures. This virtual model tracks the evolution of device behavior, assesses the long-term impact of AI-driven algorithms, and predicts device performance in diverse patient populations.[51,52]

5. Ethical and regulatory considerations for AI in materiovigilance

The use of AI-powered solutions in materiovigilance raises significant ethical and legal concerns. While AI applications in this field offer increased accuracy and efficiency in detecting and recording adverse events, they also introduce complex issues related to privacy, accountability, and equity.[53,54] The question of whether AI fits within existing legal frameworks or whether a new category should be established to address its unique features and implications remains a subject of ongoing debate.[55] The integration of AI in materiovigilance necessitates the development of robust governance frameworks to address these ethical dilemmas and guide decision-making. These frameworks should consider the distinct aspects of medical device regulation while prioritizing accountability, transparency, and privacy protection.[56]

Several regulations have been proposed in various countries to address these concerns, such as the AI Act and the Medical Device Regulation in the European Union. The AI Act represents the European Union's first comprehensive regulation of AI, classifying AI systems according to their risk levels. It establishes stringent safety and ethical standards for AI applications deemed to be of higher risk. This regulation came into effect in August 2024 across the 27 European Union member states, with full enforcement scheduled for completion by August 2027.[57] Similarly, the Medical Device Regulation, which has been in effect since May 2021, governs the safety and efficacy of medical devices within the European Union. It requires manufacturers to comply with rigorous standards for design, clinical assessment, and post-market surveillance. Together, these frameworks ensure the secure

development, approval, and oversight of AI technologies and medical devices in the European Union.[58]

Nowadays, AI is increasingly being incorporated into medical devices. In Japan, the "Improvement Design within Approval for Timely Evaluation and Notice" (IDATEN) system was introduced in September 2020 to streamline the approval process for medical devices, particularly Software as a Medical Device (SaMD) that utilizes AI. Conventionally, any modifications to approved medical devices require a comprehensive review, which could be time-consuming. The IDATEN system allows for partial modifications to be approved more swiftly, facilitating continuous improvements throughout a product's lifecycle. This feature is particularly advantageous for AI-based SaMD, which may require frequent updates to enhance performance.[59]

The full potential of AI in health-care requires addressing four key ethical issues: algorithmic biases and fairness, safety and transparency, consent to use data with patient information, and data privacy.[55] One major concern with AI-enabled materiovigilance systems is legal accountability. As these systems become more autonomous, the question of who is responsible for errors or regulatory violations becomes more complex. The challenges AI poses in monitoring medical devices may require modifications to current legal frameworks, including data protection laws and liability statutes.[60] In addition, materiovigilance must adhere to ethical guidelines that prioritize transparency, fairness, and the protection of patient privacy when utilizing AI.[61,62]

A comprehensive strategy is essential for addressing these complex issues effectively. This strategy should involve the development of explicit regulatory guidelines, the enhancement of transparency programs, and the establishment of accountability frameworks.[27,58] To ensure responsible AI application in materiovigilance, ethicists, policymakers, technology developers, and legal professionals must collaborate closely.[63] Furthermore, ongoing research and open dialog are essential for staying up to date with emerging ethical concerns and ensuring the ethical use of AI technologies in materiovigilance.[54,64] By fostering a balanced framework that prioritizes patient safety, privacy rights, and ethical AI development, stakeholders can maximize the benefits of AI in materiovigilance while reducing potential risks and maintaining public trust in the health-care system.[61,62,65]

6. Conclusion: The future of AI in health-care vigilance

AI has the potential to revolutionize health care, particularly materiovigilance, by improving patient safety and outcomes through advanced data collection, real-time monitoring, and proactive risk detection. However, the implementation of AI in health care comes with significant challenges, including ethical concerns, regulatory requirements, and technological limitations. Addressing these challenges necessitates a comprehensive approach, guided by collaborative efforts among technologists, medical professionals, regulators, and ethicists. To maximize AI's benefits, the health-care industry must prioritize ongoing research, robust regulations, professional training, and a human-centered approach that complements clinical expertise. This coordinated strategy will ensure that AI-driven materiovigilance enhances, rather than replaces, human judgment, ultimately advancing health-care safety and efficacy.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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   doi: 10.4103/picr.PICR_26_18

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ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

REVIEW ARTICLE

Application of robotics in modern surgery and critical operations: Current status, challenges, and future directions

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Abstract

The field of modern surgery has undergone significant transformation with the integration of robotics, which offers unprecedented precision, reduced invasiveness, and improved surgical outcomes. Robotic-assisted surgery has gained popularity across various specialties, including neurosurgery, orthopedics, urology, and cardiac surgery, with systems such as the da Vinci Surgical System serving as a key example. These robotic platforms enhance surgical performance by providing greater control, three-dimensional visualization, and improved dexterity, which collectively reduce operating fatigue, minimize human error, and shorten patient recovery times. Despite these advancements, challenges remain, including high operational costs, the need for specialized training, and the limitations of robotic systems in handling complex or unforeseen situations. This review explores the current state of robotic applications in surgery, addressing both their potential and their limitations. It also discusses future developments, particularly the role of enhanced sensory feedback, machine learning, and artificial intelligence in advancing robotic surgery. While robotic technologies hold the promise of improving patient outcomes, reducing complications, and increasing accessibility, ethical, financial, and technological challenges still need to be addressed. As robotic technologies continue to evolve, they have the potential to reshape the landscape of essential procedures and surgeries.

Keywords: Machine learning; Medical science; Artificial intelligence; Robotic surgery

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Citation: Rahman MA, Bristi SA, Yesmin T, Islam MT. Application of robotics in modern surgery and critical operations: Current status, challenges, and future directions. *INNOSC Theranostics and Pharmacological Sciences*. 2025;8(3):12-20.
doi: 10.36922/itps.4664

Received: August 27, 2024

Revised: January 3, 2025

Accepted: January 16, 2025

Published online: February 6, 2025

1. Introduction

Robots are autonomous machines capable of perceiving their surroundings, executing computations to make decisions, and performing various tasks based on given instructions.[1] The field of health and medical science is on the brink of a transformative

shift driven by robotic technologies. The development of artificial intelligence, miniaturization, and increased computing power are expanding the range of medical applications for robots. Over 34 years ago, an industrial robot and computed tomography navigation were first used successfully to insert a probe into a patient's brain to obtain a biopsy sample, marking the beginning of the field of medical robotics. This breakthrough was followed by the development of robots designed to perform certain urological treatments and total hip arthroplasty. However, these autonomous robots did not initially gain widespread acceptance among surgeons, leading to the development of later models that were designed to be controlled by doctors. Today, medical robots are recognized for their significant contributions to critical surgeries. More specifically, the use of robots, computers, and software to manipulate surgical tools with high precision through one or more incisions is one of the most prominent applications of medical robotics, particularly in fields such as orthopedic surgery, cardiac surgery, neurosurgery, and telesurgery.[1] Robotic manipulators in medicine are not limited to surgery; for example, a brain-machine interface (BMI) and a visual guiding system can provide shared control of a robotic arm in autonomous robotic grasping systems, as described by Dzedzickis *et al*.[2] A BMI is utilized to determine a user's intention to grasp or transfer an object, while visual assistance helps with tasks that involve low-level control, short-range motions, and the precise alignment of the robot's end-effector for grasping. Experiments have demonstrated that shared control of robotic movements leads to more precise, effective, and straightforward task completion compared to using BMI alone. In critical surgeries, achieving precision can be challenging; however, the ability to view the surgical area in three dimensions (3D) with high clarity allows surgeons to perform procedures with greater accuracy and control. It is estimated that the da Vinci system, which received the United States Food and Drug Administration (FDA) approval in 2000, has been used in more than six million surgical procedures worldwide, becoming the first robotic surgical system.[3] Robotic systems offer several advantages for both patients and surgeons, such as shorter recovery times, reduced blood loss, and smaller incisions. The long-term outcomes of robotic surgery are comparable to those of conventional surgery, although occasional malfunctions may occur. When compared to traditional laparoscopy, advanced laparoscopic techniques offer surgeons enhanced ergonomics and dexterity. However, the high cost of robotic systems and the need for specialized training for physicians and surgical teams remain significant drawbacks.[4] Robotic surgery reduces operator fatigue and human error, as it is less physically demanding than traditional surgery. Cardiac surgeries, for example, can last for many hours and are highly stressful for surgeons. Robotic systems allow surgeons to perform operations while seated comfortably, and unlike humans, robots do not experience fatigue. Their "hands" are typically rigid and steady, allowing them to maintain stability and precision during prolonged procedures. One of the main advantages of robotic surgery is the exceptional precision and accuracy it provides in catheter-based or surgical operations.[5] The primary aim of our review is to explore the application of robots in modern surgery and critical operations.

2. Application of robotics in critical surgeries

2.1. Robotic-assisted cardiac surgery

The most common method of myocardial protection in robotic-assisted surgery (RAS) for intracardiac repair involves placing an aortic cross-clamp and administering cardioplegia, similar to traditional open-heart surgery. However, installing an aortic cross-clamp may complicate surgery and perioperative outcomes in patients who have previously undergone cardiac surgery with mediastinal adhesions or those with severe aortic calcification.[3] Robotic assistance may offer additional benefits for tricuspid valve replacement or repair without the need for cross-clamping. The emergence of an atrioventricular block can help identify and promptly treat damage to the atrioventricular node caused by sutures placed through the septal portion of the tricuspid annulus, thus eliminating the need for pacemaker implantation.[4] Furthermore, the da Vinci Surgical System (Intuitive Surgical, United States of America) is one of the most advanced robotic systems used in cardiac surgery. This system consists of a console (from which the surgeon operates), a high-definition 3D vision system capable of magnifying images up to 10 times, and a side robotic cart with four robotic arms that the surgeon can control from the console. Three of the robotic arms are used for surgical instruments, while the fourth controls the camera. In laparoscopic or video-assisted thoracic surgery, the 3D endoscope provides better depth perception than conventional two-dimensional endoscopes. The surgeon at the console has complete control over the camera's orientation and positioning, which is not the case for the table-side surgical team. The da Vinci S® and da Vinci Si® systems, the second and third generations of this technology, offer easier arm movements and broader instrument reach compared to the first-generation model. The da Vinci Xi® is the fourth-generation model,[6] featuring additional enhancements such as a laser targeting system for easier robotic arm setup, thinner arms and instruments with longer reach, a movable endoscope that can be attached on any of the four robotic arms, and an overhead architecture/patient-

side cart with a boom mount for multi-quadrant surgery without requiring extensive repositioning.[7]

2.2. Robotic-assisted transplant surgery

The use of robotics in transplant surgery is rapidly rising, particularly in procedures requiring high accuracy, precision, and efficacy. Despite concerns about ischemic time, the complexity of the surgical procedures, and the level of training required, robotic transplants have yielded successful outcomes, particularly in obese patients.[8]

In the 1990s, substantial advancements in robotic surgery were made in the field of kidney transplant surgery. The development of accurate, minimally invasive robotic surgical systems, such as the da Vinci system (Intuitive Surgical, United States of America) has expanded the range of operations that can be performed, with kidney transplants showing particularly promising results. This method provides significant operational benefits, especially when the operating field is deep and limited, and delicate dissection and micro-suturing are required.[9] Several surgeons have attempted to perform robotic-assisted kidney transplantation in various ways, marking it as a new form of minimally invasive surgery. However, robotic surgery in organ donation is an advanced application of the technique, requiring a high level of expertise from any surgeon undertaking it.[10] Musquera Felip *et al.*[11] conducted a prospective analysis of the 1st 5 cases of robotic kidney transplantation through transvaginal access in 2021, utilizing an Alexis retractor. There were no procedural complications and conversion to an open approach was not required. Both the uretero-vesical anastomosis and vaginal closure were performed robotically. After the surgery, the kidney was placed extra-peritoneally. The median operation time was 220 min, with an average rewarming ischemia time of 53 min. Diuresis occurred immediately, and there were no intraoperative issues. The average hospitalization duration was nine days, with a mean serum creatinine level of 1.5 mg/dL at discharge. Research from the past 20 years indicates a growing use of robotic technology in kidney transplants, with encouraging and optimistic outcomes. Since the first case, various surgical procedures have been described. Initially, ureter implantation was performed using the conventional open technique, while robotic platforms were primarily utilized for vascular anastomosis and dissection.[12]

2.3. Robotic-assisted head and neck surgery

According to estimates, head and neck cancer is responsible for approximately 350,000 deaths and 65,000 new cases annually worldwide, making it the sixth most frequent type of cancer.[13] Prior research on robotic pediatric surgery has demonstrated its safety and low conversion rates to open surgery, where neonates weighing as little as 2.2 kg can undergo treatments using this surgical technique. There have been reports of adult patients who underwent head and neck surgery using robotic systems. Transoral robotic surgery (TORS) for ablative cancer treatment of the larynx and hypopharynx is gaining popularity.[13]

Head and neck squamous cell carcinomas account for approximately 4% of all malignant neoplasms in the United States. Transoral laser microsurgery has been employed as a minimally invasive technique in head and neck surgeries. Since the introduction of the first surgical robot in 1985, numerous medical specialties, including cardiac surgery, urology, general surgery, and gynecology, have found robotic systems to be valuable for enhancing surgical outcomes. TORS is a relatively new, minimally invasive approach in head and neck surgery, offering reduced patient morbidity and mortality.[9] TORS has been used for several years to treat oropharyngeal tumors in the early stages (T1-2) at selected tumor centers.[14] The technique provides advantages such as enhanced visualization, better tumor access, and reduced morbidity, all of which contribute to improved functional outcomes for patients.[10] A retrospective analysis of patients treated with TORS revealed positive outcomes in terms of survival rates, organ function, and quality of life. However, comparisons between TORS and conventional treatment procedures are infrequently addressed in clinical trials in this particular field. Several studies have found that patients who underwent TORS experience a good quality of life.[15] In a more recent study, comparing open transcervical surgery with TORS for patients with T1-4 hypopharyngeal tumors. The results showed no significant difference in overall survival after 5 years between the two patient groups. However, TORS-treated patients experienced improved post-operative swallowing function, shorter hospital stays, and faster recovery. In another retrospective study, patients treated with TORS had longer disease-free survival than those treated with transoral laser microsurgery.[16]

2.4. Robotic-assisted lung surgery

Robotic-assisted thoracic surgery (RATS) has become a significant focus in modern thoracic surgery. RATS is a minimally invasive technique that offers improved visibility, precise movements, and superior ergonomics for the surgeon.[17] RATS has been successfully used to treat early-stage non-small cell lung cancer.[18] RATS is considered one of the most advanced, minimally invasive surgical platforms for lung resection, offering several advantages over other minimally invasive surgical systems, such as enhanced visual haptics, seven degrees of freedom, and three-dimensional visualization. The use of robotic systems in lung resection has been advocated as a means

of improving surgical outcomes by reducing discomfort and accelerating recovery. A pooled analysis of 14 studies with 7,438 patients found that RATS is an effective and safe alternative to video-assisted thoracic surgery, resulting in lower 30-day mortality and conversion rates.[19] One type of robotic thoracic surgery is pure uniportal-RATS (U-RATS), which is performed through a single intercostal incision without rib spreading, using a robotic camera, dissecting tools, and staplers. U-RATS offers advantages such as easier management of potential intraoperative bleeding compared to multiport approaches, due to its quick undocking and the surgeon's required experience with the uniportal technique. U-RATS provides a potential solution for lung resections that is both comfortable for surgeons and promotes rapid recovery for patients. Additional benefits of RATS include 3D visualization and maneuverability. While current robotic systems are designed for multiport surgery (3 – 5 incisions),[20] a modification of the Davinci Xi® system has been developed specifically for the U-RATS technique. This adaptation allowed the execution of the world's first pure robotic cases in September 2021 in Spain. Since then, over 100 anatomic resections, including carinal resections, sleeve resections, multiple sleeve resections, and all segmentectomies, have been performed using this approach.[21]

2.5. Robotic-assisted thoracic esophagectomy

Esophageal cancer is ranked as the eighth most prevalent cancer globally and the sixth leading cause of cancer-related deaths by the World Health Organization. Minimally invasive esophagectomy (MIE) was first performed in the early 1990s, followed by the introduction of robotic-assisted procedures after the turn of the millennium. Over the past 15 years, the development of robotic-assisted MIE (RAMIE) has been facilitated by advancements in robotic platforms. While recent evidence suggests that RAMIE reduces post-operative morbidity and improves quality of life compared to open esophagectomy, no randomized trials have directly compared RAMIE with standard MIE. At our hospital, the hybrid robotic-assisted thoracoscopic procedure has demonstrated that there is a lower bar for patients to meet before they can undergo surgery. When comparing robotic-assisted esophagectomy to open esophagectomy, there were no statistically significant differences in post-operative complications or early oncological outcomes. Therefore, we believe that both methods are safe and effective.[9] The use of RAMIE for esophageal cancer has rapidly expanded globally, and this narrative review aims to clarify the current state and potential future directions of RAMIE. References were gathered from PubMed and Embase for papers published up until April 8, 2023, using search terms "robot," "robotic," or "robotic-assisted," "esophagectomy," and "esophageal cancer." In esophageal surgery, the robot can be utilized in various ways. Overall, complications from RAMIE are comparable to those from open esophagectomy and traditional thoracoscopic MIE, with some evidence suggesting lower rates of complications. RAMIE may help reduce pulmonary complications, according to several meta-analyses, although two randomized controlled studies showed similar incidences of these issues. Notably, RAMIE may facilitate the dissection of lymph nodes, particularly in the region of the left recurrent laryngeal nerve.[22] In addition, 10 cases of bilateral transcervical esophagectomy with robotic assistance performed at the National Cancer Center Hospital East in Japan between February and August of 2023 were examined. The study assessed the viability and effectiveness of the procedure, along with short-term surgical outcomes. The results demonstrated the safety and feasibility of robot-assisted bilateral transcervical esophagectomy for thoracic esophageal cancer. This procedure is expected to reduce the incidence of recurrent nerve palsy, a condition associated with mediastinoscopic esophagectomy and transcervical esophagectomy.[23] RAMIE has also been linked to significantly lower rates of wound infections (odds ratio [OR]: 0.20, 95% confidence interval [CI]: 0.07 – 0.57), atrial fibrillation (OR: 0.53, 95% CI: 0.29 – 0.98), pneumonia (OR: 0.39, 95% CI: 0.26 – 0.57), and overall pulmonary complications (OR: 0.38, 95% CI: 0.26 – 0.56). It also results in less blood loss (weighted mean difference [WMD]: −187.08 mL, 95% CI: −283.81 – −90.35), shorter hospital stays (WMD: −9.22 days, 95% CI: −14.39 – −4.06), but longer operative times (WMD: 69.45 min, 95% CI: 34.39–104.42). No other statistically significant differences in short-term oncological and surgical outcomes were observed. Comparisons between entirely robotic operations and open esophagectomy also yielded similar results. Overall, RAMIE is a safe and effective procedure that reduces blood loss, wound infections, cardiopulmonary morbidity, and hospital stays compared to open esophagectomy.[24]

2.6. Robotic-assisted neurosurgery

In the mid-1980s, surgeons began using the first robotic device for performing precise biopsies in neurosurgery. Initially, robotic surgery was used predominantly in neurosurgery but has since spread to other fields such as urology, gynecology, gastrointestinal surgery, and orthopedics. The use of robotic devices in neurosurgery offers the potential to improve surgical precision and enable more complex procedures for neurosurgeons. A retrospective review of 41 patients who underwent robotic-assisted frameless brain biopsies using the SurgiScope system demonstrated the safety, feasibility,

and excellent diagnostic yield (97.8%) of the technique.[25] Robotic applications in skull-based neurosurgery provide advantages such as better lighting and 3D visualization, replication of conventional gesture-based actions, and the capacity for precise movements in narrow operating corridors. However, the limitations of the robot include its enormous size, restricted angulation, high cost, and the lack of drilling components for fully robotic operations. Robotic endoscope holders have proven particularly useful in situations where a second surgeon or surgical assistant is not available.[26] Robotic assistance in neurosurgery is particularly beneficial for procedures that involve extremely small operating spaces. Examples of robotic use in neurosurgery include pedicle screw placement in spinal procedures, anatomical localization, the surgeon's hand stabilization, and anatomical access plan to deep brain targets.[27] Several robotic systems are commonly utilized in neurosurgery, including Neuromate, Pathfinder, NeuroArm, SpineAssist, and Renaissance. Although other surgical specialties may use robotic assistance more frequently, neurosurgery is well-suited for the integration of robotic technology due to its technical and microsurgical procedures, as well as its history of invention in stereotaxy.[28] Since 2019, our hospital has performed over 100 robot-assisted stereoelectroencephalography and deep brain stimulation depth electrode implantations. Residents and fellows are actively involved in the surgeries and participate in all aspects of surgical planning and execution. It is emphasized that didactic seminars conducted by experienced faculty members are essential learning resources prior to gaining practical experience in the operating room. Survey findings suggest that residents receive more intraoperative training than formal training sessions, while trainees gain more knowledge from educational cadaveric simulation sessions.[29]

2.7. Robotic-assisted trauma surgery

The stabilization of severely injured patients through teleoperative robotic assistance or autonomous robotic surgery is gaining popularity, particularly among military personnel. The trauma pod, a semi-automated telerobotic surgical device, and its proof of concept were introduced by Garcia *et al.*[10] The use of teleoperation to perform bowel anastomosis and shunt insertion in major vessels, along with the ability to facilitate intraoperative computed tomography scanning, was demonstrated on a mannequin patient. This robot's autonomous robotic arms could potentially function as circulation and scrub nurses, which is a significant advantage. Although this prototype is still in its early stages, it highlights the progress yet to be made before surgical robots can be used in clinical settings. In trauma surgery, robots are also useful for navigating to entry points and locking distal bolts.[30] In clinical trauma orthopedics, surgical robots are categorized into three categories: TiRobot, electromagnetic navigation surgical robots, and miniature medical robots developed by Beijing Jishuitan Hospital. Most trials have shown that the robotic group outperformed traditional methods in terms of blood loss, fluoroscopy time, and fluoroscopy frequency. The benefits of robot-assisted surgery are evident: it is more accurate, stable, and reduces radiation exposure during procedures.[31] In the context of open and minimally invasive spinal fusion, robotic assistance has been demonstrated to improve the precision of instrumentation placement. These improvements are achieved without increasing hospital stays, blood loss, or operating time. Nevertheless, most research has focused on the degenerative population, and the effectiveness of robotic assistance in treating spinal injuries remains unknown. A study involving 42 patients (mean age 61.3 ± 17.1 years; 47% female) who underwent robot-assisted spinal surgery was conducted. The patients were stratified based on the number of operation levels: 2 (n = 10), 3 – 4 (n = 11), 5 – 6 (n = 13), or >6 (n = 8). This initial experience suggests that robotic assistance can be safely used in the population with spine trauma. Further studies with larger patient groups are needed to identify which traumatic conditions are most suitable for robotic support.[32]

2.8. Robotic-assisted obstetric and gynecologic surgery

The invention of robotic surgery has enabled gynecologic surgeons to provide laparoscopic options to a much larger percentage of their patient population. Surgeons with advanced laparoscopic skills can improve operative efficiency for numerous procedures involved in benign gynecologic treatments, particularly after performing 50 or more cases.[33] Robotic-assisted laparoscopic procedures in gynecology include lymph node dissections, sacrocolpopexies, benign hysterectomy, myomectomy, tubal reanastomoses, and radical hysterectomy. Numerous recent studies feature case studies of various robotic procedures. Comparative retrospective and prospective studies have shown that this specific form of surgery is feasible. While robot-assisted gynecologic surgery is frequently linked to longer operating room times, it generally results in similar clinical outcomes, reduced blood loss, and shorter hospital stays, although individual studies vary. As more gynecologic surgeons receive training and as patients increasingly seek minimally invasive surgical options, robot-assisted gynecologic surgery is expected to continue advancing.[18] Robotic-assisted laparoscopic hysterectomy is most commonly used in gynecological oncology, particularly for endometrial cancer, though it is

also occasionally employed for restaging early ovarian and cervical cancer.[34] Compared to traditional keyhole and open abdominal surgery, the advantages of 3D viewing include enhanced depth perception, reduced tremor, improved precision, better tissue discrimination, a shorter learning curve, and increased surgeon comfort. When compared to traditional keyhole surgery, robotic-assisted keyhole surgery reduces blood loss and intra- or post-operative complications, enhances surgical performance without extending operating time, and decreases the need for abdominal surgery.[35] One study found that the introduction of robots in the United Kingdom led to a decrease in the number of open radical hysterectomies. A recent meta-analysis reviewing nine studies demonstrated widespread acceptance of robots for cervical cancer surgery, not only for hysterectomy but also for trachelectomy.[36]

2.9. Robotic-assisted pediatric surgery

For the past 20 years, children have been undergoing RAS, a novel technology with various potential clinical benefits, such as decreased post-operative discomfort, shorter hospital stays, and better cosmetic outcomes. As the demand for this cutting-edge technology in pediatric surgery continues to grow, pediatric anesthesiologists will be required to provide anesthetic care for patients undergoing procedures with unique intraoperative requirements and potential complications.[37] Robots have been used to assist in a huge number of pediatric surgical procedures, with success rates comparable to those of traditional laparoscopy.[19] RAS is safe and effective for children, and its application in pediatric surgical specialties has been steadily increasing. It is anticipated that its current limitations will be addressed in the future through further evaluation of robotic systems and surgical expertise. For instance, some adult-focused studies and recent pediatric case reports indicate that 3 mm devices represent a promising technological advancement in minimally invasive pediatric surgery.[38] Over the past few years, laparoscopic techniques have been proven beneficial and safe for both adult and pediatric populations. As

Table 1. Applications of robotics in modern surgery and critical operations

Aspect	Present status	Expectations	Challenges
Robotic systems	Robotic-assisted surgery (e.g., da Vinci surgical system) is widely used in minimally invasive procedures	Development of more specialized robots for specific organs, procedures, or tasks	High initial costs of robotic systems
Applications	Commonly used in urology, gynecology, cardiac surgery, and orthopedics	Expansion into neurosurgery, pediatric surgery, and other complex areas	Limited access to advanced robotic systems in low-resource settings
Precision and minimally invasive surgery	Robots provide enhanced precision, stability, and range of motions in surgery	Further precision improvements to reduce human error and enhance outcomes in complex procedures	Potential over-reliance on technology, reducing human surgical skills in the long term
Tele-surgery	Tele-surgery is becoming more viable with advancements in 5G and communication technologies	Remote surgery capabilities, allowing experts to operate from afar	Reliance on high-speed internet and the risk of delays during critical procedures
Training and simulation	Robotic surgery training systems exist but are not universally accessible	Advanced simulators for safer, risk-free training without the need for patient involvement	Unequal access to training, particularly in developing countries
Patient recovery and outcomes	Robotic surgery has been shown to reduce blood loss, pain, and recovery time	Continued improvement in patient outcomes through even less invasive techniques	Uncertainty about long-term benefits and potential complications
Integration with AI	AI and machine learning are integrated into some robotic systems to provide decision support	Full AI integration to assist surgeons in diagnosis, planning, and real-time decision-making	Ethical concerns and the need for regulations regarding AI involvement in critical decisions
Cost-effectiveness	High upfront cost and maintenance requirements of robotic systems	Reduced costs over time through widespread adoption, improving cost-effectiveness	High initial costs may limit widespread adoption, especially in public healthcare systems
Safety and error reduction	Improved safety through precision, but risks remain due to robot malfunctions or user errors	Reduction of human error and more reliable robotic systems	Risk of malfunctions or system failures during critical surgeries
Personalized medicine	Robots enable precision medicine by adapting to each patient's unique anatomy	Tailoring robotic procedures to individual patient's needs, reducing risks, and improving recovery	Need for greater customization of robotic systems for different body types and medical conditions

Abbreviations: AI: Artificial intelligence; 5G: The fifth generation of cellular network technology.

previously demonstrated, laparoscopy offers shorter hospital stays, smaller surgical scars, and faster recovery. In some cases, it has even replaced open surgery as the preferred approach. However, more complex procedures, particularly intracorporeal anastomosis or extensive reconstruction, have hindered its widespread adoption.[39] Studies comparing robotic surgery to traditional open or laparoscopic procedures found that robotic surgery resulted in shorter hospital stays and produced comparable outcomes in the adult population.[40] The first robotic surgical system, da Vinci (Intuitive Surgical, United States of America), received FDA approval in 2000. In a more recent study, reported that 20 out of the 24 patients who underwent robot-assisted interventions were successfully treated using the da Vinci. Procedures included eight antegrade continence enemas, six bladder neck repair surgeries, and 16 appendicovesicostomies.[35] These findings highlight that the robot provides functional outcomes similar to those of open or laparoscopic surgeries, while also offering the benefits inherent in minimally invasive surgery. Although robotic surgery is reliable and effective for complex procedures in children, its advantages must be weighed against the increased costs and longer operating times associated with this technology.[19] Notably, challenges remain, such as the mismatch between large instruments and the small thorax, the higher risk of skin scarring compared to a thoracoscopic technique, and the high cost of robotic surgery for cardiothoracic disorders. Robotic cardiothoracic surgery in children remains challenging, with only a small number of patients being suitable candidates, and the indications for its use must be closely monitored.[41] Applications of robotic technology in modern surgery and critical operation are summarized in Table 1.

3. Conclusion and future perspectives

Robotic technology has overcome many of the challenges associated with conventional endoscopic surgery, ushering in a new era in medical science that has contributed to a reduction in mortality rates. However, there are concerns that the widespread adoption of advanced robotic technology could lead to job displacement for doctors and other healthcare professionals.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

REVIEW ARTICLE

The impact of benzodiazepine use on treatment retention in opioid agonist treatment: A literature review

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Abstract

It is well established that there is an elevated overdose risk with benzodiazepine (BZD) use during opioid agonist treatment (OAT). However, further studies regarding other aspects of how BZDs influence OAT are necessary. This review summarizes the literature on concurrent BZD use with medications for opioid use disorder (MOUD) and how they affect treatment retention in OAT. EMBASE (Ovid), PubMed, and Google Scholar database search tools were used to search for studies that examined the effect of concurrent BZD and MOUD on treatment retention in OAT. Studies published up to January 30th, 2024, were included with no restriction applied other than English language. The criteria for included literature were the presence of both BZD and at least one MOUD as a variable and treatment retention or MOUD adherence as an outcome. Fourteen articles met the criteria for review: eleven retrospective studies and three observational studies. Methadone was utilized in seven studies, buprenorphine in five, naltrexone in one, and suboxone (buprenorphine + naloxone) in one study. The included studies indicated that when BZDs are taken as prescribed and for shorter periods in conjunction with OAT, subjects are retained in their MOUD programs just as well as patients who do not take BZDs. Any potential benefits of increased treatment retention must be balanced against potential harmful effects of BZD use, such as drug overdose and addiction. Further studies must be performed to validate the results of treatment retention and to evaluate other factors that might affect OAT.

Keywords: Benzodiazepine; Opioid agonist therapy; Treatment retention

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Citation: Lawrence C, McLellan-Carich R, Barr AM. The impact of benzodiazepine use on treatment retention in opioid agonist treatment: A literature review. *INNOSC Theranostics and Pharmacological Sciences*. 2025;8(3):21-34.
doi: 10.36922/itps.5151

Received: October 15, 2024

1st revised: December 30, 2024

2nd revised: January 25, 2025

Accepted: February 11, 2025

Published online: February 27, 2025

1. Introduction

North America and many other parts of the word are currently in an opioid crisis,[1-3] which is compounded by comorbid conditions for many opioid users.[4-8] For patients with opioid use disorder (OUD), the standard of care includes opioid agonist treatment/therapy (OAT),[9] where medications for opioid use disorder (MOUD), such as methadone, buprenorphine and naltrexone are substituted in place of more potent and harmful opioids.[10-13] The use of MOUD can help alleviate withdrawal symptoms and/or cravings that can be experienced as a result of discontinuing opioid usage.[10,14,15] Furthermore,

OAT has demonstrated effectiveness in supporting long-term abstinence, aiding in rehabilitation, and lowering the risk of overdosing and relapse.[10,16,17]

Polysubstance use, both prescription and illicit, is extremely common during OAT.[18-21] The concurrent use of MOUD with benzodiazepines (BZDs) has raised concerns due to its high potential for abuse and dependence.[18] BZDs are a class of anticonvulsant/anxiolytic drugs that are prescribed to treat insomnia, anxiety, depression, muscle spasms, panic attacks and seizure disorders, many of which are common opioid withdrawal symptoms.[22,23] When BZDs are taken alone, overdose risk is minimal.[24] However, when taken alongside MOUD, both drugs act synergistically to enhance GABA receptor function, significantly increasing the risk of respiratory depression, opioid-related overdose and other substance-related overdose, hospitalization and death.[24-27] It has been reported that the mortality rate for concurrent administration of both medications is ten times higher than those taking only opioids.[28] The risk of respiratory depression, overdose and death can be exacerbated due to the prevalence of illicit BZDs laced with synthetic opioids.[29] As such, BZD use is often present in opioid overdoses, with one recent study identifying BZD use in 80% of fatal opioid overdoses.[30] Despite the increased overdose risk with BZD, they are often prescribed alongside OAT.[31] Current recommendations are for short-term BZD use, however, many patients consume BZDs illicitly, where the dose, frequency, and duration of BZD use are not defined or regulated.[31] In the face of increasing concerns over BZD use in OAT, the US Food and Drug Administration has stated that patients should not be turned away from OAT if they have a history of BZD use or are currently using BZD.[32]

Currently, the association between concurrent BZD and MOUD use and increased overdose risk is well established.[14,15,26] However, the association between BZD use and retention in OAT programs remains less well known. This literature review therefore aims to evaluate how the concurrent use of BZD and MOUD specifically influences treatment retention in OAT programs, which is an important part of recovery. In this review, we explore the impact of important variables in BZD use, including the prevalence of BZD use in the MOUD-using population, as well as BZD use at intake into the treatment program, licit versus illicit BZD use, frequency of BZD use, and where BZD prescribed use was considered as a variable in treatment outcomes.

We note however, *a priori*, that it is important to consider that any such benefits from improved treatment retention must be balanced against a possible greater risk of drug poisoning from BZDs when combined with MOUD and potential concurrent use of either licit or illicit opioids.

2. Methods

2.1. Search strategy

When selecting the articles for this review, the authors started by making a comprehensive selection of terms that could be used to describe opioids, OAT, BZDs, and treatment retention. These terms were chosen with the help of a university librarian and by looking through articles in PubMed and Google Scholar to see what terms were commonly used. For the final analysis, studies were searched for that reported the effect of concurrent BZD and MOUD on treatment retention in OAT. EMBASE, PubMed, and Google Scholar (Ovid) were searched for studies from inception until January 30, 2024. The search strategy used keywords that described opioids, BZDs, OATs, and treatment retention (Table 1).

