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REVIEW

CURRENT ADVANCES AND FUTURE VISION OF DRUG DELIVERY SYSTEMS FOR CANCER

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ABSTRACT: conventional cancer treatments often damage both tumour and healthy cells, leading to significant systemic toxicity. This has created an urgent need for the advancements of targeted drug delivery systems that can selectively reach cancer cells or tissues and release the drug precisely at the intended site. Recent advancements in anticancer therapy have highlighted the importance of innovative drug delivery strategies in enhancing treatment efficacy and improving patient outcomes. This review provides an analysis of advanced drug delivery systems, exploring their mechanisms of action, key developments, and therapeutic applications. Particular relevance is given to self-assembling nanosystems, with a focus on bioinspired nanomaterials, such as self-assembled peptide nanosystems, which constitute promising drug delivery tools due to their biocompatibility and potential lack of toxicity. The review also addresses the advantages, challenges, and future potentials of drug delivery systems in cancer treatment.

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Impact statement: advanced drug delivery systems, including self-assembling peptide nanosystems, offer targeted and biocompatible solutions for cancer therapy, reducing the systemic toxicity of chemotherapy drugs and improving treatment efficacy.

Key words: *drug delivery systems; cancer; active targeting; supramolecular assembly; artificial intelligence.*

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INTRODUCTION

Drug discovery represents an important clinical challenge. Unfortunately, despite significant advancements, many approved drugs still face critical limitations, including poor absorption, distribution and metabolism, which often hinder their clinical effectiveness (1, 2). Furthermore, the complex pathophysiology of many diseases remains poorly understood, impairing the development of precise therapeutic intervention (3-5). This gap in knowledge continues to hamper the development of effective therapeutic interventions with the advancement of innovative drug delivery systems (DDS) becoming increasingly vital to deliver therapeutic agents effectively, safely, and precisely to their intended sites of action (6-8).

DDSs are also designed to control the rate, timing, and location of drug release, optimizing efficacy while minimizing side effects and enhancing patient compliance (9). A well-designed DDS should take into account multiple factors, including the properties of the drug, the characteristics of the disease, and the specific therapeutic target thus requiring a thorough understanding of critical molecular features, alongside their interrelations which can help researchers to develop more effective and safe therapies (10, 11). The integration of advanced technologies, such as nanotechnology, biomaterials, and molecular biology, is redefining the boundaries of drug delivery. Nanotechnology plays a crucial role, offering innovative solutions to overcome the limitations of traditional therapies through the control of building blocks at the nanoscale level (12). This strategy involves the development of nanosized entities, typically ranging from 1 to 100 nm, which possess unique physicochemical properties distinct from their bulk counterparts. These properties have made nanotechnology a vital tool in advancing personalized medicine and addressing the limitations of traditional therapies (13-15), as also seen in the recent Covid pandemic with the successful exploitation of lipid nanoparticles for the development of mRNA vaccines (16-20). In fact, biomaterials enable controlled release and reduced toxicity (21), and are essential to assist recent breakthroughs in molecular biology, such as RNA-based therapeutics and CRISPR gene editing, which demand sophisticated delivery systems to achieve intracellular precision (22). Moreover, understanding disease-specific characteristics, such as pH, enzyme activity, or the hypoxic microenvironment, allows researchers to develop stimuli-responsive DDS that release drugs on demand in targeted tissues (23-25). Also, exploitation of active targeting strategies using ligands, antibodies, or aptamers enable precision delivery to diseased cells, sparing healthy tissues and minimizing systemic toxicity (22).

The future of drug delivery lies in integrating these advanced approaches to meet the demands of precision medicine. The possibility of addressing these challenges, not only will improve the effectiveness and safety of treatments but also improve patient compliance, paving the way for more accessible and impactful therapies.

In cancer treatment, conventional therapies, including chemotherapy and radiation, are often limited by significant drawbacks, (26) including a lack of specificity and severe off-target toxicity (7, 27); these problems stem from the heterogeneous and adaptive nature of cancer, where tumours evolve mechanisms to evade treatment, develop resistance to drugs, and continuously metastasize to different organs. Emerging approaches, such as nuclear medicine which employs the use of radiopharmaceuticals, like chemotherapy and radiation, aim to selectively target cancer cells while minimizing systemic toxicity (28). However, these newer strategies also struggle with issues such as poor tumour penetration, non-specific uptake, and variable patient response to treatment (29). Consequently, research in this area increasingly recognizes the need for advanced DDS to overcome these barriers, ensuring more precise targeting, sustained drug release, and improved therapeutic outcomes while minimizing unintended side effects.

DDSs IN THE CANCER ERA

DDSs have emerged as a ground-breaking solution, offering innovative ways to enhance the effectiveness of cancer therapies while reducing damage to healthy tissues (10). Traditional chemotherapy cannot differentiate between healthy and cancerous cells and often cancer cells develop resistance to chemotherapy following the long-term treatments (27, 30, 31). In addition, the tumour microenvironment, characterized by abnormal blood vessels, hypoxia, and high interstitial pressure, can create physical and biochemical barriers that hinder drug delivery and penetration (32). It should also be considered that many therapeutics, especially biological drugs, have short half-lives or poor stability, making it difficult for them to reach and act on tumours effectively (31, 33). DDSs offer a cutting-edge approach to overcoming these challenges, providing a more precise and controlled way to deliver therapeutics and opening new doors for personalized therapies. DDSs such as liposomes, micelles, lipid nanoparticles (LNPs) and nanoparticles from various origins improve drug solubility and protect drugs from degradation caused by enzymes, pH, and other factors during blood circulation (8, 34). Additionally, their tuneable size, shape, and structure allow for significant drug loading capacity and enable precise delivery (**Table 1**). Since their size is comparable to that of human cell organelles, they can efficiently interact with a range of hydrophilic and hydrophobic ligands, target specific cells, and access intracellular compartments (10).

Table 1. *Examples of drug delivery strategies and clinical progress.*

DRUG DELIVERY SYSTEMS	ADVANTAGES	EXAMPLES
Nanoparticle-Based DDSs	Tumours' leaky vasculature allows nanoparticles to accumulate in cancerous tissues through the enhanced permeation and retention (EPR) effect	Doxil: A liposomal formulation of doxorubicin that reduces cardiac toxicity (39)
	Nanoparticles protect drugs from degradation in the bloodstream, prolonging their circulation time and improving stability	Abraxane: Nanoparticle-bound paclitaxel that improves solubility and efficacy (40)
	Surface modifications, such as PEGylation, prevent off-target effects, enhancing bioavailability	Onivyde: A nanoliposomal formulation of irinotecan, which protects the drug active lactone configuration, prolonging circulation time (41)
Stimuli-Responsive DDSs	Targeted activation ensures the drug is released only at the tumour site, minimizing side effects	ThermoDox: An experimental formulation of doxorubicin encapsulated in thermosensitive liposomes (42)
	Adaptability to the acidic, enzyme-rich, or hypoxic conditions of tumours	Jelmyto: an FDA-approved gel-based chemotherapy for treating low-grade upper tract urothelial carcinoma (43)
Targeted DDSs	High specificity reduces damage to surrounding healthy tissues	Trastuzumab emtansine (T-DM1): An ADC designed for HER2-positive breast cancer, combining targeted action with a cytotoxic payload (44)
	Improves the therapeutic index of potent drugs, such as antibody-drug conjugates (ADCs)	Brentuximab (Adcetris): An ADC that delivers the potent cytotoxic agent monomethyl auristatin E (MMAE) specifically to CD30-expressing cancer cells (45)
Immunotherapy	Protect sensitive biologics like proteins or RNA during transport	Moderna and BioNTech: COVID-19 vaccine (46)
	Enable targeted delivery to immune cells or tumour tissues for heightened efficacy	Immunoliposomes: currently being investigated in ongoing Phase II studies (47)
Gene Therapy and RNA-Based Treatments	Enable precise silencing of oncogenes or correction of genetic mutations driving cancer	Onpattro (patisiran): LNPs to deliver siRNA targeting transthyretin for hereditary amyloidosis treatment (48)
	Overcome delivery barriers using lipid nanoparticles or viral vectors	Luxturna: Adeno-associated virus AAV2 vector to deliver RPE65 cDNA into retinal cells, restoring enzyme function (50)
Combination therapy	Target multiple pathways simultaneously, reducing the likelihood of drug resistance	Vocimagene amiretrorepvec/flucytosine: in phase-II clinical trials, is a retroviral vector to deliver the gene cytosine deaminase gene, converting the prodrug into 5-fluorouracil (5-FU) (49)
	Combine chemotherapy, immunotherapy, or RNA therapies in a single formulation	mRNA-4157/V940 and pembrolizumab: A personalized mRNA-based cancer vaccine encapsulated in lipid nanoparticles under Phase III clinical trial (50)

DDSs can be designed to: i) extend the circulation time of drugs in the bloodstream, ensuring they remain active and available for longer periods; ii) facilitate penetration through biological barriers, such as cell membranes or the blood-brain barrier (BBB); iii) increase the accumulation of the drug in the target tissues; iv) enhance cellular uptake, ensuring the drug enters the appropriate cells more efficiently (6, 35).

To release drugs specifically to diseased tissues or cells, the surface of DDSs may be decorated with ligands, antibodies, or aptamers, that target specific receptors on cancer cells, inflamed tissues, or other pathological sites, reducing off-target effects and toxicity (36). The ability to provide a platform for controlled and sustained drug release reduces the frequency of dosing and maintains therapeutic drug levels in the body for extended periods, enhancing treatment adherence (21, 37, 38).

SUPRAMOLECULAR PLATFORMS: A PARADIGM FOR DDS IN CANCER

Supramolecular platforms, inspired by naturally occurring self-assembly processes, are widely employed in DDS. These systems exploit the spontaneous organization of constituent molecules into intricate organic and inorganic structures (51). This organization is governed by non-covalent forces, including electrostatic interactions, π-π stacking, hydrogen bonding, and van der Waals forces. While individually weak (2-250 kJ/mol) relative to covalent bonds (100-400 kJ/mol), these non-covalent interactions collectively generate robust and stable functional materials (52). Nature is rich in examples of self-assembled structures, including double-stranded DNA, the triple helix of collagen, viral structures, metal ions and membrane bilayers (53). The specific order and magnitude of these interactions dictate the shape, function, and size of the resulting assemblies. Researchers have designed and synthesized a diverse array of building blocks including but not limited to metal ion, amphiphiles, peptides, dendrimers, polymers, fullerenes, calixarenes, cyclodextrins, and lipids, to explore structure-assembly relationships and create novel architectures (53-55). Indeed, understanding, controlling, and predicting the complex interplay between individual components and the final thermodynamic state of the assembly is essential to develop the next generation of complex materials.

For instance, Liu *et al.* (56), introduced a facile and universal strategy to construct a DNA nanostructure-based co-delivery system containing a linear tumour therapeutic gene (*TP53*) and a chemotherapeutic drug (doxorubicin, DOX) for combined therapy of multidrug resistant tumour (MCF-7R). Furthermore, Lv *et al.* (57) developed a dual-targeting nanoplatform for cancer therapy, combining a hyaluronic acid-paclitaxel (HA-PTX) prodrug with marimastat (MATT)-loaded, thermosensitive liposomes (MATT-LTSLs). The HA-PTX prodrug spontaneously assembles into both positively and negatively charged liposomes, creating hybrid nanoparticles (HNPs) approximately 100 nm in size. Upon application of mild hyperthermia, these HNPs (HA-PTX/MATT-LTSLs) rapidly release their drug payloads. The released HA-PTX then efficiently enters 4T1 cancer cells via CD44-mediated binding with hyaluronic acid. The collective behaviour of these self-assembled nanostructures can lead to functions and properties beyond those of the individual building blocks and can be further tailored by incorporating additional functional molecules (51, 58). Supramolecular materials are typically constructed using a bottom-up approach, starting from various building blocks such as atoms, small molecules, or macromolecules (59). Additionally, novel building blocks can be engineered by altering the chemical composition, length, and directionality of interactions in existing units that already possess the inherent capacity for self-assembly (54). The spontaneous formation of self-assembled structures represents a system reaching a state of minimum free energy, often achievable by manipulating environmental variables (60). Given that non-covalent interactions are the driving forces, self-assembly is inherently a reversible process, sensitive to environmental conditions (61), enabling the design of materials with on-demand properties through controlled manipulation of the self-assembly process.

A variety of morphologies, including vesicles, micelles, ribbons, helices, spherical nanoparticles, nanorods, nanotubes, and nanofibers, can be obtained depending on the chosen building blocks. The complexity of these structures, and consequently their potential function, generally increases with size (**Table 2**).

TARGETED SUPRAMOLECULAR DDSs

DDSs can be targeted to tumours through two primary mechanisms: passive and active targeting. Passive targeting takes advantage of the enhanced permeability and retention (EPR) effect, while active targeting exploits the specific interaction between ligands on the DDS and receptors overexpressed on tumour cells (35, 70). In cancer treatment, nanoparticles with diameters ranging from 10-100 nm are particularly effective because they can exploit the EPR effect. Particles smaller than 1-2 nm may leak from normal vasculature and damage healthy cells, while particles larger than 100 nm are more likely to be cleared from circulation by the immune system's phagocytes (71-73).