Keywords included plural options whenever possible and were adapted to the requirements of the database. Additional studies were identified by searching the reference list of relevant publications and by using EMBASE, PubMed, and Google Scholar, as previously.[33,34] There were no limitations placed on language, year, age, sex, ethnicity, research location, or trial length during the literature search, but only studies in English were included. A study was included if it: involved both BZD and one of the MOUDs (methadone, buprenorphine, or naltrexone adherence) and treatment retention as one of the primary or secondary outcomes. All literature must have been published in a peer-reviewed journal. It is worth noting that in the title and abstract search, many of the articles lacked the treatment retention aspect of our search criteria. This was in part because the database matched a part of the terms that we included: "drug" in "drug adherence" or

Table 1. Search terms for literature review

Major category	Search terms
Benzodiazepine	Benzodiazepine(s), benzodiazepine dependence
Opioids	Opioid(s), opioid/opiate agonist, medication for opioid use disorder(s), MOUD(s), buprenorphine(s), buprenorphine plus naloxone, methadone(s), naltrexone(s)
Opioid agonist treatment	Treatment for opioid use disorder, opioid agonist treatment(s), buprenorphine treatment(s), methadone treatment(s), methadone maintenance treatment(s), medication assisted treatment(s), OAT(s), MAT(s), MMT(s)
Treatment retention	Treatment retention, treatment response(s), program retention(s), drug adherence(s), medication compliance(s)

"treatment" in "treatment retention" as two examples. As such, many of the selected articles were not included after the initial abstract reading.

2.2. Data extraction and analysis

Information taken from the studies included: author information, date of publication, journal of publication, location of study, study design, trial duration, sample size, subject demographics (age, sex, polysubstance use, and psychiatric comorbidities), MOUD used for treatment (and dose if available), definition of treatment retention, percentage of BZD users in each cohort, and methods for evaluating opioid and BZD use. We followed the definition of treatment retention provided in each study. Due to the large variability of each study's design and OAT program, we did not perform a meta-analysis of the study results. Instead, we provided a narrative summary of the key findings according to similarities in study design and methodology, as previously.[35-37]

3. Results

3.1. Search results and characteristics of studies

The search identified 141 publications and 136 articles were screened after removing duplicates (Figure 1).

Twenty-seven full-text articles were evaluated for eligibility. Fourteen papers were selected for analysis: eleven retrospective cohort studies and three observational studies. Table 2 outlines the main study design of each study, while Table 3 provides study characteristics about the subjects and the study location.

Methadone was used in seven studies, buprenorphine in five, naltrexone in one, and suboxone in one study. All studies utilized urine drug testing (UDT) to evaluate the presence of opioids, BZDs, and other substances at intake and/or during OAT. Some studies performed immunoassays to distinguish between different opioids, including whether they were illicit or prescribed. Statistical methods utilized to compare BZD's effect on OAT retention included Cox regression analyses, adjusted hazard-ratios, adjusted odds-ratios, and Z-scores.

3.2. BZD use within each study

Thirteen of the included studies reported on the percentage of BZD-positive patients within their cohort. The study by Ray-Griffith *et al.*[38] was the only included study that did not include this outcome. In the retrospective study by Morford *et al.*,[39] 18% of patients tested positive at intake. The retrospective cohort study by Brands *et al.*,[40] found that 70.9% of patients used BZD in the past year and 33% had consumed BZD for 30 out of the 90 days before treatment initiation. Similarly, the observational study by Durand *et al.*,[41] found that 65.5% of patients possessed a BZD prescription 90 days before treatment. In the retrospective cohort study by Franklyn *et al.*,[42] 15% of patients were BZD-positive at intake. The observational cohort study by Dayal *et al.*[43] noted that 16% of study patients used BZD. In the retrospective cohort study conducted by Hallowell *et al.*,[44] 18% of subjects were found to have overlapping BZD and buprenorphine prescriptions during a 12-month treatment period. In the retrospective study conducted by Schuman-Olivier *et al.*,[45] 48% of participants were found to

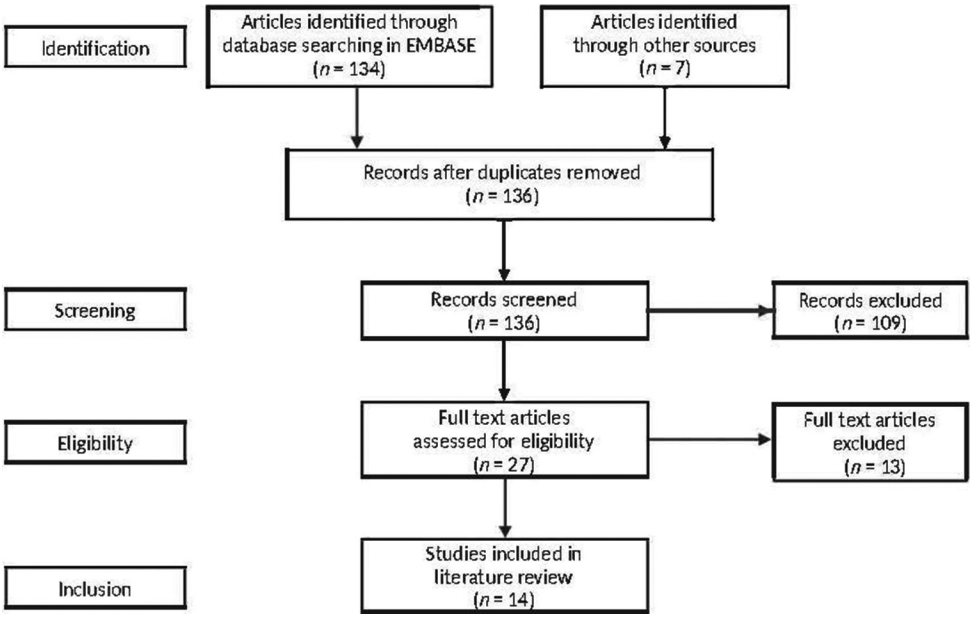

Figure 1. Flow design

Table 2. Study design

Study	Publication Year	Journal	Study Design and Methodology
Raffa *et al.*[47]	2007	*Drug Alcohol Dependence*	The primary outcome was methadone adherence. Methadone adherence was monitored through urine toxicology testing for methadone in addition to other opioids, BZDs, amphetamines, and cocaine. The schedule of urinalysis testing was not included. A patient was considered to have discontinued treatment if the records indicated no methadone doses between two scheduled urinalysis tests. Multiple linear regression analysis and two-term interactions were performed for each illicit drug class and any additional cofounders.
Brands *et al.*[40]	2008	*Journal of Addictive Diseases*	Patients were divided into 3 groups based on the nature of their BZD use: non-users, occasional users (<30 days of use during the 90 days before intake), and regular/problem users (more than 30 days). During intake, patients were surveyed and their demographics (age, sex, marital status, employment status, and education), opioid use history, and other drug history were recorded. For the first 3 months of treatment urine toxicology was performed twice a week. The frequency of drug screening following 3 months was left to the physician's discretion. Treatment retention of MMT was the primary outcome. Secondary outcomes consisted of BZD's effect on psychiatric morbidity, opioid reduction, and cocaine reduction. Univariate and Multiple regression analyses were used to examine the difference in treatment retention between the three groups at different MMT durations (1 – 3 months, 4 – 6 months, and 7 – 12 months).
Schuman-Olivier *et al.*[45]	2013	*Drug Alcohol Dependence*	Through urine toxicology screening, BZD misuse history, and whether they possessed a BZD prescription, patients were classified into four consorts: BZD misuse history with BZD prescription, BZD misuse history with no prescription, no BZD misuse history with a BZD prescription, and no BZD misuse history and no prescription. BZD misuse was defined as having evidence of inappropriate use of a BZD prescription. Urine toxicology was conducted at intake, then twice a week for a month. Frequency decreased to once a week until month 6 when patients considered clinically stable were transitioned to once a month for the remainder of treatment. Primary Outcomes included 12-month treatment retention, urine toxicology for illicit opioids, total emergency department (ED) visits, and odds of an ED visit related to an opioid overdose or accidental injury during treatment. Their definition of treatment retention was not provided.
White *et al.*[48]	2014	*Journal of Psychoactive Drugs*	The authors compared patients' treatment retention with drug usage. Drug testing frequency varied but ranged from weekly to monthly random urine toxicology screens. Patients were included in the study if three or more drug screenings were performed. A distinction was made between prescription and illicit drug use during analysis. The primary outcome was treatment retention; however, they did not define the term.
Peles *et al.*[50]	2014	*Israel Journal of Psychiatry and Related Sciences*	The primary outcome of interest is the prevalence among MMT patients, BZD's effect on long-term treatment retention, and evaluate how possible it is to discontinue MMT treatment following a year. Patients performed urine toxicology at intake, then at random throughout MMT treatment (range 1 – 11 a month). A patient was defined as if one of the samples was positive. Kaplan Meier with log-rank was used to calculate treatment retention for the cohort.
Dayal *et al.*[43]	2016	*Journal of Substance Use*	The outcomes of interest in this study are uptake rate, retention, and compliance with naltrexone therapy. Retention was defined as the duration of time in therapy from initiation to discontinuation. Compliance is defined by the extent a patient adheres to naltrexone dosage and schedule. Logistic regression was used to evaluate the predictors of naltrexone therapy compliance and retention.
Franklyn *et al.*[42]	2017	*Harm Reduction Journal*	Two different comparisons were performed: (1) Patients were separated into the BZD group if they had a BZD-positive urine sample in their 1st month of treatment; (2) Patients were separated into 4 groups based on the frequency of BZD-positive urine samples: 0 – 25, 25 – 50, 50 – 75, and 75 – 100%. Urine toxicology screening was conducted one to two times a week. One-year retention was characterized by the Cox proportional hazard model. The primary outcome was methadone or Suboxone (buprenorphine+naloxone) retention. Patients were considered to be retained in treatment based on seeing evidence of a dose of medication within 30 days.
Eibl *et al.*[49]	2019	*Journal of Addiction Medicine*	Patients were separated into four groups depending on whether they processed a BZD prescription and the frequency of BZD-positive urine samples (UDS+was indicated if the frequency was over 0.3). Patients were screened for BZD bi-weekly. Methadone treatment retention was the primary outcome. Treatment retention was defined as a prescription refill within 30 days of the previous prescription.

(Cont'd...)

Table 2. ***(Continued)***

Study	Publication Year	Journal	Study Design and Methodology
Montalvo *et al.*[51]	2019	*The American Journal of Addictions*	The primary outcome of the study was adherence to buprenorphine treatment≥1 year. Secondary outcomes included 2-year buprenorphine treatment retention. Among patients kept on continuous buprenorphine treatment, Bivariate analysis was performed to see if there was a significant difference in buprenorphine adherence between those who were and weren't co-prescribed BZDs.
Park *et al.*[46]	2020	*Addiction*	Authors compared characteristics between individuals with at least one BZD prescription filled during the study to those with no BZD prescription. They performed outcome event rates and Kaplan-Meier curves for four outcomes: Fatal opioid overdose, non-fatal overdose, all-cause mortality, and buprenorphine discontinuation. The primary outcome was time for fatal opioid overdose. Secondary outcomes included: time to non-fatal overdose, all-cause mortality, and buprenorphine discontinuation. Buprenorphine discontinuation was defined as a patient going more than 30 consecutive days without a prescription filled.
Durand *et al.*[41]	2021	*Drug Alcohol Dependence*	By following methadone prescription records, authors were able to follow a patient's engagement in the MMT program. A patient was considered to be retained in treatment in their methadone prescriptions coverage was maintained with no interruptions longer than 7 days. The primary outcome was the time of dropout of MMT at 3 months and 1 year. Determinants include sex, age, median methadone dose, history of co-prescription (BZDs, antidepressants, antipsychotics), and incarceration. The author examined prescription records for BZDs during MMT and up to 90 days before initiating treatment,
Ray-Griffith *et al.*[38]	2021	*The American Journal on Addictions*	Participants were split into two groups: postpartum treatment retention and postpartum treatment dropout. A bivariate analysis was performed on both groups to identify factors affecting treatment retention at 12 weeks postpartum. Treatment retention was defined as attending an appointment 10 – 14 weeks postpartum. In this article, treatment retention was not dependent on a prescription for buprenorphine. The presence of other substances (including BZD) was evaluated using urine drug toxicology results.
Morford *et al.*[39]	2022	*Drug Alcohol Dependence*	Based on a urine toxicology screening at intake, they separated patients into BZD and no-BZD groups for comparison. Kaplan-Meyer analysis was used to characterize treatment retention. The primary outcome of methadone discontinuation was defined as a patient going more than 30 consecutive days without a methadone dose shown in their electronic medical records.
Hallowell *et al.*[44]	2022	*Drug Alcohol Dependence*	The primary outcome was buprenorphine retention over a 12-month OAT period. Treatment retention was defined as having a medication possession ratio (total days supply of a medication/number of days in the period) during the patient's follow-up period. Multivariable logistic regression models were used to characterize treatment retention and buprenorphine prescription.

have a history of BZD misuse, with 17% of the BZD misuse group possessing a BZD prescription and 83% not having a prescription. Conversely, 52% had no BZD misuse history, with 19% of the no BZD misuse group possessing a BZD prescription and 83% not having a prescription.

In the retrospective cohort study conducted by Park *et al.*,[46] 24% of participants filled at least one prescription for BZDs while receiving buprenorphine therapy. In the study by Raffa *et al.*,[47] 61.7% of participants tested positive for BZDs. In the retrospective cohort study conducted by White *et al.*,[48] 20% of participants were found to have illicit baseline BZD use. From the BZD-positive group, 62% had prescriptions. In the retrospective study by Eibl *et al.*,[49] 75% of subjects had no BZD prescription and tested below the 30% BZD urine screen threshold. Six percent of subjects had no BZD prescription and tested above the 30% BZD urine screen threshold. Thirteen percent of subjects had no BZD prescription and tested below the 30% BZD urine screen threshold. Finally, 6% of subjects had no BZD prescription and tested above the 30% BZD urine screen threshold. In the study conducted by Peles *et al.*,[50] BZD prevalence fluctuated from year to year, with a peak of 61% and a low of 25.4%.

3.3. Studies that distinguish between BZD-positive and BZD-negative at treatment intake

Three retrospective and one observational cohort studies were conducted on treatment retention in OAT where the authors separated subjects into BZD-positive and BZD-negative groups based on UDT during treatment intake.

Table 3. Study characteristics

Study	Number of Subjects	Age (±SD)	Sex (%)	MOUD dose (±SD)	Setting	Polysubstance use	Psychiatric Comorbidity	Methodology and Location
Raffa *et al.*[47]	60	39.2 (6.4)	35% M; 65% F	Methadone 88.5 (56.8) mg/day	Community health center	Amphetamine 47% Cocaine 90%	ND	Retrospective cohort study of patients in methadone maintenance treatment in Vancouver, Canada.
Brands *et al.*[40]	172	31.9 (1.1) – 38.2 (1.2)	26 – 48% F	ND	Outpatient hospital clinic	Alcohol 60 – 84% Cannabis 52 – 68% Cocaine 48 – 63%	Depression 42 – 68% Anxiety 18 – 52%	Retrospective chart review of patients in methadone maintenance treatment in Toronto, Canada.
Schuman-Olivier *et al.*[45]	386	35.6 (10.5) – 37.7 (11.6)	35 – 64% F	ND	Academic community healthcare system	Cocaine 10 – 25% Cannabis 22 – 33% Amphetamine 4 – 8%	ND	Sequential-admission retrospective study in buprenorphine treatment programs in Boston, USA
White *et al.*[48]	604	53 (range 20 – 79)	61% M	ND	Private, non-profit addiction treatment center	Cocaine 29% Cannabis 27% Amphetamine 43%	ND	Retrospective chart review on patients in methadone maintenance treatment in Washington, DC, USA
Peles *et al.*[50]	787	ND	ND	ND	MMT clinic	ND	ND	Retrospective cohort study that investigated the prevalence of BZD usage in Tel Aviv, Israel.
Dayal *et al.*[43]	140	30.4 (10.1)	99% M	ND	Tertiary care drug dependence treatment center	Alcohol 21% Cannabis 26%	Excluded Ss with DSM Axis 1 disorder	Retrospective chart review of patients who were prescribed naltrexone treatment in Northern India.
Franklyn *et al.*[42]	3850	30.8 (10.2) – 34.3 (10.9)	56 – 60% M: 40 – 44% F	Methadone 75 – 85 (33 – 36) mg/day Suboxone 8 – 12 (7 – 8) mg/day	58 addiction treatment centers	ND	ND	Retrospective cohort study for patients initiating OAT in Ontario, Canada
Eibl *et al.*[49]	3692	31.3 (9.7) – 37.5 (10.8)	50 – 57% M: 43 – 50% F	Methadone 64 (30) – 69 (31) mg/day	52 addiction treatment centers	ND	Dx of mood/anxiety disorder 36-81%	Retrospective cohort analysis using urine drug screening data and prescription information in Ontario, Canada.

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Table 3. ***(Continued)***

Study	Number of Subjects	Age (±SD)	Sex (%)	MOUD dose (±SD)	Setting	Polysubstance use	Psychiatric Comorbidity	Methodology and Location
Montalvo *et al.*[51]	321	38 (10)	62% M: 38% F	ND	Behavioral Health Clinic	Other substance use disorder 60%	Depression 85% Anxiety 70% Other mood disorder 61%	Retrospective chart analysis of patients who received at least one buprenorphine prescription in Boston, USA.
Park *et al.*[46]	63345	38 (11)	38% F	Buprenorphine 0>16 mg/day	Multiple	ND	Depression 27% Anxiety 22% Bipolar/psychosis 7%	Retrospective cohort analysis linking five collections of data sets from Massachusetts, USA of buprenorphine users.
Durand *et al.*[41]	2035	34.4	68% M	Methadone range<60 – >120 mg/day	Primary care and specialist addiction services	ND	Antidepressants 48% Antipsychotics 24%	Observational cohort study in Ireland
Ray-Griffith *et al.*[38]	64	28.1 (4.4)	100% F	Buprenorphine 17.8 (6.1) mg/day	Women's Mental Health Program at university hospital	Nicotine use 80% Other substance use disorder 77%	DSM Axis 1 disorder 84% Depression 57% PTSD 48%	Retrospective chart review of postpartum women with OUD treated with buprenorphine at Women's Mental Health Program in Arkansas, USA
Morford *et al.*[39]	2968	37 (11)	63% M: 37% F	ND	Not-for-profit, community-based opioid treatment program	Cannabis 29% Cocaine 34%	ND	Retrospective cohort study of patients in an open-methadone program in Connecticut, USA
Hallowell *et al.*[44]	4898	18 – 65+	61% M 37% F	Buprenorphine dose range 0 to 24+mg/day	Multiple	ND	ND	Retrospective cohort study of Rhode Island, USA residents who initiated buprenorphine use.

Abbreviations: BZT: Benzodiazepine; DSM: Diagnostic and Statistical Manual of Mental Disorders; F: Female; M: Male; MMT; Methadone maintenance treatment; ND: Not determined; OAT: Opioid agonist treatment; OUD: Opioid use disorder; PTSD: Post-traumatic stress disorder; Ss: Subjects.

doi: 10.36922/itps.5151

In the retrospective study by Mortford *et al.*,[39] 31% of participants in both BZD and non-BZD groups remained in OAT after 12 months. Multivariable Cox regression showed no significant difference in treatment duration between the two groups. Similarly, Cox regression analysis by Brands *et al.*[40] indicated that BZD was not a predictor of 1-year treatment retention. Treatment retention was found to be 60% within the cohort.

Conversely, in the retrospective cohort study by Franklyn *et al.*,[42] BZD use was found to be a negative predictor of treatment retention, with a lower median retention of 215 days, compared to 265 days compared to non-users. BZD-positive subjects were found to be more likely to drop out from treatment compared to non-users using baseline urine toxicology, with an adjusted hazard ratio of 1.15. The observational study by Dayal *et al.*[43] reported a significant increase in treatment retention among patients who did not report concurrent BZD use at intake.

3.4. Studies that did not distinguish between illicit and prescription BZD use

Five retrospective and two observational studies were conducted on treatment retention in OAT where the authors did not distinguish between illicit and prescription BZD use. All four studies separated subjects into groups based on intake UDTs[39,40,42,43] and did not include BZD prescription as an independent variable. In the retrospective study by Peles *et al.*,[50] subjects who were BZD-negative upon admission were found to stay in treatment significantly longer compared to their BZD-positive counterparts, respectively. The observational study by Durand *et al.*[41] reported an increased risk of treatment dropout at 12 months associated with BZD use, with a hazard ratio of 1.22. In contrast, the retrospective study by Raffa *et al.*[47] noted a small (3.25%) but significant increase in methadone adherence associated with BZD use.

3.5. Studies that include BZD prescription as a variable

Seven articles included BZD prescription as an independent variable when comparing BZD's effect on OAT treatment. A retrospective study by Eibl *et al.*[49] reported that subjects with a BZD prescription had a statistically similar likelihood of treatment retention, regardless of the frequency of the BZD use. In the retrospective study by Park *et al.*,[46] a BZD prescription during buprenorphine treatment was associated with significantly decreased risk of treatment discontinuation (HR=0.78). In the study by White *et al.*,[48] 42% of patients who used illicit BZDs left the program during the follow-up period, compared to only 10% of subjects with no drug use. The retrospective study by Montalvo *et al.*[51] reported that a BZD prescription was a statistically significant predictor of treatment retention at 1 year, with an adjusted multivariate-OR=2.44. However, at 2 years, a BZD prescription was no longer a significant predictor of treatment retention. Buprenorphine adherence as a secondary outcome was high in both 1-year (95.8%) and 2-year (97.3%) treatment retention groups, with no difference in buprenorphine adherence between the BZD prescription group and no-BZD prescription.

A retrospective study conducted by Hallowell *et al.*[44] showed that individuals who were dispensed overlapping BZD and buprenorphine prescriptions (30 days or more supply) had higher odds of successful treatment retention than those who were prescribed supplies of 7 days or less (adjusted OR = 1.99). It is important to note, though, that in clinical practice, patients given larger drug prescriptions of drugs with potentially harmful side-effects are more likely to be medically stable,[49] thus providing a possible cofound. Conversely, in this study, it is possible that those given the shorter-duration prescriptions may have been less medically stable. The observational study by Durand *et al.*[41] reported that a BZD prescription within 90 days before OAT initiation showed no significant effect on treatment retention. In the retrospective study by Schuman-Olivier,[45] the 12-month treatment retention was 40% among all participants. The study found that retention in treatment or the use of illicit opioids was not linked to either prescription or past abuse of BDZs.

3.6. Studies that consider the frequency of BZD use

Two studies included a comparison of BZD's effect on OAT treatment retention by grouping patients by the frequency of BZD UDT screens. In the study by Franklyn *et al.*[42] subjects had an increased risk of treatment discontinuation with higher frequency of BZD use. Compared to subjects with a BZD frequency of <25%, subjects who were 25–50% BZD-positive were 26.6% more likely to discontinue treatment. Patients who had 50 – 75% BZD-positive frequency were 37.4% more likely to terminate treatment. Strikingly, patients with a BZD frequency >75% were 174.4% more likely to terminate treatment. Similarly, the study by Eibl *et al.*[49] showed a two-fold greater likelihood of treatment discontinuation of patients without a BZD prescription but were heavily using BZD. Comparing the BZD-/UDS+ group to the reference group (BZD-/UDS-) had an adjusted OR=0.38. Other BZD use groups were not statistically different than the BZD-/UDS- group concerning treatment retention.

3.7. OAT program for postpartum women

A retrospective study by Ray-Griffith *et al.*[38] investigated factors affecting treatment retention in an OAT program

for pregnant women. Seventy-three percent of subjects were retained in treatment and 26% dropped out of treatment by 12 weeks postpartum. The percent positive UDT BDZ tests were significantly greater for the drop-out group (30%) than the retained group (9%). A BDZ positive UDT did not differ significantly between the groups at enrollment (29% versus 17.0%) or proximate to delivery (24% versus 13%), but a positive UDT for BDZ any time during the third trimester was significantly more likely in the dropout group (47% versus 16%).

4. Discussion

4.1. Interpretation of results

The increased overdose risk with concurrent BZD and MOUD use is well established,[26,52,53] but further exploration is necessary to understand other aspects of OAT that are affected by concurrent BZD and MOUD use. This review included studies that examined how concurrent BZD and MOUD use in OAT influences treatment retention. Our review encompasses 11 retrospective cohort studies[38-40,42,44-47,49-51,54] and three observational studies.[41,43,48]

The reviewed studies are consistent with previous reports of high polysubstance use among subjects in OAT.[19,55-58] BZD-positive subjects ranged from a frequency of 15% at intake[42] to a high of 61.7% at intake,[47] similar to the range of BZD use (18 – 50%) reported by Lintzeris *et al.*[59] in OATs. Furthermore, evidence of high BZD and opioid co-use before the initiation of OAT[40] has been documented. Overall, these findings underscore the relevance of our review, as BZDs are commonly consumed alongside opioid agonists in OAT programs.

Disparate results were observed in studies that separated subjects into BZD and no-BZD groups, based on intake toxicology results. Two studies[42,43] indicated that BZD use is a predictor of treatment discontinuation whereas two other studies[39,40] found no association between BZD use at intake and treatment retention. A similar relationship is seen with the seven studies that separated the participants into BZD-positive and BZD-negative groups without distinguishing between prescription and illicit BZD use39 – 43,47,50. Four studies showed that BZD use is a predictor of treatment discontinuation,[40,42,43,50] while two studies[42,43] indicated no relationship between BZD use and treatment retention. Finally, Raffa *et al.*[47] showed increased treatment retention with concurrent BZD and MOUD use. These inconsistent findings may suggest the relationship between concurrent BZD and MOUD use on treatment retention is complex and varies based on additional variables that do not remain constant between studies and require further evaluation.

Studies that examined the impact of having a BZD prescription on treatment retention found no significant difference in treatment retention between individuals who were BZD-negative and those with a BZD prescription.[41,45,49] However, there was a substantial increase in treatment retention in individuals with a BZD prescription compared to participants who used illicit BZD.[44,46,48,51] These findings highlight potential importance of prescriptions for individuals in OAT programs who are known to use illicit BZD, as BZDs are regulated substances of known purity and concentration monitored and adjusted by medical professionals. In contrast, illicit BZD use is self-regulated by participants, potentially resulting in increased dosage and side-effects, especially if impure drugs are consumed.[60] Overall, these findings trend to suggest that retention may be greater in OAT programs where BZDs – if used - are under the management of a clinical expert. However, the evidence is far from conclusive, and any modest increases in OAT program retention must be considered in balance with other risks associated with concurrent MOUD and BZD use. Further studies will be required to address this balance in more depth.

When analyzing whether the frequency of concurrent BZD use affects treatment retention, studies indicated an increased risk of treatment discontinuation with prolonged BZD use.[42,49] The increased risk of treatment discontinuation may stem from the appearance of withdrawal effects from continued BZD use, subsequently resulting in more OAT discontinuation. In addition, comparing the effects of a BZD prescription and the frequency of BZD use, Eibl *et al.*[49] noted that subjects with a BZD prescription had a statistically similar likelihood of treatment retention regardless of their frequency of BZD use. This suggests that a BZD prescription may be a stronger predictor of treatment retention than the frequency of BZD use. However, it is worth noting that the study grouped participants into high BZD frequency and low BZD frequency using a fraction of positive urine drug screening for BZD of 30%, a threshold they believe denoted a high versus low frequency of BZD use. Future studies in this area would benefit from considering alternate possible thresholds, based on clinically relevant criteria. For instance, implementing a similar grouping to Frankyn *et al.*,[42] where subjects were divided into four groups based on the percentage of BZD-positive urine samples would allow for a more complex analysis, if sufficiently well statistically powered.

In addition to the primary outcome of 1-year treatment retention, Montalvo *et al.*[51] included secondary outcomes of 2-year treatment retention and buprenorphine adherence. The study indicated that a BZD prescription

increases the likelihood of 1-year treatment retention but was not statistically significant at 2 years. This suggests that BZD's effect on treatment retention may be more impactful at the start of OAT. This relationship could be present for two reasons: (1) BZD use is helpful with alleviating opioid withdrawal symptoms[15] which are much more prevalent at the start of OAT (2) BZDs are typically intended for short-term use, and if used for longer periods it may be prescribed at lower doses or tolerance may develop,[61,62] diminishing their positive impact on treatment retention. Montalvo *et al.*[51] did not report the BZD doses, thus further experiments are required to test our hypotheses. Another conclusion by the authors is that the increase in treatment retention seen with a BZD prescription was not a result of a change in MOUD adherence.[51] This finding is consistent with prior reports that BZD use, specifically under a prescription, is to alleviate comorbidities caused by opioid withdrawal.

The study by Ray-Griffith *et al.*[38] addressed the complex issue of BZD use in OAT programs by pregnant and postpartum women. To our knowledge, this is the only study of its kind, and so comparison is not possible. Nonetheless, this study provides an important investigation, specifically for the effects of illicit BZD use history on treatment retention during pregnancy. Two participants within the cohort had a BZD prescription. Following the removal of the two participants from the statistical analysis, the treatment dropout group had a relatively high rate of BZD use compared to the treatment retained group. The treatment dropout group was statistically more likely to be BZD-positive during the first trimester. The correlation between BZD use in the third trimester and postpartum treatment retention suggests that BZD use history is a predictor of treatment retention. Thus, BZD use might be only beneficial to OAT during the treatment itself, where regulation and monitoring are present. Further analysis is required to evaluate if the increased treatment dropout is solely due to having a history of BZD use, or if having BZD use specifically in the third trimester is significant to OAT retention.

5. Limitations

It is important to note that this literature review was conducted using data from 14 different studies. Some methodologies and primary outcomes were frequently used to provide additional opportunities for comparison, while others were limited to one or two articles on the topic. Thus, more studies must be conducted to validate present experimental results. In addition, while our included studies have investigated the effects of concurrent BZD use in OAT on treatment retention, there is a gap in how specific aspects of individual OAT programs might influence treatment retention. There was also a significant lack of information in the studies about the patterns of illicit BZD use among subjects, and the psychological reasons for its use. Concurrent BZD use with opioids can occur for diverse reasons, including self-medication of opioid withdrawal symptoms, concomitant mental health issues such as underlying anxiety, to increase the euphoric effects of each drug, and as a broader pattern of polysubstance abuse.[24,63] More detailed information about motives for BZD use with opioids would be clinically informative for healthcare providers and could help guide clinical management. In addition to more detailed information about patterns of drug use, it would be informative for studies to include more granular details about the patients and treatment characteristics, as these are critical factors in treatment retention. Patient characteristics including age, sex, psychiatric comorbidities, and substance use all heavily influence treatment adherence,[64-66] while treatment characteristics including MOUD dose, dosing flexibility, and treatment setting similarly influence adherence.[67-69] From Table 3, it was evident that these details are frequently not included in relevant publications.

It must also be noted that the studies we evaluated were conducted across many different regions and countries, where drug protocols and laws for BZD, MOUD, and OAT programs differ, although approximately half of the studies were completed in the North East US (Table 3). Given the widespread use of opioids throughout North America (and increasingly elsewhere) studies in more diverse regions would be a welcome addition to the literature. The included studies also had slightly varying definitions of treatment retention (Table 2). Standardizing the definition of treatment retention may help prevent the presence of biases within the data.

6. Conclusion

The existing literature on this topic consists of diverse studies of substantially different methodologies and outcome measures. Due to the intrinsic complexity and variability of many of the outcome measures, in a typically polysubstance-using population, and a lack of standardized instruments with which to measure these outcome variables, drawing firm conclusions that can be generalized to this field as a whole is not yet possible. Future studies should include a more comprehensive and precise methodology in the evaluation of BZD use during OAT programs, using more rigorous methodological evaluations if possible. Thus, until there are interventional, randomized clinical trials with head-to-head comparisons of treatment interventions, the benefits of different approaches to BZD management can only be indirectly inferred from the existing literature. It may be helpful for

clinicians to identify which studies most closely resemble their own patients and healthcare practices in trying to better consider which approaches may have clinical utility. Furthermore, any potential benefits from greater retention in OAT programs must be considered as part of the "big picture" where other factors, such as the risk of BZD toxicity and addiction represent significant health challenges to those enrolled in OAT programs. Finally, further research needs to be conducted to continue expanding the scope of BZDs' other effects on OAT. This should include evaluating polysubstance tapering or how transitioning patients from illicit BZD use to prescription might impact treatment retention.

Acknowledgments

None.

Funding

The present review was supported in part by a NSERC Discovery grant to AMB.

Conflict of interest

The authors declare they have no competing interests.

Author contributions

Conceptualization: Caitlin Lawrence
Formal analysis: All authors
Writing – original draft: All authors
Writing – review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

REVIEW ARTICLE

Precision medicine and beyond: Evolving roles of targeted therapy, immunotherapy, and artificial intelligence in oncology

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Abstract

Precision medicine in oncology is an evolving therapeutic approach that leverages genetic, clinical, and biomarker data to tailor treatments to individual patients. This review explores the three core pillars of modern precision oncology: targeted therapy, immunotherapy, and the integration of artificial intelligence (AI) into clinical practice. Targeted therapies, including monoclonal antibodies and antibody-drug conjugates, selectively inhibit molecular pathways involved in tumor growth. While conventional chemotherapy remains the backbone of treatment and has improved remission rates, its cytotoxic nature limits broader applicability and increases the risk of comorbidities. Immunotherapies, particularly immune checkpoint inhibitors and chimeric antigen receptor T-cell therapies, have transformed treatment for hematologic malignancies and are now being adapted for solid tumors such as colorectal, pancreatic, and hepatocellular carcinomas through novel combination regimens. This review also highlights the therapeutic potential of modulating the tumor microenvironment and introduces emerging modalities such as neoantigen vaccines and microRNA-based therapies. Furthermore, we outline the expanding role of AI in enhancing cancer diagnosis, drug development, and clinical decision-making. By integrating computational tools with molecular therapies, precision medicine rapidly advances toward individualized data-driven care. This review provides an overview of established therapies in the current clinical practice, novel regimens, and emerging AI technologies. Despite ongoing challenges, such as resistance and toxicity, precision medicine demonstrates significant promise in improving oncologic outcomes and transforming cancer care.

Keywords: Precision medicine; Targeted therapy; Immunotherapy; Artificial intelligence; Cancer treatment; Oncology

1. Introduction

Precision medicine is a medical approach focused on patient stratification using large-scale data, including clinical, lifestyle, genetic, and biomarker information, thus going beyond

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Citation: Li L, Doppalapudi A, Escamilla J, *et al.* Precision medicine and beyond: Evolving roles of targeted therapy, immunotherapy, and artificial intelligence in oncology. *INNOSC Theranostics and Pharmacological Sciences.* 2025;8(3):35-58.
doi: 10.36922/ITPS025140018

Received: April 1, 2025

Revised: June 18, 2025

Accepted: June 30, 2025

Published online: July 30, 2025

the classical "signs-and-symptoms" approach.[1] Targeted therapy, as defined by the National Cancer Institute, involves chemotherapy that blocks the action of specific molecules (enzymes, proteins, etc.) in pathways involved in neoplasm proliferation.[2] Cancer immunotherapy can be classified as active or passive. Active immunotherapies aim to induce specific immune responses against tumors and generate durable antitumor immune memory, whereas passive approaches involve the administration of immune components, such as monoclonal antibodies, without necessarily inducing immune memory.[3] The current chemotherapies have improved remarkably, with improved remission and cure rates. Traditional chemotherapy has undeniably established its importance regarding its precedence in treating cancer, its lower cost, and easier accessibility compared with targeted therapy. However, while there are abundant treatment options for chemotherapy, its cytotoxic nature limits the possibility of applying all potential options without inducing more comorbidities.[4] In addition, gaps remain in understanding the best approach to integrate novel targeted therapies and immunotherapies into routine clinical practice. As a result, this review summarizes both established targeted therapies currently approved for clinical use and those undergoing trials. Moreover, this review discusses the potential of incorporating artificial intelligence (AI) into discovering new targeted therapies and designing individualized treatment regimens, which can help tailor the most suitable treatment to achieve optimal outcomes.