Active targeting is essential for maximizing the specificity and effectiveness of cancer therapies. Tumour cells often exhibit altered gene and protein expression profiles compared to normal cells, which can be exploited as markers for targeted drug delivery (74). Many tumours overproduce certain molecular components that support their growth and metastasis, including G-protein-coupled receptors, growth factor

Table 2. *Examples of self-assembled nanocarriers.*

CARRIER TYPE	SIZE RANGE	FEATURES	SHAPE	PAYLOAD	APPLICATIONS	REFERENCES
Lipids	10–1000 nm	Small unilamellar (20–100 nm), large unilamellar (100–500 nm), multilamellar (500–5000 nm). Surface-modified with peptides for targeting	Monolayers, bilayers, micelles, vesicles, giant vesicles	Hydrophilic & hydrophobic drugs	Liposomal Doxil, first FDA-approved liposomal formulation for tumours	(34)
Dendrimers	1.5–10 nm	Monodispersed, multibranched core-shell structure, enabling controlled drug release & selective toxicity	Nanoparticles, nanofibers	Multitherapeutic cargo loading for targeted delivery	Cancer therapy, gene delivery	(55, 62, 63)
Hydrogels/ Nanogels	100 nm–100 µm	3D polymeric network, physically/ chemically crosslinked, high porosity	Hydrogels, microspheres, nanogels	Small molecules, polymers, nanoparticles	Tissue engineering, wound healing, drug delivery	(64, 65)
Peptides	Nano to micrometer scale	Biodegradable, modular, composed of natural amino acids	Nanoparticles, nanofibers	Insulin, glucagon, chemotherapeutics	Drug delivery, regenerative medicine	(66)
Virus-Like Particles (VLPs)	20–200 nm	Self-assembled protein structures mimicking viruses, strong immune activation	Spherical	Encapsulates nucleic acids, chemotherapeutics, antigens	Cancer vaccines, targeted drug delivery	(67)
Polymersomes	10 nm–µm	Amphiphilic polymer vesicles with membranes (5–10 nm thick), tunable stability	Spherical, tubular, donut-shaped, cucurbit-shaped	Proteins, nucleic acids, anticancer drugs	Chemotherapy, immunotherapy, antigenic vaccine development	(68, 69)
Nucleic Acids	1–100 nm	Self-assembled DNA/RNA nanostructures for precise targeting & stimuli-responsive release	DNA origami, tiles, modular assemblies	Proteins, small molecules, nano/ nucleic acid drugs	Gene therapy, chemotherapy, immunotherapy	(53)

receptors, interleukins, transferrin receptors, folate receptors, and polysaccharide moieties (75-77). While monoclonal antibodies have been the cornerstone of targeted cancer therapies, alternative targeting moieties such as small molecules and peptides are increasingly being explored to improve tissue selectivity (78). Aptamers, which are single-stranded DNA or RNA molecules, can bind specific targets with high affinity and specificity, offering advantages such as biostability, versatile chemical modification, low

immunogenicity, and rapid tissue penetration (79). Nanobodies derived from camelid heavy-chain antibodies provide benefits like enhanced tumour penetration and the ability to reach cells in poorly perfused tumour areas (80). Antibody-drug conjugates (ADCs) have also proven a highly effective strategy, combining the specificity of monoclonal antibodies with the potency of cytotoxic agents. Recent innovations include optimized linkers, novel payloads, and bispecific ADCs targeting multiple tumor antigens (81, 82). In fact, Synan *et al*. (82) further validated this strategy by demonstrating that bispecific antibodies with enhanced cross-arm binding affinity effectively target dual-positive cells, improving specificity and therapeutic potential.

Targeting peptides are typically composed of 3-15 amino acids (aa) and are designed to specifically bind to tumour cells or tumour vasculature, allowing for targeted delivery to the tumour and its microenvironment (83, 84). These peptides can be used to decorate the surface of DDSs, enhancing their ability to target tumours (85). Common targeting peptide motifs include the RGD sequence (Arg-Gly-Asp), which binds to integrins on the tumour vasculature, and the NGR sequence (Asn-Gly-Arg), which binds to aminopeptidase on endothelial cells (86). The epidermal growth factor receptor (EGFR), which is overexpressed in many types of cancer, is another important target (87). In addition, a variety of other peptides targeting different receptors overexpressed in tumours or blood vascular endothelium can be employed to improve the specificity and efficacy of cancer therapies (83).

Tumour targeting can greatly enhance the precision of cancer treatments, leading to improved drug accumulation at tumour sites while minimizing the impact on healthy tissues (**Figure 1**).

ENHANCEMENT OF PENETRATION OF SUPRAMOLECULAR DDSs

Poor tumour penetration is one of the biggest hurdles in cancer treatment. Typically, nanoparticles accumulate near tumour blood vessels but fail to penetrate deep into the tumour tissue, which often leads to drug resistance and reduced therapeutic efficacy (88). This limitation underscores the need for more effective DDSs. One promising solution to overcome this obstacle and reduce drug resis-

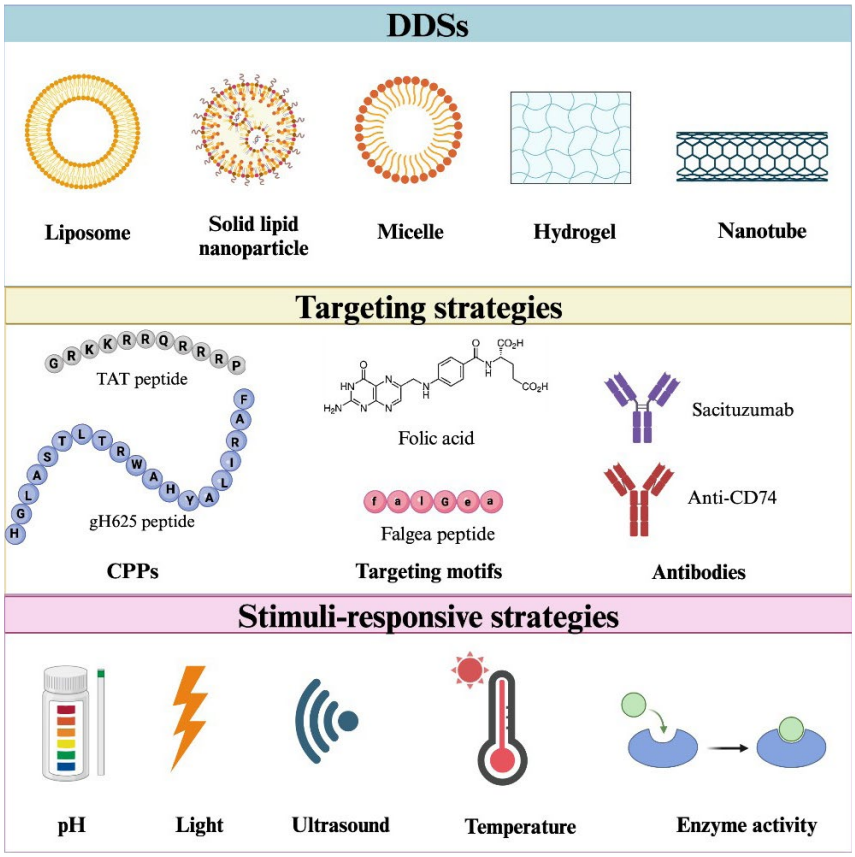

Figure 1. *Examples of different drug delivery systems strategies.*

tance is the exploitation of cell-penetrating peptides (CPPs) (89).

CPPs are short peptides, typically up to 40 aa, known for their ability to cross cellular membranes and are particularly attractive due to their low cytotoxicity, high efficiency, and the absence of limitations regarding the size or amount of the cargo (88). These peptides have been widely explored for delivering a variety of therapeutic molecules, especially in cancer treatment. The potential of CPPs to enhance drug delivery to tumours while sparing healthy tissues has been demonstrated across several tumour models. Currently, over 30 CPPs are undergoing clinical trials, though only a few have reached Phase III (90).

The most well-known cationic CPP is TAT, derived from HIV-1 proteins (91, 92). Cationic CPPs bearing amino acids like arginine and lysine are positively charged, which gives them a strong affinity for the negatively charged cell membrane (92). This electrostatic interaction facilitates internalization through a receptor independent mechanism which involves endocytic pathways (93). Amphipathic CPPs, have both polar and non-polar domains. Their amphipathic nature allows them to interact with both hydrophilic and hydrophobic environments, facilitating their integration into cellular membranes. Examples include Penetratin and MAP, which have distinct structural characteristics in aqueous and membrane environments, changing from random coil to secondary structures such as α-helical or β-sheet conformations; the modification of the secondary structure is essential in cellular uptake (94-96). Hydrophobic/membranotropic CPPs contain a high number of hydrophobic residues that enhance their affinity for lipid membranes, though the content of hydrophobic residues is low enough to avoid haemolysis of healthy cells. These peptides are capable of penetrating deeper into the membrane hydrophobic core compared to cationic CPPs (97). Notably, viral fusion peptides are examples of membranotropic CPPs that effectively penetrate cellular membranes (98, 99).

Despite the potential of CPPs, factors such as the peptide concentration, structure, charge, and length, as well as the cell type and the properties of the associated cargo (size, type, and charge), influence the efficiency of cellular uptake (100, 101). The two main internalization pathways are energy-independent (direct translocation) and energy-dependent (endocytosis) mechanisms. In both cases, peptides initially interact electrostatically with cell surface glycosaminoglycans, which facilitates their entry into cells (102). The subsequent transduction models, such as barrel-stave and toroidal pore models, or carpet models, explain how some peptides may destabilize the membrane or form pores, aiding their internalization (103).

However, one of the key challenges is overcoming endosomal entrapment, as the endocytosis process often leads to cargo being trapped in endosomes or lysosomes, hindering its release into the cytoplasm. As said, cationic peptide exploits essentially endocytosis mechanism of internalization, while membranotropic CPPs are thought to bypass this issue by directly penetrating the plasma membrane, allowing the cargo to be immediately bioavailable (104). Galdiero *et al.* developed a membranotropic peptide, namely gH625 (105) that interacts with the cell membranes and spontaneously inserts into the bilayer forming an amphiphilic α-helical structure. The N-terminal histidine residue is strongly involved in the fusogenic activity of the peptide, while the hydrophobic residues of tyrosine and tryptophan are fundamental for the structural stability during the interaction with the lipidic membrane. gH625 was proved to be able to carry different cargos inside several cell lines and to enhance internalization across the BBB (106, 107).

Studies have shown that combining CPPs with chemotherapeutic agents can improve drug delivery, even in drug-resistant cancer cells (108-110). Doxo, when coupled with Penetratin and Tat, showed increased cytotoxicity in both drug-sensitive and drug-resistant cancer cells. Liposomes conjugated to gH625 were able to overcome Doxo resistance in lung adenocarcinoma cell lines (111).

ON-DEMAND STRATEGIES FOR DRUG RELEASE

The development of stimuli-responsive DDSs has become a significant area of scientific research, offering new approaches for targeted drug delivery. These systems exploit the unique features of the tumour microenvironment to enhance the specificity and effectiveness of treatment (78, 111). For instance, cancer cells rely on anaerobic glycolysis for energy, leading to an accumulation of lactate and protons, which makes the surrounding tumour microenvironment more acidic (111). This acidic environment not only contributes to tumour growth and metastasis but also plays a key role in drug resistance. To exploit this, pH-responsive peptides can be conjugated to DDSs. pHLIPs

is a pH-low insertion peptide which can change its structure in response to acidic conditions. In particular, at lower pH, the acidic aa bind protons, lose their negative charge, and can more effectively penetrate cell membranes, promoting internalization and drug delivery into tumour cells (112). Chen *et al*. reported a new pH-responsive nano-drug delivery system using boronic acidester chemistry (23). They synthesized nanoparticles (PTXPBA NPs) from hydrophobic phenylboronic acid-modified polyesters and hydrophilic PEGs, loading them with the chemotherapy drug paclitaxel. The resulting nanoparticles were tested *in vitro* and *in vivo*, showing improved drug release, encapsulation efficiency, anticancer activity, pharmacokinetics, and reduced toxicity compared to traditional methods.

Another hallmark of cancer cells is the redox-active environment, which can be exploited to design redox-sensitive DDS. The intracellular levels of glutathione (GSH) in cancer cells are much higher than in normal cells and this difference can be used to trigger drug release from DDSs that are conjugated through disulfide bonds or di-selenide bonds, which are cleaved by GSH (113).

Many enzymes that are upregulated in cancer cells can also be used to modify DDSs and trigger drug release. For instance, matrix metalloproteinases (MMPs), which are enzymes involved in tumour invasion and metastasis, are overexpressed on the surface of cancer cells (114). MMP-sensitive DDSs can be designed to release drugs when cleaved by these enzymes. Nagel *et al.* (115) developed MMP-sensitive, peptide-crosslinked nanogels (pNGs) for improved drug delivery to solid tumours using a dendritic polyglycerol scaffold and strain-promoted click chemistry. The pNGs were stable in physiological conditions but degraded by MMP-7, leading to size reduction and enhanced penetration in agarose matrices and multicellular tumour spheroids (MCTS) (115).

Guarnieri *et al.* (116) developed tumor-activated prodrug-conjugated nanoparticles (TAP-NPs) that release Doxo in MMP2-rich tumor environments. In a similar study, Del Genio *et al.* (58) designed self-assembled peptide-based nanofibers for TNBC therapy, incorporating a CCP and an MMP-9-responsive sequence. Characterization confirmed efficient drug loading, release, and in vitro cytotoxicity. Also, Bellavita *et al.* (117) functionalized peptide-based nanofibers with a CPP and EGFR-targeting peptide to enhance Doxo specificity in TNBC. MTT assays showed superior efficacy compared to free Doxo, demonstrating the potential of peptide-decorated nanofibers in targeted cancer therapy.

Interestingly, Ramezanian *et al.* (24) created a DDS by grafting poly(dimethylaminoethyl methacrylate) (PDMAEMA) onto silica nanoparticles with two different lengths using an in-situ atom transfer radical polymerization, obtaining a pH- and temperature-sensitive shell. The drug release followed the polymer shell protonation at pH 5 while a critical temperature of 41 °C aided rapid solvation of the shell polymers in the blood. Wang *et al.* (8) reported the creation of photocleavable hydrogels by directly gelling 4-arm thiol-terminated polyethylene glycol with 3,6-dichloro-1,2,4,5-tetrazine, forming S, S-tetrazine linkages. They stated that these hydrogels degraded efficiently when exposed to ultraviolet or green light, with a possibility of hydrogen peroxide significantly catalysing the degradation. Furthermore, they reported that loading the hydrogels with either calcium peroxide microparticles or glucose oxidase/catalase enzymes allowed for precise and efficient *in vivo* photocontrol of both gel breakdown and drug release for cancer treatment.

Regarding drug delivery methods generated by ultrasound, lipid-supported mesoporous silica nanoparticles (MSNPs) were used by Amin *et al.* (118) to create a stimuli-responsive DDS that would activate drug release at the target location while preventing an early release into the systemic circulation. They used perfluoropentane (PFP), a US responsive material, as a model drug and created MSNPs with a release profile that complied with FDA-approved US-irradiation, which is characterised by a larger drug loading capacity and extremely gradual release. They reported these MSNPs to provide stable and non-toxic delivery of anticancer drugs, with ultrasound triggering the release of PFP gas to disrupt the lipid coating and release the drug.

The development of stimuli-responsive DDS offers great potential for improving the specificity and effectiveness of cancer therapies (119). The combination of multiple responsive triggers, such as pH, temperature, and enzymatic activity, enables more precise control over drug release, improving both the therapeutic outcome and reducing systemic toxicity.

EMERGING SUPRAMOLECULAR DDSs

Peptides offer functional diversity based on their sequence, making them excellent candidates for creating self-assembling systems with distinct properties (54). The nature of their amino acid side chains is responsible for key noncovalent interactions that make them ideal components for constructing com-

plex supramolecular assemblies (120). Peptides are relatively simple to synthesize using solid-phase techniques, allowing researchers to explore various designs and understand how molecular changes affect their characteristics (121). Additionally, peptides possess high biocompatibility, biodegradability, and low immunogenicity, which are advantageous when developing functional nanomaterials (120).

Peptides can spontaneously self-assemble into various nanostructures, such as vesicles, nanotubes, fibers, sheets, micelles and nanospheres according to their sequence length, amino acid side chains, and external conditions like pH and ionic strength (54, 122). Researchers can create diverse nanostructures with desired features by modifying these parameters and these nanostructures can be exploited as DDSs, opening the path to the modification of existing self-assembly sequences to optimize their functions. It is possible to easily incorporate cell-targeting sequences into these structures enhancing the specificity of drug delivery and reducing the impact on healthy tissues (58). Additionally, peptides can be modified to produce structures that respond to environmental changes, such as low pH or elevated temperature, making them ideal for on-demand drug release (123). In the context of cancer theranostics, self-assembling peptides offer dual benefits by delivering anticancer drugs while also providing a means for bioimaging and diagnosis (124). Peptide-based carriers are thus designed to bind specific tumour markers, allowing for targeted therapy and non-invasive monitoring of the treatment's effectiveness. Biological distribution of peptide self-assembled DDSs is influenced by their size and shape. While spherical nanoparticles have been commonly used in carrier designs, recent research has shifted toward anisotropic nanoparticles, which differ in shape depending on the direction (125). These anisotropic nanoparticles tend to exhibit greater resistance to non-specific elimination by cells, making them "stealthier" compared to spherical ones and have also been proved to have improved penetration efficiency. For example, Ben-Akiva *et al.* (126) synthesized spherical PLGA nanoparticles and then stretched them above the glass transition temperature of PLGA. These anisotropic nanoparticles-maintained fluidity and stability similar to spherical ones and could be coated with naturally derived cell membranes. When coated with red blood cell membranes, anisotropic nanoparticles were better able to evade macrophage clearance, leading to a longer half-life compared to spherical nanoparticles. In fact, around 50% of mice treated with ellipsoidal nanoparticles remained healthy a week after being administered alpha toxin (126).

Galdiero Group developed self-assembled peptide-based nanofibers from two amphiphilic peptides P1 and P2 bearing both an hydrophobic (C19 lipid tail and hexa-alanine sequence) and a hydrophilic (-GDDS- and -GKRS-) domain (58). The two peptides were the building blocks used to obtain the nanofiber structure, while the nanofiber surface was decorated with gH625 and Doxo, as delivery peptide and anticancer drug, respectively (**Figure 2**). Doxo was released due to the presence of a peptide sequence cleaved by the MMP-9 enzyme for on demand delivery into Triple-negative breast cancer lines (58).

Similarly, this self-assembled peptide-based nanofibers can be used for gene therapy; in fact, the functionalization of nanofiber surfaces with R9 peptides allowed to electrostatically bind a siRNA, targeting and silencing epidermal growth factor receptor (EGFR) gene overexpressed in triple-negative breast cancer (127). This evolution would expand the system's application to combined approaches, including

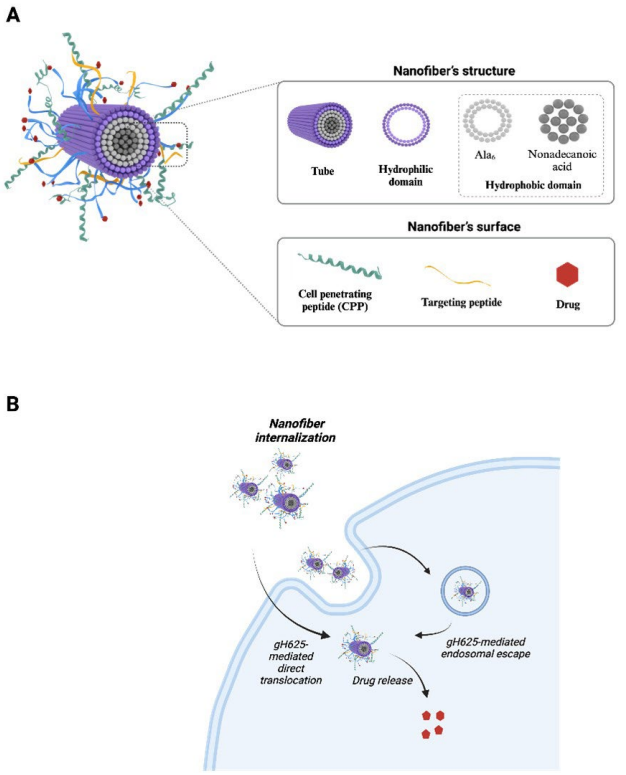

Figure 2. *(**A**) Schematic representation of self-assembled nanofiber composed of amphiphilic peptides, which can be functionalized with specific ligands for targeted delivery and conjugated with chemotherapeutic drug; (**B**) Mechanism of nanofiber internalization: the system, decorated with the cell penetrating peptide, facilitates cellular uptake and drug release in cancer cells.*

both pharmacological treatment and gene therapy, particularly relevant in resistance subtypes of cancer.

ARTIFICIAL INTELLIGENCE AND DDSs

The development of increasingly sophisticated and accurate artificial intelligence (AI) systems has in recent years enabled the integration of machine learning and bioinformatics algorithms for the design and development of DDSs. These algorithms allow the optimization of the property of delivery systems and predict both the behaviour in the microenvironment in which they will be inserted through external solvent simulations and the properties of interactions of lipid or polymeric components in the case of lipid- and polymer-based particles, with a predictive index of, for example, the critical aggregation. Dong *et al*. have designed a web platform namely FormulationAI that allows in silico pharmaceutical formulation design, with data collected regarding used and known six DDS and solubility of drugs in different solvents reported from public databases and literature data referring to a time range of 10 years prior to writing the project (128), while another strategy combines AI methods and target fishing that allows the rapid identification of biological targets, an important aspect for linking novel compounds (129). To fully unlock the potential of nanomedicine, the integration of artificial intelligence plays a crucial role. In particular, the use of artificial neural networks (ANNs) can aid in predicting how the characteristics of a designed nanosystem influence its interactions with target tissues and cells (130). In addition, machine learning methods of this kind would facilitate rapid assessments of both therapies and patients, strengthening the connection between scientific research and clinical practice while advancing the development of highly precise personalized treatments (131). These conceptual tools can provide theoretical and predictive support during the design phase of a delivery system, simulating both the interaction of the nanosystem constituents with the microenvironment and the interaction of the drug against the specific therapeutic target.

FUTURE PERSPECTIVE

In summary, with the promising advancements in DDS, the horizon of possible treatments is expanding, enabling the development of novel therapies, the delivery of previously inaccessible treatments, and ultimately paving the way for precision medicine. At the clinical level, these advancements have led to improved patient compliance, reduced exposure to frequent dosing, and alternative administration routes. One of the most significant impact of DDSs in cancer is the site-specific delivery of cytotoxic payloads to tumoral cells, and patients with metastatic cancer now have access to therapies that can specifically target metastatic zones. For instance, Antibody-Drug Conjugates (ADCs) have been developed to deliver cytotoxic agents directly to cancer cells, minimizing systemic exposure and enhancing treatment efficacy (132). Moreover, DDS are particularly beneficial for diabetes patients, reducing the need for repeated insulin injections and improving overall disease management (133).

In addition, the rapid advancement of focuses on minimizing chemotherapeutic side effects by utilizing biocompatible and biodegradable materials that do not accumulate in the body but instead provide beneficial effects through their metabolization; for instance, substances like chitosan, gelatine, alginate, peptides are used for oral administration (54, 134). Solid lipid nanoparticles and liposomes have gained widespread acceptance, especially after the success of lipid-based COVID-19 vaccines, though concerns about immune responses to certain materials remain. Fully peptide-based delivery systems, like those developed in our lab, present a promising strategy for creating biodegradable and biocompatible nanoparticles (54). Ultimately, the development of innovative requires a multidisciplinary approach that integrates clinical needs with chemical and biological expertise. Interdisciplinary collaboration can help overcome challenges in traditional therapies and bridge the gap between research and medical application. Continued innovation in DDS design and manufacturing could improve automation, reduce costs, and enhance healthcare accessibility (10).

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Authors' contributions

SB, OEA, SP, LEI, were responsible of the initial draft; RB, AF, LA, revised the manuscript; AG and SG conceived the content of the manuscript.

Ethical approval

Ethical approval was not necessary for this study.

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Plagiarism

The article provides a comprehensive review of the latest studies in the field, with accurate citations.

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The writing and contents of the article are entirely original and were developed entirely by the authors.

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RESEARCH ARTICLE

ENDOMETRIOSIS: A CANCER-MIMICKING DISEASE AND THE NEED FOR A TRANSLATIONAL PERSPECTIVE

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ABSTRACT: Endometriosis, defined by the presence of endometrial-like tissue beyond the uterine cavity, afflicts over 190 million young women worldwide and often significantly reduces quality of life. Despite being historically classified as a benign gynecologic disorder, endometriosis can mimic cancer in imaging findings, serum tumor markers, and molecular signature. Increasing evidence suggests endometriosis encompasses multiple biologic subtypes rather than representing a single uniform disease, which may explain divergent presentations, from extensive lesions in some patients with minimal pain to smaller implants in others with severe symptoms. Current management relies heavily on empirical hormonal therapies, repeated surgeries, and symptomatic treatment. Inadequate diagnostic tools and incomplete mechanistic understanding contribute to misdiagnosis, delayed intervention, and suboptimal outcomes. Without deeper elucidation of its complex biology, especially at the molecular level, substantial therapeutic breakthroughs will likely remain elusive. Notably, pathways commonly implicated in malignancy are aberrantly activated in ectopic endometrial tissue, driving proliferation, angiogenesis, and immune evasion. To address heterogeneous endometriosis phenotypes, a rigorous translational framework is essential. Through such structured investigation, novel data and non-hormonal therapies targeting core molecular events could emerge, reducing both protracted diagnostic timelines and lowering the incidence of overtreatment. In recognizing endometriosis as potentially comprising distinct pathologies under one umbrella, the field may advance truly individualized, biology-guided interventions.

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Impact statement: Endometriosis, affecting over 190 million people worldwide, displays clinical and molecular profiles that closely resemble malignancies. Framing endometriosis as a "cancer-mimicking" disease highlights why current models, diagnostic tools, and empirical therapies fail to adequately address prolonged diagnostic delays, high recurrence rates, and inconsistent treatment outcomes. This perspective advocates a structured translational approach, integrating meticulous preclinical validation, phase-appropriate clinical trials, and rigorous safeguards in artificial intelligence and biomarker development, to bridge critical gaps in understanding disease biology. Such a bidirectional pipeline, guided by real-world clinical feedback and clear mechanistic insights, aims to optimize pain management, fertility preservation, and overall disease control. Reconceptualizing endometriosis as a systemic condition with cancer-mimicking features underscores the urgency and the opportunity to develop targeted therapies beyond traditional hormonal suppression and empirical surgeries, ultimately enhancing patient quality of life worldwide.

Key words: *endometriosis; cancer-mimicking; molecular medicine; translational research.*

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INTRODUCTION

Endometriosis, broadly defined as the presence of endometrial glands and stroma outside the uterine cavity, affects over 190 million young women worldwide and severely compromises the quality of their lives (1-3). Historically considered predominantly gynecological, current evidence positions endometriosis as a multisystem disorder driven by inflammatory, hormonal, genetic, and neurobiological drivers (2-5). Notably, its capacity for tissue invasion, recurrence, and resistance to standard therapies has led many experts to characterize it as "cancer-mimicking," reflecting both its clinical severity and complex underlying biology. However, despite sharing several molecular and clinical features with malignant conditions, endometriosis itself is not classified as cancer. Nevertheless, endometriosis does carry a recognized, albeit relatively low, risk of malignant transformation, to endometriosis-associated ovarian cancer (EAOC) (6) with recent studies indicating approximately a two-fold increase in lifetime risk (from about 1% in the general population to roughly 2% in women with endometriosis) (7-9). Critically, it remains uncertain which patients with endometriosis are at risk of progressing to ovarian cancer. Endometriosis imposes a substantial lifelong burden on women, primarily because prolonged diagnostic delays, often exceeding seven years due to nonspecific symptoms and overlap with other conditions, mean that many affected individuals live for years unaware of their underlying disease (10). Furthermore, the absence of a unifying theory (spanning retrograde menstruation, stem-cell hypotheses, or coelomic metaplasia) limits our understanding of why endometriosis emerges in some individuals, or why disease trajectories are heterogeneous (10-17). Treatment remains largely empirical, involving hormonal suppression, analgesics, and repeated surgeries, offering only transient or partial relief (18). In parallel, emergent technologies, refined imaging, artificial intelligence diagnostics, and novel molecular biomarkers, offer potential (4, 18). Nevertheless, premature implementation of these technologies, especially AI-driven diagnostics, without sufficient biological understanding, risks embedding biases, exacerbating inequalities, or restricting accessibility for marginalized populations (19, 20). Therefore, a patient-centered translational framework becomes paramount, allowing rigorous biologic characterization to shape early-phase validations, rather than deploying large-scale AI-driven strategies that might misdirect care. Indeed, parallels between endometriosis and malignancy, including shared molecular pathways and invasive properties, highlight the necessity for strategic, stepwise integration of laboratory findings and clinical observations. Leveraging the concept of endometriosis as "cancer-mimicking," we illustrate how ambiguous pathogenesis, diagnostic uncertainty, and nonspecific treatments hinder patient outcomes. First examine immunologic and molecular mechanisms underlying these cancer-mimicking traits, emphasizing the importance of elucidating these pathways for targeted intervention development. Next, we propose a bidirectional translational framework, anchored by phase 0/preclinical studies and informed clinical trials, aiming to advance diagnostics, management, and, potentially, preventive strategies.