This review explores current precision medicine practices in chemotherapy, focusing on targeted therapies and immunotherapy options, and potential AI applications. The review commences with a discussion on all established targeted therapies in standard care, specifically monoclonal antibodies or antibody-drug conjugates (ADCs) that target specific genes. The following section discusses monoclonal antibodies, including established therapies and combination regimens currently in clinical trials. It also covers preclinical trial models, exploring various strategies to enhance drug delivery and modulate the tumor microenvironment (TME), primarily focusing on hepatocellular carcinoma (HCC), pancreatic cancer, and colorectal cancer (CRC). Chimeric antigen receptor (CAR) T-cell therapy is featured in both sections due to its established success in hematological cancer and its expanding application to other advanced-stage solid tumors.[5] This is followed by a section exploring the potential of incorporating AI algorithms into precision medicine in various aspects, including diagnosis, drug development, and clinical practice.

2. Methodology

This review was first conceptualized by outlining the key topics to be discussed. It was then organized into subsections corresponding to each topic (established therapies, targeted therapies, and AI). To maintain a focused scope, the selection of publications prioritized studies addressing the pharmacological implications, treatment response, and adverse effects of targeted therapies. Commonly used treatments were identified, and a targeted, retrospective literature search was conducted to gather relevant information. Notably, this review does not follow a formal systematic review protocol.

This review also discusses the emerging targeted therapies currently in the pre-clinical or clinical trial phases for HCC, CRC, and pancreatic cancer. These cancers were selected due to their high resistance to treatment, poor response rates, and significant global prevalence. Tables were included to present summarized data from the reviewed publications more clearly.

3. Targeted therapies in clinical practice

Compared to traditional chemotherapy and radiation that indiscriminately damage both cancerous and healthy cells, targeted therapies concentrate on specific molecular pathways driving tumor growth, limiting systemic toxicity and adverse effects. These advancements have significantly improved clinical outcomes, with many targeted drugs achieving superior response rates and prolonged survival in patients with specific genetic mutations. Table 1 shows representative clinical trials highlighting the therapeutic application of targeted agents and immunotherapies across multiple cancer types.

3.1. Human epidermal growth factor receptor 2 (HER2)

HER2, a protein involved in normal cell growth, may be produced in excess by certain types of cancer cells, including those in breast, ovarian, bladder, pancreatic, stomach, and esophageal cancers. HER2 belongs to the epidermal growth factor receptor (EGFR) family, which comprises four types: ErbB1 (EGFR/HER1), ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4). Overexpression of HER2 proteins leads to the formation of homodimers or heterodimers that promote cell growth and proliferation through the phosphatidylinositol 3-kinase/protein kinase B and rat sarcoma/mitogen-activated protein kinase (MEK) pathways, which ultimately contribute to oncogenesis, leading to cancer development. Targeted therapies that bind to the extracellular domain of HER2 prevent heterodimer formation and inhibit cancer growth.[16] Trastuzumab deruxtecan (T-DXd), an ADC consisting of a monoclonal antibody linked through a cleavable tetrapeptide to a membrane-permeable topoisomerase I inhibitor, ensures high drug delivery and cytotoxic effects within HER2-expressing cells, particularly targeting

Table 1. Drug list for established targeted therapies

Drug name	Target	Biomarker	Method of detection	Clinical use	Adverse effects	Sample size	Reference	Combinations
Trastuzumab-deruxtecan	HER2	HER2	Biopsy+IHC	Breast, bladder, ovarian, gastric cancers, NSCLC	Nausea, vomiting, constipation, diarrhea, anorexia, fatigue, alopecia, anemia	Synthesized in review[a]	Martín *et al.* [6]	Antibody - drug conjugate (trastuzumab + deruxtecan)
Pembrolizumab	PD-1	N/A	Biopsy+flow cytometry	NSCLC, HCC,	Diarrhea, autoimmune hepatitis, type 2 diabetes mellitus, and immune reactions	154	Reck *et al.* [7]	
Osimertinib	EGFR	N/A	Biopsy+flow cytometry and serum	NSCLC	Rash, acne, diarrhea, dry skin, interstitial lung disease, QT prolongation (0.33%), one patient each had fatal pneumonia, cerebral infarction, myocardial infarction, pulmonary embolism	279	Soria *et al.* [8]	Osimertinib platinum - based chemotherapies
Palbociclib	CDK4/6	N/A	Biopsy+flow cytometry	Hormone-positive breast cancer, HCC	Neutropenia, leukopenia, thrombocytopenia, fatigue, nausea, headache, upper respiratory infections	417	Turner *et al.* [9]	Palbociclib + fulvestrant
Letrozole	Aromatase	N/A	Biopsy+IHC	ER or PgR-positive breast cancer	Arthralgia, hot flashes, weight gain, insomnia, vaginitis, fatigue	164; 666	Ingle *et al.* [10]; Finn *et al.* [11]	Palbociclib; letrozole
Fulvestrant	ERa	HER2	Biopsy+flow cytometry	ER-positive breast cancer	Hot flashes, headache, nausea, vomiting, constipation, increased LFTs, UTIs, rashes, vaginitis	347 patients	Cristofanilli *et al.* [11]	Palbociclib + fulvestrant
Dabrafenib	BRAF V600E	N/A	Biopsy+flow cytometry	NSCLC	Fatigue, pyrexia, headache, arthralgia, hyperkeratosis, retinal vein occlusion, pneumonitis, interstitial lung disease, cutaneous SCC	36 patients	Planchard *et al.* [12]	Dabrafenib + trametinib
Trametinib	MEK1, MEK2, BRAF	Ki67	Biopsy+flow cytometry	Melanoma, NSCLC	Dermatitis acneiform, peripheral edema, fatigue, nausea	Synthesized in review[a]	Salama *et al.* [13]	Dabrafenib + trametinib
Sorafenib	Raf-1, BRAF, VEGFR1-3, PDGFR-β	ACSL4	Serum	HCC	Fatigue, weight loss, desquamation, hand-foot skin reaction, alopecia, diarrhea, anorexia, nausea, vomiting, voice change	227 patients	Llovet *et al.* [14] and Feng *et al.* [15]	

Note: N/A refers to not available. [a]Indicates data sourced from reviews Martín *et al.* [6] and Feng *et al.*,[15] which synthesize evidence rather than report original sample size.

Abbreviations: ACSL4: Acyl-CoA synthetase long chain family member 4; BRAF: V-Raf murine sarcoma viral oncogene homolog B1; CDK4/6: Cyclin-dependent kinase 4/6; EGFR: Epidermal growth factor receptor; ER: Estrogen receptor; HCC: Hepatocellular carcinoma; HER2: Human epidermal growth factor receptor 2; IHC: Immunohistochemistry; LFTs: Liver function tests; MEK: Mitogen-activated protein kinase; NSCLC: Non-small cell lung cancer; PD-1: Programmed cell death protein 1; PDGFR-β: Platelet-derived growth factor receptor beta; PgR: Progesterone receptor; Raf-1: Rapidly accelerated fibrosarcoma 1; SCC; Squamous cell carcinoma; UTIs: Urinary tract infections; VEGFR: Vascular endothelial growth factor receptor.

extracellular region IV. This combination therapy may reduce platelet count, fatigue, and anemia.[6] While earlier HER2-directed agents targeted tumors with high HER2 expression, T-DXd has demonstrated activity beyond these, including HER2-positive (immunohistochemistry [IHC] 3+ or IHC 2+/*in situ* hybridization [ISH]+) and HER2 low diseases (IHC 1+ or IHC 2=/ISH-). In HER2-low breast cancer patients (IHC 1+ or IHC 2+/ISH-negative), T-DXd received the Food and Drug Administration's (FDA) approval based on the DESTINY-Breast04 trial, which showed a median progression-free survival (PFS) of 9.9 months compared to only 5.1 months with physician's choice of chemotherapy, corresponding to a hazard ratio (HR) of 0.05.[17] Furthermore, in the DENSITY-Breast06 trial, T-DXd showed efficacy in HER2 ultra-low breast tumors (IHC 0 with membrane staining of ≤10%), with a median PFS of 13.2 months compared to 8.1 months in the physician's choice group (HR: 0.72).[18] In HER-mutated non-small cell lung cancer (NSCLC) with activated ErbB2 exon 20 insertions, T-DXd achieved an objective response rate (ORR) of 49% and a median duration of response of 16.8 months in the DESTINY-Lung02 trial.[19] In addition, compared to trastuzumab emtansine, another ADC, T-DXd features lysable linkers with an increased drug-to-antibody ratio, enhancing its antitumor effects.[20,21] With leading success rates in multiple trials and studies, HER2-targeting drugs have demonstrated remarkable efficacy in treating breast, bladder, NSCLC, ovarian, gastric, colon, cervical, and endometrial cancers.[16]

3.2. Programmed cell death protein 1 (PD-1)/ programmed death ligand 1 (PDL1)

PD-1 and PDL1 are transmembrane proteins belonging to the immunoglobulin superfamily. PD-1 can be found on activated T-cell membranes, while PDL1 typically acts as the ligand.[22] The interaction between PD-1 and PDL1 inhibits lymphocyte proliferation through the T-cell receptor, supporting immunosurveillance. However, many tumors exhibit elevated expression of PDL1, allowing uncontrolled proliferation.[23] Pembrolizumab, a monoclonal antibody, binds to these PD-1 receptors on T cells, prevents further conjugation with PDL1 ligands, and restores the immune response against tumor cells. The KEYNOTE-024 trial demonstrated significant overall survival (OS) benefits of pembrolizumab as monotherapy in NSCLC patients with a tumor total proportion score (TPS) of PDL1 ≥50%.[7] KEYNOTE-042 revealed a significant OS benefit in tumors with PD-L1 TPS ≥1% (median OS 16.7 versus 12.1 months; HR: 0.81) and in the 1–49% subgroup (HR 0.88).24 In addition, large-scale clinical trials such as KEYNOTE-189[25] and 407[26] have shown that pembrolizumab, when combined with platinum-based chemotherapy, improves survival in patients with advanced NSCLC regardless of PDL1 expression level. Similarly, the KEYNOTE-189 trial demonstrated improved OS across all PDL1 strata, including the <1% (HR: 0.59), the 1–49% (HR: 0.55), and the ≥50% (HR: 0.36) groups, by adding pembrolizumab to pemetrexed-platinum chemotherapy.25 KEYNOTE-407 reported similar results.26 Despite the common use of general platinum-based chemotherapies, targeted therapies like pembrolizumab have resulted in an increase in OS irrespective of *EGFR* or *ALK* sensitizing mutations, regardless of administering it in combination or as a monotherapy.

Atezolizumab is an immunoglobulin G1 (IgG1) antibody derived from phage display technology. It binds and blocks PDL1 on tumor cell surfaces and has shown significant curative results in kidney, bladder transitional cell carcinoma, and breast cancer.[27] In a study of 3,336 patients who had previously received platinum-based chemotherapies, those receiving atezolizumab were associated with a significantly improved OS compared to other treatments, including docetaxel and nivolumab.[28]

3.3. Tyrosine kinase inhibitor (TKI)-EGFR

TKIs target enzymes that are critical in various cellular processes, including signaling, growth, and proliferation. In several cancer types, these kinases are dysregulated due to mutations or overexpression, leading to unchecked cell growth. Targeting these kinases can inhibit tumor progression. Osimertinib is a third-generation EGFR-TKI that selectively targets both *EGFR*-sensitizing mutations and the T790M resistance mutation in patients with advanced NSCLC. In the FLAURA trial, osimertinib, used as first-line treatment in advanced *EGFR*-mutated NSCLC, demonstrated a median PFS of 18.9 months.[8] It also showed a favorable safety profile with lower incidences of grade ≥3 adverse events, such as rash, diarrhea, and interstitial lung disease. Current clinical guidelines recommend molecular testing for *EGFR* mutations in patients with advanced NSCLC to determine eligibility for EGFR-TKI targeted therapy.[29] Osimertinib is currently the preferred first-line treatment for patients harboring these *EGFR* mutations due to its strong efficacy, central nervous system penetration, and safety profile.[30] In addition, for patients who develop resistance to first- or second-generation EGFR-TKIs – particularly those with the T790M resistance mutation – osimertinib remains the recommended treatment option. In February 2024, the FDA approved osimertinib in combination with platinum-based chemotherapy for patients with locally advanced or metastatic NSCLC harboring *EGFR* exon 19 deletions or exon 21 L858R mutations.[31,32] Furthermore, the FDA approved osimertinib for adults with locally

advanced, unresectable stage III NSCLC whose disease has not progressed during or following platinum-based chemoradiation therapy, provided their tumors have specific *EGFR* mutations.[33] These advancements highlight the crucial role of osimertinib in the management of EGFR mutant NSCLC, offering improved survival outcomes and enhanced quality of life through personalized therapeutic strategies.

EGFR-inhibiting monoclonal antibodies such as cetuximab[34] and panitumumab[35] are also standard-of-care for *RAS* wild-type metastatic CRC. Cetuximab, a chimeric IgG1 monoclonal antibody, and panitumumab, a fully human IgG2 monoclonal antibody, both target the extracellular domain of EGFR to inhibit downstream signaling. However, their efficacy is restricted to patients with *RAS* wild-type tumors, as activating mutations in *KRAS* or *NRAS* result in constitutive downstream signaling that renders EGFR inhibition ineffective. Thus, *KRAS* mutation serves as a predictive biomarker of resistance to anti-EGFR therapy. Molecular profiling to assess *RAS* mutation status is essential before initiating treatment with cetuximab or panitumumab. Multiple clinical trials and meta-analyses have demonstrated that these therapies significantly improve PFS and OS in eligible patients.[36,37]

3.4. Cyclin-dependent kinase (CDK) 4/6

CDK4 and CDK6 regulate cell cycle progression by operating within the G1 to S phase transition; their enzymatic performance is based on D-type cyclins that are expressed in response to signals, including mitogens, cytokines, and estrogen. Once activated, CDK4/6 holoenzymes phosphorylate retinoblastoma tumor suppressor proteins that repress early 2 transcription factors responsible for DNA replication and mitosis. CDK4 and 6 inhibiting therapies can avert retinoblastoma phosphorylation and block the transcription of early 2 factor target genes, thereby inhibiting both estrogen- and mitogen-mediated cell growth.[38] Palbociclib and ribociclib can selectively inhibit CDK4/6, while abemaciclib is a more distinct pyrimidine scaffold that further enhances selectivity and pharmacokinetic properties that are more effective at lower doses and suited for long-term administration.[39] Palbociclib (pyridopyrimidine) showed a prolonged PFS across PDL1 strata; however, no statistically significant OS was observed in the PALSOMA-2/3 trial.[40] Ribociclib demonstrated a superior OS as the first-line treatment in the MONALEESA-2 trial (63.9 versus 51.4 months; HR: 0.76) and as an adjuvant in the NATALEE trial (HR: 0.74 in stage II/III). It efficiently modulated tumor immunity and was successfully combined in CDK4/6 plus immunotherapy regimens.[41] In addition, abemaciclib exhibited a higher selectivity for CDK4 over CDK6, enabling continuous dosing, brain penetration, and approval as a single agent for endocrine-refractory conditions. Consequently, studies investigating CDK4/6 inhibition in *ESR1*-mutant tumors have been conducted.[42,43]

While the PALOMA-2 and PALOMA-3 trials demonstrated a significant improvement in PFS with the addition of palbociclib to endocrine therapy – such as letrozole or fulvestrant – in hormone receptor-positive, HER2-negative advanced breast cancer, neither study showed a statistically significant OS benefit.[44-47] Nonetheless, the favorable PFS and manageable toxic profiles have led to the widespread use of palbociclib in clinical practice.

3.5. V-Raf murine sarcoma viral oncogene homolog B1

V-Raf murine sarcoma viral oncogene homolog B1 (BRAF) inhibitors block a specific protein called BRAF, a kinase enzyme that helps control cell growth and signaling. Dabrafenib is a BRAF inhibitor combined with trametinib, a MEK inhibitor. Together, they inhibit the BRAF V600E mutation and the downstream MEK pathway. Combination therapy has shown an overall response rate of approximately 64% in patients with *BRAF* V600E-mutant SCLC with minimal adverse effects such as pyrexia, hypertension, and vomiting.[12] Based on these findings, the FDA approved the combination of dabrafenib and trametinib for the treatment of metastatic NSCLC harboring the *BRAF* V600E mutation.[48] In clinical practice, identifying patients with *BRAF* V600E mutations is crucial for selecting appropriate targeted therapies. Comprehensive molecular profiling of tumors is recommended to detect actionable mutations, including *BRAF* V600E, to guide treatment decisions.[49] The combination of dabrafenib and trametinib offers a valuable treatment option for patients with this specific genetic mutation, providing significant clinical benefits with a tolerable safety profile.

3.6. Vascular endothelial growth factor receptor (VEGFR)

VEGFR inhibitors prevent the formation of new blood vessels required for tumor growth and may also induce cancer cell death. Sorafenib is an oral multi-kinase inhibitor of VEGFR, platelet-derived growth factor receptor, and rapidly accelerated fibrosarcoma. In patients with advanced HCC, it extends OS by about 3 months compared to placebo (median OS ~10.7 months).[14] Increasing levels of the enzyme DEAD box protein 5 (DDX5) in liver cancer cells improved the effectiveness of sorafenib. Higher DDX5 levels enhanced sorafenib's ability to reduce tumor growth, suggesting that therapies boosting DDX5 could potentiate sorafenib's anticancer effects.[50] Used as a second-line treatment for patients who have progressed

on sorafenib, regorafenib inhibits multiple kinases involved in tumor angiogenesis, oncogenesis, and the TME. The RESORCE trial reported a median OS of 10.6 months for regorafenib compared to 7.8 months for placebo.[51]

3.7. Anaplastic lymphoma kinases (ALK)

ALK belongs to the insulin receptor superfamily. These genes play crucial roles in alternative splicing, mutations, and amplifications linked to inflammatory myofibroblastoma and NSCLC. *ALK* rearrangements can also induce T cell activation, cytokine release, and immune surveillance in tumors. TKIs such as crizotinib, alectinib, and lorlatinib have gained approval for advanced ALK+ NSCLC due to their ability to significantly improve efficacy compared to standard chemotherapies. Second-generation ALK-TKIs, like alectinib, demonstrate greater clinical efficacy in terms of median PFS, ORR, duration, and higher central nervous system response rates compared to crizotinib, a first-generation ALK-TKI.[52] Alectinib functions as a selective inhibitor and substrate that easily penetrates through the blood–brain barrier and prevents downstream tumor survivability, avoiding the collateral damage of chemotherapy regimens.[53]

3.8. Rearranged during transfection gene fusions inhibitors

RET (rearranged during transfection) gene fusions are oncogenic drivers found in a small subset of solid tumors, including NSCLC and thyroid cancers. These gene rearrangements result in constitutive kinase activity that promotes tumorigenesis, making them actionable targets for precision therapies.

Selpercatinib and pralsetinib are highly selective RET inhibitors that have demonstrated significant clinical efficacy in RET fusion-positive NSCLC and thyroid cancers. The LIBRETTO-001 trial showed that selpercatinib achieved an ORR of 64% in previously treated NSCLC patients and 85% in treatment-naive patients.[54] In medullary thyroid cancer with RET mutations, selpercatinib also showed durable responses with manageable toxicity profiles, including hypertension and elevated liver enzymes.[55]

Given its clinical activity and favorable safety profile, RET inhibition represents a critical component of precision therapy in cancers harboring RET rearrangements. Genomic profiling for RET fusions or mutations is essential for patient selection, and ongoing trials are exploring combination strategies to enhance response and mitigate resistance.

3.9. Neurotrophic tyrosine receptor kinase (NTRK) inhibitors

NTRK gene fusions are rare but actionable alterations found across various adult and pediatric solid tumors, including salivary gland tumors, thyroid cancer, and some sarcomas. These fusions result in constitutive activation of TRK proteins, driving uncontrolled cell growth and survival.

Larotrectinib and entrectinib are first-generation TRK inhibitors approved for tumor-agnostic use in NTRK fusion-positive cancers. Larotrectinib demonstrated an ORR of 75% in a pooled analysis of adult and pediatric patients, with durable responses and minimal toxicity.[56] Entrectinib, which also targets c-ros oncogene 1 and ALK, has shown particularly strong efficacy in central nervous system-involved tumors due to its ability to penetrate the blood–brain barrier.[57]

Routine comprehensive genomic testing is critical for detecting NTRK fusions, and these therapies underscore the potential of histology-agnostic treatment strategies. Clinical trials continue to explore mechanisms of resistance and next-generation inhibitors.

3.10. CAR T-cell therapy

CAR T-cell therapy involves collecting a patient's T cells and genetically modifying them to express CARs that recognize specific antigens on cancer cells (Figure 1). Once infused back into the patient, these engineered T-cells can identify and destroy malignant cells expressing the target antigen. Tisagenlecleucel (Kymriah®), the first FDA-approved CAR T-cell therapy, is used for treating relapsed or refractory B-cell acute lymphoblastic leukemia in patients up to 25 years old.[58] Clinical trials have demonstrated high remission rates, offering hope to patients with limited treatment options. Axicabtagene ciloleucel (Yescarta®) and tisagenlecleucel have been approved for adult patients with relapsed or refractory diffuse large B-cell lymphoma after two or more lines of systemic therapy, showing significant response rates.[59] CAR T-cell therapies targeting B-cell maturation antigen, such as idecabtagene vicleucel (Abecma®), have been approved for patients with relapsed or refractory multiple myeloma after four or more prior lines of therapy.[60] Real-world data on idecabtagene vicleucel for relapsed/refractory multiple myeloma showed a 69% response rate with manageable toxicity,[61] though prolonged hematologic toxicity remains a challenge, highlighting the need for optimizing CAR T-cell expansion.

4. Therapies in preclinical/clinical trials

After the success of targeted therapies and immunotherapies in lung cancer, breast cancer, and hematological cancer, the use of targeted therapies has been expanded to other types of advanced-stage cancer with high metastatic potential, such as HCC, CRC, and pancreatic ductal adenocarcinoma (PDAC). For HCC, the primary tumor generated from

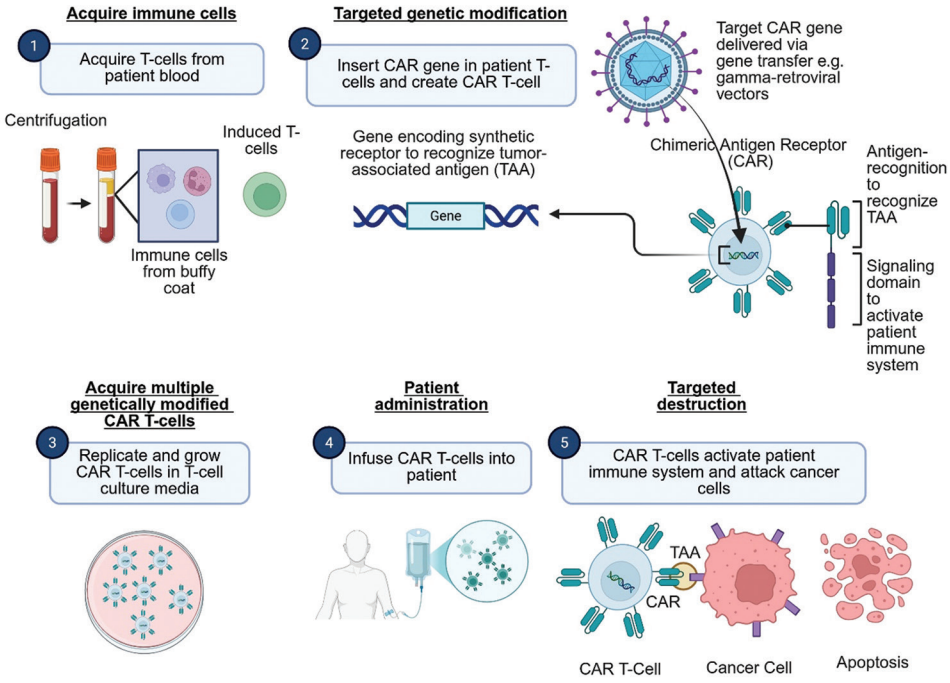

Figure 1. General mechanism of action for chimeric antigen receptor (CAR) T-cell therapy. Created in BioRender. Mito, S. (2025) https://BioRender.com/gqcdtct.

hepatocytes accounts for 90% of liver cancers. Although early-stage detection and subsequent surgical resection can increase the 5-year survival rate (50–75%), the recurrence rate can reach 50%. As a result, targeted therapy is one of the established therapies to suppress the rate of recurrence and enhance the 5-year survival rate.[62]

Comparably, CRC is the third most common cancer diagnosis and is ultimately the second leading cause of cancer-related death in the United States. The 5-year survival rate for early-stage CRC is 90%; however, the declining survival rate in metastatic CRC is heavily associated with minimal advancements in colorectal screening and therapeutics.[63] PDAC is acknowledged as one of the most challenging cancers to approach with management, as it is often diagnosed in its advanced stage due to difficulty in screening and detection. With precision medicine growing and newer targeted therapies taking the lead in promising clinical trials and research studies, PDAC has become one focus of such investigations.[64]

The major approaches in clinical trials have two directions: (i) to test the efficacy of a combined regimen between monoclonal antibodies targeting different genes and (ii) to explore options that can potentiate targeted therapies and reduce chemotherapy resistance in tumor cells. This section primarily discusses clinical trials testing combination regimens in HCC, CRC, and PDAC and their efficacies, along with their toxicity profiles. It also includes other candidates in Phase I/II clinical trials or in preclinical settings that show significant potential in enhancing the drug regimen. The drug list is summarized in Table 2.

4.1. PD-1/PDL1 combination therapy

In patients with CRC, characterized by DNA mismatch repair deficiencies and high microsatellite instability, immune checkpoint inhibitors (ICIs) have been effectively researched as therapies against solid tumors and other cancers as monotherapies. However, these patients are only a fraction of the total number of individuals with cancers that cannot be effectively treated in the same way and require combination therapies instead.[81] Therefore, PD-1/PDL1 has been tested in future clinical trials in combination with other therapies and slowly integrated into clinical practice.[82]

The treatment of HCC has slowly transitioned from molecular therapies, such as sorafenib and lenvatinib, toward immunotherapy as the first-line approach.[83] For example, nivolumab and pembrolizumab have been approved for late-stage HCC.[84] Pembrolizumab (Keytruda®) has demonstrated moderate efficacy as monotherapy in patients with higher PD-1/PDL1-expressing tumors but poor efficacy in other cancer types. However, when combined with gemcitabine (Gemzar®) and nab-paclitaxel in intermittently-scheduled doses, results have been shown to improve significantly with longer median PFS

Table 2. Drug list for preclinical/clinical therapies

Drug name	Target	Biomarker	Clinical use	Method of detection	Adverse effects	Sample size	Reference	Combination
Sotorasib	KRAS	N/A	CRC, PDAC, endometrial cancer, NSCLC, melanoma	Biopsy+flow cytometry (mainly serum for CRC progression)	Diarrhea, fatigue, nausea, anemia, elevated LFTs, hepatitis, hyponatremia	129 patients	Hong *et al.* [65]	Sotorasib + panitumumab
Adagrasib	KRAS	N/A	NSCLC, CRC, PDAC	Biopsy+flow cytometry	Fatigue, nausea, vomiting, diarrhea	25 patients	Ou *et al.* [66]	Adagrasib + docetaxel + cetuximab
CAR T-cell	CD19, GCC	N/A	Metastatic CRC, B-cell ALL, DLBCL, multiple myeloma	Serum	Cytokine release syndrome, diarrhea	15 patients	Chen *et al.* [67]	
Regorafenib	VEGFR	N/A	CRC, liver metastasis,	Serum	Hand-foot syndrome, rash, fever, hoarseness, diarrhea, hypertension, hepatotoxicity, chest distress, myalgia, headache, thrombocytopenia, fatigue	42 patients	Wang *et al.* [68]	Regorafenib + toripalimab
Toripalimab	PD-1	N/A	CRC, liver metastasis, nasopharyngeal carcinoma	Serum	Leukopenia, hypothyroidism, pruritus, pneumonia, and immune myocarditis	289 patients	Mai *et al.* [69]	Regorafenib + Toripalimab
Cabozantinib	VEGFR, c-MET	N/A	CRC	Serum	Acneiform rash, fatigue, diarrhea	25 patients	Strickler *et al.* [70]	Cabozantinib + panitumumab
Panitumumab	EGFR	N/A	CRC	Serum	Nausea, neutropenia, dermatitis acneiform, hypomagnesemia, diarrhea, hepatotoxicity	160 patients	Fakih *et al.* [71]	Cabozantinib + panitumumab and sotorasib + panitumumab
Nivolumab	PD-1	N/A	Melanoma, squamous cell lung cancer, NSCLC, HCC	Serum	Maculopapular rash, erythema, hepatitis, infusion reactions		Abedi Kiasari *et al.* [72]	
Camrelizumab	PD-1	N/A	Esophageal squamous cell carcinoma, HCC	Biopsy+flow cytometry	Neutropenia, hepatitis, reactive cutaneous capillary endothelial proliferation	596 patients	Xu *et al.* [73]	Camrelizumab + apatinib
Apatinib	VEGFR2	N/A	HCC	Serum	Hypertension, hand-foot syndrome, and thrombocytopenia	400 patients	Qin *et al.* [74]	Camrelizumab + apatinib
Cadonilimab	PD-1, CTLA-4	N/A	Cervical cancer	Serum	Neutropenia, lymphopenia, and anemia	445 patients	Wu *et al.* [75]	

(Cont'd...)

Table 2. ***(Continued)***

Drug name	Target	Biomarker	Clinical use	Method of detection	Adverse effects	Sample size	Reference	Combination
Tisotumab	Transcription factor	N/A	Cervical cancer	Biopsy+flow cytometry	Anemia, diarrhea, nausea, and thrombocytopenia	142 patients	Vergote *et al.*[76]	
Atezolizumab	PDL1	N/A	Kidney, bladder, transitional epithelial, breast, and cervical cancer, HCC	Biopsy+flow cytometry	Diarrhea, arthralgia, pyrexia, rash, hypothyroidism, hyperthyroidism, constipation, myalgia, infusion reaction	410 patients	Oaknin *et al.*[77]	
Sintilimab	PD-1	TMB, circulating tumor DNA	Hodgkin lymphoma, HCC	Serum	Pneumonia, diarrhea, colitis, hepatitis, nephritis, endocrine disease, infection reactions, rashes	146 patients	Zhang *et al.*[78]	Sintilimab+ bevacizumab
Durvalumab	PDL1	N/A	SCLC, HCC,	Serum	Febrile neutropenia, anemia, leukopenia, thrombocytopenia,	805 patients	Al-Salama *et al.*[79]	
Tremelimumab	CTLA-4	$CD4^+$, $CD8^+$	Melanoma, HCC, PDAC	Biopsy+flow cytometry	Increased LFTs, diarrhea, increased lipase, and amylase	332 patients	Kelley *et al.*[80]	Tremelimumab+ gemcitabine

Note: N/A refers to not available.
Abbreviations: ALL: Acute lymphoblastic leukemia; c-MET: Cellular-mesenchymal epithelial transition factor; CRC: Colorectal cancer; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; DLBCL: Diffuse large B-cell lymphoma; EGFR: Epidermal growth factor receptor; GCC: Guanylyl cyclase C; HCC: Hepatocellular carcinoma; KRAS: Kirsten rat sarcoma virus; LFTs: Liver function tests; NSCLC: Non-small cell lung cancer; PD-1: Programmed cell death protein 1; PDAC: Pancreatic ductal adenocarcinoma; SCLC: Small-cell lung cancer; TMB: Tumor mutational burden; VEGFR: Vascular endothelial growth factor receptor.

time (median of 9.1 months) for PDAC, suggesting that chemotherapy and immunotherapy combinations are a promising direction for further investigation, rather than relying on immunotherapy alone.[85]

Adjunct therapy combining VEGFR2 inhibitors and PD-1/PDL1 inhibitors has become an important strategy to treat HCC. For example, atezolizumab-bevacizumab has recently been approved by the FDA to treat HCC.[86] However, resistance to atezolizumab-bevacizumab, such as high *HES1* (transcriptional target of NOTCH pathway) expression, has been observed in clinical settings, highlighting the need for alternative therapeutic strategies.[87] First-line camrelizumab plus apatinib (VEGFR2 inhibitor) has shown remarkable efficacy and has since been used as a first-line therapy for unresectable HCC in a Phase 3 trial.[74] In addition, camrelizumab and apatinib are employed in a Phase I trial for advanced gastric cancer, and they showed favorable clinical outcomes with an overall response rate of 76.5%.[88] Sintilimab has shown superior clinical benefits compared to sorafenib when combined with bevacizumab.[89]

In addition, ADCs, the novel agents designed to deliver cytotoxic drugs into tumors, can further increase the efficacy of targeted therapies (Figure 2). The structure of ADC includes monoclonal antibodies that recognize specific markers expressed by tumor cells, linked to monoclonal antibodies with cytotoxic drugs that induce tumor cell death upon binding. Among several biomarkers, tumor mutational burden is currently the most widely accepted.[90,91] While efficacy is remarkably stronger, combination regimens generally induce stronger Grade 3 adverse events than single monoclonal antibody therapy; the most common side effects include hypertension and palmar-plantar erythrodysesthesia syndrome.