MATERIALS AND METHODS

This manuscript is a perspective rather than a systematic review. We conducted a structured literature search using PubMed to address three thematic queries: (i) clinical manifestations of cancer-mimicking features in endometriosis (Section Clinical Parallels), (ii) molecular pathways shared with malignancy (Section Molecular Parallels), and (iii) translational research gaps and unmet clinical needs (Section Gaps in Understanding). The search strategy included original research articles and comprehensive reviews published in English, French, Spanish and Italian, identified using combinations of predefined keywords ("endometriosis," "cancer mimicry," "molecular pathway," "translational research"). Identified articles underwent initial screening based on title and abstract. Subsequently, selected publications were reviewed and refined by author consensus, considering direct relevance to the specified thematic areas. This process resulted in the selection of 35 articles for Sections Clinical Parallels and Molecular Parallels each, and eight articles for Section Gaps in Understanding. The selected references were not intended to represent an exhaustive review but rather to illustrate critical clinical observations, molecular insights, and translational opportunities pertinent to this perspective.

CANCER MIMICRY

Clinical Parallels

Clinical evidence consistently indicates that endometriosis, particularly in complex or atypical pre-

sentations, closely mimics malignancy in clinical, biochemical, radiological, and pathological findings. This resemblance includes elevated tumor markers, invasive imaging characteristics, and lesions at atypical anatomical sites, complicating diagnostic accuracy and often leading to overtreatment.

Elevated Tumor Markers

A frequent diagnostic pitfall arises when markedly elevated tumor markers, such as CA-125 or CA-19.9, which ordinarily raise suspicions of gynecologic or gastrointestinal malignancies, occur in endometriosis patients. Numerous reports document significantly elevated CA-125 levels, sometimes surpassing 1000 U/mL, prompting urgent oncologic evaluations (21-25). For example, an extremely elevated CA-125 level of 1386.50 U/mL, coupled with a large ovarian mass, led directly to surgery under suspicion of ovarian cancer (25). Similar elevations of CA-125 or CA-19.9 are also observed in extrapelvic lesions, including subcutaneous and abdominal wall endometriosis (26, 27). Although indicative of malignancy, these elevations lack specificity, underscoring the need for cautious interpretation.

Imaging Findings Suggestive of Invasive Disease

Advanced imaging modalities, including ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), and even positron emission tomography (PET), frequently detect masses with irregular margins, heterogeneous enhancement, and restricted diffusion, hallmarks of malignancy (28-30). Reports describe "ill-defined," "stellate," or "irregular" soft-tissue masses with enhancement patterns indistinguishable from malignancies (31-33). For instance, bladder-infiltrating endometriosis has appeared on MRI as a heterogeneous, solid mass with restricted diffusion, closely resembling bladder carcinoma (34). Similarly, ovarian polypoid endometriosis has been misdiagnosed preoperatively as advanced carcinoma due to papillary structures, solid components, or extensive "peritoneal" disease (25, 30). Also lesions in atypical locations, such as beyond the pelvis, because of discrete enhancing lesions in the lumbar plexus (35) or renal parenchyma (36-38) have led radiologists to suspect nerve sheath or renal cell tumors, respectively.

"Metastatic" or Disseminated Disease Patterns

Beyond local invasion, endometriosis may present as multifocal implants throughout the peritoneum, bowel serosa, and omentum, mimicking peritoneal carcinomatosis (23, 24). Some cases include extensive nodularity, ascites, and pleural effusions, features characteristic of advanced intra-abdominal or thoracic malignancies (32, 39). Widespread peritoneal dissemination, as observed in polypoid endometriosis (26, 30, 34, 40, 41), can be mistaken for metastatic dissemination. Even endometriotic lymph node involvement has been reported, raising suspicion of metastatic carcinoma (30, 42). One report detailed extrapelvic endometriosis with progressive abdominal distension and cachexia, two clinical indicators that triggered an oncology referral for presumed metastatic cancer (24).

Overlapping Symptom Profiles

Endometriosis frequently manifests with alarming symptoms classically linked to malignancy, such as rectal bleeding, hematuria, or large bowel obstruction (36, 40, 43). Rectal bleeding and weight loss in conjunction with a rectal mass have led clinicians to suspect colorectal cancer (43). Likewise, recurrent hematuria and flank pain associated with ureteral or bladder involvement have initially suggested urological malignancies (34, 36, 37). Similarly, uterine bleeding and pelvic masses often suggest gynecological cancers; however, polypoid endometriosis can produce an indistinguishable clinical picture (25).

Extensive Surgical Intervention due to Suspected Malignancy

Cancer-mimicking presentations sometimes prompt surgeons to undertake aggressive interventions, including radical hysterectomy, bilateral salpingo-oophorectomy, bowel resection, or omentectomy (38, 39, 44). In one striking example, a patient was scheduled for hyperthermic intraperitoneal chemotherapy to address presumed pseudomyxoma peritonei, only for intraoperative findings to reveal endometriosis (24). Such extensive procedures carry considerable morbidity, particularly if a benign process is overtreated (27). Frozen-section biopsies may also fail to definitively exclude malignancy, reinforcing diagnostic confusion (24, 32, 42).

Histopathological Pitfalls

Pathologists also face challenges, particularly with atypical variants such as polypoid endometriosis, decidualized endometriosis, or Müllerianosis in lymph nodes (31, 43-47). These entities may demonstrate glandular crowding, papillary architectures, or cytologic atypia, making intraoperative differentiation from malignancy difficult (30-32, 46). Immu-

nohistochemistry is frequently indispensable to confirm endometrial derivation and rule out higher-grade carcinomas or metastatic lesions (31, 35, 43, 48, 49). Thoracic endometriosis, in particular, poses diagnostic challenges distinct from pelvic lesions. Unlike pelvic endometriosis, which has been more extensively studied and characterized, thoracic endometriosis demonstrates unique clinical and histological features that set it apart. These differences include variations in lesion appearance, behavior, and tissue composition. In particular, there is growing recognition of the importance of stromal endometriosis, lesions composed predominantly of endometrial-type stromal cells without accompanying glands, in the thoracic cavity. This form of endometriosis may be underdiagnosed or misclassified due to its subtle histological presentation, contributing to inconsistencies in diagnosis and classification across different anatomical sites. Understanding the distinct nature of thoracic endometriosis, especially the role of stromal components, is essential for improving diagnostic accuracy, guiding treatment strategies, and advancing a more comprehensive understanding of the disease's pathophysiology (50).

Clinical Consequences and Need for Vigilance

Taken together, these clinical and radiological parallels have significant implications for patient care. Suspicion of cancer prolongs diagnostic workups, increases patient anxiety, and may result in excessive therapy. Conversely, dismissing endometriosis prematurely may delay essential interventions and allow disease progression (24, 28, 37, 40, 46, 51). Consequently, clinicians should maintain a high index of suspicion for endometriosis in atypical presentations, extrapelvic masses, or cancer-like imaging profiles, regardless of reproductive age.

Clinical observations across multiple organ systems (21-49, 51-56) illustrate how endometriosis can reliably mimic malignancy, with raised tumor markers, suspicious radiographic features, multifocal dissemination, and deceptive histology (**Table 1**). These overlapping features underline the urgent need for enhanced education, training, and effective dissemination of existing diagnostic criteria for endometriosis. Treatment strategies should consistently adopt a multidisciplinary approach, applying current guidelines appropriately yet adapting them individually to each patient's specific clinical presentation, disease phenotype, and personal therapeutic objectives.

Table 1. *Endometriosis Mimicking Malignancy in 35 References.*

REFERENCE	SUSPECTED MALIGNANCY	LOCATION OF ENDOMETRIOSIS	CLINICAL CASE SCENARIO
Hu, 2021	Primary rectal aden\ ocarcinoma	Deep rectal wall/ rectosigmoid colon	Clinical: Rectal bleeding, weight loss Imaging (CT/MRI/EUS): 4.5–5 cm rectal mass (T3/T4 suspicion), restricted diffusion Pathology: Rectal Mucosal Biopsies showed mucosal hemorrhage with associated hypercellular stroma; Scattered atypical glands were present deep in the muscularis mucosa, rimmed by hypercellular stroma; The deep glands and surrounding stroma were strongly positive for Estrogen Receptor: The cellular stroma was strongly positive for CD10 3; These histological and immunohistochemical findings confirmed the diagnosis of endometriosis
Stuparich, 2020	Peritoneal carcinomatosis/ Gynecologic malignancy	Multiple peritoneal nodules, abdominal wall, omentum	Clinical: Postmenopausal on estrogen Imaging (CT): Multiple nodules suspicious for carcinomatosis Laparoscopy: Irregular nodules, neovascularization Pathology: An initial CT-guided biopsy demonstrated endometriosis, but malignancy could not be definitively excluded. Intraoperative pathology during the laparoscopic procedure demonstrated only endometriosis. The final pathology report, after the surgical removal of all disease, showed polypoid endometriosis without cancer.
Gargan, 2023	Ovarian malignancy (multiple solid masses)	Right ovary (adjacent to endometrioma)	Clinical: Premenopausal, worsening pelvic pain Imaging (US/MRI): Several solid, echogenic, vascular lesions with homogeneous enhancement Pathology: Polypoid endometriosis mimicking neoplastic masses

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REFERENCE	SUSPECTED MALIGNANCY	LOCATION OF ENDOMETRIOSIS	CLINICAL CASE SCENARIO
Kaymaz Gezer, 2016	Malignant mesothelioma (deciduoid) in differential	Cesarean section scar (abdominal wall)	Clinical: Mass in previous C-section scar Pathology: Decidualized endometriosis with large polygonal cells resembling deciduoid mesothelioma
Mota, 2020	Colorectal cancer	Upper rectum, middle sigmoid colon	Clinical: Changes in bowel habits, intermittent hematochezia Imaging (CT): Irregular parietal thickening with contrast enhancement, stenosis Colonoscopy: Concentric stenosis, friable mucosa (negative biopsies) Pathology: proctosigmoidectomy specimen revealed intestinal wall endometriosis, compromising submucosa and internal and external muscular layers, with fibrosis.
Sarofim, 2018	Primary sigmoid malignancy	Distal sigmoid colon, rectum, pericolic LNs	Clinical: Acute large bowel obstruction Imaging (CT): Thickened distal sigmoid mass causing obstruction Operative: Dense adherence to pelvic sidewall Pathology: Endometriosis in pericolic nodes mimicking metastatic spread
Rodrigues, 2015	Recurrent perianal abscess/fistula (non-malignant)	Perianal region (episiotomy scar)	Clinical: Anal itching, pain, discharge Endorectal US: Irregular hypoechoic lesion Pathology: Confirmed perianal endometriosis
Uno, 2014	Nuck cyst/ femoral hernia (benign differential)	Right groin (femoral ring)	Clinical: Painful, enlarging groin mass Imaging (MRI): Cystic structure with hemorrhagic features, elevated CA-125 Histology: Mesothelial cyst with endometrial stroma
Foulon, 2021	Crohn's disease (perforating)	Bowel (ileum, colon), pelvis	Clinical: Diarrhea, abdominal pain, abscesses Imaging: Ileitis, colitis, multiple abscesses, sigmoid stricture. Magnetic Resonance Imaging (MRI): Revealed findings more characteristic of endometriosis, such as sigmoid wall thickening with infiltration of the perisigmoid fat, adhesions, a retractile endometrial nodule, and a left endometrioma 1. Computed Tomography (CT) Colonography: Confirmed the sigmoid stricture but also showed nodular lesions in the mesorectum, compression of the left ureter by a nodule, a right ovarian cyst, and a small left ovarian cyst 1. Endoscopic Sonography of the Rectum: Showed a 32 mm lesion that was suggestive of rectal endometriosis Labs: Elevated CRP/WBC; actually, endometriosis mimicking Crohn's disease Diagnosis: Based on these collective imaging findings (MRI, CT colonography, Endoscopic Sonography), which revealed features highly suggestive of endometriosis (endometrial nodule, endometrioma, specific lesions, ovarian cysts), the clinical team changed the diagnosis from Crohn disease to complicated deep endometriosis
Kourouma, 2017	Keloid (though malignancy sometimes considered)	Umbilicus (cutaneous)	Clinical: Painful, enlarging umbilical nodule on dark skin Initially treated with steroids as keloid Cyclical bleeding indicated endometriosis Pathology: Under an ulcerated epidermis, the presence of endometrial glands lined by cylindrical epithelium was observed. Endometrial stroma composed of small round cells was also present.
Mahiou, 2024	Invasive pelvic cancer (gynecologic or colorectal)	Vaginal stump, rectovaginal septum, rectum, pelvic peritoneum	Clinical: 68-year-old postmenopausal, infiltrating vaginal stump mass MRI: Solid + cystic lesion, hemorrhagic components Intraop: Cauliflower-like mass; extensive resection Pathology: Endometriosis
Carvalho, 2020	Ovarian/ peritoneal malignancy	Retroperitoneal mass (17x13x16 cm), omen tum, iliac LN	Clinical: 31 y/o, large solid-cystic massCA-125: 641 U/mL Laparoscopy: Frozen pelvis, suspicious omental nodules Pathology: Hard, irregular lesion resembling tumor; final = endometriosis

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REFERENCE	SUSPECTED MALIGNANCY	LOCATION OF ENDOMETRIOSIS	CLINICAL CASE SCENARIO
Fischer, 2021	Invasive carcinoma (florid mesothelial hyperplasia)	Abdominal wall (Pfannenstiel incision)	Clinical/Path: Endometriosis with florid mesothelial hyperplasia Pathology: Florid mesothelial hyperplasia occurring within fibrous tissue associated with abdominal wall endometriosis. The lesion exhibited an infiltrative pattern and stellate architecture, mimicking an invasive carcinoma, particularly given its cytokeratin positivity 1, 2. However, the mild cytologic atypia and positive staining for mesothelial markers (calretinin, WT-1, CK5), along with negativity for markers typical of common adenocarcinomas, established the diagnosis as a benign, reactive mesothelial proliferation.
Pang, 2019	Advanced ovarian cancer	Uterus (posterior fundus), bilateral ovaries, peritoneum	Clinical: Weight loss, large pelvic massCA-125: 372.4 U/mL Imaging: Solid/cystic tumor, 2000 mL bloody ascites Pathology: Endometriosis
Rodriguez, 2017	Cervical adenocarcinoma (Pap smear AGC-NOS)	Cervix (superficial endometriosis)	Clinical: Atypical glandular cells on Pap(AGC-NOS) Concern for endocervical neoplasia Pathology: Microscopically, glandular formations with an endometrial pattern were found, surrounded by fibrous stroma. These findings were suggestive of an endometrioma
Yang, 2021	Cystic renal tumor (Bosniak III)	Lower pole of right kidney	Clinical: Intermittent gross hematuria Imaging (CT/US): Complex cystic renal mass (50% chance of malignancy) Pathology: Histopathology revealed endometriosis of the right renal parenchyma. Gross Examination: The resected mass had a diameter of approximately 1.5 cm. It contained several capsular spaces filled with brown fluid, and the cut surface of the mass was yellowish. Microscopic Examination: Confirmed the diagnosis of renal endometriosis, characterized by the presence of endometrial glands and embedded stromal cells. No atypia was observed. Im immunohistochemical Analysis: The stromal cells and epithelial cells were positive for estrogen receptor (ER), progestin receptor (PR), and vimentin, further supporting the diagnosis of renal endometriosis.
Basnayake, 2020	Possible malignant transformation of inguinal endometriosis	Inguinal canal	Clinical: 4x4 cm cystic mass in inguinal region Imaging: Benign hydrocele-like, unusual site Surgery to exclude malignancy; Pathology: Endometriosis
Molina, 2019	Cecal/colorectal cancer	Cecum, right adnexa	Clinical: Acute complete bowel obstruction, weight loss, mass Imaging: 7x7x4 cm cecal lesion + adnexal mass High suspicion of malignancy Pathology: Ce cum Mass: A 4 × 3 × 2.5 cm bluish, heterogeneous mass was identified, which occluded almost all the lumen of the cecum and the ileocecal valve. Microscopic Examination (Cecum): Microscopy showed that the colon wall was invaded by glands and endometrial stroma. The colonic epithelium displayed inflammatory changes but was negative for malignancy. Adnexal Mass (Ovary and Fallopian Tube): In the ovarian parenchyma, an endometrial cyst covered with siderophages was found. Glands and endometrial stroma were also observed in the fallopian tube. The final diagnosis based on these findings was endometriosis
Hsieh, 2023	Intra-abdominal malignancy (gynecologic)	Within uterine leiomyoma + peritoneum	Clinical: Large (~10 cm) heterogeneous tumor, ascites, severe pain CA-125: 3061, CA-19.9: 1407 Ruptured lesion with suspicious implants; Pathology: Endometriosis

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REFERENCE	SUSPECTED MALIGNANCY	LOCATION OF ENDOMETRIOSIS	CLINICAL CASE SCENARIO
Cameron, 2016	Metastatic breast carcinoma	Umbilicus (subcutaneous)	Clinical: Postmenopausal with prior invasive lobular breast CA New umbilical lesion suspicious for metastasis Pathology: Endometriosis
Yazawa, 2022	Advanced ovarian carcinoma	Right adnexa, cecum, sigmoid, omentum	Clinical: Rapid tumor growth, partial obstruction Imaging (CT/PET): Multiple solid masses, high FDG uptake Pathology: Disseminated endometriosis
Gaillard, 2022	Peritoneal surface malignancy (mesothelioma, ovarian CA)	Diffuse intraperitoneal cystic lesions, mesentery, pelvic peritoneum	Clinical: Progressive abdominal distention, cachexia Imaging: Multicystic peritoneal disease, hydronephrosis Elevated CA-125, CA-19.9; Pathology: Laparoscopic Appendectomy (Age 23): The specimen was negative for appendicitis, endometriosis, or an appendiceal neoplasm. Diagnostic Laparoscopy (Age 26): Biopsies of diffuse cystic lesions revealed abdominal cysts but were negative for endometriosis. Cytologic Examination (Age 29, from drained cyst fluid): Revealed neutrophil granulocytes (indicating infection/inflammation). Cultures were positive for Staphylococcus aureus. Diagnostic Laparoscopy (Age 29): Biopsies of a cystic wall showed fibrinoid tissue and macrophages loaded with hemosiderin (indicating clearance of old hemorrhage). These were negative for endometriosis and malignant disease. De bulking Surgery (Age 29): Frozen Section: Analysis of a sample from the wall of the largest cyst revealed numerous hemosiderin loaded macrophages. No malignancy was present in this sample. Final Histology (Post Debulking): Confirmed the presence of a large cyst (32 × 16 × 5 cm) and multiple smaller cysts containing endometrial epithelium and specialized stroma, consistent with endometriosis. Stripping specimens showed mesothelium and the presence of pigmented macrophages. Cytologic analysis revealed ligated blood cells without malignant cells.
Buder Bakhaya, 2019	Metastatic melanoma	Subcutaneous tissue, lower right abdomen	Clinical: History of melanoma, new subcutaneous lesion Imaging (MRI): Solid lesion with enhancement Pathology: Endometriosis
Iida, 2017	Ovarian carcinoma(with LN metastasis)	Left ovary (polypoid endometriosis), pelvic LN	Imaging (MRI): Papillary nodules, diffusion restriction Enlarged LN with strong enhancement Elevated CA-125, CA-19.9; malignancy not excluded intraop; Pathology: Endometriosis
Jeswani, 2011	Nerve sheath tumor (schwannoma)	Left L4 neural foramen	Clinical: Progressive radicular pain Imaging (MRI): Foraminal mass, suspected schwannoma Intraoperative: Mass involving nerve root; Pathology: endometriosis
Takeda, 2025	GIST, schwannoma, glomus tumor, or metastatic cancer	Terminal ileum/ ileocecal region	Clinical: Intestinal obstruction Imaging (CT, colonoscopy): Well-enhanced submucosal mass, inconclusive biopsies Pathology: Endometriosis
Badri, 2018	Renal malignancy	Upper pole of left kidney	Clinical: Flank pain, gross hematuria Imaging (CT/MRI): Heterogeneous enhancing renal mass Pathology: Robotic partial nephrectomy: Endometriosis
Nambiar, 2018	Metastatic breast carcinoma	Abdominal wall (sub cutaneous), suprapubic region	Clinical: Advanced breast CA; new abdominal wall mass Pathology: Endometriosis, not metastatic disease
AlSinan, 2021	Inguinal hernia *vs.* Malignant soft tissue tumor or lymphoma	Left inguinal region (round ligament)	Clinical: Painful, cyclical inguinal mass Imaging: Solid inguinal lesion Differential: Sarcoma, lymphoma; Pathology: Endometriosis

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REFERENCE	SUSPECTED MALIGNANCY	LOCATION OF ENDOMETRIOSIS	CLINICAL CASE SCENARIO
Wu, 2023	Colorectal cancer	Sigmoid colon, peri colic LNs	Clinical: Large-bowel obstruction, weight loss, constipation Imaging: Mural thickening, impassable steno sis Pathology: Macroscopic Findings: The specimen showed localized, rubbery bowel wall thickening which was compressing and distorting the lumen. The serosa appeared mottled brown, indicating previous hemorrhage and hemosiderin deposition, and also showed greyish-white fibrous puckering. A cross section showed a rubbery, pale tan appearance consistent with hyperplastic smooth muscle compressing the lumen. Patches of congested, mottled, and brown serosa overlay sites of endometriosis. No features suggestive of malignant transformation were found . Microscopic Findings: The presence of endometrial glands and stroma scattered throughout the bowel wall (submucosa and muscularis propria) confirmed the diagnosis of Deep Infiltrating Endometriosis (DIE). There was marked smooth muscle hyperplasia, expanding the bowel wall. The muscularis mucosae was seen blending with the muscularis propria, and the hyperplastic smooth muscle contained scattered endometrial-type glands. Ectopic endometrial epithelium was discovered within two pericolic lymph nodes. Within the affected lymph node(s), there was a cystically dilated gland lined by endometrial epithelium, contain ingblood/fibrin, surrounded by lymph node parenchyma showing reactive follicles.
Ledezma, 2021	Bladder malignancy	Bladder dome (infiltrating), contacting remnant cervix & sigmoid	Clinical: Chronic pelvic pain, severe hematuria Imaging (US/CT/MRI): Infiltrative bladder mass, restricted diffusion CA-125: 93.9 Pathology: Endometriosis
Medlin, 2016	Pseudomyxoma peritonei (appendiceal/ peritoneal CA)	Diffuse peritoneal implants, large cystic masses, endometriomas	Clinical: Diffuse abdominal pain, weight gain, ascites Imaging (CT): Multi-loculated fluid, bowel centralization, adnexal mass Elevated CA-125 (223) Pathology: Endometriosis
Fan, 2025	Ovarian/ peritoneal malignancy	Left ovary (polypoid endometriosis), pelvic side wall, uterus, right adnexa	Clinical: Severe pelvic pain, recurrence Imaging (CT/ MRI): Complex cystic-solid pelvic masses, infiltration, hydronephrosis CA-125: 1386.5 Pathology: Polypoid endometriosis
Zhao, 2018	Rectal cancer; also suspected cervical cancer or GIST	Rectal wall (4 cm from anus)	Clinical: Postcoital bleeding, constipation, narrow stool Imaging (US, CT, PET): Rectal mass with FDG uptake Initial biopsy: Mesenchymal tumor suspicion; Pathology: Endometriosis
Umair, 2020	Renal tumor	Right kidney (interpolar region)	Clinical: Paroxysmal flank pain in pregnancy Imaging (MRI): ~ 6 cm heterogeneous renal mass Radical nephrectomy for presumed malignancy; Pathology: endometriosis

Molecular Parallels

Clinical reports indicating that endometriosis frequently mimics cancer in its presentations have found corroboration at the molecular level, where substantial parallels have emerged. Multiple canonical malignancy-associated pathways (PI3K/AKT/mTOR, MAPK (ERK, p38, JNK), NF-κB, Wnt/β-catenin, and JAK/STAT) demonstrate aberrant activation in endometriotic lesions. These similar pathways sustain proliferation, invasive capacity, angiogenesis, and an anti-apoptotic state, fostering an environment in which endometriosis can behave much like a neoplasm although endometriosis does not fulfill all the hallmarks of cancer (57). Further amplifying these

malignant-like behaviors are hormonal signaling disturbances (notably estrogen-dependent growth and progesterone resistance), persistent inflammatory drivers, and various epigenetic modifications often also implicated in tumor pathogenesis **(Figure 1)**.

Molecular Pathways

- PI3K/AKT/mTOR Pathway: Extensive work has established that the PI3K/AKT/mTOR axis is persistently overactive in eutopic and ectopic endometrial cells, evidenced by high levels of phosphory-

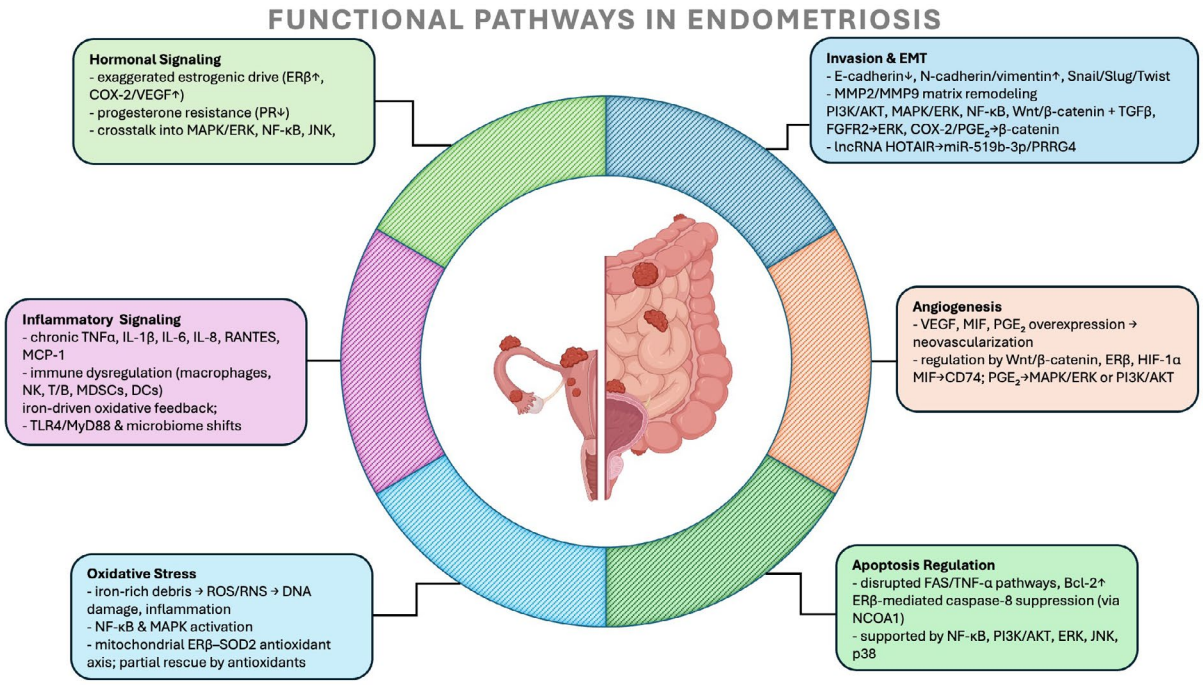

Figure 1. *This figure highlights six interrelated functional pathways that underlie the pathophysiology of endometriosis, depicted around a central image showing ectopic endometrial lesions dispersed across pelvic and intestinal structures. Each color-coded sector summarizes evidence-based mechanisms contributing to lesion establishment and survival:*