Numerous molecular targets have been identified in HCC, and targeting them may enhance the efficacy of ADCs. For instance, in an HCC mouse model, diacylglycerol kinase gamma was found to promote tumor

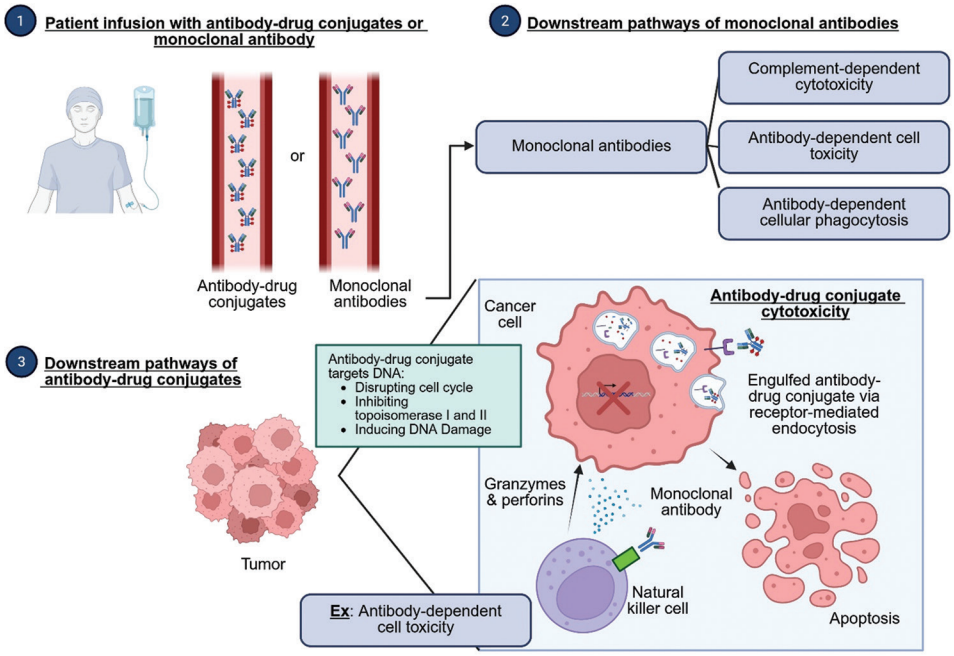

Figure 2. General mechanism of action of antibody-drug conjugate to potentiate the effects of monoclonal antibodies. Created in BioRender. Mito, S. (2025) https://BioRender.com/gqcdtct.

angiogenesis and immunosuppressive regulatory T-cell differentiation in HCC treated with camrelizumab and apatinib.[92] As more pathways and clinical data continue to emerge, genomic and transcriptomic sequencing have been employed to personalize treatment for HCC patients based on specific genetic mutations. For example, patients with *TSC2* inactivation have been treated with everolimus, while patients with *CDK4* amplification were treated with palbociclib.[82]

Another example of this chemo-immunotherapy combination effectiveness is explored in a Phase I trial combining tremelimumab (Imjudo®), a cytotoxic T-lymphocyte-associated protein 4 monoclonal antibody, and gemcitabine. The trial suggested that increasing the dosage of the immunotherapy will result in a prolonged OS. However, the severity of Grade 3 toxicities also increased with increasing drug dose, including thrombocytopenia, nausea, diarrhea, anemia, neutropenia, and general weakness; nonetheless, the regimen was generally well-tolerated under close management.[93] However, a Phase II trial investigating tremelimumab combined with durvalumab (Imfinzi®), a PD-1/PDL1 inhibitor, reported minimal efficacy in PDAC patients, with a median PFS and OS of 1.5 months and 3.1 months, respectively, and an ORR of only 3.1%. It was speculated that the active T-cell suppression and nitric oxide synthase overexpression created by the dysregulated immune signaling cells produced a resistance to the antitumor response of the combined therapy. Thus, although this combination therapy was found to be well-tolerated, the results did not meet the efficacy threshold and highlighted the need for further investigation on both tremelimumab and durvalumab as plausible ICI choices when treating cancers like PDAC.[94]

A similar area of ongoing research for drug therapy combinations is the use of ipilimumab (Yervoy®), a cytotoxic T-lymphocyte-associated protein 4 monoclonal antibody, combined with GVAX (a granulocyte-macrophage colony-stimulating factor-secreting vaccine cancer therapy) to achieve a greater tumor response and survival rate. Although the study revealed no improved OS in patients with metastatic PDAC, it demonstrated promising biological effects, including increased T-cell differentiation and M1 macrophage infiltration.[95] Another Phase I study of metastatic castration-resistant prostate cancer patients treated with the same combination of ipilimumab and GVAX found a notable decline in prostate-specific antigen levels along with adequate clinical activity and tolerability.[96]

While anti-PD-1 therapy is especially helpful in targeting tumors with high microsatellite instability and DNA mismatch[97] – which are significantly associated with immunogenic subtypes of CRC, such as Lynch syndrome – combination therapy is crucial in treating microsatellite stable CRC with low immune cell infiltration

and is resistant to therapies targeting PD-1/PD-L1.[68] The use of multi-kinase inhibitor regorafenib with anti-PD-1 monoclonal antibody toripalimab has recently progressed to early phases of clinical trials to treat metastatic CRC. A Phase I/IIb trial analyzing the ORR and disease control rate in CRC patients treated with this therapy reported a 15.2% response rate of the 42 patients who were treated with the combination therapy; however, lower ORRs were seen in patients with liver metastases (8.7%). This reduction in response in CRC patients with liver metastases is likely due to resistance to checkpoint blockade, as the liver harbors a significant proportion of immunosuppressive cells that mediate this resistance.[68] High levels of the anaerobic species *Fusobacterium* are also associated with decreased response to the combination of regorafenib and toripalimab, and its presence can be used as a marker in monitoring treatment resistance.[68] In addition, the disease-control rate was 36.4% in patients who were specifically treated with the recommended dose of 80 mg of regorafenib plus toripalimab.[68] The study also noted that while 94.9% of patients in the trial experienced an adverse event due to the treatment, only 38.5% experienced a significant Grade 3 or higher event.[68] This response suggests that the combination treatment has a safety profile comparable to that of the individual drugs, while providing the added benefit of improved efficacy.

Another clinical trial also investigated the efficacy and safety of combination treatment with regorafenib and toripalimab in 33 patients with microsatellite instability subsets of CRC, a malignancy typically considered non-immune-responsive due to resistance to ICIs, which is often attributed to poor infiltration of immune cells into the TME.[98] A response to the treatment was seen in 16 patients, with 12 patients experiencing stability in their disease, with further analysis using a Kaplan–Meier survival curve displaying a median PFS of 113 days and a Cox regression model demonstrating a 12.12% objective remission rate.[98] Safety assessment of the therapy showed an incidence rate of Grade 3/4 adverse reactions of 9.09%, with the majority of adverse events (33.3%) being hand-foot syndrome. Altogether, the current studies are exploring PFS, OS, and safety outcomes associated with different combination therapies, with the goal of optimizing the use of ICIs as targeted cancer therapies in specific patient populations.

4.2. CAR T-cell therapy in colorectal cancer

CAR T-cell therapy is showing promise in the treatment of hematological malignancies but has scarce clinical data for solid tumors. However, there have been recent pre-clinical studies investigating the effect of CAR T-cell therapy in the treatment of CRC. One investigational approach involving engineered T-cells incorporated modifications that promote the targeted expression of interleukin 6, interferon gamma, and interleukin 2 toward guanylate cyclase-c (GCC19), a target expressed in 70% to 80% of CRC.[67] A study analyzing the adverse effects of GCC19 CAR T-cell in patients with metastatic CRC revealed that 93% of participants experienced a Grade 3 or higher adverse event, primarily related to cytokine release syndrome (CRS). However, these adverse events were transient. The median PFS was significantly longer in patients who received higher doses (2×10^6 cells) of GCC19 CAR T-cell compared to those receiving a lower dose (1×10^6 cells), with a PFS of 6 months versus 1.9 months, respectively. Although the sample size was small, the results suggest potential clinical activity of GCC19 CAR T-cell therapy in patients with advanced-stage CRC.

Another antigen under investigation is carcinoembryonic antigen, commonly overexpressed in CRC. A Phase I trial evaluating carcinoembryonic antigen-specific CAR T-cell therapy in 10 patients with metastatic CRC revealed that seven achieved stable disease, with two showing a reduction in liver metastases.[99] Moreover, CRS was reported in only three patients, with one experiencing a severe adverse event.[99] While overall safety appeared manageable, CRS remains the most common adverse event and requires vigilant monitoring during trials to prevent systemic complications.

CAR T-cell therapy has also been used to target mesothelin (MSLN), a differentiation antigen normally expressed in the mesothelium but also highly expressed in solid cancers, including in 48–61% of CRC cases.[100] In a pre-clinical study using MSLN-targeted CAR T-cells with irinotecan in patient-derived xenograft mouse models, significant antitumor activity was observed in MSLN-positive cells – particularly with the CAR_R47 construct, which targets the Region 1 epitope of MSLN – while no effects were observed in MSLN-negative cells.[101] Complete tumor regression was noted in two of the five mice receiving the combination treatment. Although these results are encouraging, further validation should be considered in future clinical trials.

Despite early promise, CAR T-cell therapy in CRC faces multiple significant external factors that impact the potency and overall safety profile of CAR T-cell therapy, including inherent immunosuppressive barriers; secretion of immunosuppressive cytokines; heterogeneity of CRC tumors; and on-target, off-tumor effects that can lead to severe toxicity and unintended damage in normal tissue.[100] These limitations have hindered clinical translation. Future directions in overcoming these challenges include optimizing T-cell persistence, identifying new CRC-specific antigens, and designing combinatorial or

checkpoint-modified CAR constructs to improve efficacy and safety. For now, CAR T-cell therapy in CRC should be regarded as a promising but investigational modality under active clinical evaluation.

4.3. Novel therapies targeting Kirsten rat sarcoma viral oncogene homolog mutations

Kirsten rat sarcoma viral oncogene homolog (KRAS) is a guanosine triphosphatase (GTPase) molecular switch that is active when bound to guanosine triphosphate and inactive when bound to guanosine diphosphate; this cycling is controlled by guanine nucleotide exchange factors and GTPase-activating proteins. In its active form, KRAS controls a signaling cascade of over 80 effector proteins and kinases, including nuclear transcription factors involved in cell growth, proliferation, survival, migration, and cell differentiation. When mutated at codon 12, the KRAS GTPase is unable to convert guanosine triphosphate to guanosine diphosphate, causing it to remain in the activated state and continuously stimulating the downstream cancerous cellular process.[102] Hypomethylation of CpG islands in the promoter sequence of the *KRAS* gene can lead to overexpression of *KRAS* and ignite the cascade of p53 mutations and associated overexpression of cyclooxygenase-2.[103] Consequently, *KRAS* mutations are strongly associated with CRC and PDAC.

The current standard-of-care therapy for metastatic CRC associated with wild-type *KRAS* typically involves the use of EGFR inhibitors, such as panitumumab and cabozantinib. Nevertheless, resistance to monotherapy still arises, particularly due to amplification of the *MET* oncogene.[70] To combat EGFR treatment resistance, combination therapy with the TKI cabozantinib has been investigated in early clinical trials to assess for any changes in clinical activity or safety profile compared to monotherapy. A Phase Ib trial treating 25 patients with a combination therapy of cabozantinib and panitumumab reported an ORR of 16% with a median PFS of 3.7 months. In regard to the safety profile, 20% of the patients discontinued treatment due to experiencing adverse events relating to toxicity; however, these adverse events were reduced with lower doses of cabozantinib.[70] With a PFS that is higher than that of monotherapy, early clinical outcomes of cabozantinib and panitumumab prove to be a promising regimen in the management of KRAS-mediated metastatic CRC. Further research is needed to determine the appropriate dosing to improve the safety profile and overall tolerability of this combination therapy.

The oncogenic *KRAS* mutation at codon 12 results in different subtype allele frequencies, which have been found to play a significant role in the aggressive advancement and worsening prognosis of PDAC. Sotorasib (Lumakras®), a drug targeting *KRAS* G12C mutations in solid tumors, is recognized as a useful therapy for PDAC. At present, in Phase I/II trials, this drug restrains the activation of the KRAS signaling cascade in cancer development and cell differentiation by keeping the molecule in a guanosine diphosphate-bound inactive state.[65] Low-grade toxic effects of diarrhea and fatigue were frequently observed in trials evaluating safety, making it a plausible option for PDAC compared to other existing therapies. Out of 38 patients, between both Phase I and Phase II trials, diarrhea and nausea were reported in nine patients, and eight patients experienced vomiting. The most common Grade 3 adverse events observed were diarrhea and fatigue in two patients, with no reported Grade 4 or 5 adverse events.[104] Moreover, sotorasib demonstrated an increased success rate when combined with panitumumab, an EGFR inhibitor, as a chemorefractory cancer therapy in patients without previous treatment. The treatment resulted in a median PFS of 5.6 months with a 96 mg dose and 3.9 months with a 240 mg dose. The standard care group in this trial demonstrated a median PFS of only 2.0 months and was used as the reference.[71] Further trials with larger patient cohorts and investigations into combination therapies as second-line therapy in previously treated patients are underway, reflecting growing confidence in the efficacy of sotorasib as a solid tumor cancer therapy.

Adagrasib (Krazati®) has been identified as a selective, covalent inhibitor of the *KRAS* G12C mutation, with known favorable pharmacokinetic properties and efficient bioavailability. With a similar mechanism of action to sotorasib, sustained levels of adagrasib above a determined threshold have been shown to suppress the synthesis and rebound of KRAS-dependent signaling in solid tumors, especially in NSCLC. The study resulted in a median PFS of 11.1 months for eight out of the 15 patients who were determined to have a confirmed partial response to the drug.[66] Nausea, diarrhea, vomiting, and fatigue are some of the classically presenting adverse effects with the use of adagrasib, similarly seen with other chemotherapies. Moreover, adagrasib acts as an inhibitor of cytochrome P450 3A4, the enzyme responsible for its metabolism along with other drugs, causing concern for drug–drug interactions when co-administering other therapies.[105] After promising Phase I/Ib trials, Phase III trials observing adagrasib in combination with drugs such as docetaxel and cetuximab to treat *KRAS* G12C-mutated solid tumors in NSCLCs and CRCs are underway.[66]

Studies comparing the efficacy and toxicity of sotorasib and adagrasib have been synthesized in meta-analyses to

analyze differences in their drug profiles and how these relate to patient characteristics and medical history.[106] For example, sotorasib was observed to be associated with significantly lower rates of gastrointestinal adverse effects such as diarrhea and nausea (around 40% and 55% lower prevalence of each, respectively), when compared to adagrasib. Hepatotoxicity with increased alanine aminotransferase levels was also found to be associated more with adagrasib use than sotorasib, suggesting that sotorasib is a better option in patients with prior gastrointestinal or liver-related health issues. For example, the prevalence of diarrhea and nausea associated with adagrasib (70.7% and 69.8%, respectively) was higher than in sotorasib (34% and 14%). Moreover, the overall Grade 3 adverse effects were 89.1% with adagrasib and 20% with sotorasib. In addition, although adagrasib was found to have a slightly higher therapeutic efficacy in sustaining cancer control, most studies suggested similar efficacies between the two drugs. The choice between them often hinges on patient group-specific considerations regarding adverse effects profiles. Importantly, the efficacy of both drugs overall was partially dependent on wild-type *RAS* feedback reactivation induced by Src homology-2 domain-containing protein tyrosine phosphatase-2, known to be the primary resistance mechanism of KRAS inhibitors.[106]

4.4. Poly(ADP-ribose) polymerase inhibitors in PDAC

In cancers with *BRCA* mutations, including some pancreatic adenocarcinomas, poly-ADP-ribose polymerase (PARP) inhibitors such as olaparib and talazoparib are being investigated as potentially effective therapies. Olaparib (Lynparza®) was studied in Phase II trials with results suggesting a well-tolerated response in patients with long-standing ovarian, breast, pancreatic, and prostate cancers, with a significant efficacy (about a 26.2% tumor response rate overall) in genetically targeting PARP enzymes in *BRCA* 1/2-mutated circumstances.[107] Upon further investigation of DNA damage repair genes in trials with olaparib, cross-resistance with platinum analogues was identified. However, after modifying the study accordingly, the drug was ultimately determined to be consistent in its efficacy, supported by parallel studies in Israel and the United States, with only minor expected toxic effects such as anemia, fatigue, anorexia, and nausea.[108]

Another PARP inhibitor of newfound importance in cancers with *BRCA* 1/2 mutations is talazoparib (Talzenna®), with a significant improvement in PFS and efficacy compared to other chemotherapies. The results of the recent controlled Phase 3 EMBRACA trial demonstrated a double response rate and 46% risk reduction for cancer progression or death with talazoparib. Minor myelotoxicity and hematological complications, such as anemia, are conveniently managed with dose modifications.[109]

In contrast, olaparib carries a higher risk than talazoparib in terms of drug–drug interactions, as it is primarily metabolized by cytochrome P450; thus, it is less preferred as an option in patients taking multiple medications. In addition, the recommended oral dosage of talazoparib (1 mg) is lower than olaparib (300 mg) and is taken only once daily, whereas olaparib is often prescribed to be taken twice a day.[110] In different trials observing drug efficacy, the EMBRACA trial (for talazoparib) determined a slightly longer median PFS than the OlympiAD trial (for olaparib) in similar patient populations.[111] However, in terms of safety profiles, olaparib has less severe adverse effects, but with more gastrointestinal changes like vomiting, whereas talazoparib is associated with hematological toxicity, including more severe forms of anemia and neutropenia.[112] Overall, both olaparib and talazoparib are being studied and have so far been determined to be effective PARP inhibitor therapies for *BRCA* 1/2-mutated cancers, with carefully monitored dose management and symptom monitoring.

4.5. TME

TME plays a pivotal role in tumor progression, therapeutic resistance, and immune evasion. Composed of immune cells, stromal components, vasculature, signaling molecules, and extracellular matrix, the TME interacts dynamically with tumor cells to influence treatment outcomes. As a result, strategies aimed at remodeling the TME have emerged as a critical complement to conventional and targeted therapies.

4.5.1. Modulating the TME through immunogenic vaccines

One of the primary approaches to overcoming TME-associated immunosuppression is the use of cancer vaccines in combination with ICIs.

Vaccines can also be combined with ICI to potentiate their efficacy. For instance, combining the alpha-fetoprotein vaccine with ICIs has been shown to elicit strong $CD8^+$ cytotoxic T-cell responses and hinder HCC progression in pre-clinical models.[113] Interestingly, engineered oncolytic viruses capable of inducing hyperacute rejection were administered in 20 patients with refractory cancer and reached a 90% response without any Grade 4 adverse event.[114] Neoantigen-based vaccines, personalized formulations derived from somatic mutations identified through whole-exome or RNA sequencing, have demonstrated the ability to provoke highly specific antitumor immunity when combined with ICI therapy.[115]

4.5.2. Epigenetic and microRNA-mediated reprogramming of the TME

MicroRNAs (miRNAs) also influence the immunological and angiogenic features of the TME. In HCC, certain miRNAs, such as miR-139-5p, directly target *WTAP* to suppress epithelial-mesenchymal transition, thereby reducing metastasis and altering the TME toward a less invasive phenotype.[116,117] In addition, miR-126, another critical regulator, is stabilized and processed to interact with METTL14 in a N^6-methyladenosine-dependent manner, ultimately, to exert anti-angiogenic effects and inhibit the metastatic potential of HCC.[118] As they modulate tumorigenesis, metastasis, or suppression, they can be valuable biomarkers predicting prognosis or treatments. Regarding treatments, inhibitors targeting genes modulating N^6-methyladenosine regulators such as *METTL3*, *FTO*, and *ALKBH5* have been developed.[119] Notably, STM2457, a potent methyltransferase-like 3 inhibitor, has been shown to reduce N^6-methyladenosine modification levels, disrupt leukemogenic gene expression programs, and recondition the immune TME in multiple malignancies, including acute myeloid leukemia, intrahepatic cholangiocarcinoma, prostate cancer, and Sonic Hedgehog medulloblastoma.[120-123] In HCC, STM2457 enhances the efficacy of lenvatinib by modulating both tumor-intrinsic signaling and the microenvironment,[124] representing a bridge between miRNA regulations, epigenetic remodeling, and TME reprogramming. However, research in this area remains in its early stages and is currently limited to pre-clinical settings. Other newer therapeutic approaches include gene therapy, proteolysis-targeting chimera, and the targeting of upstream regulators in cancer.[119] So far, several proteolysis-targeting chimeras have entered Phase I and Phase II trials, marking a promising new chapter in targeted cancer treatment.

4.5.3. Targeted delivery and localized modulation of the TME

Additional strategies to overcome physical and metabolic barriers in the TME include localized chemotherapy and nanotechnology. Hepatic arterial infusion chemotherapy, which allows high-dose drug delivery to the liver, reduces systemic toxicity and has shown efficacy in advanced infiltrative HCC when used alongside targeted agents.[125] Nanoparticles offer another promising platform to directly deliver small interfering RNA, clustered regularly interspaced short palindromic repeats-associated protein-9 nuclease-synthetic guide RNA, or immunostimulatory agents to tumor or immune cells. By silencing the oncogenes through the delivery system, the efficacy of anti-PD-1 therapy against CRC has markedly improved.[126,127] Nanoparticles can also help deliver substances to tumor cells or immune cells to induce tumor cell death or boost antitumor immunity, such as fat mass and obesity-associated protein inhibitors and tumor-associated antigens, into HCC.[128] Despite their strong potential, nanoparticles are still under consideration due to concerns regarding long-term safety and biodegradability.[129]

Interestingly, treatment targeting TME can significantly reduce the relapse rate. For pediatric patients with high-risk acute lymphoblastic leukemia, blinatumomab (bispecific antibodies) showed a significantly higher event-free survival than consolidation chemotherapy (31% versus 57%, $p<0.001$), with an HR of 0.33 (95% confidence interval: 0.18–0.61).[130] Given the significant role of TME in relapse and metastasis, therapies targeting the TME are expected to yield superior clinical outcomes.

5. Precision medicine and AI

Precision medicine is heavily reliant on big data sets, yet biomedical knowledge is often fragmented across manuscripts and non-standardized data repositories, making the effort of parsing through the existing literature a time-consuming and intensive task.[131] While the implementation of AI in biomedical research and clinical practice is not without its challenges, AI shows promise as a powerful investigative and diagnostic tool in cancer research. AI-based summary tools can be used by clinicians to stay updated on the most recent developments in cancer diagnosis and treatment. Specifically, this section will focus on three key areas where AI is being applied: cancer diagnosis, drug development, and clinical practice, and its various applications, as summarized in Figure 3.

5.1. AI applications in histopathology and radiology for cancer diagnosis

Histopathologic grading and radiologic imaging are often key elements of obtaining a cancer diagnosis. AI has shown promise in augmenting the detection and assessment of cancerous lesions found in these images. Conventionally, histological preparation and assessment of tumor samples can be labor-intensive and prone to observer variability.[132] Certain tumors, such as those derived from breast or prostate tissue, have diverse presentations and thus are more susceptible to observer variability than others.[133,134] While errors in pathological diagnosis are relatively low and do not pose clinically significant concerns at the population level, the movement toward precision medicine may make these faults increasingly decisive in the care of individual patients.[135,136] Recent advances in imaging and storage technology have allowed the routine digitization of conventional glass slides in pathology laboratories. Machine learning algorithms have been developed to

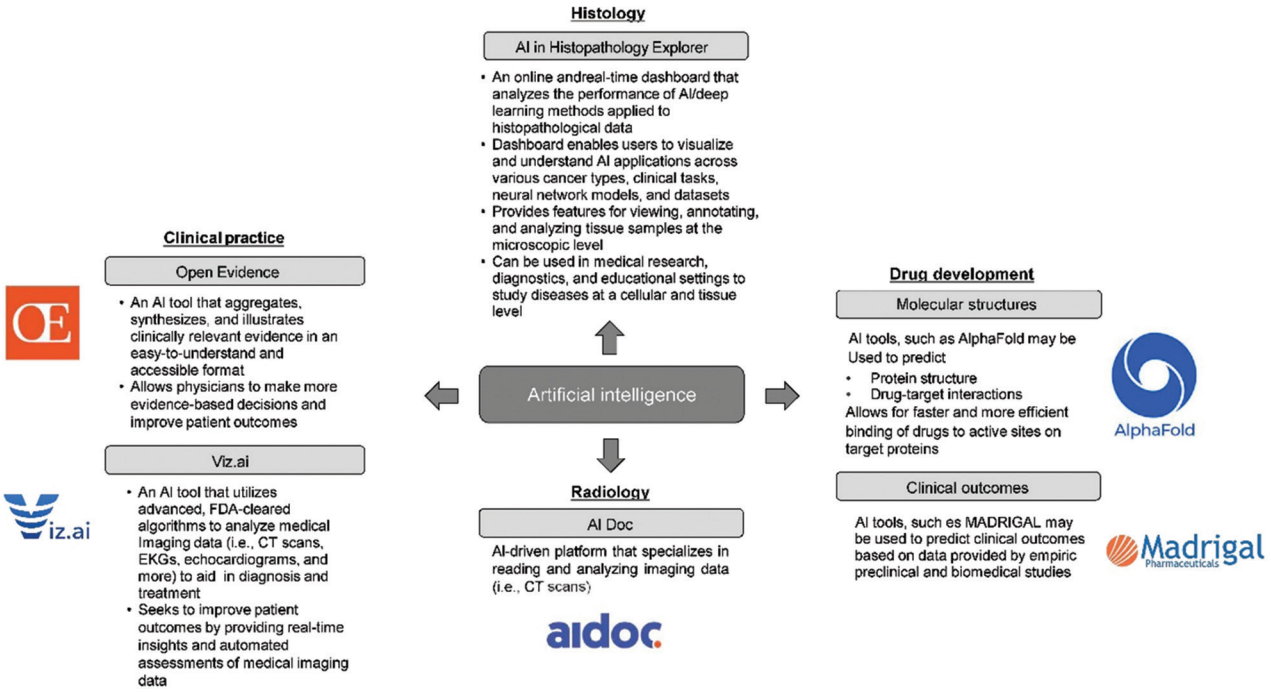

Figure 3. Current summary of diverse applications of artificial intelligence
Abbreviations: CT: Computed tomography; EKGs: Electrocardiograms; FDA: Food and Drug Administration.

automate the process of detecting and labeling tumor markers in cancers such as HCC and mesothelioma.[132] In addition, numerous studies have demonstrated the diagnostic power of deep learning in histopathology.[137-139] For example, Ko *et al.*'s[139] study revealed how AI can be used to increase efficiency and accuracy in diagnosing gastrointestinal cancers.[136] In the study, pathologists used an AI-based tool instead of human pathologists to double-check the screening of gastric and colorectal biopsy specimens. By incorporating AI into their quality control protocol, they were able to increase the number of slides reviewed in the same period by 7–10 times. The accuracy rates of the gastric and colorectal models were 93.08% and 95.03%, respectively. These findings align with broader trends in the field: a meta-analysis of 48 AI-assisted diagnostic pathology studies found a mean sensitivity of 96.3% (confidence interval: 94.1–97.7) and a mean specificity of 93.3% (confidence interval: 90.5–95.4) in disease detection across all studies.[140]

Similar principles driving AI innovations in pathology can be applied to developments in radiology. AI has demonstrated promise toward aiding the detection and diagnosis of cancerous lesions in radiological imaging. A 2025 study compared breast cancer detection rates between two groups of radiologists: those who used AI-supported double reading and a control group who used the conventional double reading method. Radiologists in the AI-supported group had a detection rate that was 17.6% (95% confidence interval: 5.7, 30.8) higher than those in the control group.[141] In the diagnosis of prostate cancer, AI can be used to identify clinically significant lesions to allow more targeted biopsy procedures.[142] This enables the clinician to focus on specific areas of the prostate, potentially reducing the risk of under- and overtreatment.

5.2. AI in drug development

AI-based tools can also be used to investigate the progression of carcinogenesis and predict the fitness of potential anticancer targets. For example, AlphaFold 2 uses AI to obtain a protein sequence, predict its backbone shape and side-chain conformations, and subsequently generate a model of the overall protein structure.[143] Increasing the accuracy of structure prediction can help researchers better understand factors, such as ligand binding and molecular function, that contribute to drug-target interactions. Other AI tools attempt to streamline the process of identifying the most promising treatments for specific cancers. PINNED is one such machine learning model that can be used to assess potential anticancer therapies and evaluate the druggability of potential target proteins by assigning scores based on the proteins' structure, sequence, localization, biological function, and network information.[144] In addition, Huang *et al.*'s[145] MADRIGAL is a multimodal AI

model that uses genomic profile and xenograft model data from pre-clinical and biomedical studies to predict clinical outcomes of drug combinations for cancer patients with comorbid conditions such as type II diabetes.[146] Although this particular tool is in its early stages of development, innovations such as PINNED, MADRIGAL, and AlphaFold are indicative of AI's potential in pharmaceutical research.

5.3. AI in clinical practice

Some studies have explored the idea of using AI to inform clinical decision-making. A recent study from China assessed the impact of an AI-based clinical decision support system on the treatment of breast cancer patients.[147] A group of physicians was asked to provide treatment recommendations for an average of 198 patients before and after viewing individualized AI-generated treatment plans. Researchers found that adherence to National Comprehensive Cancer Network guidelines increased slightly (0.5%; p=0.003) after the implementation of AI support for the treatment of patients with stages I–III breast cancer.[147]

Clinicians can also use AI-based knowledge graphing and summary tools to stay updated on the most recent developments in cancer treatment. Chandak *et al.*[148] presented an AI-based multimodal knowledge graph, PrimeKG, which synthesized data from 20 primary databases to map relationships between the proteins, genes, phenotypes, and risk factors associated with over 17,000 diseases, including cancers.[148] PrimeKG's integrative network also describes indications, contraindications, and off-label uses of drugs used to treat these diseases.[148]

Several large language model AI tools capable of generating human-like text are in development for clinical applications. However, this particular area of AI research is still in its infancy and requires further investigation before large language model tools can be integrated into clinical workflows.[145]

6. Conclusion

This review discussed the potential of precision medicine in the field of oncology. The review has explored first-line targeted therapies and immunotherapies that have been well-established in the current standard care. Furthermore, the review discussed current targeted therapies in HCC, CRC, and PDAC and various trials of different targeted therapies and immunotherapies to achieve a more efficacious regimen. Finally, this review has examined the emerging avenues in the field of precision medicine in cancer diagnosis, drug development, and clinical practice. We chose to discuss the possibility of including AI in our review, as we have seen the immense potential for AI as a tool to be more patient-specific with improved clinical outcomes. We also believe that AI will eventually be incorporated into medical practices. However, we would like to acknowledge significant challenges, such as logistical challenges to digital pathology, data quality concerns, risk of bias, ethical implications, and potential compromise to patient trust. The current advancement of AI in medicine accompanies a worrying lack of legislative framework, making it susceptible to breaches of patient rights. Therefore, rash adoption of powerful technology is like building a new story on a foundation of quicksand, which will lead to an ultimate collapse. We are optimistic that with careful planning and thoughtful implementation, AI can be incorporated in a way that truly benefits patients while minimizing unintentional harm.

Acknowledgments

The authors acknowledge the Biorender.com website used to create Figures 1 and 2.

Funding

None.

Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

ORIGINAL RESEARCH ARTICLE

Knowledge, perception, and practices of ecopharmacovigilance among government and private medical students in Bangladesh

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Citation: Sonia SBA, Chowdhury IH, Nahar K, *et al*. Knowledge, perception, and practices of ecopharmacovigilance among government and private medical students in Bangladesh. *INNOSC Theranostics and Pharmacological Sciences*. 2025;8(3):59-70. doi: 10.36922/itps.7678

Received: December 16, 2024

1st revised: February 24, 2025

2nd revised: March 25, 2025

Accepted: April 1, 2025

Published online: April 17, 2025

Abstract

Given the rapid growth of the pharmaceutical industry and the increasing use of medication, ecopharmacovigilance (EPV) has become an effective approach for managing and reducing the environmental impact of pharmaceuticals. EPV addresses and explains the undesired environmental effects of pharmaceutical use. This study aimed to evaluate the knowledge, perceptions, and practices related to the disposal of unused and expired medications among undergraduate medical students in Bangladesh. A questionnaire-based cross-sectional study was conducted over a period of 3 months, from August to October 2024, at Armed Forces Medical College, Mugda Medical College and Hospital, and Shahabuddin Medical College in Dhaka. A total of 300 3rd- and 4th-year medical students from these medical colleges in Bangladesh completed a self-administered questionnaire. In this study, knowledge of EPV was found to be only 27%. Overall, medical students' perceptions of environmental medication contamination and EPV were encouraging. Among those interviewed, 54.3% "strongly agreed" that leftover medications could have a negative impact on the environment. Despite knowledge of the environmental risks posed by pharmaceuticals, the common practice of storing medications at home until they expire and then discarding them persists. This highlights both a lack of knowledge and the absence of safe disposal procedures. Thus, greater efforts are needed to improve medical students' knowledge of EPV. Respondents also expressed a preference for evidence-based and environmentally friendly methods for disposing of unwanted medications.