- ***Hormonal Signaling***
  Exaggerated estrogenic drive (elevated ERβ expression, COX-2/VEGF induction) and reduced progesterone receptor signaling jointly sustain inflammatory and proliferative cascades, in part through crosstalk with MAPK/ERK, p38, NF-κB, JNK, and Wnt/β-catenin (59, 70, 71, 75, 78).
- ***Inflammatory Signaling***
  Persistent elevation of proinflammatory cytokines (TNFα, IL-1β, IL-6, IL-8, RANTES, MCP-1) and immune cell dysregulation (macrophages, NK cells, T/B lymphocytes) drive lesion progression and pain. Iron overload from retrograde menstruation intensifies oxidative damage, while TLR4/MyD88 and microbiome shifts exacerbate localized and systemic inflammation (59, 69, 71, 78, 81-83).
- ***Oxidative Stress***
  Repeated hemorrhage into the peritoneal cavity and iron-rich debris trigger excess reactive oxygen/nitrogen species, fueling DNA damage and inflammation. Mitochondrial ERβ-mediated responses, along with NF-κB and MAPK activation, reinforce lesion viability; partial amelioration is possible through antioxidant strategies in model systems (65, 67, 69, 71, 75, 78, 84).
- ***Apoptosis Regulation***
  Aberrations in FAS and TNF-α pathways, coupled with increased Bcl-2 expression, enable endometriotic cells to evade apoptosis. Multiple pathways, including NF-κB, PI3K/AKT, ERK, JNK, and p38, further sustain cell survival, while mitochondrial ERβ can suppress caspase 8 (via NCOA-1), mirroring chemoresistance observed in malignancies (59, 69, 71, 78, 85).
- ***Angiogenesis***
  Overexpression of VEGF, MIF, and PGE_2 drives formation of new vascular networks critical for lesion nourishment. Regulation by Wnt/β-catenin, ERβ, and HIF-1α converges on MAPK/ERK and PI3K/AKT/mTOR, while NF-κB signaling amplifies the production of pro-angiogenic mediators (69, 71, 83, 85).
- ***Invasion and Epithelial–Mesenchymal Transition (EMT)***
  Decreased E-cadherin and heightened markers such as N-cadherin and vimentin, together with Snail/Slug/Twist transcription factors, promote tissue invasion and migration. Mechanistic drivers, PI3K/AKT, MAPK/ERK, NF-κB, Wnt/β-catenin, act in concert with TGFβ and COX-2/PGE_2, while lncRNA HOTAIR fosters EMT through miR-519b-3p/PRRG4 (59, 60, 68-70, 76).

lated AKT (p-AKT), PI3K, AKT1, 4EBP1, and mTOR-activating proteins (AXL, SHC1), together with diminished PTEN-mediated inhibition (58–64). Notably, hotspot mutations in *PIK3CA* and *PTEN* have been reported in deep infiltrating variants, implicating these genetic defects in advanced disease. Through this pathway, endometriosis lesions gain proliferative, pro-angiogenic, and pro-survival functions, potentially contributing to both progesterone resistance and heightened risk of EAOC (58-65). Regulatory control is multifactorial: cytokines (TNFα), growth factors (FGFR2, ERBB2/3), and estrogen (via PTEN suppression) jointly activate PI3K/AKT. Non-coding RNAs such as miR-92a and miR-135a/b further amplify the pathway, whereas miR-194-5p attenuates it. LncRNAs, notably HOXA-AS2, interface with miR-4459/IGF2BP2 to enhance cell proliferation via AKT, and ENPP3, commonly hypomethylated, fuels the AKT/mTOR/4EBP1 axis. In addition, endostatin-expressing endometrial stem cells may counter angiogenic signals via miR-21-5p/TIMP3 within this cascade (58, 60-62, 62-64, 66).

- MAPK Pathways (ERK, p38, JNK): Enhanced activation of ERK, p38, and JNK MAPKs is evident in endometriotic lesions relative to normal endometrial tissue (59, 67, 68). These MAPKs govern proliferation, survival (through Bcl-2), migration, invasion, angiogenesis, inflammation, and pain hypersensitivity. Their activation arises from diverse stimuli, TNFα, IL-1β, FGFR2, leptin, or TGFβ, and proceeds via the Ras–Raf–MEK cascade (59, 62, 66). Specific miRNAs (e.g., miR-340-5p) modulate MAPK activity and pharmacological inhibition of Raf, VEGFR, p38, or JNK suppresses lesion growth in preclinical models (55, 64).

- NF-κB Pathway: Chronic NF-κB activation occurs in ectopic stromal cells and peritoneal macrophages, diverging from normal cyclic regulation (61, 69). This persistent activation drives inflammatory mediators (IL-6, IL-8, RANTES, MCP-1, GM-CSF, MIF), matrix remodeling via metalloproteinases (MMPs), angiogenesis (VEGF), and resistance to apoptosis (59, 61, 62, 65, 66, 69, 70). NF-κB activation is triggered by TNFα, IL-1β, TSLP, iron overload, or TLR4/MyD88 signaling, whereas miR-16 negatively regulates the pathway by targeting IKKβ (65, 66, 69, 71).

- Wnt/β-catenin Pathway: Aberrant Wnt/β-catenin signaling in endometriosis, characterized by altered β-catenin expression and SFRP2 hypomethylation, promotes invasive growth, fibrosis, and epithelial–mesenchymal transition (EMT) (65, 72, 73). Progesterone usually inhibits Wnt/β-catenin, but progesterone resistance diminishes this protective effect. Dysregulated factors (estrogen, FOXP1, WEE1, MMP9) sustain Wnt signaling, while miRNAs (miR-33b, let-7a/g, miR-532-3p) and COX-2/PGE2 influence pathway intensity and EMT induction (58, 70, 71, 73).

- JAK/STAT Pathway: Research focusing on the JAK2/STAT3 arm identifies IL6ST (gp130) hypomethylation and overexpression in ectopic tissue, magnifying IL-6 signaling (74). Enhanced JAK2/STAT3 contributes to lesion proliferation, invasion, and anti-apoptotic phenotypes analogous to tumorigenic growth (74). Simultaneously, downregulation of STAT1 by miR-194-5p removes a moderating effect on mTOR, further bolstering JAK2/STAT3 (58, 74).

- Epigenetic and Non-coding RNA Regulation: The role of epigenetic derangements, DNA methylation shifts (e.g., SFRP2 hypomethylation or aberrant IL6ST methylation), histone modifications (HDAC upregulation), and dysregulated miRNA/lncRNA expression, in driving ectopic lesion resilience (64, 65, 72, 74–76) have been raised attention. Genes mediating steroid hormone action (ESR2, PR), inflammatory signaling (IL6ST), or tumor suppression (RASSF1A, E-cadherin) are frequently abnormally silenced or expressed. As seen in oncologic processes, such epigenetic alterations provide cellular plasticity, allowing endometriotic lesions to endure fluctuations in hormones and cytokines. Concomitant aberrations in specific miRNAs (e.g., miR-135b or miR-194-5p) or lncRNAs (HOXA-AS2, HOTAIR) can intensify these adaptive capabilities (64, 65, 71, 74, 74-79).

Critical Functional Modifications

- Hormonal Signaling (Estrogen/Progesterone): Endometriosis characteristically shows an exaggerated estrogenic drive and impaired progesterone receptor signaling, driving persistent lesion growth and inflammatory responses (59, 71, 75, 77, 78, 80). Notably, ERβ is abundant, even within mitochondrial compartments, supporting enhanced bioenergetics and oxidative stress defenses. Estrogen triggers COX-2 and prostaglandin upregulation, as well as angiogenic mediators (VEGF), while progesterone resistance, encompassing reduced PR expression, disrupts physiologic tissue remodeling (59, 70, 71, 75, 77). Hormonal crosstalk also activates MAPK/ERK, p38, NF-κB (through PTEN attenuation), JNK (via TSLP), and Wnt/β-catenin, with epigenetic modifiers such as Betulinic Acid (ERβ suppression) and miR-23a/b (SF-1) further refining these networks (59, 66, 67, 69).

- Inflammatory Signaling: Chronic inflammation remains a hallmark of endometriosis, reflected by heightened TNFα, IL-1β, IL-6, IL-8, RANTES, and MCP-1 in peritoneal fluid and lesions. Concomitant dysfunction occurs in macrophages, NK cells, T and B cells, MDSCs, and dendritic cells (59, 69, 71, 77, 81-83). This proinflammatory milieu helps establish lesions, promotes new vessel formation, and drives fibrosis and pain. Iron overload arising from retrograde menstruation can intensify inflammatory and oxidative damage, while broad molecular routes (NF-κB, MAPK, JAK/STAT, PI3K/AKT/mTOR) orchestrate extended neuroinflammatory cascades. Moreover, gut microbiome shifts and TLR (TLR4/MyD88) perturbations appear to escalate systemic and localized inflammation (59, 69, 71, 77, 81-83).

- Oxidative Stress Pathway: Repeated episodes of retrograde bleeding deposit iron-rich debris, fueling reactive oxygen and nitrogen species that injure DNA, heighten inflammation, and potentially initiate precancerous changes (65, 69, 71, 75). Mitochondrial ERβ may modulate aspects of antioxidant responses (e.g., SOD2). Chronic oxidative stress reciprocally activates NF-κB and MAPK, reinforcing lesion viability. Model systems demonstrate that antioxidant interventions can partially mitigate these detrimental effects (66, 69, 75, 77, 84).

- Apoptosis Regulation: Another defining feature is the ability to evade programmed cell death through disrupted FAS or TNF-α–mediated pathways, bolstered by elevated Bcl-2 (59, 69, 71, 85). This evasion allows ectopic tissue to persist through cyclical hormonal changes. Investigations underscore pivotal roles for NF-κB, PI3K/AKT, ERK, JNK, and p38 in maintaining these cells, while estrogen (ERβ) can suppress caspase 8 by means of cofactors such as NCOA-1 (59, 69, 71, 77). Such anti-apoptotic mechanisms mirror chemoresistance in various malignancies.

- Angiogenesis: Multiple studies reveal that VEGF, MIF, and PGE2 are consistently overexpressed in endometriosis, forming an aggressive neovascular network critical for lesion support (69, 71, 83, 85). This shift into enhanced vessel formation mirrors tumor biology, delivering nutrients and oxygen to ectopic cells. Wnt/β-catenin, ERβ, and HIF-1α can govern VEGF expression, whereas MIF (acting via CD74) and PGE2 converge on MAPK/ERK or PI3K/AKT/mTOR routes (69, 71, 83, 85). NF-κB likewise induces pro-angiogenic mediators.

- Cell Invasion, Migration, and EMT: Endometriotic tissue often manifests lowered E-cadherin, heightened N-cadherin and vimentin, and transcription factors (Snail, Slug, Twist) that define EMT (67-69, 71-73, 86). Metalloproteinases, such as MMP2 and MMP9, remodel extracellular matrices, fostering deeper tissue infiltration. These invasive traits are orchestrated by pathways including PI3K/AKT, MAPK/ERK, NF-κB, and Wnt/β-catenin, often with TGFβ serving as a central pro-invasive factor. FGFR2 augments migration via ERK, while COX-2/PGE2 interacts with β-catenin. LncRNA HOTAIR drives EMT through miR-519b-3p/PRRG4, aligning with malignant-type metastatic processes (59, 60, 67-70, 76).

GAPS IN UNDERSTANDING

Despite substantial progress in elucidating clinical and molecular aspects of endometriosis, significant uncertainties persist regarding its pathogenesis, accurate diagnosis, and effective application of emerging insights to patient care.

Methodological and Translational Limitations

Numerous research teams emphasize the shortcomings of existing in vitro and in vivo models, which fail to mirror the complexity of the human disease. Most rodent models insufficiently capture the varied pain phenotypes, particularly non-evoked, chronic components, that characterize endometriosis in patients. While such models remain key to preclinical testing, they often focus on reflex-based endpoints alone, thereby underrepresenting clinically relevant pain features (87). Furthermore, many preclinical and clinical studies inadequately or inconsistently address pain endpoints, limiting translational relevance (88). Parallel issues exist in the validation and clinical translation of biomarkers. Although numerous genomic, epigenomic, proteomic, and metabolomic candidates have been proposed, few have demonstrated sufficient sensitivity, specificity, or reproducibility to enter clinical practice reliably (89). Variability across studies, limited sample sizes, and a lack of robust validation studies prevent broader implementation, leaving invasive diagnostic methods and empirical management as current standards of care (90).

Clinical Challenges and Unmet Needs

Pain Mechanisms: A crucial unresolved issue is the poor correlation between lesion burden and pain severity; some patients experience minimal symptoms despite extensive disease, while others suffer

severe, debilitating pain from minor lesions. Emerging evidence suggests that neuroinflammatory pathways and central sensitization mechanisms might decouple pain from lesion size or anatomical staging (91). As conventional surgical or hormonal therapies target primarily visible lesions, many patients remain undertreated for persistent or recurrent pain. Without clearer insights into these overlapping neuronal and inflammatory processes, current approaches may miss a substantial subset of patients who continue to experience pain despite standard treatments.

Lesion Biology Heterogeneity: Significant variability in lesion morphology, invasive behavior, hormonal responsiveness, and recurrence risk underscores inherent biological heterogeneity in endometriosis. Evidence suggests stem-like or progenitor cell populations contribute substantially to lesion resilience and therapeutic resistance (92). However, direct identification of these stem-like cells in clinically pertinent models, particularly for deep infiltrating endometriosis, is lacking. Improved techniques to isolate and characterize such cells could enable more precisely targeted therapies that reduce recurrence without the broad side effects characteristic of hormonal suppression.

Fertility and Reproductive Outcomes: Fertility-related research remains notably deficient. Emerging single-cell transcriptomic and proteomic analyses reveal disruptions in oocyte maturation pathways linked to oxidative stress and abnormal molecular regulation, correlating with reduced reproductive outcomes in patients with ovarian endometriosis (93). While these data highlight potential molecular pathways affecting ovarian function, translation into clinical practice, such as methods to restore typical oocyte function, remains largely unaddressed. Equally puzzling is why some endometriosis patients maintain robust fertility, whereas others encounter severe, treatment-refractory infertility.

Limitations of Current Hormonal Therapies: Management of endometriosis remains predominantly reliant on broad hormonal suppression, posing significant drawbacks for patients with contraindications or fertility goals. Despite preclinical promise, therapies targeting local estrogen biosynthesis, inflammatory signaling pathways, or dysregulated neuroimmune interactions remain limited in their clinical adoption and validation (94). Moreover, no consensus exists for targeted therapies aimed at local estrogen synthesis, inflammatory pathways, or dysregulated neuroimmune mechanisms without broad hormonal suppression. Novel interventions (for example, lncRNA or circRNA modulators and agents targeting stem-like cells) show promise in preclinical investigations but lack rigorous testing in phase I/II clinical trials (87, 90-92, 94).