Keywords: Ecopharmacovigilance; Medical students; Knowledge; Perception; Environment; Pharmaceuticals

1. Introduction

The modern world is experiencing an unprecedented increase in drug consumption and production due to population growth, increased disease burden, and the rapid advancement of the pharmaceutical industry. As a result, pharmaceuticals have increasingly entered the environment.[1,2] A wide range of medications, including antibiotics, steroid hormones, analgesics, anti-inflammatory drugs, anti-hypertensives, and anti-depressants, have been detected in various environmental compartments, such as soil, sediments, groundwater, surface water, drinking water, and living organisms.[3-6] Pharmaceuticals are commonly referred to as pseudo-persistent pollutants due to their widespread use, continuous release into the environment, and the ineffective clearance of pharmaceutical residues.[7]

These pollutants enter the environment through multiple pathways, including pharmaceutical manufacturing, improper disposal by consumers, hospital waste, pharmacy practices, veterinary use, and other sources.[8-11] Improper disposal of pharmaceuticals leads to the release of harmful chemicals and various pollutants, which can pose risks to human health.[12-14] At present, over 600 pharmaceuticals have been detected in the environment worldwide.[15] Given the widespread use of over-the-counter medications, it is essential to raise public awareness regarding proper disposal procedures.[16] Although pharmaceutical residues are often found in small concentrations, their persistent release into the environment can have a negative impact on the ecosystem, wildlife, and human health.[3,8,11,17] For example, veterinary use of diclofenac has significantly endangered Asian Gyps vulture species,[8] while antibiotic residues contribute to the emergence of antibiotic resistance, which is a serious public health concern.[18-21] In addition, studies have shown that exposure to trace amounts of 17α-ethynylestradiol, an estrogen commonly found in birth control pills, at concentration of 5 – 6 ng/L in lake water can cause feminization and near extinction certain fish species.[22,23] In addition, exposure to animal antimicrobial residues in contaminated food or drinks has been linked to a higher risk of overweight and obesity among school children.[18]

Despite being a major category of emerging pollutants, pharmaceuticals are not regulated under existing laws for detection, notification, or environmental control.[24] Furthermore, the removal of pharmaceuticals using current sewage treatment systems is ineffective.[3,8,15] The environmental effects of drugs have received insufficient attention over time, with few studies examining their impact. Meanwhile, the use of pharmaceuticals in both humans and animals continues to rise. According to one study, by 2030, more than 100,000 tons of veterinary antibiotics will be consumed worldwide.[25] Pharmaceuticals were found in water bodies in previous investigations, most of which came from high-income nations. However, one of the largest studies assessing the prevalence of drugs in 1,052 rivers across104 countries revealed that the most contaminated rivers were located in low- and middle-income nations in South America, South Asia, and sub-Saharan Africa.[26] Despite the growing importance of safe pharmaceutical disposal, both consumers and healthcare professionals remain largely unaware of this issue. In response, ecopharmacovigilance (EPV) has been introduced. EPV is defined as "the science and activities associated with the detection, evaluation, understanding, and prevention of adverse effects of pharmaceuticals in the environment." It aims to address important environmental problems associated with drugs and ensure that they are effectively managed.[11,27,28]

The main goal of EPV is to regulate the causes of pharmaceutical exposure.[8,29] EPV monitors, assesses, and regulates pharmaceutical pollutants, taking into account elements including degradation, wastewater, and the effectiveness of drinking water treatment plants.[30] EPV encourages the reduction of production leftovers, rational utilization, reliability, and the safe disposal of unwanted medications.[31] EPV strategies should focus on drugs known to have negative environmental consequences, with emphasis to areas with elevated risks and significant pollution issues.[32] To minimize the amount of medicinal products in the ecosystem, some EPV strategies have been proposed, including the development and production of green medications, carefully management of pollutants from drug production, the return and disposal of unused medications, and the proper use of medicines. However, EPV has not yet established a formalized implementation model.[3,8,11] Practical application of EPV is already underway. For instance, before a medication can be marketed in the European Union (EU), an environmental risk assessment (ERA) must be completed.[11] According to regulations set by the European Medicines Agency (EMA), every medication intended for human use undergo an ERA before being marketed.[33] In terms of EPV legislations, the United States (US), EU, and Canada lead the world.[17] Pharmaceutical companies like Astra Zeneca have created environmental risk management plans to simplify the monitoring of environmental impacts.[34] According to the Drug Enforcement Administration's 2014 Finalized Rule on the Disposal of Controlled Pharmaceuticals,[35] patients can choose to mail leftover drugs to a registered collector with proper labeling or return them during take-back program. To effectively address the issue of pharmaceutical waste, EPV must be integrated with a medication take-back system, together with additional financial and logistical

involvement from drug manufacturers.[36] The findings of numerous research on how the public and healthcare professionals view EPV have highlighted the need for a clear educational plan on the topic.[28]

According to a Chinese survey, the public expects healthcare professionals, preferably doctors and pharmacists, to educate them about pharmacovigilance.[37] Efforts to include EPV into the education of future medical practitioners are already underway. For example, pharmacy students at the University of Groningen participated in a course titled "To Decrease Pharmaceuticals in Groundwater."[38] Furthermore, a postgraduate degree on pharmaceutical contamination is offered in Spain, and four EU universities have launched a 2-year program named "Sustainable Drug Discovery."[39,40] During manufacturing, use, and disposal, active pharmaceutical ingredients leak into the environment, contributing to an estimated 58,000 neonatal deaths each year from drug-resistant diseases.[41] In the US, approximately 30 billion doses of non-steroidal anti-inflammatory drugs are prescribed annually.[42] Furthermore, leftover pharmaceuticals can lead to poisoning, both accidental and intentional and may encourage self-medication.[43] Irrational prescribing can contribute to pharmaceutical waste; thus, future prescribers should be aware of proper disposal methods and the potential consequences of improper pharmaceutical disposal.[11] According to a study in Nepal, even pharmacists lack sufficient knowledge and awareness of safe pharmaceutical disposal, highlighting the necessity of a safe pharmaceutical take-back program.[44] The pharmaceutical take-back program is a safe and environmentally responsible way to dispose of leftover and expired medications. The drugs are returned to pharmacies or law enforcement agencies and destroyed without causing a threat to the environment.[45] In nations such as Australia and Canada, medicine take-back programs are well-established and authorized by the government. Likewise, nations such as Sweden and the US have effective pharmaceutical waste management system in place.[46] There are several ways to dispose pharmaceutical waste, including chemical, physical, biological, and recycling techniques. According to the World Health Organization (WHO) guidelines on pharmaceutical waste management, healthcare waste should not be burned outdoors, as it may pose environmental hazards.[47] Various modern end-of-pipe technologies, such as photo-assisted processes, biodegradation, ozonation, and extended filtration, have been developed by environmental specialists to improve the removal of pharmaceutical pollutants from environmental matrices. However, factors such as practical stability, cost-effectiveness, and ease of use restrict their implementation in traditional sewage treatment plants.[48,49]

Research conducted in Bangladesh, Nepal, and other regions of the world highlights a lack of knowledge among medical, dental, nursing, and pharmacy students regarding the proper storage and disposal of pharmaceuticals. Approximately 58% of students reported disposing of liquid pharmaceuticals in sinks or toilets, which finally enter wastewater systems. Pharmacies, where students obtain their medications, would be ideal locations for education on this matter. The curricula for medical, dental, nursing, and pharmacy graduates should address safe and appropriate pharmaceutical disposal. In addition, legislative authorities should take these matters into consideration.[50,51] Pharmaceutical consumption in Bangladesh is increasing daily.[52] Bangladesh's annual pharmaceutical consumption is likely among the highest in the world due to its dense population, numerous widely recognized pharmaceutical organizations, and affordable access to almost all sorts of medications. However, many prescribed drugs often go unused for various reasons, including brand name replacements in prescriptions, adverse drug reactions, and patient recovery leading to treatment discontinuation.[53] However, there are still very few regulations governing the regular monitoring, management, and control of pharmaceuticals in the environment as emerging pollutants, which would lead to the continuous discharge of pharmaceuticals into the environment.[17,54] At the 2015 International Conference on Chemicals Management, non-governmental organizations and the pharmaceutical industry recognized the urgent need to protect the environment from "pharmaceutical pollution" due to the widespread presence of pharmaceutical residues and their possible environmental risks.[55,56] The purpose of this research is to evaluate the current understanding of this crucial issue among Bangladeshi medical students. As future healthcare professionals, these students play an important role in both applying EPV principles in their professional practice and educating the public on the subject. Healthcare professionals, particularly prescribers and dispensers, are responsible for promoting rational prescription policies that assure the safe and efficient use of medications, with a focus on the prescriber's role. Given the rapid advancements in the healthcare industry, it is equally necessary for emerging healthcare professionals to mentor and guide their seniors. Since EPV affects both environmental sustainability and public health, its significance cannot be underestimated.

2. Materials and methods

2.1. Study design

This was a questionnaire-based and cross-sectional study.

2.1.1 Ethical approval

The study protocol and final questionnaire were approved by the Ethics Committee of Armed Forces Medical College, Dhaka. Informed consent was obtained from all willing students before they participated in the study. The study adhered to the principles of the Helsinki Declaration on human subjects in medical research.

2.1.2. Study site

The study was conducted at Armed Forces Medical College, Mugda Medical College and Hospital, and Shahabuddin Medical College, Dhaka.

2.1.3. Study duration

The study was conducted over a period of 3 months, from August to October 2024.

2.1.4. Study population

All 3rd-and 4th-year undergraduate medical students were invited to participate in the study. Informed consent was obtained from all interested students before the study began.

2.1.5. Sample size

Approximately400 medical students were initially included in the sampling frame, with 200 students from the 3rd-year and 200 from the 4th-year from each medical college. From this list, 300 medical students were randomly selected to participate in the study, with 160 students from the 3rd year and 140 from the 4th year. Respondents were provided with hard copies of the questionnaire. The first page included an explanatory letter describing the survey's objectives. They were instructed to complete the questionnaire and return it to the researchers within 1 h. Respondents who refused to participate in the study or failed to return the completed questionnaire within the given time frame were excluded.

2.1.6. Sampling method

Enrolment was based on voluntary participation from those willing to participate.

2.2. Data collection

2.2.1. Development of the structured questionnaire

A structured, self-administered questionnaire was developed to address the research question, focusing on several aspects of medical personnel's knowledge, perceptions, and practices regarding the storage of unused pharmaceuticals and the improper disposal of expired and unused medications, which can ultimately affects the ecosystem. To finalize the content, a thorough literature review of published studies on medicine storage and disposal methods, as well as information on EPV, was conducted, along with consultations with subject matter experts.[57-59] The questionnaire was developed in English.

2.2.2. Questionnaire contents

The final questionnaire consisted of several sections and 25 structured questions. The first section included four questions about the respondents' socio-demographic information, such as age, gender, year of study, and whether they had any first-degree relatives in the medical field. Furthermore, four more questions were designed to assess the respondents' understanding of EPV and environmental contamination from drugs, including safe drug disposal procedures, their awareness of EPV, the impact of veterinary diclofenac residues on several Asian Gyps (bird) populations, and the effects of oral contraceptive pill residues on frog sterility and the feminization of male fish in aquatic environment.

The second section of the study consisted of nine questions designed to evaluate medical students' perceptions of EPV. These questions were framed using a 5-pointLikert scale (1 = strongly agree, 2 = agree, 3 = neutral, 4 = disagree, and 5 = strongly disagree). This section aimed to assess the students' opinions, attitudes, and beliefs on responsible pharmaceutical management and its ecological implications. Furthermore, the final section of the questionnaire consists of eight questions that evaluate participant's daily behavior and practices related to the management, disposal, and storage of medications. These questions were developed based on a thorough literature review and discussions among the authors.

2.2.3. Validity and reliability of questionnaire

The questionnaire's content was reviewed and verified by two selected experts in the relevant field, both of whom were medical college academicians.[60] Their comments were carefully considered, and necessary revisions were made accordingly. The questionnaire was then forwarded through private email to 30 volunteer faculty members for their comments and answers. Based on their feedback, the questionnaire was further revised to improve clarity and ensure a better understanding of the questions.

2.4. Data analysis

Data analysis was performed using descriptive statistics. Continuous data were expressed as mean ± standard deviation, while nominal data were expressed as percentages. Statistical analysis was carried out using the Statistical Package for the Social Sciences version 26.0 for Windows.

3. Results

The socio-demographic characteristics of the students are shown in Table 1. The mean age of the participants was

22 ± 0.96 years. Among the 300 respondents, females comprised a higher proportion (61.3%) compared to males (38.7%). In addition, one-third of the respondents (33%) had a first-degree relative in the healthcare field. Of the total participants, 160 (53.3%) were 3rd-year students, while 140 (46.7%) were 4th-year students.

Table 1. Socio-demographic characteristics and knowledge of EPV among study subjects (*n*=300)

Participant attribute	Responses	Number (%)
Age (mean±SD)	-	22±0.96
Gender	-	
Male		116 (38.7)
Female		184 (61.3)
Year of study	-	
Third year		160 (53.3)
Fourth year		140 (46.7)
Having any 1st degree relatives in the healthcare field	Yes	99 (33)
	No	201 (67)
Do you have any knowledge about safe drug disposal?	Yes	96 (32)
	No	204 (68)
Do you have any idea about EPV?	Yes	81 (27)
	No	219 (73)
Numerous Asian Gyps (bird) species were eradicated due to environmental animal diclofenac residue	Yes	204 (68)
	No	96 (32)
OCP residues in the water environment cause sterility in frogs and feminization of male fish	Yes	180 (60)
	No	120 (40)

Abbreviations: EPV: Ecopharmacovigilance; SD: Standard deviation; OCP: Oral contraceptive pill.

Table 2 shows that nearly 63.7% of respondents "strongly agreed" and 35.3% "agreed" that the creation and implementation of green manufacturing processes for pharmaceuticals are essential. In addition, 60.7% of respondents "strongly agreed" and 35.3% "agreed" that the potential environmental impacts of a new medicine should be evaluated before it receives authorization. Similarly, 54.3% of respondents "strongly agreed" and 44.3% "agreed" that pharmaceutical residues in the environment could have cause adverse effects on the ecosystem, wildlife species, even human health. Likewise, nearly 45.3% of respondents "strongly agreed" and 53.3% "agreed" that they considered it their professional duty to implement EPV.

Table 3 shows that 73.7% of respondents often checked the expiry date of their medications and 73.3% reported taking their medications according to the advice of a doctor or pharmacist.

Table 4 describes that 20% of private medical students were knowledgeable about safe drug disposal, whereas only 12% of government medical students had this knowledge. The difference was statistically significant ($p \leq 0.05$). The knowledge and perception scores were statistically significant ($p \leq 0.05$) for both government and private medical students, except for the topic of increased microbial resistance to antibiotics due to environmental

Table 2. Perception of EPV among medical students (n=300)

Survey questions	Responses, number (%)				
	Strongly agree	Agree	Disagree	Strongly disagree	Neutral
1. Drugs may be discharged into drainage systems and persist in the environment.	98 (31.3)	128 (42.7)	21 (7)	54 (18)	4 (1.3)
2. Drug compounds in the environment may have harmful consequences on ecosystems, wildlife species, and human health.	163 (54.3)	133 (44.3)	2 (0.7)	2 (0.7)	0
3. EPV is required for managing drug residues and associated adverse effects as part of pharmacovigilance to protect the environment.	129 (43)	167 (55.7)	3 (1)	1 (0.3)	0
4. I have an intense desire in EPV as a future physician.	112 (37.3)	165 (55)	20 (6.7)	3 (1)	0
5. Individuals should be educated on the potential environmental harm caused by pharmaceutical residues, as well as the proper consumption, disposal, and handling of medications.	189 (63)	100 (33.3)	7 (2.3)	1 (0.3)	3 (1)
6. It is essential to plan and implement environmentally friendly techniques pharmaceutical manufacturing processes.	191 (63.7)	106 (35.3)	2 (0.7)	1 (0.3)	0
7. It is necessary to assess the potential environmental risks before approval of a new drug.	182 (60.7)	110 (36.6)	4 (1.3)	1 (0.3)	3 (1)
8. I consider it my professional duty to implement EPV.	136 (45.3)	160 (53.3)	2 (0.7)	2 (0.7)	0
9. If I received proper training, I would be blessed to participate in EPV practices in the coming years.	139 (46.3)	140 (46.6)	9 (3)	3 (1)	9 (3)

Abbreviation: EPV: Ecopharmacovigilance.

Table 3. Practices related to EPV among medical students (*n*=300)

Survey questions	Responses, number (%)
1. How often do you check the expiry date of your medications?	
Often	221 (73.7)
Sometimes	57 (19)
Rarely	20 (6.7)
Never	2 (0.7)
2. How often do you take your medications according to the advice of your doctor or pharmacist?	
Often	220 (73.3)
Sometimes	66 (22)
Rarely	13 (4.3)
Never	1 (0.3)
3. When multiple medications are prescribed to you or your family members, do you use only the prescribed medications?	
Often	157 (52.3)
Sometimes	119 (39.7)
Rarely	18 (6)
Never	6 (2)
4. How often do you practice self-medication for minor illness, such as fever or headaches?	
Often	145 (48.3)
Sometimes	111 (37)
Rarely	13 (10)
Never	14 (4.7)
5. How often do you buy OTC medications that require a valid prescription?	
Often	89 (29.7)
Sometimes	132 (44)
Rarely	68 (22.7)
Never	11 (3.7)
6. What factors limit the use of medications prescribed to your family members?	
Forgetfulness	129 (43)
Medication ineffectiveness	15 (5)
Medications adverse effects	32 (10.7)
Improvement of health condition	124 (41.3)
7. What do you do with any unused medications remaining at your home or hostel?	
Throw away in household garbage	59 (19.7)
Keep at home until it gets expired	218 (72.7)
Burn them along with garbage	1 (0.3)
Return it to pharmacy shop	22 (7.3)
8. Have you ever educated your friends or family members about the safe and proper disposal of medicines?	
Yes	68 (22.7)
No	232 (70.3)

Abbreviations: EPV: Ecopharmacovigilance; OTC: Over-the-counter.

antimicrobial residues. The finding for this aspect was not statistically significant (p=0.674).

4. Discussion

The WHO defines pharmacovigilance as "the science and activities relating to the detection, assessment, understanding, and prevention of adverse effects or any other possible drug-related problems." Due to its importance in reducing the harmful effects associated with pharmaceutical residues, EPV has drawn significant interest from the research community as a significant application of pharmacovigilance in environmental science.[3,8,11] Globally, EPV should be promoted, particularly in developing nations with dense populations and often insufficient sewage treatment systems. The successful adoption of EPV by medical students and their subsequent behavioral changes depends on their comprehensive understanding, knowledge, and positive perception of the issue, which could improve the efficacy of EPV implementation.

To the best of our knowledge, this is the first study in Bangladesh to assess medical students' understanding, perceptions, and practices related to EPV and environmental pharmaceutical contamination. Only 27% of the participants were familiar with EPV, highlighting the urgent need for comprehensive education on the subject for medical students. A separate study conducted in Bangladesh revealed that pharmacy students possessed limited knowledge regarding proper medication disposal methods. Approximately 58% of students disposed of their liquid medications in sinks or toilets, where they ultimately reached the wastewater treatment system.[51] The respondents' knowledge of the ecological hazards posed by drug residues and the increased antibiotic resistance in bacterial communities, potentially caused by antimicrobial residues in the environment. This includes the destruction of many Asian Gyps vultures due to veterinary diclofenac residues, the feminization of male fish, and the sterility of frogs, which may result from hormonal compounds in water. The accuracy rates for these issues were77.7%, 68%, and 60%, respectively. These potential environmental hazards associated with antibacterials and hormonal compounds align with the usual impacts of pharmaceuticals, such as antibiotic-induced bacterial resistance and the endocrine-disrupting effects of hormones. Gyps vulture populations in the Indian subcontinent were largely destroyed due to diclofenac. Approximately 97.3% of participants expressed a desire to learn more about take-back programs, safe disposal techniques, potential environmental pharmaceutical residues, and the handling of unwanted medications, indicating an active interest in environmental protection. The study's most promising result is that 99% of students expressed interest in using eco-friendly methods for disposing of unwanted medications in

Table 4. Comparison of knowledge about EPV between government and private medical students (*n*=300)

Survey questions/Statement	Institution				*p*-value
	Government		Private		
	Yes *n* (%)	No *n* (%)	Yes *n* (%)	No *n* (%)	
Do you have any knowledge about safe drug disposal?	36 (12)	114 (38)	60 (20)	90 (30)	0.007[a]
Have you ever educated your friends or family members about safe medicine disposal?	25 (8.3)	125 (42)	43 (14.3)	107 (36)	0.018[a]
Do you have any knowledge of EPV?	33 (11)	117 (39)	48 (16)	102 (34)	0.000a
Numerous Asian Gyps (bird) species have been eliminated due to environmental residues of veterinary diclofenac.	89 (30)	61 (20)	115 (38)	35 (12)	0.002[a]
Environmental antimicrobial residues may contribute to increased antibiotic resistance in microbial populations.	115 (38)	35 (12)	118 (39)	32 (11)	0.674[a]
OCP residues in the water environment can cause sterility in frogs and feminization of male fish.	70 (23)	80 (26.6)	110 (37)	40 (13.3)	0.000[a]

Abbreviations: EPV: Ecopharmacovigilance; OCP: Oral contraceptive pill.

the future. This high degree of willingness suggests a strong potential for behavior change and collaboration toward environmental sustainability. These findings highlight the importance of providing education and resources to encourage proper medication disposal practices. Although 98.6% of respondents agreed that EPV implementation is necessary, 92.3% indicated interest in engaging with the practice. More significantly, 96% of respondents expressed willingness to use EPV in their future work, which provided that they received appropriate training.

Despite a lack of thorough knowledge of EPV, these findings indicate that most medical students show a positive attitude toward its implementation. However, the study also revealed poor practices, which is consistent with the lack of knowledge regarding EPV. For instance, 73.7% of respondents reported that they "often" checked the expiry dates of medications. In comparison, a study in Chennai, India, found that about 6% of pharmacy and medical students failed to check the expiry dates. In addition, 60% of respondents in that study agreed that medications become harmful after their expiry dates.[61] In this study, about 7.3% of participants returned their unwanted medications to pharmacies. Similarly, a study among dental students in India indicated that 61% believed expired that medications should be returned to the manufacturer.[62] In this study, 72.7% of respondents stored their medications at homes or in their hostels until they expired. A study conducted in Nepal found that 44.7% of medical and dental students also stored medications until they expired.[63] Furthermore, 19.7% of students disposed of unused medications in the trash. A survey in Bangladesh revealed that 47% of both urban and rural residents disposed of unused medications in the same way, and similar practices have been observed globally.[64-66] The reasons for having leftover medication include forgetfulness (43%), medication ineffectiveness (5%), adverse drug effects (10.7%), and perceived improvement in health (41.3%). Similar patterns of keeping extra drugs at home have been observed in other studies.[67] In this study, 68% of respondents were unaware of proper drug disposal methods. A study conducted in the Dhaka Metropolitan area revealed that over 67% of respondents were unaware about the correct way to dispose of drugs.[68] Another study in Nepal found that unwanted and expired medications were not disposed of properly, prompting the authors to recommend the establishment of a government-funded drug take-back program.[69] Although drug storage was common, self-medication was also prevalent. The storage of unused medications at home may contribute to this behavior. In this study, 48.3% of respondents reported self-medicating for common medical conditions, including headaches and fevers. Previous research in Nepal has documented the prevalence of self-medication among both students and public.[61,70-73] Many students consider self-medication acceptable, as they believe they are knowledgeable about medications and medical conditions. When comparing the respondents' knowledge of EPV with that of medical students, those from private institutions scored higher, likely indicating their greater interest and enthusiasm to learn about proper medication disposal. The curricula of these students do not include instruction on proper medication disposal. A significant 97.3% of respondents supported the implementation of a drug take-back program that allows patients to return medications to pharmacies, which would then return them to distributors. In addition, they advocate for education on the potential environmental hazards of pharmaceutical residues and the importance of rational drug use. It is possible to enhance knowledge of EPV during undergraduate studies through educational campaigns, seminars, or workshops targeted

at both students and public, promoting safe medication disposal procedures. The campaigns must emphasize the negative effects of improper medication disposal on the environment, in addition to raising public awareness about safe disposal practices.

This study establishes the basis for further research on EPV. However, the results may not be as globally applicable, as this study involved a small sample size and was limited to one region in Bangladesh. To confirm these findings, future studies with larger sample sizes and participants from other regions of Bangladesh should be carried out.

5. Conclusion

This first study on EPV among medical students in Bangladesh found that while the students had a positive perception of EPV, they lacked sufficient knowledge and experience with EPV. Although medical students were willing to engage in EPV, understanding that pharmaceutical residues in the environment could harm the ecosystem, wildlife, and human health, they still required more relevant information. Their understanding of the concept, goal, significance, and application of EPV was limited. Furthermore, the main barrier to the successful implementation of EPV in Bangladesh was identified as a lack of awareness. To improve medical students' knowledge of EPV, further works are needed.

Acknowledgments

The authors thank the student participants from Armed Forces Medical College, Mugda Medical College and Hospital, and Shahabuddin Medical College, Dhaka, for their active participation in the study.

Funding

None.

Conflict of interest

The authors declare no conflicts of interest.

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Ethics approval and consent to participate

The study protocol and the final questionnaire received approval from the Ethics Committee of Armed Forces Medical College, Dhaka. Informed consent was obtained from all participants before their involvement in the study. The research adhered to the principles outlined in the Helsinki Declaration regarding the ethical treatment of human subjects in medical research.

Consent for publication

Participants consented on the publication of their data.

Availability of data

Data supporting the study's conclusions are not publicly accessible to preserve participants' privacy but are available from the corresponding author on reasonable request.

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ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

ORIGINAL RESEARCH ARTICLE

Antibacterial activity of green-synthesized silver nanoparticles against Gram-negative bacteria and insights into potential resistance mechanisms

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Abstract

Gram-negative bacterial infections pose a serious public health challenge due to their high global mortality rates and potential to cause severe complications. Antibiotics – one of the most impactful medical innovations of the 20th century – remain vital in treating life-threatening bacterial infections. However, the increasing prevalence of antibiotic resistance has made it progressively harder to treat Gram-negative bacterial infections effectively. Therefore, nanoparticles have gained attention as a promising alternative treatment owing to their targeted antibacterial properties. Among the various synthesis methods, green synthesis is considered one of the most effective approaches for nanoparticle production. In this study, silver nanoparticles were synthesized using a green approach that utilized silver nitrate salt and an extract derived from carpenter bee wings (CBWs). The synthesized nanoparticles were characterized using spectroscopic techniques and scanning electron microscopy. Their antibacterial activity was tested against two pathogenic Gram-negative bacteria using the broth dilution method. Furthermore, whole genome sequencing was conducted to assess the mutagenic effects of the biosynthesized silver nanoparticles on the two bacterial strains. The results demonstrated that the green-synthesized silver nanoparticles exhibit notable antibacterial activity, likely through electrostatic interactions that promote cell binding and induce significant morphological alterations. Genomic analysis revealed mutations associated with efflux pump regulation, neutralization, transport, energy metabolism, cell division, biosynthetic pathways, adaptation, and invasion in the tested strains. These findings demonstrate the potential of CBWs as a novel biological resource for the green synthesis of silver nanoparticles with antibacterial properties. However, the study also raises concerns regarding the potential for bacteria to develop resistance to nanoparticles over time.

Keywords: Carpenter bee wing extracts; Genomics; Gram-negative bacteria; Green synthesis; Nanoparticles

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Citation: Ewunkem AJ, Daodu B, Williams ZJ, *et al*. Antibacterial activity of green-synthesized silver nanoparticles against Gram-negative bacteria and insights into potential resistance mechanisms. *INNOSC Theranostics and Pharmacological Sciences*. 2025;8(3):71-84.
doi: 10.36922/ITPS025080007

Received: February 17, 2025

Revised: June 3, 2025

Accepted: June 11, 2025

Published online: July 1, 2025

1. Introduction

Gram-negative bacterial infections are among the leading causes of both hospital-acquired and community-acquired infections, commonly seen in diseases such as septicemia, tuberculosis, and pneumonia. Among the most prevalent Gram-negative pathogens are *Klebsiella pneumoniae* and *Escherichia coli*.[1] These bacteria are responsible for a wide range of infections affecting various body systems, including the gastrointestinal tract, renal system, and central nervous system.[2-5] Effective treatment of *K. pneumoniae* and *E. coli* infections is crucial, as delayed or inadequate treatment can result in severe complications and potentially fatal outcomes. Therefore, timely medical intervention is critical to manage infections caused by *K. pneumoniae* and *E. coli*.

Antibiotics represent one of the most impactful medical innovations in modern history. Antibiotics – such as chloramphenicol, glycylcyclines, fluoroquinolones, cephalosporins, and aminoglycosides – play a crucial role in the treatment of *K. pneumoniae* and *E. coli* infections, saving millions of lives worldwide.[6-8] These drugs target specific processes or structures within bacterial cells, thereby disrupting essential cellular functions. Depending on their mechanism of action, antibiotics can exhibit bacteriostatic effects (inhibiting bacterial growth) or bactericidal effects (killing bacteria).[9,10] However, the widespread and reckless use of antibiotics has significantly contributed to the emergence of antimicrobial resistance.[11]

Gram-negative bacteria – including *E. coli* and *K. pneumonia* – have developed multiple resistance mechanisms against antibiotics.[12-14] One major defense is their outer membrane, which acts as a barrier to hinder antibiotic penetration.[13] In addition, the production of β-lactamases confers resistance to a wide range of antibiotics, such as penicillin, cephalosporins, and carbapenems.[15] Resistance is further enhanced by mutations or deletions in porin proteins such as OmpK35 and OmpK36, which reduce the uptake of antimicrobial agents.[16] Bacterial resistance presents a major public health threat, leading to severe infections and is projected to contribute to approximately 10 million deaths annually by 2050.[17] This highlights the urgent need to explore innovative alternatives to traditional antibiotic treatments.

Nanoparticles emerge as a promising alternative to antibiotics for treating bacterial infections, largely due to their ability to overcome microbial drug resistance.[18] They exhibit antimicrobial activity by directly interacting with and disrupting bacterial cell membranes through mechanisms such as physical penetration and generating reactive oxygen species, ultimately leading to cell damage and death.[19]

Their small size enables close interaction with bacterial membranes, causing structural damage and leakage of cellular contents, ultimately leading to cell death.[20] Furthermore, metal-based nanoparticles can penetrate bacterial cells and interact with intracellular components – including proteins, nucleic acids, and lipids – disrupting essential cellular processes. These interactions may also induce mutations and contribute to cell death due to their high surface area.[21]

The antimicrobial activity of silver nanoparticles has been extensively explored against a wide range of pathogenic bacteria, including *E. coli* and *K. pneumonia*.[22-26] Previous research has also examined the antibacterial effects of silver nanoparticles synthesized using the extract from the wings of carpenter bees (*Xylocopa virginica*) – hereafter referred to as carpenter bee wings (CBWs) – against selected Gram-negative and Gram-positive bacteria.[24] These biosynthesized silver nanoparticles have been shown to exert antibacterial effects by aggregating on bacterial cell surfaces, potentially interacting with cellular components in ways that lead to mutations. These findings raise important concerns about the long-term risk of bacterial adaptation or resistance in response to nanoparticle-based antimicrobial strategies.

However, the specific mutations associated with biologically synthesized silver nanoparticles in Gram-negative bacteria remain largely unexplored. This study hypothesizes that *X. virginica* wing extract can be used to synthesize silver nanoparticles with improved antimicrobial activity and the potential to induce genetic changes in target bacteria.

The findings from this study may provide valuable insights into the mechanisms by which biosynthesized nanoparticles exert antimicrobial effects and how they may contribute to the development of bacterial resistance. In particular, identifying mutations in genes related to cell wall integrity, DNA repair, and stress response pathways could help elucidate bacterial adaptation strategies. Understanding these mutations can guide the development of optimized nanoparticles with reduced potential to induce resistance, thereby enhancing their effectiveness in healthcare and environmental applications.

This study aims to evaluate the *in vitro* antimicrobial activity of green-synthesized silver nanoparticles produced using *X. virginica* wing extract against two pathogenic Gram-negative bacterial strains, namely *E. coli* and *K. pneumoniae*. The morphology and size distribution of the synthesized nanoparticles are characterized using spectroscopic analyses. In addition to assessing their antibacterial activity, the study investigates the potential of these biosynthesized silver nanoparticles to induce genetic

mutations in the target bacteria – an effect that raises important concerns regarding the possible development of resistance to nanoparticle-based antimicrobial agents.

2. Materials and methods

2.1. Materials

In this study, carpenter bees were obtained from Winston-Salem State University. The two Gram-negative bacterial strains – *E. coli* 1946 and *K. pneumonia* – were obtained from the American Type Culture Collection (ATCC; United States). The following analytical-grade chemicals were used: deionized water, 70% ethanol (Fisher Scientific, USA), 1 mM silver nitrate (Fisher Scientific, USA), 0.1 M sodium hydroxide (Fisher Scientific, USA), nutrient broth (Fisher Scientific, USA), phosphate-buffered saline (Fisher Scientific, USA), and glutaraldehyde solution (Fisher Scientific, USA). Additional materials included a 98-well plate (Fisher Scientific, USA), centrifuge tubes (Fisher Scientific, USA), Ziploc bags, a field emission scanning electron microscope (JEOL Ltd., Japan), a GENESYS™ 180 ultraviolet-visible (UV-vis) spectrophotometer (Fisher Scientific, United States), a DNeasy 96 PowerSoil Pro QIAcube HT Kit (QIAGEN, USA), and an Isotemp 2300 Digital Water Bath (Fisher Scientific, USA).

2.2. Extract preparation and synthesis of silver nanoparticles

Dead carpenter bees (*X. virginica*) were collected in June 2022 from Winston-Salem State University, Winston-Salem, North Carolina, United States of America, and transported to the laboratory in Ziploc bags. The wings were carefully removed using sterilized forceps, then sterilized in 70% ethanol, washed with deionized water, and air-dried at room temperature.

Silver nanoparticles were synthesized using the following protocols adapted from previous studies.[24,25] Briefly, 0.1 g of bee wings was weighed and hydrolyzed in 0.1 M sodium hydroxide at 90°C using an Isotemp 2300 Digital Water Bath for 60 min. After hydrolysis, the mixture was cooled and centrifuged at 8,000 rpm for 10 min. The supernatant pH was adjusted to neutral, and 1 mL of this extract was added to 49 mL of 1 mM silver nitrate solution in a 100 mL beaker. The mixture was incubated at 28 ± 1°C for 60 min, during which the color change from light yellow to dark brown indicated the formation of silver nanoparticles. The UV-vis absorbance spectrum of the synthesized nanoparticles was measured in the 200 – 1,000 nm range using a GENESYS™ 180 UV-vis spectrophotometer. Scanning electron microscopy (SEM) was performed using a field emission SEM to characterize the morphology and size distribution of the nanoparticles.

2.3. Antibacterial analysis

E. coli 1946 (ATCC 25922) and *K. pneumoniae* NCTC 9633 (ATCC 13883) were cultured in nutrient broth medium at 37°C for 24 h with shaking at 150 rpm in a shaking incubator. The antibacterial activity of biosynthesized silver nanoparticles from CBW at concentrations ranging from 0 to 100 μM was evaluated against *E. coli* and *K. pneumoniae*, following protocols adapted from previous studies.[24,25] After 24 h of incubation, bacterial growth was assessed by measuring the optical density at 600 nm using a 98-well plate format with a GloMax Multiplate Reader (Promega, United States). In addition, SEM was used to analyze the morphological changes in the treated bacterial strains.[24,25]

2.4. Genomic analysis

Whole genome sequencing (WGS) was performed to investigate the genetic alterations in *E. coli* and *K. pneumoniae* following exposure to biosynthesized silver nanoparticles.[25,26] Briefly, after 24 h of treatment, genomic DNA was extracted from both the control group (untreated bacteria) and the nanoparticle-treated cells using the DNeasy 96 PowerSoil Pro QIAcube HT Kit, following the instructions provided by the manufacturer. Genomic libraries were prepared and sequenced using the NextSeq2000 system (manufacturer, country) with a 300-cycle flow cell kit to generate 2 × 150 base pair paired-end reads. Read demultiplexing, trimming, and run analytics were performed using DRAGEN v4.2.7, the onboard analysis software integrated with the NextSeq2000 system.

2.5. Statistical analysis

All statistical analyses were conducted using GraphPad Prism version 8.01 (manufacturer, country). Data are presented as the mean ± standard error of the mean. Statistical comparisons between groups were conducted using the Student's *t*-test, and differences were considered statistically significant at $p<0.05$.

3. Results

3.1. Characterizations of biosynthesized silver nanoparticles from CBWs

The biosynthesis of silver nanoparticles is visually confirmed by a color change in the treated extract, turning from light yellow to dark brown. The UV-vis spectral analysis reveals a strong surface plasmon resonance (SPR) peak at approximately 420 nm, indicating the successful formation of silver nanoparticles (Figure 1).[25]

The shape, size, morphology, and composition of the synthesized nanoparticles directly influence the SPR bands. SEM images reveal agglomeration of the biosynthesized

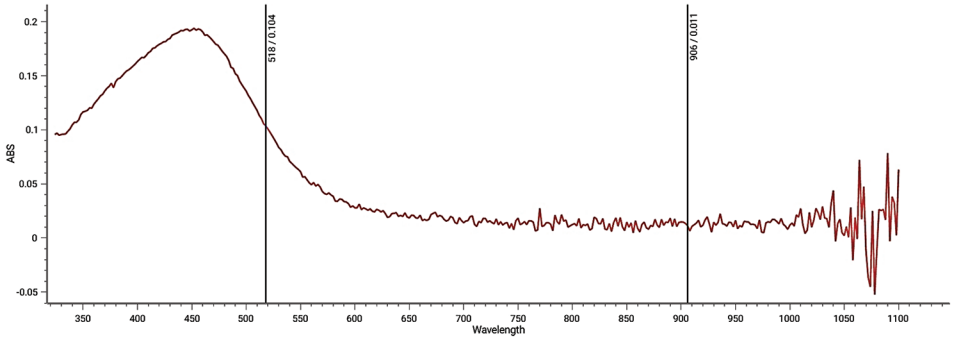

Figure 1. Ultraviolet-visible spectra of biosynthesized silver nanoparticles from carpenter bee wing extract

silver nanoparticles (Figure 2). In addition, SEM analysis shows that the nanoparticles exhibit a spherical morphology with an approximate diameter ranging from 10.0 nm to 40.0 nm (Figure 2).

A decrease in optical density with increasing nanoparticle concentration suggests that silver nanoparticles inhibit the growth of *E. coli* and *K. pneumoniae* (Figure 3). To further investigate the bacterial response to silver nanoparticles, stress indicators, cell morphology, and nanoparticle–cell interactions were examined using SEM. The results show no aggregation in the control samples (i.e., in the absence of nanoparticles) (Figures 4A-D and 5A-D). However, aggregation is observed in *E. coli* and *K. pneumoniae* cells treated with silver nanoparticles (Figures 4E-H and 5E-H).