DISCUSSION

Endometriosis displays complex cancer-mimicking characteristics that complicate biomarker development and clinical translation (7, 81, 91). Recent proteomic studies identified a promising 10-protein plasma panel achieving high diagnostic accuracy (AUC 0.997), yet its performance varies considerably across disease stages (95). Similarly, saliva-based miRNA signatures combined with artificial intelligence (AI) algorithms achieved sensitivities of 96–97% and specificities up to 95–100% (96, 97). Although these noninvasive tools are promising, false-negative results remain a concern, potentially extending diagnostic delays. Thus, balancing assay accuracy against minimally invasive surgical interventions remains critical. Comparative studies quantifying risks associated with false-negative diagnoses versus surgical morbidity are necessary to inform optimal patient management strategies (10). A fundamental barrier remains the lack of systematic preclinical and early-phase validation frameworks connecting bench research directly to clinical practice. Despite identifying numerous molecular candidates, such as dysregulated noncoding RNAs, epigenetic alterations, and immune checkpoint dysregulation, few have successfully navigated rigorous, phased evaluations in clearly characterized disease models (89). Biomarkers derived from proteomic or metabolomic platforms similarly require validation through large-scale confirmatory studies, which remain insufficient (90). Consequently, invasive diagnostic procedures, broad hormonal suppression, and repeated surgical interventions persist as the predominant standards of care (98). Although endometriosis predominantly remains a benign condition with cancer-mimicking features, a small but clinically important proportion can undergo genuine malignant transformation, particularly to EAOC. Recent studies have elucidated distinct EAOC clinical entities, notably distinguishing between endometriosis-correlated ovarian carcinoma, characterized by transitional lesions such as atypical endometriosis or borderline tumors, and endometriosis-incidental ovarian carcinoma, in which benign endometri-

osis occurs independently alongside ovarian cancer. Patients with endometriosis-correlated ovarian carcinoma tend to present at younger ages, earlier disease stages, and with different histopathological subtypes compared to ovarian cancers without associated endometriosis, underscoring critical prognostic and therapeutic differences (6). At a molecular level, the transition from benign ovarian endometriosis to carcinoma is associated with specific miRNA profiles, with recent analyses identifying miRNAs, such as hsa-miR-200a-3p, hsa-miR-141-3p, hsa-miR-183-5p, and hsa-miR-10a-5p, that are significantly upregulated during malignant transformation. These miRNA biomarkers offer promising diagnostic potential for the early identification of patients at elevated risk of progression to EAOC but they still need external clinical validation (99). Furthermore, comprehensive molecular and clinical data reinforce the need for individualized management strategies, emphasizing precise molecular diagnostics and targeted therapeutic approaches to optimize outcomes in ovarian cancer management (100). To address these shortcomings, we propose adopting a structured translational approach whereby new hypotheses undergo systematic validation in robust preclinical models before advancing to carefully staged clinical trials within rigorously stratified patient populations (101). Such an approach ensures promising molecular or immunological candidates first undergo Phase 0 trials to determine safety and biological plausibility prior to progressing to larger-scale Phase II/III evaluations. Aromatase inhibitors exemplify how structured, small-scale experimental validations can translate into targeted, non-hormonal therapeutic options (94). Employing this structured pipeline facilitates efficient conversion of laboratory discoveries into clinically applicable tools. Structured translational research also promotes refined clinical phenotyping. By stratifying endometriosis into distinct clinical subtypes (e.g., deep infiltrating, peritoneal, ovarian), novel therapies—such as immune checkpoint modulators, anti-inflammatory compounds, and epigenetic drugs—can be more precisely matched to patient subgroups most likely to respond. This targeted approach reduces reliance on empirical treatment strategies (11). Early-phase models further help define relevant clinical endpoints, such as pain alleviation, fertility restoration, and lesion regression, while incorporating advanced imaging, immunological profiling, and biomarker assessments to measure therapeutic outcomes dynamically. An emerging yet underappreciated concern involves reliance on large-scale data analytics and AI-driven methodologies for identifying molecular signatures and disease subtypes. Without robust, patient-centered translational frameworks, indiscriminate use of AI risks embedding pre-existing biases, amplifying health inequalities, and generating outcomes that neither address patient-specific clinical needs nor tangibly improve clinical care (20, 102). AI models developed on incomplete or biased datasets may further exacerbate misdiagnosis or inappropriate therapeutic decisions. To mitigate these risks, AI applications should undergo transparent, phase-appropriate clinical validation, clearly defined endpoints, and equity-focused performance assessments. Such rigor prevents the pitfalls associated with opaque algorithmic ("black-box") decision-making. Similarly, advanced imaging and machine-learning technologies must follow carefully structured validation pathways prioritizing high-quality data, equitable patient access, and continual monitoring. These measures ensure AI-enhanced strategies meaningfully advance critical clinical outcomes such as pain relief and fertility preservation, thereby safeguarding both scientific integrity and personalized patient care. Ultimately, endometriosis represents a multifaceted, systemic disorder with numerous malignant-like features (11). Establishing a translational roadmap grounded in refined phenotyping, phase-focused validation, and methodical clinical trials is vital. By instituting a well-defined pipeline, clinicians and researchers can move beyond traditional hormonal treatments, ultimately delivering targeted, effective interventions and significantly improving quality of life for women who currently endure the substantial burdens of endometriosis.

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COMPLIANCE WITH ETHICAL STANDARDS

Fundings

No funding was utilized for this study as it comprised observational research incorporating routine clinical practices.

Conflict of interests

The authors declare that there are no conflicts of interest associated with this publication.

Availability of data and materials

The data supporting the findings of this study are available upon reasonable request to the corresponding author.

Authors' contributions

CM: Conceptualization, Methodology, Formal analysis, Writing – Original Draft, Supervision, Project administration. AV: Conceptualization, Investigation, Resources, Writing – Review & Editing, Supervision. FDC: Formal analysis, Visualization, Writing – Original Draft. GM: Validation, Formal analysis, Data Curation. SEM: Investigation, Data Curation, Writing – Original Draft. LA: Formal analysis, Investigation, Validation. AE: Investigation, Resources, Validation, Writing – Review & Editing. AG: Supervision, Project administration, Funding acquisition, Writing – Review & Editing.

Ethical approval

This research adhered to the ethical standards of the World Medical Association's Declaration of Helsinki and complies with the Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals, including the inclusion of diverse human populations in terms of sex, age, and ethnicity.

Human studies and subjects

N/A.

Animal studies

N/A.

Publications ethics

The publication ethics followed by this study align with those outlined by the International Committee of Medical Journal Editors (ICMJE), regarding publishing and editorial issues in medical journals.

Plagiarism

The article provides a comprehensive review of the latest studies in the field, with accurate citations.

Data falsification and fabrication

The writing and contents of the article are entirely original and were developed entirely by the authors.

Abbreviations

AI: Artificial Intelligence
AKT: Protein Kinase B (also referred to as "PKB")
AXL: AXL Receptor Tyrosine Kinase
Bcl-2: B-cell lymphoma 2
CA-125: Cancer Antigen 125
CA-19.9: Cancer Antigen 19.9
CD74: Cluster of Differentiation 74
circRNA: Circular RNA
COX-2: Cyclooxygenase-2
CT: Computed Tomography
CTLA-4: Cytotoxic T-lymphocyte-Associated Protein 4
EAOC: Endometriosis-Associated Ovarian Cancer
EMT: Epithelial–Mesenchymal Transition
ENPP3: Ectonucleotide Pyrophosphatase/Phosphodiesterase 3
ERβ: Estrogen Receptor Beta (also written as ESR2)
ERK: Extracellular Signal-Regulated Kinase
FGFR2: Fibroblast Growth Factor Receptor 2
Gal-9: Galectin-9
GC–MS: Gas Chromatography–Mass Spectrometry
GM-CSF: Granulocyte Macrophage Colony-Stimulating Factor
HDAC: Histone Deacetylase
HIF-1α: Hypoxia-Inducible Factor 1-Alpha
HOXA-AS2: HOXA Cluster Antisense RNA 2
HOTAIR: HOX Transcript Antisense RNA
IGF2BP2: Insulin-like Growth Factor 2 mRNA-Binding Protein 2
IKKβ: IκB Kinase Beta
IL-1β: Interleukin 1 Beta
IL-6: Interleukin 6
IL-8: Interleukin 8
IL6ST (gp130): Interleukin 6 Signal Transducer (glycoprotein 130)
JAK: Janus Kinase
JNK: c-Jun N-terminal Kinase
lncRNA: Long Noncoding RNA
MAPK: Mitogen-Activated Protein Kinase
MCP-1: Monocyte Chemoattractant Protein 1 (also known as CCL2)
MDSCs: Myeloid-Derived Suppressor Cells
MIF: Macrophage Migration Inhibitory Factor
miRNA: MicroRNA

MRI: Magnetic Resonance Imaging
mTOR: Mechanistic Target of Rapamycin
MyD88: Myeloid Differentiation Primary Response 88
N-cadherin: Neural Cadherin
NCOA-1: Nuclear Receptor Coactivator 1
NF-κB: Nuclear Factor kappa B
p38: p38 Mitogen-Activated Protein Kinase
pAKT: Phosphorylated AKT
PD-1: Programmed Cell Death Protein 1
PD-L1: Programmed Death-Ligand 1
PET: Positron Emission Tomography
PI3K: Phosphatidylinositol 3-Kinase
PRRG4: Proline-Rich Gla (γ-carboxyglutamic acid) Protein 4
PTEN: Phosphatase and Tensin Homolog
Raf: Rapidly Accelerated Fibrosarcoma (proto-oncogene in the MAPK pathway)
RANTES: Regulated upon Activation, Normal T Cell Expressed and Secreted (also known as CCL5)
RASSF1A: Ras Association Domain Family Member 1
SHC1: SHC Adaptor Protein 1 (Src Homology 2 domain-containing)
SFRP2: Secreted Frizzled-Related Protein 2
SOD2: Superoxide Dismutase 2
STAT: Signal Transducer and Activator of Transcription
TGFβ: Transforming Growth Factor Beta
TIM-3: T-cell immunoglobulin and mucin-domain containing-3
TLR4: Toll-Like Receptor 4
TNFα: Tumor Necrosis Factor Alpha
TSLP: Thymic Stromal Lymphopoietin
VEGF: Vascular Endothelial Growth Factor
VEGFR: Vascular Endothelial Growth Factor Receptor
WEE1: WEE1 G2 Checkpoint Kinase
Wnt: Wingless/Integrated
4EBP1: Eukaryotic Translation Initiation Factor 4E-Binding Protein 1

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RESEARCH ARTICLE

A NOVEL POINT-OF-CARE DEVICE FOR MONITORING CIRCULATING MIRNA-107 IN PROSTATE CANCER: TOWARDS A USER-FRIENDLY LIQUID BIOPSY

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ABSTRACT: Liquid biopsy represents an advanced non-invasive cancer detection system which analyzes biomarkers found in blood and other body fluids. MicroRNAs (miRNAs) are important biomarkers because they are stable, disease-specific, and have the potential to reflect tumor dynamics. The miRNA-107 plays an important role in the development and progression of prostate cancer (PCa), which makes it a potential target for early diagnosis and monitoring.
A low-cost paper-based electrochemical platform was developed for sensitive miRNA-107 detection, using in-house synthesized gold nanoparticles (AuNPs)-modified screen-printed electrodes (SPEs) to enhance conductivity and surface area. A DNA probe labeled with methylene blue (MB) was immobilized on SPEs via Au-S bonding. It was then rinsed with deionized (DI) water, and blocked with 6-mercapto-1-hexanol (MCH) to lower nonspecific binding. The platform works on "signal-off" square wave voltammetry (SWV) mechanism which decreases current upon addition of target miRNA hybridization. The key parameters AuNPs volume, probe concentration and frequency were optimized. The sensor has achieved detection limits of 1.8 nM in PBS and 1.0 nM in serum, with strong selectivity against non-target miRNAs.
The results show that this approach has a strong potential to be a rapid, reliable and cost-effective tool for PCa detection. It is portable and easy to use, making it suitable for resource-limited settings, and thus a practical solution for early diagnosis and monitoring at the point of care (POC).

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Impact statement: POC devices have the potentiality to revolutionize the access to diagnosis and care. Liquid biopsy on chip means opportunity for all patients to get timely responses and interventions. MiRNAs represent very promising biomarkers in the field of liquid biopsy, and their decentralized monitoring can be performed at portable strips, similarly in simplicity to those utilized by diabetes patients to monitor blood glucose.

Key words: *Liquid biopsy; miRNA-107; prostate cancer; electrochemical biosensor; signal-off detection; point-of-care diagnostics.*

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INTRODUCTION

Prostate cancer (PCa) is the second most frequently diagnosed cancer among men worldwide (1-3). The disease resulted in 397,430 deaths during 2020 and researchers predict 2.9 million new cases will occur each year until 2040 (4, 5). The incidence rate increases dramatically with age. The majority of PCa

cases progress slowly but the disease can transform into an aggressive form that threatens life (6). So premature early detection and precise monitoring become crucial (7).

Current diagnostic practices for PCa include prostate-specific antigen (PSA) testing, digital rectal examination (DRE), and biopsies (8-10) . PSA testing has helped with early detection of PCa. However, it lacks specificity. Elevated PSA levels can also occur due to benign conditions like prostatitis or benign prostatic hyperplasia. This often leads to overdiagnosis and overtreatment of non-aggressive cases (11). DRE serves as a supplementary tool but has limitations in sensitivity and specificity (12). The gold standard for confirming PCa still remains tissue biopsy because it provides detailed histopathological insights. The procedure is invasive and carries risks of bleeding and infection and may miss tumors which results in false negatives (13, 14). Traditional methods have demonstrated such significant drawbacks that alternative methods have become essential to achieve precise results with low /or minimal invasiveness.

The focus of research has moved toward developing more advanced diagnostic tools which combine precision with user-friendly features and minimal invasiveness. Liquid biopsy emerges as a promising alternative among these because it provides non-invasive diagnostic capabilities through the analysis of circulating tumor cells (CTCs) and cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA) and extracellular vehicles (EVs) and non-coding RNAs including microRNAs (miRNAs) and other biomarkers found in bodily fluids (15, 16). The technique provides multiple clinical benefits because it performs minimally invasive procedures while being safer and better suited for repeated testing throughout time which makes it suitable for monitoring diseases longitudinally (17). The technology enables early detection of cancer recurrence as well as immediate identification of treatment resistance which supports personalized patients care. It shows increasing accuracy through molecular analysis advancements which makes it a promising alternative for conventional diagnostic procedures (18-21).