3.2. Genomic analysis

WGS analysis was conducted on control and treated *K. pneumoniae* and *E. coli* cells and compared against their respective reference genomes to identify potential genetic alterations and mutations resulting from exposure to biosynthesized silver nanoparticles after 24 h. The genomic variants identified in *K. pneumoniae* are presented in Tables 1 and 2. The treated cells display a total of four putative polymorphisms, three of which exceed a frequency of 0.5 (Table 1). These include mutations in the putrescine transport system adenosine triphosphate (ATP)-binding protein (*J2Y72_004072*), multidrug (MDR) efflux pump (*J2Y72_003942*), nitrate reductase beta subunit (*J2Y72_003241*), and ferric enterobactin receptor (*J2Y72_000218*).

The most significant polymorphisms identified in the control cells (Table 2) include: Staphyloferrin A export major facilitator superfamily transporter/ D-ornithine citrate ligase (*sfaA/sfaD*), adenine phosphoribosyltransferase (*KQ76_RS08360*), teichoic acid D-alanine esterase (*fmtA*), DUF3169 family protein (*KQ76_RS01520*), alpha/beta hydrolase (*KQ76_RS13020*), DNA-binding heme response regulator (*hssR*), ribosome

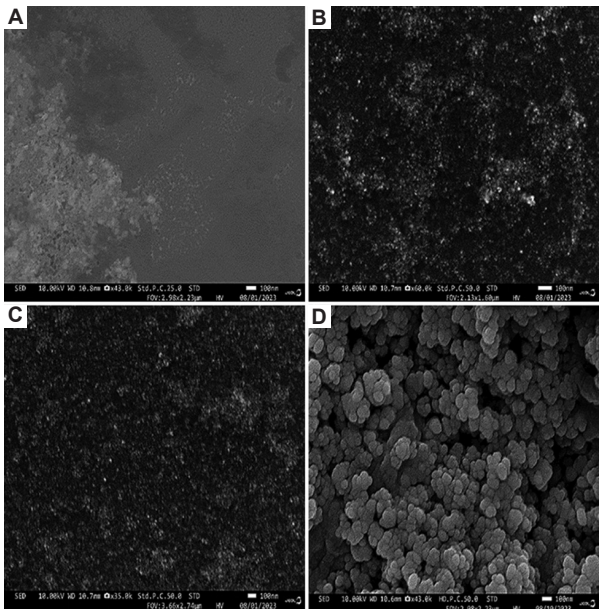

Figure 2. Scanning electron microscopy micrographs of biosynthesized silver nanoparticles from carpenter bee wing extract, each displaying a 100 nm scale bar and captured at different magnifications: (A) ×43,000, (B) ×60,000, (C) ×35,000, and (D) ×43, 000

biogenesis guanosine triphosphate (GTP)-ase (*ylqF*), peptidoglycan teichoic acid D-alanyltransferase (*dltB*), M23 family metallopeptidase/haloacid dehalogenase-like hydrolase subfamily IIB (*KQ76_RS11280/KQ76_RS11285*), general stress protein (*KQ76_RS01815*), small stable RNA A-binding protein (*smpB*), phage major capsid protein (*KQ76_RS07375*), phosphoribosylformylglycinamidine synthase subunit (*purS*), transfer RNA uridine 5-carboxymethylaminomethyl (34) synthesis enzyme (*mnmG*), D-lactate dehydrogenase (*KQ76_RS12955*), and beta-glucoside operon antiterminator protein family transcriptional antiterminator (*KQ76_RS10985*).

WGS was conducted to identify polymorphisms in both control and treated cells following 24 h of exposure to biosynthesized silver nanoparticles. All detected

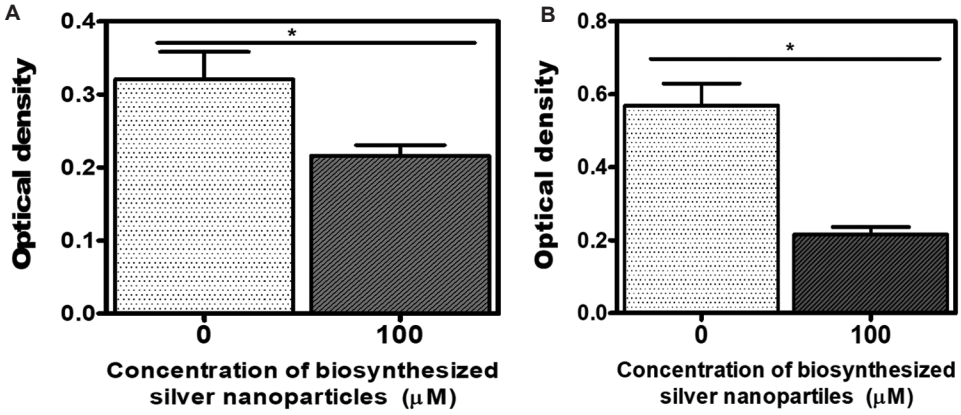

Figure 3. Antibacterial activity of biosynthesized silver nanoparticles from carpenter bee wing extract against (A) *Escherichia coli* and (B) *Klebsiella pneumoniae* after 24 h of exposure
Note: Asterisk (*) indicates statistically significant differences compared to the control ($p<0.05$).

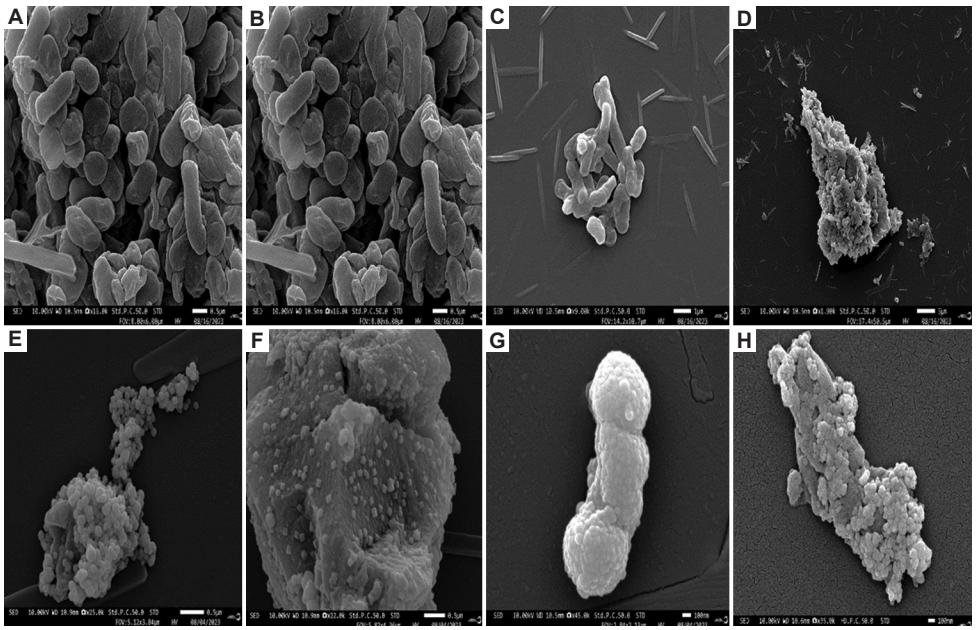

Figure 4. Scanning electron microscopy images of *Klebsiella pneumoniae* cells show interactions with biosynthesized silver nanoparticles from carpenter bee wing extract after 24 h of exposure. (A-D) Control (untreated) cells; (E-H) cells treated with the silver nanoparticles. (A and B) Scale bar = 0.5 μm, magnification = ×15,000; (C) scale bar = 1 μm, magnification = ×9,000; (D) scale bar = 5 μm, magnification = ×1,900; (E) scale bar = 0.5 μm, magnification = ×25,000; (F) scale bar = 0.5 μm, magnification = 22,000×; (G) scale bar = 100 nm, magnification = 45,000×; (H) scale bar = 100 nm, magnification = ×35,000.

polymorphisms, along with their mutation frequencies (f), are presented in Tables 2 and 3.

Single nucleotide polymorphisms identified in the control cells are presented in Table 4, along with descriptions of the de novo mutations. A total of 14 polymorphisms in the control cells are detected in the control cells, each showing a frequency increase ranging from 20% to 34%.

4. Discussion

Bacterial infections caused by *K. pneumoniae* and *E. coli* can result in serious, potentially life-threatening complications.[27,28] Antibiotics remain powerful and lifesaving agents for treating infections such as urinary tract and bloodstream infections. However, *K. pneumoniae* and *E. coli* are increasingly developing resistance to antibiotics.[29] As a result, nanoparticles have gained attention for their potential to combat bacterial resistance, owing to their unique physiochemical properties that enable multiple bactericidal mechanisms.[30] The synthesis of nanoparticles represents a significant technological advancement, offering enhanced antimicrobial performance. Nevertheless, conventional synthesis methods may raise concerns

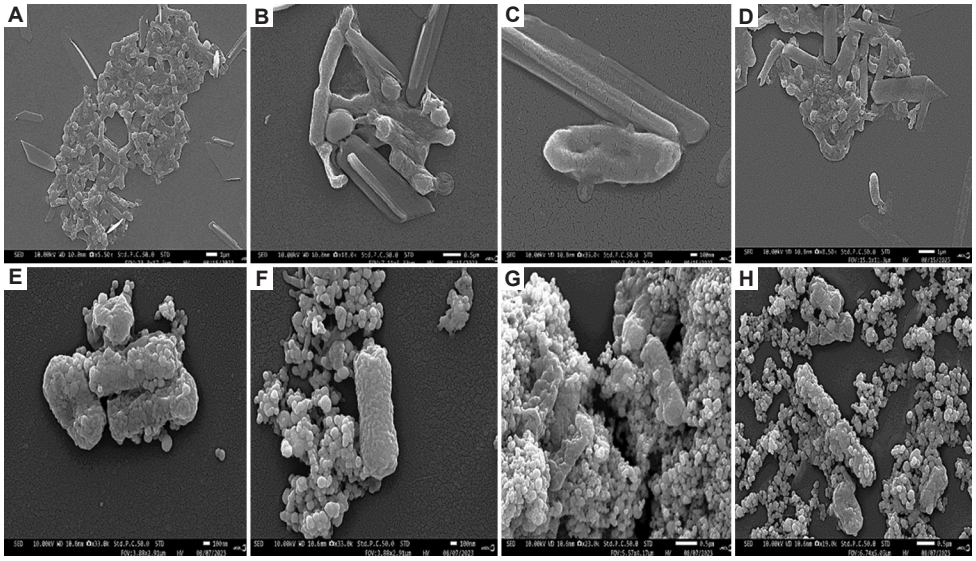

Figure 5. Scanning electron microscopy images of *Escherichia coli* cells show interactions with biosynthesized silver nanoparticles from carpenter bee wing extract after 24 h of exposure. (A-D) Control (untreated) cells; (E-H) cells treated with the silver nanoparticles. (A) Scale bar = 1 μm, magnification = ×5,500; (B) scale bar = 0.5 μm, magnification = ×10,000; (C) scale bar = 100 nm, magnification = ×33,000; (D) scale bar = 1 μm, magnification = ×8,500; (E and F) scale bar = 100 nm, magnification = ×33,000; (G) scale bar = 0.5 μm, magnification = ×23,000; (H) scale bar = 0.5 μm, magnification = ×19,000.

Table 1. Genomic analysis of *Klebsiella pneumoniae* cells after 24 h of exposure to biosynthesized silver nanoparticles from carpenter bee wing extract

Position	Frequency (%)	Annotation	Gene	Product
4,398,960	100.0	S189A* (TCG→GCG)	*J2Y72_004072*	Putrescine transport system ATP-binding protein
4,264,009	62.3	I879M* (ATT→ATG)	*J2Y72_003942*	Multidrug efflux pump
3,539,179	61.1	K215K* (AAG→AAA)	*J2Y72_003241*	Nitrate reductase beta subunit
227,265	55.6	Coding (303/2229 nt)	*J2Y72_000218*	Ferric enterobactin receptor

Notes: Asterisk (*) indicates that the annotation provides functional context to the corresponding gene sequence, facilitating interpretation and analysis; Underlined letters denote specific nucleotide or amino acid mutations identified within the sequence.
Abbreviation: ATP: Adenosine triphosphate.

related to toxicity and environmental impact. Therefore, the development of safe and sustainable nanoparticle production methods is essential.

Nanoparticles can be synthesized through various techniques, including chemical and biological (green) methods. Compared to chemical synthesis, green synthesis offers several advantages and is not associated with the limitations of chemical approaches.[31] It is environmentally friendly, cost-effective, and offers potentially higher biocompatibility.[32] In this study, silver nanoparticles were synthesized using CBWs, which act as a reducing agent for silver ions from silver nitrate. The study focuses on the synthesis, characterization, antibacterial evaluation, and mutation profiling in response to CBW-derived biosynthesized silver nanoparticles.

The mixing of CBW extract with silver nitrate results in a visible color change over time, indicating the reduction of silver ions and the excitation of the SPR peak associated with silver nanoparticles. Compounds such as aliphatic hydrocarbons in the CBW may facilitate the formation of silver nanoparticles within the size range of 20 – 40 nm.[25] The UV-vis spectra of the synthesized nanoparticles show a peak at approximately 440 nm, which is characteristic of metal nanoparticles, consistent with findings from previous studies.[25-33] Silver nanoparticles have been widely used as antimicrobial agents, demonstrating broad-spectrum efficacy against pathogens.[24,25,34] In this study, the biosynthesized silver nanoparticles exhibit excellent antimicrobial activity by inhibiting the growth of *E. coli* and *K. pneumoniae*, likely through interactions with negatively charged components on the bacterial cell wall.[35] The nanoparticles primarily adhere to the bacterial surface via electrostatic attraction and release positively charged silver ions, which disrupt cellular processes and damage DNA.[36]

Silver nanoparticles have the potential to induce mutations or polymorphisms, primarily through direct

Table 2. Genomic analysis of *Klebsiella pneumoniae* cells in the control group

Position	Frequency (%)	Annotation	Gene	Product
2,228,365	69.2	Intergenic (−35/−67)	*sfaA/sfaD*	Staphyloferrin A export MFS transporter/ D-ornithine citrate ligase
1,700,478	65.6	G59S* (GGC→AGC)	*KQ76_RS08360*	Adenine phosphoribosyltransferase
1,017,325	65.3	Q304* (CAA→TAA)	*fmtA*	Teichoic acid D-alanine esterase
342,330	62.0	I180I* (ATT→ATC)	*KQ76_RS01520*	DUF3169 family protein
2,574,726	32.2	G69A* (GGC→GCC)	*KQ76_RS13020*	Alpha/beta hydrolase
2,389,192	32.0	R188P* (CGA→CCA)	*hssR*	DNA-binding heme response regulator
1,213,003	31.3	E184D* (GAG→GAC)	*ylqF*	Ribosome biogenesis GTPase
860,322	31.3	E344K* (GAA→AAA)	*dltB*	Peptidoglycan teichoic acid D-alanyltransferase
2,252,747	29.9	Intergenic (−54/+157)	*KQ76_RS11280/KQ76_RS11285*	M23 family metallopeptidase/haloacid dehalogenase-like hydrolase subfamily IIB
391,361	29.1	S36I *(AGT→ATT)	*KQ76_RS01815*	General stress protein
810,694	29.0	M1K* (ATG→AAG)	*smpB*	SsrA-binding protein
1,536,460	27.5	Y112* (TAT→TAA)	*KQ76_RS07375*	Phage major capsid protein
1,027,271	27.0	A87P* (GCA→CCA)	*purS*	Phosphoribosylformylglycinamidine synthase subunit
2,776,116	26.7	H117Q* (CAT→CAA)	*mnmG*	tRNA uridine 5-carboxymethylaminomethyl (34) synthesis enzyme
2,574,727	26.6	G69R* (GGC→CGC)	*KQ76_RS13020*	Alpha/beta hydrolase
2,564,194	26.6	A17P* (GCA→CCA)	*KQ76_RS12955*	D-lactate dehydrogenase
2,190,680	25.7	T500S* (ACG→TCG)	*KQ76_RS10985*	BglG family transcriptional antiterminator

Notes: Asterisk (*) indicates that the annotation provides functional context to the corresponding gene sequence, facilitating interpretation and analysis; Underlined letters denote specific nucleotide or amino acid mutations identified within the sequence.
Abbreviations: BglG: Beta-glucoside operon anti-terminator protein; GTP: Guanosine triphosphate; MFS: Major facilitator superfamily; SsrA: Small stable RNA A; tRNA: Transfer RNA.

Table 3. Genomic analysis of *Escherichia coli* after 24 h of exposure to biosynthesized silver nanoparticles from carpenter bee wing extract

Position	Frequency (%)	Annotation	Gene	Product
4,935,197	43.3	Intergenic (−347/+147)	*lysO/aqpZ*	L-lysine exporter LysO/aquaporin Z
2,376,506	42.9	E119* (GAA→TAA)	*D1792_RS11465*	YtfJ family protein
4,790,571	28.2	A218P* (GCA→CCA)	*sucD*	Succinate-CoA ligase subunit alpha
1,244,541	25.4	V392L* (GTA→CTA)	*hisD*	Histidinol dehydrogenase
461,346	25.0	R159P* (CGG→CCG) ‡	*D1792_RS02575*	Helix-turn-helix transcriptional regulator
2,155,046	22.9	T314R* (ACG→AGG)	*uacT*	Urate/proton symporter UacT
641,105	21.1	E1049D* (GAG→GAC)	*D1792_RS03370*	Host specificity protein J
1,997,598	20.7	V23L* (GTA→CTA)	*ygcS*	MFS transporter

Notes: Asterisk (*) indicates that the annotation provides functional context to the corresponding gene sequence, facilitating interpretation and analysis; Double dagger (‡) indicates a variant that is flagged as potentially problematic or requires further investigation; Underlined letters denote specific nucleotide or amino acid mutations identified within the sequence.
Abbreviations: CoA: Coenzyme A; MFS: major facilitator superfamily; UacT: Uric acid transporter.

interaction with DNA and by generating oxidative stress.[37] However, the mutagenic effects of silver nanoparticles and the associated resistance mechanisms in *E. coli* and *K. pneumoniae* remain largely unexplored. This study investigates whether biosynthesized silver nanoparticles from CBW can induce genetic mutations in *E. coli and*

Table 4. Genomic analysis of *Escherichia coli* cells in the control group

Position	Frequency (%)	Annotation	Gene	Product
5,022,977	35.4	L28V* (CTC→GTC)	*pqiB*	Intermembrane transport protein PqiB
82,367	29.6	A100P* (GCC→CCC)	*mdoG*	Glucan biosynthesis protein G
5,022,975	29.2	A27G* (GCG→GGG)	*pqiB*	Intermembrane transport protein PqiB
4,935,212	28.6	Intergenic (−362/+132)	*lysO/aqpZ*	L-lysine exporter LysO/aquaporin Z
325,706	27.4	S9R* (AGC→AGG)	*sirB2*	Invasion regulator SirB2
1,664,261	27.1	T163T* (ACC→ACG)	*fryC*	PTS fructose transporter subunit IIC
1,244,541	26.2	V392L* (GTA→CTA)	*hisD*	Histidinol dehydrogenase
1,078,228	25.0	A53P* (GCC→CCC)	*D1792_RS05680*	DUF4756 family protein
2,077,731	24.0	Intergenic (−7/+65)	*D1792_RS10070/D1792_RS10075*	Phosphoglycerate dehydrogenase/SIS domain-containing protein
2,376,513	23.8	G116G* (GGC→GGA)	*D1792_RS11465*	YtfJ family protein
2,725,980	22.5	G94G* (GGC→GGG)	*chiA*	Bifunctional chitinase/lysozyme
2,936,459	21.8	L94* (TTA→TGA) ‡	*rcdB*	LysR family transcriptional regulator
2,555,115	21.5	V98L* (GTG→CTG)	*diaA*	DnaA initiator-associating protein DiaA
2,077,733	20.0	Intergenic (−9/+63)	*D1792_RS10070/D1792_RS10075*	Phosphoglycerate dehydrogenase/SIS domain-containing protein

Note: Asterisk (*) indicates that the annotation provides functional context to the corresponding gene sequence, facilitating interpretation and analysis; Double dagger (‡) indicates a variant that is flagged as potentially problematic or requires further investigation; Underlined letters denote specific nucleotide or amino acid mutations identified within the sequence.
Abbreviations: PTS: Phosphotransferase system; PqiB: Paraquat-inducible protein B; SirB2: Signal regulatory protein beta 2; SIS: Sugar isomerase.

K. pneumoniae, potentially contributing to the development of resistance.

WGS, a technique that enables comprehensive identification of genomic mutations by sequencing an organism's entire genome,[38] was employed to analyze the interaction mechanisms between the biosynthesized silver nanoparticles and the bacterial cells. A key finding of this study is the detection of mutations in several genes of *K. pneumoniae*-treated cells that may reduce the antibacterial efficacy of silver nanoparticles. These mutations are associated with defense mechanisms, efflux systems, neutralization, ion transport, energy metabolism, and siderophore production.

Notably, mutations are identified in the genes encoding the putrescine transport system ATP-binding protein (*J2Y72_004072*), MDR pump (*J2Y72_003942*), nitrate reductase beta subunit (*J2Y72_003241*), and ferric enterobactin receptor (*J2Y72_000218*). Among these, the mutation in the ATP-binding cassette (ABC) transporter gene (*J2Y72_004072*) is particularly significant, as it exhibits the highest mutation frequency (100%).

In WGS, mutation frequency refers to the proportion of a specific genetic variation observed within the studied population. ABC transporters are responsible for importing nutrients and exporting toxic substances in bacterial cells.[39] Mutations in ABC transporters can contribute to antimicrobial resistance, thereby reducing the efficacy of silver nanoparticles. This study suggests that exposure to silver nanoparticles may promote the emergence of such mutations in ABC transporter genes. Mutations in ABC transporters can significantly affect bacterial physiology by disrupting nutrient uptake or causing uncontrolled efflux of vital intracellular components. These disruptions can impair growth, alter virulence, and modulate antibiotic susceptibility.[39] Ultimately, the inability to maintain intracellular homeostasis may compromise cellular processes, leading to reduced bacterial viability or cell death.

Other genomic variants identified in *K. pneumoniae* exposed to biosynthesized silver nanoparticles are associated with transport and resistance mechanisms, including mutations in genes encoding for MDR pump (*J2Y72_003942*), nitrate reductase beta subunit (*J2Y72_003241*), and ferric enterobactin receptor (*J2Y72_000218*). MDR pumps are membrane-associated transporter proteins that expel toxic compounds from bacterial cells, enhancing survival and contributing to antibiotic resistance.[40] These pumps also protect bacteria from antimicrobial agents and harmful substances, including heavy metals and organic solvents.[41] The nitrate reductase beta subunit forms part of an enzyme complex

involved in electron transfer and energy production,[40] whereas the ferric enterobactin receptor is an outer membrane protein responsible for transporting iron into the periplasm.[42-45] Mutations affecting iron transport systems can lead to antimicrobial resistance by impairing iron uptake. This is significant because many antibiotics rely on iron transport pathways to enter bacterial cells.[46] Consequently, limiting iron acquisition can enhance bacterial resistance to antimicrobial agents.

The nutrient broth medium provides a rich source of readily available nutrients – such as carbohydrates, protein, vitamins, and minerals – that enable *K. pneumoniae* to efficiently access nutrients necessary for rapid growth and proliferation.[25] In the control group, mutations are detected in *K. pneumoniae* cells grown in this nutrient-rich medium. These mutations appear to confer advantageous traits that enhance nutrient utilization, allowing the control cells to outcompete the treated cells and display increased growth.

Several notable mutations are observed in the control cells, particularly in genes related to iron metabolism, biosynthesis, metabolism, cell growth, detoxification, cell wall integrity, structural stability, defense, and stress responses. These mutations likely provide a competitive advantage in the nutrient-rich media. This also raises concerns about stress-induced mutagenesis, as the observed mutations in the control group may reflect an elevated rate of adaptive mutation – potentially contributing to future resistance development in *K. pneumoniae.* Nevertheless, the study demonstrates that biosynthesized silver nanoparticles effectively inhibit bacterial growth, suggesting a cytotoxic effect of silver nanoparticles that interferes with essential cellular processes and disrupts normal cell function.

Mutations can arise spontaneously without exposure to external stressors and are a key driver of bacterial evolution. In untreated bacterial cells, mutations may result from natural genetic alterations during DNA replication. While many of these changes are neutral, some may confer advantageous traits – such as increased antibiotic resistance – that enhance bacterial survival in challenging environments, including exposure to antimicrobials.[12]

These findings highlight a critical concern: the presence of resistance genes may render nanoparticles ineffective. Such genes can reduce nanoparticle efficacy through several mechanisms, including actively expelling nanoparticles, modifying the cell membrane to prevent nanoparticle entry, and chemically altering nanoparticles to reduce their toxicity.

Genomic analysis reveals several mutations in the genes of control *K. pneumoniae* cells, including *sfaA/sfaD*, *KQ76_RS08360*, *fmtA*, *KQ76_RS01520*, *KQ76_RS13020*, *hssR*, *ylqF*, *dltB*, *KQ76_RS11280/KQ76_RS11285*, *KQ76_RS01815*, *smpB*, *KQ76_RS07375*, *purS*, *mnmG*, *KQ76_RS12955*, and *KQ76_RS10985*.

Describing the function of these genes is crucial for understanding how *K. pneumoniae* adapts to its environment, particularly in relation to antibiotic resistance, pathogenicity, and microbial evolution. Mutations in these genes can significantly alter bacterial traits by affecting key cellular processes such as metabolism, virulence factor expression, and drug susceptibility.

The following are the functions of the mutated genes identified in *K. pneumoniae* control cells:

(i) *sfaA/sfaD* is involved in the transport of iron from the environment into the cell, supporting essential cellular processes.[47]
(ii) *KQ76_RS08360* enables the recycling of adenine, a critical building block of DNA.[48]
(iii) *fmtA* is involved in cell division and bacterial cell wall synthesis.[49]
(iv) *KQ76_RS13020* belongs to a large enzyme superfamily with diverse catalytic functions,[50] including roles in cell growth, metabolism, and detoxification.
(v) *hssR* regulates gene expression related to iron metabolism and other cellular activities.[51]
(vi) *ylqF* assists in the assembly and regulation of ribosomes. GTPases also regulate cellular functions.[52]
(vii) *dltB* maintains cell wall integrity and regulates cation balance, contributing to resistance against cationic antimicrobial peptides.[53]
(viii) *KQ76_RS11280/KQ76_RS11285* facilitates bacterial competition for resources or consumption of other bacteria.[54]
(ix) *KQ76_RS01815* promotes bacterial survival under environmental stresses and induces virulence factor expression.[55]
(x) *smpB* is involved in tagging and degrading proteins produced from defective mRNAs and plays a role in nutrient acquisition.[56]
(xi) *KQ76_RS07375* triggers bacterial defense mechanisms.[57]
(xii) *purS* is involved in the purine biosynthetic pathway.[58]
(xiii) *mnmG* is crucial for accurate codon-anticodon pairing during protein translation.[59]
(xiv) *KQ76_RS12955* is a key enzyme in glycolysis.[60]

Mutations identified in *E. coli*-treated cells involve genes associated with transport, cell division, biosynthetic adaptation, and invasion:

(i) L-lysine exporter LysO/aquaporin Z (*lysO/aqpZ*) mediates the export of L-lysine and confers resistance to the toxic antimetabolite L-thialysine.[61]

(ii) YtfJ family protein (*D1792_RS11465*) is involved in cell division and cell wall hydrolysis.[62]
(iii) Succinate-coenzyme A ligase subunit alpha (*sucD*) plays a role in ATP synthesis.[63]
(iv) Histidinol dehydrogenase (*hisD*) is essential for bacterial survival.[64]
(v) Helix-turn-helix transcriptional regulator (*D1792_RS02575*) modulates gene expression by activating or repressing transcription.[65]
(vi) Proton symporters (*uacT*) transport substrates and protons across the cell membrane, aiding bacterial adaptation to environmental changes.[66]
(vii) Host specificity proteins (*D1792_RS03370*) contribute to bacterial infectivity and assist in evading the host immune response.[67]
(viii) Major facilitator superfamily transporters (*ygcS*) help bacteria withstand toxic metabolites, heavy metals, and environmental stressors.[68]

The control cells exhibit a distinct mutation pattern compared to the treated cells. *E. coli* control cells carry mutations in genes such as intermembrane transport protein (*pqiB*), glucans biosynthesis protein (*mdoG*), invasion regulator (*sirB2*), phosphotransferase system fructose transporter subunit (*IIC*), *hisD*, phosphoglycerate dehydrogenase/sugar isomerase domain-containing protein (*D1792_RS10070/D1792_RS10075*), bifunctional chitinase/lysozyme (*chiA*), LysR family transcriptional regulator (*rcdB*), and initiator associating protein (*diaA*). These mutations influence *E. coli* growth by altering functions related to nutrient acquisition, stress response, immune evasion, or antibiotic resistance, as supported by their known roles.

pqiB is essential for bacterial survival, pathogenesis, and antimicrobial resistance.[69] *mdoG* modulates virulence, biofilm structure, and immune evasion.[70] *sirB2* supports bacterial survival and adaptation.[71] *IIC* facilitates sugar transport across the membrane.[72] *hisD* catalyzes the final two steps in histidine biosynthesis and is vital for survival during infection.[64] *D1792_RS10070/D1792_RS10075* produces serine, a key amino acid for protein production.[73] *chiA* acts as a virulence factor by allowing *E. coli* to invade chitinous hosts – such as insects or fungi – through cell wall degradation.[74] *rcdB* contributes to metabolism, stress response, and virulence.[75] *diaA* acts as the primary "initiator" protein.[76]

Mutations in *lysO/aqpZ* and *D1792_RS11465* are shared between control and treated groups. *lysO/aqpZ* mediates L-lysine export and confers resistance to the toxic antimetabolite L-thialysine,[61] while *D1792_RS11465* is involved in cell division and cell wall hydrolysis.[72] Collectively, these mutations enhance *E. coli*'s ability to survive under challenging conditions by improving resource utilization, antibiotic resistance, and immune evasion.

The findings indicate that *K. pneumoniae* is more sensitive to silver nanoparticles than *E. coli* (Figure 2), possibly due to structural differences in their cell walls. *E. coli* possesses a relatively thicker peptidoglycan layer, which can hinder nanoparticle penetration, whereas the thinner cell wall of *K. pneumoniae* allows easier entry and interaction with the cell membrane.[77] Furthermore, variations in lipopolysaccharides (LPS) between *E. coli* and *K. pneumoniae* may influence nanoparticle aggregation and uptake. LPS, present on the surface of Gram-negative bacteria, are known to attract and bind to nanoparticles.[78] The LPS of *K. pneumoniae* typically has a more complex structure with additional sugar modifications compared to those of *E. coli*, potentially enhancing their interaction with silver nanoparticles and contributing to increased sensitivity.[79] The highly charged and hydrophilic nature of *K. pneumoniae* LPS promotes strong binding to nanoparticle surfaces, which may disrupt the bacterial outer membrane and compromise cell viability.[80]

5. Conclusion

Nanoparticles hold great promise as antimicrobial agents due to their potent antibacterial activity, particularly when synthesized using metals such as silver. CBW-derived silver nanoparticles are highly effective against *E. coli* and *K. pneumoniae* by aggregating on the bacterial cell surface. These biosynthesized nanoparticles present a suitable alternative to conventional antibiotics for addressing antibiotic resistance in *E. coli* and *K. pneumoniae* and are strong candidates for medical applications where antimicrobial activity is essential. Future studies should investigate the potential toxicity of biosynthesized silver nanoparticles on human cells to ensure their safe application for both human health and the environment.

Acknowledgments

The authors are grateful to the Department of Biological Sciences at Winston-Salem State University for their valuable suggestions and support.

Funding

The study was supported by the Professional Development Committee Research Grant (grant number: 211444), Winston-Salem State University, and The Genomic Research and Data Science Center for Computation and Cloud Computing (grant number: 211512).

Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The data that support the findings of this study are available upon request from the corresponding author.

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ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

ORIGINAL RESEARCH ARTICLE

Impact of Tianji orthopedic robot on patient satisfaction and quality of life

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Abstract

Internal fixation (IF) surgery has been promoted with the combination of robotic technology, promising increased accuracy and improved patient prognosis. This study examined the effect of IF surgery using Tianji orthopedic robot on patient satisfaction and quality of life (QoL) over a longitudinal follow-up time. A cohort of 387 patients undergoing IF guided by Tianji orthopedic robot surgery was followed from the pre-surgery phase through 12 months post-surgery. Patient-reported outcome measures, including the Oswestry Disability Index (ODI) and the Short Form Health Survey (SF-36), were administered at baseline, 6 months, and 12 months. In addition, the Newcastle Satisfaction with Nursing Care Scale (NSNCS) was also used to assess patient satisfaction. Data were analyzed using repeated measures analysis of variance. However, only 338 (87.33%) patients who underwent robot-assisted surgery completed the survey. A total of 214 (63.31%) females and 124 (36.68%) males, with an age of 63.76 ± 14 years, were included in the study. The study indicated significant progress in patient satisfaction and QoL. The mean ODI score decreased from 79.1 ± 4.76 pre-surgery to 46.2 ± 6.09 at 12 months (p<0.001), compared to the SF-36 score from 43.5 ± 4.20 to 84 ± 4.8 (p<0.05). Moreover, the NSNCS scores of 86 ± 4.32 reflected high satisfaction levels, indicating that participants were satisfied with their surgical outcomes at the 12-month follow-up. The Tianji orthopedic robot significantly improves patient satisfaction and QoL over a year. These findings confirm the significance of robotic technology and surgical procedures and highlight the essential role of nurses in using telehealth for continuous follow-up care.

Keywords: Tianji orthopedic robot; Spinal cord abnormalities; Satisfaction; Quality of life; Nursing

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Citation: Alshorman J, Altahla R. Impact of Tianji orthopedic robot on patient satisfaction and quality of life. *INNOSC Theranostics and Pharmacological Sciences*. 2025;8(3):85-92.
doi: 10.36922/ITPS025080009

Received: February 19, 2025

Revised: June 10, 2025

Accepted: June 16, 2025

Published online: July 4, 2025

1. Introduction

The advancements in surgical techniques have altered orthopedic surgery, particularly in spinal care. Internal fixation (IF) surgery, vital for stabilizing spine abnormalities, has traditionally involved complex procedures with different outcomes.[1] Recent achievements, such as robot-assisted surgery, have enhanced surgical accuracy and may improve patient prognosis.[2-6]

Spinal cord abnormalities cover a range of conditions that influence the structure or function of the spinal cord.[6-8] Common types of spinal cord abnormalities include spina bifida, tethered cord syndrome, and spinal cord tumors. These abnormalities can cause different symptoms, such as pain, weakness, sensory loss, and deteriorated mobility, significantly affecting the patient's quality of life (QoL).[9]

The prevalence of spinal cord abnormalities differs according to the specific condition.[10] For example, spina bifida occurs in about 1 in 1000 live births, while spinal cord injuries, which often occur after trauma, affect approximately 54 individuals per million annually. Increased awareness, advances in medical technology, and research innovations have improved early detection and management, which affect both incidence and outcomes.[11]

The Tianji orthopedic robot showed significant advancement, offering higher accuracy in screw placement and decreased intraoperative complications.[12] This robotic technology improves surgical efficiency and seeks to elevate the overall patient experience and prognosis.[12,13] As patient satisfaction and QoL are paramount considerations in health care, understanding the effect of such developed technologies is definitive.

The most significant contribution of robotics to orthopedics is its ability to increase surgical accuracy. The traditional surgical techniques mainly depend on the surgeon's skills, which increases the chance of inattentive errors. Moreover, robotic systems combined with advanced imaging and real-time feedback techniques allow surgeons to plan and perform surgery with high accuracy.[14]

The high accuracy level needed minimizes disruption of soft tissue, resulting in reduced post-surgery complications and recovery periods. Developing a multidisciplinary strategy requires structured communication and cooperation, where each healthcare providers share their insights into improving patient safety and surgical sufficiency.[15-17] Specifically, nurses play a fundamental role in perioperative care, providing essential assessments, monitoring, and support associated with the surgical procedure.[18,19]

Patient satisfaction covers various aspects, including pain management, functional recovery, and the overall experience of care, while QoL includes a patient's physical, emotional, and social well-being.[20,21] It is essential to highlight the importance of these factors in assessing how robotic-assisted IF surgery influences the patients over time.

This longitudinal follow-up study aims to investigate the effects of IF surgery using the Tianji orthopedic robot on patient satisfaction and QoL. By involving validated patient-reported outcome measures and conducting assessments at multiple follow-up times, this research seeks to show overall insights into the long-term advantages of robotic-assisted surgery. In addition, the study explores the role of nursing care and telehealth in assisting and facilitating continuous follow-up, further improving the patient experience and outcomes. Finally, through this investigation, the study aims to contribute beneficial knowledge to the field of orthopedic surgery and consolidate evidence-based practices that prioritize patient-centered care.

2. Materials and methods

2.1. Participants and data collection

The patient's data were collected from two hospitals between December 2023 and November 2024. Only experienced physicians and nurses were allowed to collect and record the data. This study included patients with spinal cord abnormalities, whether the patient underwent surgery with or without an assisted robot, with at least 12 months of follow-up time. The demographic data, imaging studies, fracture characteristics, and operation data were collected and analyzed.

Before the questionnaire, patients received a brief explanation of the research study and the purpose of using the collected data. Patients were required to answer all questions before submitting the survey at the pre-operation stage. A self-administered questionnaire was distributed using the Baidu forms platform and shared via Chinese social media platforms (WeChat and QQ) for patient follow-up. However, patients who received the online questionnaire were required to confirm their desire to participate in the survey. Participation in this study was voluntary, no compensation was provided, no identifying details were collected, and data collection was anonymous. Demographic and injury data were collected from patient records and imaging studies, following the principles outlined in the Helsinki Declaration. This study was approved by the ethics committee of The Second Affiliated Hospital of Hainan Medical University.

2.2. Inclusion/exclusion criteria

The inpatients who were ≥18 years old, capable of understanding the study, and agreed to participate were included. Patients who were unable to complete the questionnaire, with cognitive impairments, or did not provide consent, either personally or through a family member, were excluded.

2.3. Sample size

The Raosoft calculator (Raosoft Inc., United States) was utilized to set the sample size for our study, based on the

total number of patients who underwent spine surgery. We applied a response distribution of 50%, a confidence interval of 95%, a standard deviation of 1.96, and a margin of error of 5%. This calculation showed a required sample size of 213. In addition, we accounted for a 10% margin ($n = 22$) to address any errors in questionnaire completion. Ultimately, 338 participants voluntarily responded, completed the survey, and were included in the final analysis.

2.4. Instrument

2.4.1. Oswestry Disability Index (ODI)

The ODI consists of 10 sections, including pain intensity, personal care, lifting, walking, sitting, standing, sleeping, social life, traveling, and employment/homemaking. Participants selected statements that best describe their condition. Responses were scored from 0 to 5, with higher scores representing severe disability. The total score was then converted to a percentage, where higher percentages indicate greater disability. In the Chinese version, the Cronbach's α value is 0.81 and the validity index is 0.86.[22]

2.4.2. Short Form Health Survey (SF-36)

The SF-36 is a widely used patient-reported outcome measure that assesses health-related QoL. Moreover, it provides an overall physical and mental health status. This survey included 36 questions divided into eight sections, each scored from 0 to 100, with higher scores indicating a better health status.

The scores were then summarized to create a physical component summary and a mental component summary. In the Chinese version, the Cronbach's α value was 0.88 and the validity index was 0.94.[23]

2.4.3. Newcastle Satisfaction with Nursing Care Scale (NSNCS)

Li *et al.*[23] verified the validity and reliability of the NSNCS scale in the Chinese version. This scale is a 5-point Likert scale with 19 items; one is nursing care. The final score of the evaluation ranges from 0 to 100 points. The total score elucidates the patient's satisfaction with nursing care. The Cronbach's α value and the validity index were 0.94 and 0.93 in the Chinese version, respectively.[24] The NSNCS scale was used to assess patient satisfaction with nursing care among those who underwent spine surgery assisted with the Tianji robot.

2.5. Ethical considerations

The ethical committee of The Second Affiliated Hospital of Hainan Medical University approved the study with ethical approval number: HMU-IRB20210314. All procedures were conducted following the ethical principles outlined in the 1964 Declaration of Helsinki and its subsequent revisions. The study method abides by the relevant guidelines and regulations. Informed consent was gained from all participants and their family members. Moreover, consent was acquired from legally authorized representatives if the patient was illiterate. The researchers explained the study's purposes to the patients and their family members before collecting their data.

2.6. Data analysis

Statistical Package for the Social Sciences (29.0, IBM, United States) was used to analyze the collected data. Categorical variables were reported as frequencies and percentages, while continuous data were presented as mean ± standard deviation. One-way analysis of variance was performed to compare QoL and participant satisfaction throughout the follow-up time. Multiple linear regression analyses were performed to identify key determinants of the ODI, SF-36, and NSNCS scores. Variables were selected based on the patients' theoretical or clinical significance, as well as their statistical significance, to control for potential confounding effects. The significance level (p-value) in this study was set at 0.05.

3. Results

This study included 387 patients who underwent spine surgery assisted by the Tianji robot. However, only 338 (87.33%) patients completed the survey.

3.1. Demographic characteristics

Among 338 participants, there were 214 (63.31%) females and 124 (36.68%) males, with a 1.72:1 ratio of females versus males. The mean age of the participants was 63.76 ± 14 years. The mechanism of injury was traumatic in 260 (76.92%) and non-traumatic in 78 (23.07%) patients. Associated fractures mostly involved the lower extremities (166/338, 49.11%). The most commonly affected spine region was lumbar (190/338, 56.21%), followed by thoracic (99/338, 29.28%), thoracolumbar (33/338, 9.76%), and cervical (14/338, 4.14%) (Table 1).

The robot-assisted surgery involved in this study included robotic navigation system (76/338, 20.9%), robot-assisted closed reduction and IF (62/338, 18.34%), robot-assisted balloon (47/338, 15.7%), robot-assisted percutaneous balloon dilatation (15/338, 4.43%), 5G remote robot-assisted closed reduction (6/338, 1.77%), and robot-assisted balloon vertebroplasty (7/338, 2.07%).

3.2. Comparison of QoL and satisfaction at pre-surgery, 6-month, and 12-month follow-ups among patients who underwent Tianji robot-assisted surgery

Figure 1 shows significant changes in the ODI, SF-36, and NSNCS scores across pre-surgery and 2 follow-up times.

Table 1. Descriptive characteristics of patients who underwent spinal surgery assisted with Tianji robot

Variable	*n* (%)	Mean±SD
Gender		
Male	124 (36.68)	-
Female	214 (63.31)	-
Age (years)	-	63.76±14
Surgical type		
Robot-assisted closed reduction and internal fixation	62 (18.34)	-
Robotic navigation-assisted	70 (20.71)	-
Robot-assisted balloon	47 (15.7)	-
Robot-assisted percutaneous balloon dilatation	15 (4.43)	-
5G remote robot-assisted closed reduction	6 (1.77)	-
Robot-assisted balloon vertebroplasty	7 (2.07)	-
Associated fractures		
Upper extremity fractures	93 (27.51)	-
Lower extremity fractures	166 (49.11)	-
Others*	79 (23.37)	-
Fracture type		
Traumatic	260 (76.92)	-
Non-traumatic	78 (23.07)	-
Fracture region		
Lumbar	190 (56.21)	-
Thoracic	99 (29.28)	-
Thoracolumbar	33 (9.76)	-
Cervical	14 (4.14)	-
Admission to surgery (days)	-	3.03±1.60

Note: *Other fractures.
Abbreviation: SD: Standard deviation.

Among the 3 time points: Pre-surgery, 6-month, and 12-month post-surgery, there were significant changes in the ODI scores over different categories, including pain intensity, personal care, lifting, walking, sitting, standing, traveling, and employment/homemaking. In addition, the physical component summary subscale of the SF-36 scores showed significant differences throughout the three follow-up periods (p=0.014). In contrast, the mental component summary did not show any significant changes. The NSNCS scores showed significant changes throughout the follow-up periods (p=0.034) (Table 2).

3.3. Predictors of QoL and satisfaction using multiple regression analyses

Multiple linear regression analyses were performed to recognize the key factors affecting QoL and satisfaction scores. To control for potential confounding factors and

Table 2. Comparison of quality of life and patient satisfaction at pre-surgery and two follow-up points among those who underwent Tianji robot surgery

Scales	Pre-surgery	6-month follow-up	12-month follow-up	*p*-value
ODI				
Pain intensity	76±2.76	48±7.98	32±4.28	0.012
Personal care	87±6.98	35±5.90	22±8.54	<0.001
Lifting	83±7.46	62±3.75	58±6.73	0.045
Walking	79±3.65	58±9.65	44±3.54	0.005
Sitting	77±4.98	54±2.65	49±6.32	0.032
Standing	88±4.43	76±8.56	63±5.42	0.028
Sleeping	70±4.21	66±7.35	59±5.82	0.087
Social life	504±206.98	43±8.65	32±9.54	0.531
Traveling	93±1.76	80±4.66	58±3.94	0.018
Employment/ homemaking	88±4.43	71±6.32	45±6.82	0.021
SF-36				
PCS	34±5.87	51±3.23	79±4.78	0.014
MCS	53±2.54	75±5.87	89±4.82	0.067
NSNCS	48±7.43	65±6.09	86±4.32	0.034

Abbreviations: MCS: Mental component summary; NSNCS: Newcastle Satisfaction with Nursing Care Scale; ODI: Oswestry Disability Index; PCS: Physical component summary; SF-36: Short Form Health Survey-36.

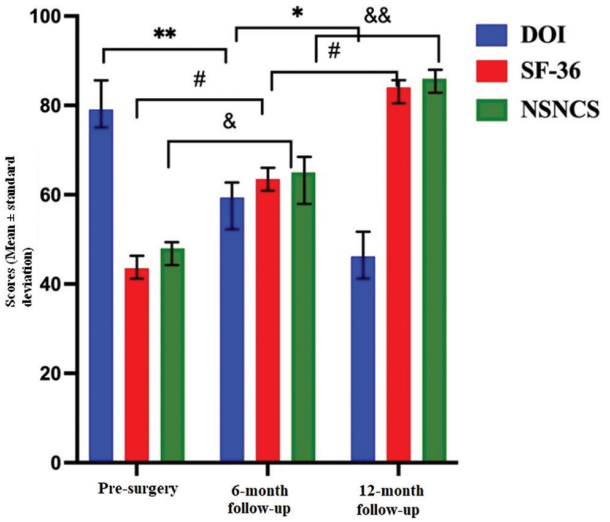

Figure 1. The ODI, SF-36, and NSNCS scores of pre-surgery and two follow-up sessions
Notes: *, #, and & indicate significant differences among ODI, SF-36, and NSNCS across different time points, with p<0.05; **, ##, and && indicate significant differences among ODI, SF-36, and NSNCS across different time points, with p<0.01.
Abbreviations: NSNCS: Newcastle Satisfaction with Nursing Care Scale; ODI: Oswestry Disability Index; SF-36: Short Form Health Survey-36.

ensure statistical significance, variables were selected according to their theoretical and clinical relevance.

The analyses revealed that several predictors, including age, surgical method, surgery type, fracture type, fracture region, and admission time, were significantly associated with the ODI, SF-36, and NSNCS scores (Table 3).

4. Discussion

Spinal cord injuries are common health issues that show significant physical, psychological, and economic challenges.[25] Spinal cord abnormalities can cause mobility issues, chronic pain, and neurological symptoms, which affect an individual's overall QoL. In addition, the psychosocial impacts include increased anxiety, depression, and social isolation, frequently aggravated by societal prejudice.[26]

The involvement of robots in IF surgery has emerged as a significant achievement in orthopedic surgery and care.[27,28] Robotic technology like the Tianji orthopedic robot ensures high surgical accuracy, which lead to high accuracy level of screw placement.[28] This accuracy reduces tissue damage, operative time, and risk of complications.[29] There was a significant reduction in the ODI scores, indicating that patients experienced less pain and high functional ability. This finding supports the advancements in surgery that lead to sensible improvements in recovery and QoL, consistent with previous studies.[30,31] In contrast, Liow *et al.*[32] reported no significant differences in the physical function throughout the follow-up periods.

The significant increase in the SF-36 scores shows a considerable improvement in patients' QoL and well-being. This improvement includes psychological and social dimensions of health. Robot-assisted surgeries may contribute to these QoL enhancements by improving recovery and reducing long-term effects, consistent with findings from previous studies.[33-35]

Table 3. Multiple regression analyses of predictors influencing quality of life and satisfaction among patients who underwent Tianji robot surgery

Characteristics	ODI		SF-36		NSNCS	
	β	*p*-value	β	*p*-value	β	*p*-value
Gender	3.31	0.103	0.85	0.667	14.67	0.393
Age	5.38	<0.001	6.58	0.027	5.38	0.039
Surgical type	0.37	0.44	1.88	0.203	1.28	0.486
Associated fractures	3.07	0.044	−0.02	0.989	2.36	0.147
Fracture type	3.92	0.038	5.98	0.017	7.69	0.011
Fracture region	5.76	0.032	7.32	0.050	7.96	0.025
Admission to surgery	4.19	<0.001	0.30	0.799	−2.07	<0.001

Abbreviations: NSNCS: Newcastle Satisfaction with Nursing Care Scale; ODI: Oswestry Disability Index; SF-36: Short Form Health Survey-36.

The high levels of satisfaction in the NSNCS scores highlight the essential role of both surgical and nursing care in the overall patient experience. The positive effect of robot-assisted surgery on patient care may arise from various factors, including reduced pain, faster recovery times, and improved post-operative assistance, which aligns with previous research findings.[36,37] Furthermore, the use of telehealth for persistent follow-up underscores the role of nurses in providing continuous care and monitoring, which can improve patient satisfaction. However, factors such as patient age, associated fractures, fracture type, fracture region, and admission to surgery time are significant predictors of lower QoL and patient satisfaction.

This study confirms the importance of multidisciplinary cooperation in patient care. Collaboration between surgeons, nursing staff, and rehabilitation staff is essential in optimizing patient prognosis. The involvement of nurses in the telehealth follow-up time allows for timely interventions, addresses any concerns that may arise during recovery, and helps create a supportive environment for patients.

As robot technology continues to develop, further research is required to assess its long-term effects across diverse patient populations and different types of orthopedic surgery. Effectively integrating robot systems into clinical practice will be substantial for maximizing their benefits. In addition, future studies should explore the cost of robot-assisted surgeries compared to traditional methods, as well as the training and support required for healthcare professionals to confirm the advantages of these technologies.

This is the first study to evaluate changes in patient satisfaction and QoL following IF surgery with the Tianji orthopedic robot, with assessments estimated by nursing staff using telehealth services. This study has some limitations. The primary limitation is the dependence on patient-reported outcome measures, such as the ODI and the SF-36. This reliance may introduce response bias, as patients might overestimate or underestimate their satisfaction and QoL. The second limitation is the lack of a control group, making it difficult to attribute improvements in the robot-assisted surgery. The third limitation is that other unmeasured factors may affect patient outcomes, complicating the interpretation of the outcomes.

5. Conclusion

The Tianji orthopedic robot-assisted IF surgery significantly enhances patient satisfaction and QoL over a 12-month follow-up period, by analyzing patient-reported outcomes including the ODI, SF-36, and NSNCS scores. Moreover,

the Tianji orthopedic robot represents a transformative tool in spinal surgery, combining technological innovation with nursing-led continuous care to enhance patient-centered outcomes. These results underscore the importance of robotic technology and multidisciplinary collaboration in modern orthopedics.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: All authors
Formal analysis: Ruba Altahla
Investigation: Jamal Alshorman
Methodology: Ruba Altahla
Writing – original draft: All authors
Writing – review & editing: All authors

Ethics approval and consent to participate

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of The Second Affiliate Hospital of Hainan Medical University (protocol code HMU-IRB20210314). Informed consent was obtained from all individual participants included in the study.

Consent for publication

All participants provided informed consent for the publication of the findings derived from this study. Where applicable, participants gave explicit permission for the publication of any data, images, or information that could potentially reveal their identity. The authors affirm that all relevant consent forms have been obtained and are available upon request.

Availability of data

Data are available upon request via the corresponding author.

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MINI-REVIEW

Early identification of cardiovascular health risks in pre-eclampsia: A biomarker-centric perspective

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Abstract

Pre-eclampsia is a complicated hypertensive pregnancy condition that has a major effect on the health of both the mother and the fetus and puts the affected women at risk for long-term cardiovascular (CV) problems. Despite advances in understanding its etiology, early detection of pre-eclampsia and its associated CV risks remains challenging. This mini-review emphasizes the critical role of biomarkers and advanced diagnostic techniques in addressing this gap. Emerging biomarkers, including angiogenic factors (soluble fms-like tyrosine kinase-1/placental growth factor ratio), metabolic and lipidomics markers, inflammatory cytokines, and exosomal components, provide promising pathways for early identification and risk stratification. Diagnostic techniques can be further improved by classifying these biomarkers according to their capacity to predict long-term CV risks. Technological advancements, such as omics platforms, molecular imaging, wearable health devices, and artificial intelligence (AI) and machine learning, further improve real-time detection and personalized management of pre-eclampsia. By focusing on biomarker-centric predictors of CV risks, this review highlights the integration of multi-biomarker panels and AI-driven algorithms to optimize risk prediction. The transition from association to action is explored, with an emphasis on translating knowledge into effective prevention strategies and improved risk assessment protocols. Structured postpartum follow-up is advocated to monitor and mitigate long-term CV risks in pre-eclamptic women. Practical applications, including targeted interventions and personalized risk management strategies, are discussed. By bridging cutting-edge research and clinical practice, this review aims to enhance maternal health outcomes and advance preventative measures for CV diseases in women with a history of pre-eclampsia.

Keywords: Pre-eclampsia; Hypertension; Pregnancy; Cardiovascular disease; Biomarkers; Health risk

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Citation: Gupta A, Shah K, Gupta V. Early identification of cardiovascular health risks in pre-eclampsia: A biomarker-centric perspective. *INNOSC Theranostics and Pharmacological Sciences*. 2025;8(3):93-100.
doi: 10.36922/itps.7839

Received: December 17, 2024

Revised: January 23, 2025

Accepted: February 24, 2025

Published online: March 6, 2025

1. Introduction

Pre-eclampsia is a complex and potentially fatal pregnancy hypertensive condition that manifests as proteinuria and new-onset hypertension after 20 weeks of pregnancy.[1-4] It affects 5 – 15% of pregnancies worldwide and is a leading cause of maternal and perinatal morbidity and mortality.[5,6] This condition increases the risk of long-term cardiovascular

(CV) problems for the affected individuals, in addition to making pregnancy more challenging. Compared to women with normotensive pregnancies, individuals with a history of pre-eclampsia had a 2 – 4-fold increased lifetime risk of having heart failure, ischemic heart disease, stroke, and chronic hypertension.[7] Morbidity and death can be considerably decreased by early identification of pre-eclampsia and the CV health risks it entails. Pre-eclampsia is therefore a sentinel occurrence that can be used to identify women who are later at risk of CV diseases (CVDs).

Pre-eclampsia has a complicated pathogenesis that includes diminished placental angiogenesis, oxidative stress, endothelial dysfunction, and immunological maladaptation.[8] Although our understanding of the molecular pathways has advanced significantly, early diagnosis and risk assessment remain difficult. Improving the outcomes for mothers and newborns requires the search for novel and trustworthy diagnostic techniques and the exploration of biomarkers for identifying and controlling CV risks related to pre-eclampsia.[9]

In this mini-review, we focus on biomarkers and state-of-the-art diagnostic methods in pre-eclampsia that are also predictive of long-term CV risk, with a focus on their role in early intervention and prevention of CVDs. By integrating new advancements in omics technology, artificial intelligence (AI) and machine learning (ML), and molecular imaging, this review aims to provide insights into the most important indicators associated with long-term CV risk in women with pre-eclampsia.

2. Biomarker predictors of long-term CV risk

One of the most important indicators of a woman's long-term CVD is pre-eclampsia. A promising method for identifying CV health problems early on is biomarker profiling, which enables focused preventative measures.[10] Strong predictive value for CV outcomes has been shown by biomarkers, such as soluble fms-like tyrosine kinase-1 (sFlt-1), placental growth factor (PlGF), endothelin-1, and asymmetric dimethylarginine (ADMA). Endothelial dysfunction is reflected in elevated sFlt-1/PlGF ratios during pre-eclampsia,[11] whereas vascular injury and decreased nitric oxide generation are associated with ADMA. Particularly vulnerable are women who have severe pre-eclampsia or early-onset pre-eclampsia, as CV problems may appear 5 – 10 years after giving birth. These indicators' continuous to rise after pregnancy indicates ongoing inflammation, metabolic dysregulation, and endothelial damage, all of which greatly heighten the risk of CVDs. Early subclinical atherosclerosis and increased rates of hypertension, dyslipidemia, and diabetes are common in women with severe pre-eclampsia, and these conditions hasten the degradation of the CV system.[12] Crucially, the severity and timing of each biomarker during pregnancy should be taken into consideration when performing risk stratification. During and following pre-eclampsia, a number of new biomarkers/predictors have been identified (Table 1).

3. Emerging biomarkers in pre-eclampsia and CV risk

Our understanding of the pathophysiology of pre-eclampsia and its consequences on the CV system has radically transformed as a result of biomarker research. Angiogenic factors, metabolic indicators, and endothelial dysfunction markers are biomarkers that have shown promise in the early identification and risk classification of pre-eclampsia.[13,14] Below is a list of some significant novel biomarkers and descriptions of how they relate to CV health problems:

3.1. Metabolomics and lipidomics biomarkers

Metabolomics and lipidomics are emerging as groundbreaking methods for the early identification of CV health risks in pre-eclampsia, a pregnancy syndrome marked by hypertension and organ failure. Novel metabolic and lipid biomarkers that indicate the state of CV health have been discovered by advanced omics technologies.[15] Modern omics methods enable comprehensive lipid and metabolite profiling, revealing subtle biochemical alterations connected to the onset and progression of pre-eclampsia. Information regarding disrupted vascular and metabolic pathways can be obtained through metabolomics, including organic acids and amino acid derivatives, as well as specific lipids and lipid ratios.[16]

(i) Lipid peroxidation products. Pre-eclampsia is associated with increased levels of isoprostanes and malondialdehyde, which are indicators of oxidative lipid damage
(ii) Ceramides and sphingolipids. Atherosclerosis and the risk of CVD are linked to dysregulated lipid metabolism in pre-eclampsia.

By enabling early diagnosis, risk assessment, and customized treatment, these biomarkers may enhance maternal and fetal outcomes. Combining metabolomics and lipidomics with state-of-the-art analytical technologies, such as mass spectrometry and ML improves the predictive accuracy and mechanistic understanding of CV risks linked to pre-eclampsia.

Table 1. Categorization of pre-eclampsia biomarkers/predictors

Pre-eclampsia	During pre-eclampsia	After pregnancy
Biomarkers/Predictors	• Soluble fms-like tyrosine kinase-1 • Placental growth factor • Proteinuria • Blood pressure	• Persistent hypertension • Dyslipidemia • Insulin resistance • Increased body mass index (BMI)
Timing for assessment	Evaluated during pregnancy (when preeclampsia first appears or is diagnosed)	Evaluated after pregnancy (usually 6 weeks after delivery and during annual follow-ups)
Associated risk insight	Determines the severity and biomarker levels of women who are at immediate and long-term cardiovascular risk	Represents ongoing cardiovascular risk; changes might be brought on by interventions, aging, or changes in lifestyle
Intervention plan	• Enhanced maternal monitoring during pregnancy • Stabilization of blood pressure and proteinuria • Early delivery if necessary	• Lifestyle modifications (diet, exercise) • Pharmacological intervention (e.g., antihypertensives, statins) • Regular cardiovascular screenings
Instigation time	During pregnancy, immediately upon diagnosis of preeclampsia	Post-partum, beginning with the 6-week check-up and continuing with follow-ups every year afterward
Potential impact	Aids in predicting long-term risk and minimizing pregnancy problems	Focuses on preventing long-term cardiovascular disease and modifying risk

3.2. CV stress and myocardial injury biomarkers

Early detection and management of CV health risks in pre-eclampsia depend on biomarkers for CV stress and myocardial injury. Pre-eclampsia poses major risks to the mother's and the fetus's health and may have long-term CV consequences. Brain natriuretic peptide (BNP), high-sensitivity C-reactive protein (hs-CRP), and cardiac troponins are biomarkers that provide information on endothelial dysfunction, inflammation, and myocardial stress.

(i) N-terminal pro-B-type natriuretic peptide (NT-proBNP). The risk of heart failure is indicated by elevated NT-proBNP in pre-eclampsia, which implies myocardial strain.
(ii) Troponins. In situations of severe pre-eclampsia, cardiac troponins are investigated due to their capacity to detect myocardial damage.

These markers facilitate early identification, risk assessment, and tracking the evolution of disease, allowing for timely action to prevent adverse outcomes. Innovative diagnostic technologies that enhance the accuracy of biomarker assessment and offer real-time clinical decision support include point-of-care assays and advanced imaging modalities.[17] Improved CV health outcomes for pre-eclamptic women are made possible by predictive algorithms, tailored medicine approaches, and biomarker screening. This combination of biomarkers and technology highlights the potential for transformative advancements in CV risk management and maternal-fetal medicine.

3.3. Angiogenic and anti-angiogenic factors

Pregnancy-specific pre-eclampsia, which is marked by organ malfunction and hypertension, poses serious health risks to both the mother and the fetus. The vascular remodeling necessary for a healthy pregnancy depends on angiogenesis and anti-angiogenic factors, such as vascular endothelial growth factor and PlGF.[18] Because these variables are dysregulated and anti-angiogenic proteins such as sFlt-1 are elevated, pre-eclampsia is linked to endothelial dysfunction and reduced placental blood flow. Decreased PlGF and elevated sFlt-1 are early indicators of placental dysfunction, which is crucial to the pathophysiology of pre-eclampsia and reflects an imbalance between the two factors.[19] An anti-angiogenic condition linked to endothelial dysfunction and unfavorable outcomes in pre-eclampsia is also reflected by an elevated sFlt-1/PlGF ratio.[20] Elevated levels of soluble endoglin, a co-regulator of angiogenesis, have been connected to hypertension and vascular inflammation, making it a possible biomarker for identifying women who are at risk of CVDs. The sFlt-1/PlGF ratio has demonstrated usefulness in predicting CV problems after pregnancy and differentiating pre-eclampsia from other hypertensive disorders.

Early detection of anomalies in these biomarkers can lead to timely treatments and useful information regarding CV risks. Emerging technologies, such as sophisticated biosensors and point-of-care diagnostic tools, provide accurate quantification of angiogenic factors, aiding in risk assessment and customized treatments. Incorporating these indications into predictive algorithms may also improve outcomes for both mothers and fetuses and revolutionize the assessment of CV risk. Further investigations into new medicines that target these pathways to mitigate the long-term effects of pre-eclampsia on CV health are warranted.

3.4. Inflammatory and oxidative stress biomarkers

The disease's defining characteristics include systemic inflammation and elevated oxidative stress, which worsen

vascular endothelial dysfunction and increase the risk of CVDs. For risk assessment and early detection, reliable biomarkers associated with these processes must be found. Tumor necrosis factor-alpha, interleukins (IL-6, IL-8), and hs-CRP are important inflammatory indicators that reveal status about the chronic inflammatory milieu of pre-eclampsia.[21] At the same time, increased levels of oxidative stress indicators such as malondialdehyde, nitric oxide, endothelin-1, ADMA and 8-isoprostane, along with reduced antioxidant defenses, such as glutathione levels, indicate oxidative burden in affected individuals.[22]

The assessment of CV risk in pre-eclampsia could be revolutionized by predictive algorithms, advanced imaging, and innovative diagnostic techniques that utilize these indicators. By integrating these biomarker profiles with clinical data, healthcare providers can implement targeted interventions that will ultimately enhance maternal CV health outcomes and reduce long-term problems.

Monitoring indicators of endothelial dysfunction facilitates the assessment of the long-term CV risks in affected women. These biomarkers may be used to predict CV risks following childbirth and to shed light on systemic inflammation. Together, these biomarkers demonstrate the complex relationship between CV risks and pre-eclampsia, opening the door to focused prevention measures.

3.5. Exosomal, epigenetic, and proteomic biomarkers

Exosomal, epigenetic, and proteomic biomarkers have become novel approaches to address this problem. Cells release exosomes, which are nanovesicles containing bioactive substances, such as lipids, proteins, and RNA. These molecules reflect physiological parameters and offer information on pathological alterations. Placenta-derived exosomes contain proteins, lipids, and microRNAs that reveal the placenta's health and dysfunction.[23] Past research highlights the role that epigenetic changes play in the CV risks linked to pre-eclampsia:

(i) miRNAs or microRNAs. Circulating and specific miRNAs, including miR-210 and miR-155, control immunological and angiogenic pathways and serve as non-invasive biomarkers for pathophysiology, pre-eclampsia, disease development, and CV risk prediction. Examples of epigenetic biomarkers that shed light on gene-environment interactions that raise the risk of pre-eclampsia development include DNA methylation and histone modifications.

(ii) Proteomic signatures. By analyzing plasma proteomically, distinct protein signatures associated with endothelial dysfunction and CV risk have been found. Across the proteomics approach, dysregulated pathways and potential therapeutic targets can be revealed by identifying and quantifying proteins in biological materials.

Together, these biomarkers increase the precision of CV risk assessment in pre-eclampsia, enabling personalized treatment and early intervention. Their integration into clinical practice has the potential to revolutionize maternal healthcare by improving diagnosis accuracy and promoting creative preventative strategies for CV problems in pre-eclampsia.

4. From association to action: Translating knowledge into prevention and risk assessment

Pre-eclampsia is becoming more widely acknowledged as a risk factor for women's long-term CV health. A thorough framework for risk assessment and prevention is necessary to close the gap between this association and workable solutions. To convert scientific discoveries into significant public health results, this framework should include risk categorization, preventative measures, and healthcare integration.

(i) Risk stratifications. Utilizing clinical and biochemical indicators identified during pregnancy is essential for identifying patients at increased CV risk after pre-eclampsia.[24] Future risk can be inferred from markers including dysregulated lipid profiles, proteinuria, and high blood pressure. Categorization into high- and low-risk groups allows for customized post-partum treatment. Women who have severe or recurring pre-eclampsia, for example, may be deemed high-risk and require close observation.[25] Risk prediction models can be further improved by emerging biomarkers, such as angiogenic factors, such as PlGF and sFlt-1. Predictive analytics and electronic health records can expedite this stratification procedure, guaranteeing the early and methodical identification of high-risk individuals.

(ii) Preventative interventions. Specific prophylactic actions can reduce long-term CV morbidity in high-risk women. The cornerstone tactics are lifestyle changes, such as diet adjustments, exercise, and stress reduction. In accordance with individual risk profiles, pharmacological therapy with medications such as statins or antihypertensives can be used to treat chronic dyslipidemia or hypertension. Establishing routine CV screenings after pregnancy is also essential.[26] Early identification of endothelial dysfunction, subclinical atherosclerosis, and other risk factors for CVDs should be the main goal of such examinations. Effective implementation of evidence-

based therapies can be ensured by collaboration with specialized care teams and cardiologists.

(iii) Healthcare integration. Transforming information into practice requires the incorporation of CV follow-up measures into post-partum care programs. It is crucial to implement a systematic strategy that includes post-partum visits specifically aimed at CV health. Scheduled screenings, lifestyle counseling, and risk stratification evaluations may be included in these sessions. It is equally crucial to educate primary care physicians, midwives, and obstetricians on the long-term CV consequences of pre-eclampsia. Support at the policy level to guarantee the long-term viability of these integrated care models can improve results and compliance. Longitudinal monitoring can be further facilitated by telemedicine and digital health platforms, which can close gaps in treatment delivery and access.

5. Pragmatic approaches

Several pragmatic approaches are presented in the following:

(i) Proposed program of testing. It is crucial to evaluate CV risk factors, such as blood pressure, lipid profiles, and glucose tolerance as soon as possible after giving birth. The link between delivery and long-term CV health can be closed with annual physicals that concentrate on these metrics and the use of home blood pressure monitoring for early identification.

(ii) Financial implications. It is important to stress how cost-effective early detection and preventative care are. Evidence indicates that systematic post-partum follow-up could significantly lower the long-term financial burden associated with controlling CVDs, despite the initial investment appearing hefty. Existing post-partum care programs' insights highlight how feasible such a strategy is.

(iii) Implementation and oversight. For implementation to be successful, an integrated care route involving cardiologists, obstetricians, and primary care doctors is essential. To ensure fair access and participation, community health workers and digital health platforms can help with adherence and continuity of care.

(iv) Future trial. To validate these suggested therapies, extensive, long-term studies are necessary. Pre-eclampsia women's existing registries are a useful tool for obtaining sufficient samples and carrying out long-term follow-up research. Evidence-based guidelines would be informed by these trials, improving post-partum care and lowering long-term CV morbidity in this susceptible group.

6. Practical applications for post-partum care

This section describes useful applications that close the knowledge gap between science and practical application, with an emphasis on enhancing post-partum care for women who have previously experienced pre-eclampsia.

(i) Home blood pressure monitoring. All women with pre-eclampsia who receive a home blood pressure monitor are better equipped to monitor their CV health on their own. Accurate readings are guaranteed and early hypertension detection can be realized through sufficient training on correct usage and interpretation. By providing prompt medical advice based on patient-reported data, telehealth services can further complement this strategy.

(ii) Post-partum care packages. Long-term CV health can be improved with a structured care package designed for pre-eclamptic women. These packages ought to contain structured physical activity suggestions, mental health support, and dietary instructions that prioritize cardioprotective diets and lower sodium intake. Regular check-ins can be made easier with the help of telehealth services, guaranteeing adherence and quickly resolving new issues.

(iii) Community-based follow-up programs. Collaborations with local healthcare professionals provide regular and easily accessible follow-up care. Programs could consist of community support groups, educational courses, and recurring health evaluations. These programs encourage participation and remove obstacles to care, especially for marginalized groups.

7. Innovative diagnostic tools

7.1. Point-of-care testing (POCT)

A major development in early diagnostic techniques is POCT, which provides quick and accurate identification of biomarkers linked to pre-eclampsia and CV health risks. Real-time evaluation of vital parameters, such as blood pressure, proteinuria, and new biomarkers, such as PlGF and sFlt-1 is made possible by POCT equipment, which are portable and easy to use.[27] Previous research has demonstrated the effectiveness of POCT in environments with low resources, where traditional laboratory infrastructure might not be available.

To mitigate maternal and fetal problems, for example, the incorporation of POCT for sFlt-1/PlGF ratio testing has shown promise in stratifying the severity of pre-eclampsia. POCT promotes personalized medicine methods by promoting early detection and intervention, which improves outcomes for women who are at risk of pre-eclampsia and related CV disorders.[27] POCT will

become more useful in the future as biosensor technology and digital health integration advance, guaranteeing prompt, precise, and easily accessible diagnostics for maternal healthcare.

7.2. AI and ML

With their potential for early detection and risk assessment of CV problems linked to pre-eclampsia, AI and ML have become revolutionary tools in the healthcare industry. For prompt intervention, CV risks must be identified early. Large and complex datasets, such as biochemical, genetic, proteomic, clinical biomarker, and imaging data, can be analyzed with algorithms driven by AI and ML to predict the development and course of pre-eclampsia. Furthermore, integrating wearable health monitoring technology with AI-powered platforms improves the monitoring of blood pressure and heart rate variability in real-time, resulting in a personalized risk profile. AI models that take into account biomarker levels, blood pressure trends, and maternal history have shown superior predicted accuracy.[28] In addition, ML models, such as random forests and support vector machines have shown excellent accuracy in predicting hypertensive disorders during pregnancy by analyzing patient history and physiological data.[29] These tools outperform traditional statistical methods by detecting non-linear patterns and interactions between variables. AI and ML developments have the potential to revolutionize pre-eclampsia care by enabling the early detection of CV risks, enhancing maternal-fetal outcomes, and reducing medical expenses.

7.3. Advanced imaging techniques

Cutting-edge imaging methods have become essential for the early detection and risk assessment of CV problems in pre-eclampsia. In patients with pre-eclampsia, early indicators of CV dysfunction are detectable using methods including echocardiography (ECG) and cardiac magnetic resonance imaging (MRI). For example, Doppler ultrasound is frequently used to evaluate anomalies in uteroplacental blood flow, which are early markers of pre-eclampsia and its development into CV disorders.[5] In addition, high-resolution insights into the placental function and structural alterations linked to pre-eclampsia have been demonstrated using MRI.[30] Furthermore, a thorough assessment of the hemodynamic changes and cardiac remodeling in afflicted patients is made possible by three-dimensional ECG.

Recent advancements, such as the integration of computed tomography angiography for vascular imaging, have enhanced the precision of identifying endothelial dysfunction and vascular stiffness, which are both critical biomarkers of long-term CV risks. Innovations in imaging-based AL algorithms further enable predictive modeling, improving early detection and personalized management strategies. Collectively, these advanced imaging techniques offer a non-invasive, reproducible, and highly sensitive approach for identifying CV risks in pre-eclampsia, significantly improving maternal and fetal outcomes.

7.4. Wearable health technology

In the early detection of CV health problems, wearable health technology has become a game-changer, especially in circumstances, such as pre-eclampsia. These cutting-edge tools make it easier to continuously monitor physiological indicators and provide real-time data that helps identify risk biomarkers. Wearable sensors have been shown to be useful in monitoring heart rate variability and blood pressure.[31] Furthermore, improvements in non-invasive biosensors have made it possible to assess circulating biomarkers, such as sFlt-1 and PlGF, improving predictive powers.

ML algorithms have been incorporated into smart wearable devices, such as smartwatches and patches, to forecast the course of diseases and send out tailored notifications. A recent analysis showed that by identifying minute hemodynamic changes weeks before clinical symptoms, wearable devices could enhance early diagnosis and treatment results.[31] This demonstrates how wearable technology may help prevent long-term CV problems by changing the management of pre-eclampsia from reactive to proactive. When paired with developments in biomarker research, these technologies constitute a major breakthrough in the treatment of problems related to maternal and fetal health.

8. Conclusion

A paradigm change in maternal healthcare is provided by the early detection of CV health risks in pre-eclampsia through the development of biomarkers and creative tools, which also present new chances for risk prediction and appropriate risk management. Combining these indicators with cutting-edge diagnostic technologies, such as wearable technology and ML algorithms, has the potential to completely transform personalized medicine methods in CV and maternal-fetal health. To ensure prompt and efficient therapy of pre-eclampsia and associated CV after-effects, translational efforts are crucial in bridging the gap between research and clinical practice.

The integration of multi-biomarker panels, advanced diagnostic tools, and AI-driven technologies holds promise for revolutionizing the management of pre-eclampsia. Further research is required to: (i) validate

novel biomarkers in large, diverse populations, and explore their potential to guide targeted interventions; (ii) enhance the accessibility and affordability of innovative tools in low- and middle-income countries; and (iii) investigate the long-term CV outcomes in women with a history of pre-eclampsia using advanced diagnostics.

Acknowledgment

The authors acknowledge the ongoing efforts in research and clinical advancements in maternal and CV health.

Funding

None.

Conflict of interest

The authors declare no competing interests.

Author contributions

Conceptualization: All authors
Writing–original draft: Abhishek Gupta
Writing–review & editing: All authors

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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CASE SERIES

Persistent smell and taste disorders following COVID-19 vaccination: A report of three cases and review of the literature

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Abstract

Persistent smell and taste disorders following COVID-19 vaccination are rare adverse effects. Herein, we reported three cases in which patients developed smell and taste disorders 9 – 20 days after receiving their second dose of the AstraZeneca/Oxford COVID-19 vaccine in 2021. These symptoms persisted for 1 – 3 years. All patients underwent nasal endoscopy, imaging of the nasal and olfactory structures, as well as Sniffin' Odor along with flavor and taste identification tests. Case 1 was a 37-year-old male who presented in December 2022 with persistent dysgeusia for 18 months. Case 2 was a 40-year-old male who presented in February 2023 with persistent anosmia and parosmia for 20 months. Case 3 was a 48-year-old male who presented in August 2024 with persistent hyposmia for 3 years. These persistent disorders may be due to immune responses triggered by the vaccine, potentially affecting the olfactory neuroepithelium. Recognition and reporting of such adverse effects are important to acknowledge among physicians and for future studies and treatment trials targeting related disorders.

Keywords: COVID-19 vaccine; Anosmia; Ageusia; Parosmia; Dysgeusia

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Citation: Hamed SA, Ahmed AEMAA, and Ahmed MAA. Persistent smell and taste disorders following COVID-19 vaccination: A report of three cases and review of the literature. *INNOSC Theranostics and Pharmacological Sciences*. 2025;8(3):101-106.
doi: 10.36922/ITPS025140020

Received: April 03, 2025

Revised: May 29, 2025

Accepted: May 30, 2025

Published online: June 17, 2025

1. Introduction

Several vaccines were rapidly developed and approved to combat COVID-19 in the early stages of the pandemic. The common and worldwide distributed vaccines included Pfizer-BioNTech, Moderna, AstraZeneca/Oxford, and Johnson and Johnson (J and J or Janssen). The Pfizer-BioNTech and Moderna vaccines are messenger RNA (mRNA) delivered to host cells to express the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) spike protein, which is used by the virus to gain entry into host cells. In contrast, the AstraZeneca/Oxford and Janssen vaccines are viral vectors encoding the SARS-CoV-2 spike protein. The expression or delivery of spike protein into the host cells by the vaccines will elicit an immune response, production of antibodies against the spike protein, activation of T-cells, and generation of memory cells to combat future SARS-CoV-2 infection, thereby preventing viral manifestations or decreasing their severity and duration.

Several studies have documented the significant contribution of COVID-19 vaccines in the reduction of morbidity and mortality.[1] However, as with other vaccines, adverse

effects have been reported. Common adverse effects such as fever, fatigue, muscle soreness, malaise, chills, arthritis, and headache occur more frequently than the otolaryngological ear, nose, and throat (ENT) effects, which include anosmia/hyposmia, ageusia/hypogeusia, parosmia, phantosmia, and dysgeusia. According to the COVID-19 vaccine analysis report released in the UK, there were 70 cases of anosmia, 58 parosmia, and six hyposmia out of the 100,809 recipients of the Pfizer-BioNTech vaccine;[2] 802 cases of anosmia, parosmia, and hyposmia among 25 million recipients of the AstraZeneca/Oxford vaccine; and 402 cases of parosmia were recorded among 842,270 recipients of the AstraZeneca vaccine.[3] Studies have suggested a higher incidence of ENT adverse effects with mRNA vaccines compared to viral vector vaccines (78.3% vs. 70.4%, p=0.064), while systemic adverse effects were more common with viral vector vaccines (87.2% vs. 61%, p=0.001).[4] Some studies reported a higher frequency of ENT adverse effects with the Oxford-Pfizer-BioNTech (BNT162b2) vaccine than with the Moderna COVID-19 vaccine (mRNA-1273) (Ageusia: 0.755% versus 0.608%, p=0.001; Dysgeusia: 0.681% versus 0.486, p=0.001).[5] Most of these reports estimated that the time elapsed from administration of the COVID-19 vaccine to the development of smell and taste disorders ranged from 1 to 20 days (mainly 1 – 9 days). They also reported favorable outcomes with complete resolution of manifestations within 4 – 8 weeks.[4,5] However, to date, there are six published case reports of long-term or persistent smell and taste disorders.[6-11]

Herein, we reported three Egyptian cases with post-COVID-19 vaccine olfactory and gustatory disorders. Vaccination was obligatory in their workplace. Smell and taste disorders developed within days following administration of the second dose of the AstraZeneca/Oxford vaccine in 2021. In some cases, these disorders persisted for years. Repeated nasal swabs and polymerase chain reaction (PCR) tests for SARS-CoV-2 were negative at symptom onset, excluding the possibility of COVID-19 infection. There was no history of comorbid medical, neurological, or other conditions that might cause smell and taste disorders, such as allergies, infections, sinusitis, trauma, dry mouth, medications, and specific treatments. Multiple ENT consultations were performed. Nasal endoscopy, computed tomography of the nasal cavities, anterior cranial fossa, and sinuses, and magnetic resonance imaging (MRI) of the brain, including olfactory bulbs and tracts, revealed no abnormalities. They presented to the ENT outpatient clinic because of persistent disorders lasting several years after the initial onset. Clinical evaluation at the presentation included neurological evaluation and objective testing of olfactory and gustatory function using Sniffin' Odor' identification test (SOIT), taste, and flavor identification tests, as previously described.[12,13]

2. Case presentation

2.1. Case 1

A 37-year-old male developed anosmia and ageusia 9 – 10 days after receiving the second dose of the AstraZeneca/Oxford vaccine (June 2021, administered in Egypt). No systemic manifestations were reported after vaccination. After 3 months, parosmia and dysgeusia (described as smoked or burned-out smell and taste) developed and resulted in weight loss, depression, and insomnia. Improvement in smell and taste were reported several months after onset, along with resolution of parosmia. Past medical history included a confirmed COVID-19 infection in May 2020, verified by a positive PCR test for SARS-CoV-2. Viral manifestations included fever, fatigue, cough, expectoration, and loss of smell and taste, all of which resolved completely within 30 days. Presentation in December 2022 was due to persistent severe dysgeusia for 18 months. Sniffin'odor, flavor, and taste identification tests revealed intact smell, flavor, and taste sensations.

2.2. Case 2

A 40-year-old male developed anosmia 20 days after receiving the second dose of the AstraZeneca/Oxford vaccine (June 2021, administered in Saudi Arabia). Parosmia (described as a rotten odor) developed 4 months later. No systemic manifestations were reported after vaccination. Initial treatment included local steroids and vitamin B complex. No history of prior COVID-19 infection was documented. Only minimal improvement in olfactory function was reported over the past 2 years. Presentation in February 2023 was due to persistent anosmia and severe parosmia for 20 months. The SOIT score showed anosmia with a result of 4 out of 16. Both flavor and taste sensations remained intact.

2.3. Case 3

A 48-year-old male developed anosmia 10 days after receiving the second dose of the AstraZeneca/Oxford vaccine (August 2021, administered in Kuwait). A history of myalgia and fatigue after vaccination was reported, which resolved completely within 1 week. Two previous episodes of COVID-19 infection were documented. The first episode occurred in February 2020 and was manifested by fever, flu-like symptoms, myalgia, cough, and loss of smell and taste. The second episode occurred in January 2021 and presented with fever, myalgia, fatigue, flu-like symptoms, and cough. Viral manifestations in both episodes resolved completely within 7 – 15 days. Presentation in August 2024

Table 1. Case reports of persistent smell and taste disorders following COVID-19 vaccination

References	Causative vaccine	Onset after vaccination	Manifestations	Persistent manifestations and duration	Previous COVID-19 infection and course
Zamzami *et al.*[6]	Second dose of the AstraZeneca/Oxford vaccine (August 2021)	7 days	- A 38-year-old male - Sudden onset of parosmia (unpleasant smoke-like odor) - No smell or taste loss - Treatment included oral and nasal steroids, omega-3 supplement	Parosmia for more than 4 months	- Yes - Smell and taste loss - Complete resolution within 10 – 12 days
Fantin *et al.*[7]	First dose of the AstraZeneca/Oxford vaccine	2 days	- A 76-year-old male - Hyposmia, dysgeusia, parosmia, left aural fullness, and tinnitus - Treatment included nasal steroids, multivitamins, and olfactory training - MRI showed mild atrophy of the olfactory bulbs	Hyposmia for more than 3 months	N/A
Ogata *et al.*[8]	Second dose of the Pfizer-BioNTech vaccine	1 day	- A 70-year-old Japanese man - Acute onset of weakness and paresthesia in all four limbs, impaired proprioception, sensory ataxia (acute inflammatory demyelinating polyneuropathy, more commonly known as GBS), tongue paresthesia, and dysgeusia (bitter taste sensation) - Normal smell, sweet, sour, and salt sensation - Seronegative for post-infective GBS antibodies - Partial improvement of dysgeusia and motor and sensory manifestations with corticosteroids, not IVIG. - The authors suggested that the improvement with steroids, rather than IVIG, might be due to that the mechanism of GBS after COVID-19 vaccination is related to immune-mediated inflammation rather than molecular mimicry	Dysgeusia for several months	No
Barter and Bagnato[9]	Johnson and Johnson (Janssen) vaccine	3 weeks	- A 39-year-old male - Phantosmia (burning or smoke odor): Initially daily for~1 h, then decreased in duration, frequency, and intensity over 11 months (1 to 2 times per week)	Phantosmia for more than 21 months	No
Shin and Tam.[10]	First dose of the Moderna vaccine (March 2021)	Several days	- A 74-year-old male - Hyposmia and dysgeusia - History of ESKD (on hemodialysis), peripheral neuropathy, hypertension, hyperlipidemia, atrial fibrillation, cardiomyopathy, heart failure, and cerebrovascular stroke. - Prior dysgeusia improved with dialysis (pre-pandemic) - Post-vaccine hyposmia and dysgeusia did not improve with dialysis - Normal blood glucose, zinc, vitamin B12, thyroid function, and Sjogren's disease workup	Hyposmia and dysgeusia for more than 2 years	No

(Cont'd...)

Table 1. ***(Continued)***

References	Causative vaccine	Onset after vaccination	Manifestations	Persistent manifestations and duration	Previous COVID-19 infection and course
Keir *et al.*[11]	Second dose of the Pfizer's vaccine	Several days	- A 57-year-old female - Constant phantosmia and hyposmia (smoke smell) - MRI showed edema of olfactory bulbs and tracts, clumping of olfactory nerve filia (suggestive of inflammation) - No history of parosmia or taste loss	Phantosmia and hyposmia	No

Abbreviations: ESKD: End-stage kidney disease; GBS: Guillain–Barré Syndrome; IVIG: Intravenous immunoglobulins; MRI: Magnetic resonance imaging.

was due to persistent hyposmia for 3 years. The SOIT score was 9 out of 16, indicating hyposmia. Both the flavor and taste sensations remained intact.

3. Discussion

In this study, three adult males were reported to have developed smell and taste disorders between 9 and 20 days after the second dose of the AstraZeneca vaccine was administered in 2021. Persistent disorders, including dysgeusia, anosmia, parosmia, and hyposmia, lasting 1 – 3 years were observed. A temporal relation between the vaccination and the development of the disorders, together with repeatedly negative nasal swabs and PCR tests for SARS-CoV-2 and the exclusion of alternative causes, further confirms that these disorders were adverse effects of the vaccine. To date, only six case reports have been published describing persistent smell and taste disorders, which lasted more than 3 months to over 3 years following COVID-19 vaccination. These disorders included parosmia, hyposmia, dysgeusia, and phantosmia[6-11] (Table 1).

The pathogenic mechanisms of post-COVID-19 vaccine smell and taste disorders have not yet been understood and remain speculative. Some mechanisms have been hypothesized: (1) It has been proposed that the vaccine may have similar effects to the attachment of SARS-CoV-2 to the olfactory epithelium and perivascular angiotensin-converting enzyme type 2 receptors, causing inflammation of the olfactory neuroepithelium. This has been supported by findings of olfactory edema, blocked olfactory clefts, and clumping of olfactory filia in some patients with post-vaccination anosmia.[7,14] However, this mechanism has not been considered applicable because most cases had normal MRI at the acute condition. (2) It has been suggested that the humoral immune response triggered by the spike protein induced by the mRNA vaccine may directly damage the olfactory neuroepithelium without replication of the virus.[15] It has been observed that most patients developed the disorders after the second dose of the vaccine. Studies have observed that inactivated viral and viral vector-based vaccines induced a stronger immune response after the second dose compared to the weak cellular immune responses against spike protein after the first dose of the vaccine, which has been considered compatible with an antibody-dependent enhancement mechanism.[15] (3) It has been suggested that the local expression of the spike protein after vaccination and its interaction with the α7 nicotinic acetylcholine receptors in macrophages may cause deregulation of the cholinergic pathway, release of proinflammatory cytokines, and activation of the inflammatory reflex. Signals could be produced and transmitted via neural pathways from the local injection site to the distant one.[16,17] (4) It has been suggested that these disorders may be due to demyelination of the chemosensory nerves. Demyelination of the central and peripheral nervous systems may be triggered by the vaccines.[8,18] (5) Reduction in olfactory bulb volume has been suggested as a cause of persistent post-COVID-19 vaccine anosmia/hyposmia.[7,19] In support of this, a reduction in the volume of the olfactory bulbs has been reported in patients with persistent post-COVID-19 olfactory disorders.[20] (6) Activation of COVID-19 infection by the vaccine in asymptomatic carriers has been recommended. However, this suggestion is not currently applicable because all reported cases demonstrated negative repeated PCR and repeated nasal swabs for SARS-CoV-2 at the onset of the conditions, which ruled out COVID-19 infection as a cause of smell and taste disorders.

4. Conclusion

Persistent smell and taste disorders may occur as complications following post-COVID-19 vaccination. Reports of COVID-19 vaccine-related smell and taste adverse effects should not be ignored and must be recognized by otolaryngologists and different medical specialties, including neurologists. These adverse effects should also be considered in future studies on vaccine

complications and in clinical trials targeting persistent post-COVID-19 vaccine disorders.

Acknowledgment

None.

Funding

None.

Conflict of interests

The authors declare that they have no competing interests.

Author contributions

Conceptualization: All authors
Investigation: All authors
Writing – original draft: All authors
Writing – review & editing: All authors

Ethics approval and consent to participate

This study was done in accordance with the revised Helsinki Declaration (2013) and its amendments and approved by the local ethics committee of Assiut University Hospitals, Assiut, Egypt (ID: AUFM_COVID_00020).

Consent for publication

We declare that we have obtained written informed consent from all patients for releasing their data and the results of their evaluations in this paper.

Availability of data

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

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ACCSCIENCE PUBLISHING

INNOSC Theranostics and Pharmacological Sciences

SHORT COMMUNICATION

In silico analysis for drug repurposing in cholesteatoma: A novel approach to address an unmet medical need

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Abstract

Cholesteatoma presents a significant clinical challenge with limited pharmaceutical treatment options. This paper aims to explore the potential of drug repurposing for managing cholesteatoma through advanced bioinformatics analysis of high-throughput genetic data. For this purpose, we conducted a systematic search of published high-throughput genetic studies related to cholesteatoma and used functional and literature enrichment analysis of multiple sets, a validated web-based bioinformatics platform, for analysis. We employed common pathway enrichment analysis and cross-referenced data from DrugBank and gene list automatically derived for you drug annotations to identify potential medical treatments. Our analysis covered eight high-throughput genetic studies, with extended gene lists available for five of them. Enrichment analysis identified common pathways, including matrix metalloproteinases, interleukins, apoptosis, and the phosphoinositide 3-kinase-AKT pathway, shedding light on both expected and less-studied aspects of cholesteatoma pathogenesis. In addition, the analysis proposed several medications, including anti-tumor necrosis factor-α (TNF-α), zinc, and marimastat, as potential treatments. In conclusion, drug repurposing is a potential cost-effective approach to address the unmet medical need for cholesteatoma management. The identified medications, especially anti-TNFα and zinc, offer promising options. Given the limited research funding in this field, this bioinformatic approach holds great promise, highlighting specific molecular pathways that hold the greatest potential to be implicated in the pathogenesis of cholesteatoma and offering a faster route for future trials to reduce cholesteatoma recurrence.

Keywords: Cholesteatoma; Enrichment pathways; Drug repurposing; Bioinformatics platform

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Citation: Vlastos IM, Almomani M, Hajiioannou J, Drimalas N, Gkouskou K. *In silico* analysis for drug repurposing in cholesteatoma: A novel approach to address an unmet medical need. *INNOSC Theranostics and Pharmacological Sciences*. 2025;8(3):107-112. doi: 10.36922/itps.2571

Received: December 29, 2023

Revised: February 17, 2024

Accepted: March 20, 2024

Published online: April 11, 2025

1. Introduction

Cholesteatoma, a keratinizing epithelial lesion within the middle ear, poses a significant clinical challenge due to the lack of pharmaceutical treatments. At present, surgical

intervention remains the primary strategy for managing cholesteatoma, but it is far from ideal. Recurrence rates, soaring up to 40%, continue to be a challenging problem and the invasive nature of surgery places the structures of the temporal bone at risk.[1-3] This underscores the pressing need for a more effective, non-invasive medical approach to cholesteatoma management.

The pharmaceutical industry's prioritization of drug development has left cholesteatoma largely overlooked. To date, there are no investigational drugs specifically designed for this condition. However, signs of hope emerge through the field of drug repurposing, also known as drug repositioning. This innovative approach seeks to identify new therapeutic applications for existing drugs, whether approved for other indications or previously failed in clinical trials.[4] By investigating this unexplored opportunity in the field of pharmaceuticals, we may uncover a medical treatment that could revolutionize cholesteatoma management.

Even though the cholesteatoma's pathophysiology remains largely unclear, a surge in genetic research, driven by high-throughput techniques, has yielded a wealth of data. Thousands of gene expression profiles and non-coding microRNAs have been studied.[5] Yet, the underlying mechanisms of cholesteatoma development and progression remain largely unknown.[1,2] At this point, bioinformatics, based on the advances of computer, have the potential to make sense of all this genetic information.

In this regard, this study aims to utilize advanced bioinformatics to carefully examine the extensive genetic data accessible for cholesteatoma. By thoroughly exploring this abundance of information, our goal is to identify the likely molecular pathways responsible for cholesteatoma formation, progression, and recurrence. Armed with these insights, we seek to propose the repurposing of drugs associated with these identified pathways. This interdisciplinary approach merges genetics, bioinformatics, and pharmacology, offering a promising avenue for cholesteatoma treatment innovation.

2. Methods

Published high-throughput genetics studies were searched in the PubMed database. Genes or other genetic factors that can be retrieved by these publications and their corresponding databases were then introduced in functional and literature enrichment analysis of multiple sets (FLAME), a novel validated web bioinformatics platform, which is freely available to public.[6,7] FLAME excels in the simplicity of use, providing extensive flexibility in importing and managing multiple lists, executing advanced state-of-the-art functional enrichment analysis pipelines, and merging and prioritizing results through sophisticated interactive visualizations. In our study, FLAME was utilized to correlate gene lists at a network level, leveraging resources such as aGOtool, g:Profiler, WebGestalt, and EnrichR. Common enrichment pathways between the high-throughput cholesteatoma genetic studies were retrieved by utilizing the aforementioned tools. The number of times a pathway was found, and the higher enrichment scores were extracted. In addition, the option to enrich the provided gene lists from DrugBank and gene list automatically derived for you (GLAD4U) drug annotations were chosen to find potential medical treatment options. The statistical significance of the combined results is estimated by applying Fisher's combined probability test represented by X^2 and combined P-value metrics for each enrichment term identified by two or more tools.

Studies on specific genetic factors and pathways were excluded from the analysis since the aim of this *in silico* analysis is to enrich current knowledge, or in other words, to suggest possible new molecular targets in cholesteatoma research and treatment.[8]

3. Results

Of the nine high-throughput genetic studies that were identified, extended gene lists were available in six.[8-13] In three studies,[14-16] a full list of genetic factors could not be retrieved in the published paper and after contacting the corresponding author.

Overlapping enriched that can be found in at least two studies is shown in Table 1.

Drugs or substances proposed following this *in silico* analysis as potential robust modulators of the enriched metabolic pathways are shown in Table 2.

4. Discussion

In the present study, we focused on utilizing high-throughput studies as they provide a multifaceted set of genetic data. This approach was chosen because studies analyzing only a few genes may introduce selection bias and may not require advanced bioinformatic techniques to reveal potential pathways. By employing high-throughput data, we aimed to reduce the risk of bias and gain a more complete understanding of the genetic factors associated with cholesteatoma.

The limitation of this analysis is that we primarily relied on expression studies. While these studies offer valuable insights, they cannot definitively establish whether the altered gene expression is a primary cause or a secondary effect of cholesteatoma. Consequently, it remains uncertain

Table 1. Enriched pathways found in common in at least two studies

Pathways	$-\chi^2$	log10 (*p*-value)
Pathways common in four studies		
Matrix metalloproteinases and their activation	4.25E−06	5.37
PI3K-Akt signaling pathway*	0.00000446	5.35
Apoptosis	7.12E−06	5.15
Collagen degradation	1.12E−05	4.95
Degradation of extracellular matrix	1.24E−05	4.91
Extracellular matrix organization	7.48E−05	4.13
Vitamin D receptor pathway	0.0017	2.77
Cell surface interactions at vascular wall	0.00629	2.2
Pathways common in three studies		
Innate immune system	1.23E−10	9.91
Metal sequestration by antimicrobial proteins	2.27E−06	5.64
Antimicrobial peptides	0.000133	3.88
Formation of cornified envelope	0.0268	1.57
Pathways common in two studies		
IL-18 signaling pathway	2.2E−08	7.66
Keratinization	5.71E−07	6.24
Metabolism of lipids	6.62E−07	6.18
CCKR signaling map ST	1.09E−06	5.96
Cytokine signaling in immune system	3.22E−06	5.49
Neutrophil degranulation	4.33E−06	5.36
Signaling by interleukins	1.12E−05	4.95
Interleukin-4 and interleukin-13 signaling	1.23E−05	4.91
IL-1 and megakaryocytes in obesity	9.09E−05	4.04
Focal adhesion	0.000217	3.66
Toll-like receptor 4 (TLR4) cascade	0.000559	3.25
Lung fibrosis	0.000873	3.06
Cell junction organization	0.00219	2.66
Signaling by receptor tyrosine kinases	0.00568	2.25
Signaling by nuclear receptors	0.0103	1.99
Type I hemidesmosome assembly	0.0193	1.71
Estrogen signaling pathway	0.0209	1.68
MyD88:MAL (TIRAP) cascade initiated on plasma membrane	0.0248	1.61
Fibronectin matrix formation	0.028	1.55
Collagen formation	0.0374	1.43

*The only pathway found in common in five studies.
Abbreviation: IL: Interleukin.

whether the identified pathways are directly implicated in the disease's pathogenesis or are simply induced by the inflammation associated with cholesteatoma.

Table 2. Potential useful substances for the cholesteatoma management proposed by our primary *in silico* analysis

Study	Source	Function	-log10 (*p*-value)
Lee *et al.*[12]	DRUGBANK	Zinc	5.1
	GLAD4U_DRUG	Collagenase	4.87
	GLAD4U_DRUG	Protease inhibitors	4.55
	GLAD4U_DRUG	Papain	4.53
	GLAD4U_DRUG	Ustekinumab	4.12
	GLAD4U_DRUG	Alteplase	3.87
	GLAD4U_DRUG	Urokinase	3.83
	GLAD4U_DRUG	Interleukin inhibitors	3.8
	DRUGBANK	Calcium	3.67
Klenke *et al.*[13]	GLAD4U_DRUG	Infliximab	4.95
	GLAD4U_DRUG	Heparins or heparinoids for topical use	4.87
	GLAD4U_DRUG	Heparin	4.87
	GLAD4U_DRUG	Nystatin	3.92
	GLAD4U_DRUG	Other hormones	3.89
	DRUGBANK	Zinc	3.85
	GLAD4U_DRUG	Etanercept	3.82
Kwon *et al.*[15]	DRUGBANK	Marimastat	6.7
Tokuriki *et al.*[10]	GLAD4U_DRUG	Interleukin inhibitors	15.65
	GLAD4U_DRUG	Heparins or heparinoids for topical use	5.13
	GLAD4U_DRUG	Collagenase	4.56
	DRUGBANK	Marimastat	3.4
	GLAD4U_DRUG	Other hormones	3.13
	GLAD4U_DRUG	Protease inhibitors	3.43

Note: The study of Yoshikawa *et al.*[8] was the only high-throughput study targeting specific cells (fibroblasts) of cholesteatoma. An extensive list of potential therapeutic agents was retrieved by our analysis. Only common agents with the rest of the studies are reported here.
Abbreviation: GLADU: Gene list automatically derived for you.

Nevertheless, certain findings, such as the involvement of matrix metalloproteinases (MMPs) and interleukin (IL) pathways, which have been previously observed in cholesteatoma research, are not unexpected from this analysis. Several published studies since the middle 1990s indicate that MMPs could play an active role in the molecular mechanisms of cholesteatoma invasion into the temporal bone.[17] Since then, animal models,[18] *in vitro*

cultures[19], and direct expression studies on cholesteatoma tissues[20-23] have been utilized to decipher the complex molecular interplay of proinflammatory pathways in relation to metalloproteins, osteoclasts activation, and bone resorption. Most of the above indicative references conclude that various MMPs are related to disease aggressiveness or recurrence. There are indications of dysregulation of local immune status in cholesteatoma, as suggested by the expression studies of several ILs and other cytokines[24,25] or the study of various signaling pathways such as those involving triggering receptor expressed on myeloid cells-2 and toll-like receptor 4.[18] Nevertheless, a partial understanding of the proinflammatory mediators and MMPs in bone resorption has been achieved, as indicated by, for example, the conflicting results published regarding the promising role of the RANKL/orthopantomogram pathway.[26,27]

In addition, our approach enriched less well-studied pathways of cholesteatoma pathogenesis, such as apoptosis and the PI3K-AKT pathways and provided some less-expected insights, such as the finding of interactions at vascular wall. There is an increasing body of evidence that epithelial keratinocytes in cholesteatoma are protected against apoptosis.[28-30] There are also some indications of the presence of drivers of angiogenesis,[31] although the exact role of these factors in the perimatrix of cholesteatoma is largely unknown. The notable aspect of these studies is that they provide a molecular framework and do not dispute common mechanical theories of cholesteatoma formation, such as the retraction pocket mechanical theory.

Our analysis also led to the identification of medications that either have previously been suggested for cholesteatoma treatment, such as anti-tumor necrosis factor α (TNFα), or are unexpected, such as zinc or heparin. Notably, there is a case report suggesting the potential effectiveness of anti-TNFα in cholesteatoma management.[32] TNFα, a multifunctional proinflammatory cytokine implicated also in lipid metabolism, coagulation, insulin resistance, and endothelial function, seems to be involved in several of the proposed molecular pathways extracted from our analysis.

In addition to these findings, our analysis proposed other substances, such as zinc and marimastat, which hold promise in cholesteatoma treatment. Of note is that the bioinformatic programs utilized here for the discovery of potential medical treatments are well-known and freely available, but they do not utilize artificial intelligence for the elaboration of the expanding knowledge at molecular level. At present, several companies are developing their own proprietary algorithms for drug repurposing and have already provided important results in other diseases. Here, a primary reading of the suggested molecular pathways can lead to the proposal of various other medications such as duvelisib, an anti-PIK3 medication. This inhibitor has been suggested previously,[3] since the PI3K-Akt signaling pathway enriched by our method has also been proposed in cholesteatoma pathogenesis in a separate study focusing only on molecules involved in this pathway.[28]

This information is particularly valuable given the limited research funding allocated to cholesteatoma. The discovery of a dedicated medical treatment for cholesteatoma within the next few years is unlikely. However, drug repurposing offers an efficient and cost-effective pathway toward this goal. Relying on drug repositioning for the development of cholesteatoma management strategies is a pragmatic approach, considering the challenging research landscape.

Furthermore, some of the medications identified in our analysis could potentially be applied topically during initial treatment to assess their impact on reducing cholesteatoma recurrence. For example, incorporating zinc into materials used in cholesteatoma procedures, such as bone-alive or gelfoam, could serve as a practical trial to evaluate its effectiveness in reducing recurrence rates.

Finally, our study highlighted the utility of FLAME, a validated open-source program capable of integrating complex genetic data. While FLAME's application extends to various diseases, it particularly proves invaluable in the context of cholesteatoma, as demonstrated in our research. This tool has the potential to advance our understanding of the genetic underpinnings of the pathological condition and may have broader applications in medical research beyond cholesteatoma.

5. Conclusion

Functional enrichment analyses are particularly promising for underfunded research areas, as they can suggest potential avenues for drug repurposing. Our study highlights the value of high-throughput approaches and the integration of bioinformatic tools in elucidating cholesteatoma pathogenesis, both by confirming known pathways and identifying novel therapeutic targets.

Acknowledgment

We would like to thank G. Pavlopoulos for his valuable comments on FLAME bioinformatic tool.

Funding

None.

Conflict of interest

The authors declare no conflict of interest.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author upon reasonable request.

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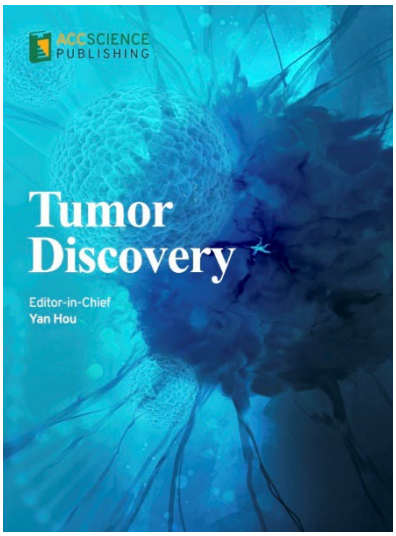

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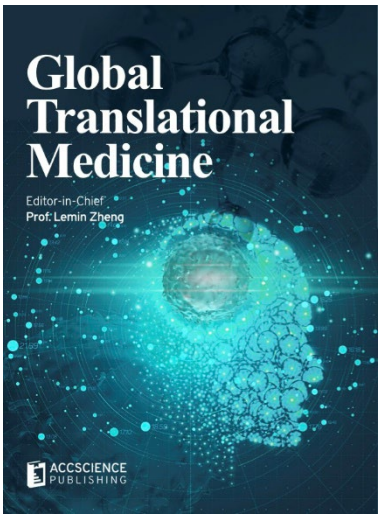

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