Small non-coding RNAs have emerged as promising biomarkers among the numerous biomarkers. The miRNAs show particular advantages because they are highly stable in bodily fluids, easily detectable, and often exhibit cancer-specific expression patterns. These advantages make them ideal candidates for non-invasive PCa diagnostics (22). PCa shows specific miRNA associations with its disease behavior. The progression of PCa together with its tumor aggressiveness is linked to miRNA-141 (23) and miRNA-21. The metastatic potential of prostate cancer is associated with miRNA-375 (24). Similarly, the miRNA-107 shows promise as a non-invasive biomarker for PCa diagnosis. The diagnostic potential of miRNA-107 is supported by its elevated levels in PCa patient in serum and urine although most studies present relative expression values instead of absolute concentrations (25, 26). Its high levels in castration-resistant prostate cancer (CRPC) and its association with advanced disease stages make it a a promising tool for early-stage diagnosis. Recent studies have demonstrated that circulating miR-107 levels are significantly elevated in patients with CRPC. For instance, Puente-Rivera *et al.* (2024) reported that miR-107 expression in liquid biopsies was significantly higher in CRPC patients compared to non-castration-resistant prostate cancer (NCRPC) patients, with a p-value <0.005, suggesting its potential as a non-invasive diagnostic biomarker for identifying CRPC patients (27). The diagnostic value of miRNA-107 has been expanded through recent research which demonstrates its therapeutic potential to improve PCa treatment results. The radiosensitivity of PCa cells increases when miRNA-107 targets granulin (GRN) because this protein promotes tumor growth and resistance thus improving radiotherapy outcomes (28).

Similarly, other miRNAs such as miRNA-107 have also been identified as potential non-invasive biomarkers. Its high levels in castration-resistant prostate cancer (CRPC) and its association with advanced disease stages make it a potential biomarker for early detection and monitoring (27). The diagnostic value of miRNA-107 has been expanded through recent research which demonstrates its therapeutic potential to improve PCa treatment results. The radiosensitivity of PCa cells increases when miRNA-107 targets granulin (GRN) because this protein promotes tumor growth and resistance thus improving radiotherapy outcomes (28).

The clinical implementation of miRNA-107 shows great promise to revolutionize PCa management through its ability to provide non-invasive blood tests which detect PCa early and predict patient outcomes and tailor treatment plans. This approach improves patient outcomes, reduces the need for invasive biopsies, and provides more targeted and effective care by monitoring treatment responses and refining risk assessments. The clinical application of miRNA-based assays for PCa diagnosis and

prognosis faces challenges because conventional analytical platforms have certain limitations. Northern blotting techniques have limitations for clinical use because they lack sensitivity and require extensive time which prevents their use in routine diagnostics (29). The short sequences and high sequence homology of miRNAs make it difficult to design primers and achieve accurate quantification in real-time quantitative polymerase chain reaction (RT-qPCR) (30, 31). Microarrays and next-generation sequencing (NGS) provide broad profiling capabilities but demand costly infrastructure and extensive sample preparation, limiting their use outside specialized laboratories (32, 33). The NanoString nCounter system provides amplification-free quantification but its high costs and complex normalization procedures limit its use (34). The emerging tool of digital droplet PCR (ddPCR) provides exact miRNA quantification but requires trained personnel and sophisticated equipment (35). Loop-mediated isothermal amplification (LAMP) technique demonstrates potential for field applications yet requires further optimization to detect miRNAs because of sequence constraints (36). While these systems offer strong analytical performance, they often require complex fabrication and instrumentation. So, the focus has shifted to diagnostic formats which offer accessibility and speed and require minimal infrastructure without losing clinical relevance.

The demand has shifted toward decentralized testing through Point-of-Care (POC) innovations which enhance diagnostic access worldwide. The increasing worldwide demand for POC diagnostics demonstrates the critical requirement for quick and affordable testing solutions particularly in resource-limited areas. The latest developments in decentralized testing have demonstrated its worthwhile propelling POC innovations that transform disease diagnosis methods (37, 38). Building on this progress, the mChip represents a practical application of POC technology because it functions as a credit card-sized microfluidic device which performs HIV and syphilis detection from a single drop of blood. The technology proved its effectiveness by reaching 100% accuracy for HIV detection and 94% accuracy for syphilis detection during Rwanda trials while each chip cost approximately $1 which makes it ideal for low-resource settings (39-41). Complementing these advancements, SD Biosensor's dual HIV/syphilis rapid diagnostic test was developed with support from MedAccess. The test has been adopted by more than 100 low- and middle-income countries since 2022. The test uses a finger-prick sample to deliver results within 20 minutes. The test operates at a cost below $1 per unit which makes it suitable for locations with restricted laboratory facilities (42). The widely acceptance of patient-centered care has been further enhanced by basic diagnostic tools including glucose meters, pregnancy tests and COVID-19 kits. These tools provide fast and precise results while enabling testing at the exact location where it is needed (43). These technologies enhance this transformation by both filling critical diagnostic gaps and enabling a decentralized patient-centered care system with better responsiveness. The broad range of applications from infectious diseases to cancer demonstrates how POC tools are increasingly reshaping healthcare delivery systems.

The development of POC devices for miRNA detection has tumor dynamics. transform cancer management in a similar direction. These tools offer timely and cost effective diagnostic solutions, bridging the gap between complex traditional methods and the need for accessible real time testing (44). Extending this potential further, stability and specificity of miRNAs make them valuable biomarkers for cancers including prostate, breast and lung (45). The approach can be further developed by integrating miRNA detection into portable POC platforms that utilize electrochemical sensors and paper-based microfluidic systems to enable early diagnosis and dynamic disease monitoring and tailored therapy outside traditional laboratory settings. This represents a vital advancement in precision oncology that delivers accessible patient-centered care.

Responding to the critical need for rapid and accessible cancer diagnostics, our research group continuously focuses on developing paper-based electrochemical platforms with screen-printed electrodes (SPEs) for cancer detection. The research focuses on converting precise analytical methods into deployable field formats which will enhance point of care biosensing capabilities for resource limited and decentralized clinical settings. Building on this foundation, Cimmino and colleagues developed an electrochemical device for miRNA-224 detection which serves as a biomarker for lung cancer. The system used methylene blue (MB) labeled DNA probes with SPEs on wax patterned paper. The system reached a detection limit of 0.6 nM in spiked human serum which demonstrated both high sensitivity and field deployability (46). Raucci *et al.* developed a biosensor using paper to detect miRNA-652 which serves as a biomarker for triple negative breast cancer. The

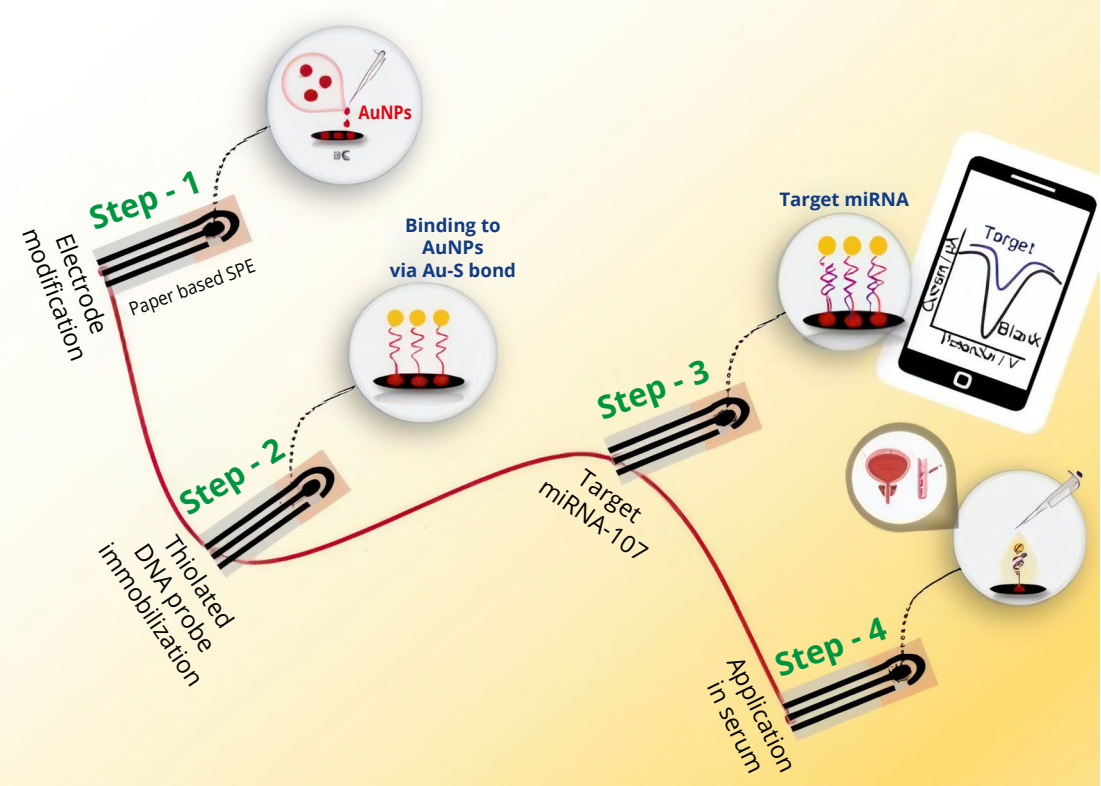
device utilized screen printed electrodes that were modified with gold nanoparticles (AuNPs) on office paper. The system used wax-assisted chromatographic preconcentration as its analytical method. The sensor reached a detection limit of 0.4 nM in human serum while demonstrating good repeatability (47). Martino *et al.* further showed that nanostructured gold composites on microfabricated electrodes enable sub-nanomolar detection of cancer-related miRNAs (48).

The diagnostic application of miRNA detection benefits from AuNPs modified electrodes because they provide both high sensitivity and adjustable surface chemistry. The research presents a portable paper-integrated biosensor, which detects miRNA-107 specifically because this biomarker appears in different cancer types. The sensor combines AuNPs synthesized in-house with SPEs made from standard office paper to create a cost-effective and scalable detection system. The working electrode contains thiolated DNA capture probes which are labeled with MB. The immobilized DNA probe recognizes its target miRNA 107 sequence through hybridization. The molecular interaction between the target and the probe changes the electrochemical properties of the redox label which allows square wave voltammetry (SWV) to obtain electrochemical readout. The platform demonstrates excellent potential for POC applications because it provides high sensitivity and electrochemical readout functionality. Further, it also offers a simple and reliable operation which makes it ideal for decentralized cancer diagnostics particularly in resource limited settings. Our method unites proven sensitivity with home-based manufacturing and inexpensive materials to enhance both accessibility and scalability for clinical and point-of-care applications. The step-by-step fabrication and operation of the paper-based electrochemical biosensor for miRNA-107 detection is illustrated in **Scheme 1** following the detailed description of the platform.

EXPERIMENTAL SECTION

Materials and apparatus

The target sequence for hsa-miR-107 (5'-AGCAGCAUUGUACAGGGCUAUCA-3') and its complementary thiolated DNA probe (5'-TGATAGCCCTGTACAATGCTGCT-3') were obtained from Metabion GmbH



Scheme 1. *The paper-based electrochemical sensor fabrication and working steps for miRNA-107 detection:* ***(1)*** *electrode surface modification using AuNPs;* ***(2)*** *immobilization of a thiolated MB-labeled DNA probe;* ***(3)*** *detection of target miRNA-107 by SWV by signal-off mechanism; and* ***(4)*** *application in serum.*

(Steinkirchen, Germany). Phosphate-buffered saline (PBS) tablets (containing 140 mM NaCl, 10 mM phosphate buffer, and 3 mM KCl), tris(2-carboxyethyl)phosphine (TCEP), MCH, chloroauric acid ($HAuCl_4$), sodium citrate, and sodium borohydride were procured from Sigma-Aldrich (St. Louis, MO, USA). All reagents used were of analytical grade. The fabrication of sensors required AuNPs which were synthesized in our laboratory through a standard method that we use regularly and stored at 4 °C. These AuNPs have been routinely used in our lab across similar biosensor platforms. The synthesized batch was characterized by dynamic light scattering (DLS) and scanning electron microscopy (SEM), confirming a monodisperse distribution with an average diameter of 196.2 ± 20.65 nm and a polydispersity index (PDI) of 0.13 ± 0.07, consistent with previous reports (49). The nanoparticles served as a modification for working electrode surfaces to improve their performance.

All electrochemical measurements were conducted using a PalmSens 4 portable potentiostat (PalmSens, Netherlands) connected to a multi-12 channel reader and operated via PSTrace 5.10 software, ensuring precise control and reproducibility across all experiments.

Fabrication of Paper-Based SPEs: Custom-designed paper-based SPEs were fabricated in-house on Fabriano office paper (80 g/m²) using standard screen-printing techniques. A hydrophobic layout was printed with a Xerox ColorQube 8580 and thermally treated at 100°C for 1 minute to form fluidic barriers. A three-electrode configuration was achieved by first printing silver ink (Loctite, Italy) for the reference, followed by graphite ink (Sun Chemical, USA) for the working (4 mm diameter) and counter electrodes. Each layer was kept at 60°C for 30 minutes. The resulting electrochemical strips measured 2.5 cm × 1 cm, offering a simple, scalable platform for biosensor integration (50).

Optimization of experimental parameters

All the experimental parameters were optimized for the development of highly effective and reliable electrochemical biosensor for detecting miRNA-107. For this, one of the first parameters were amount of AuNPs on the surface of the working electrode, the concentration of the anti-miRNA-107 DNA probe and the frequency of SWV.

Optimization of AuNP volume

Various volumes (2, 4, 8, and 10 µL) of AuNPs were tested to identify the optimal volume for the detection of the electrochemical signal on the electrode surface. This conductive nanostructured layer was formed by depositing AuNPs through drop casting onto the working electrode area of paper-based SPEs, allowing for electron transfer and acting as a high-density binding surface for the immobilization of thiol-functionalized DNA probes.

Concentration of DNA probe

In order to obtain a high-efficiency hybridization interface for miRNA-107 detection, we optimized the concentration of DNA probe. Several probe concentrations (50-200 nM) were tested to obtain the optimal probe density that exhibited maximum signal change.

Optimization of SWV frequency

To determine the optimum condition with the maximum signal intensity as well as a well-defined voltammetric peaks, frequencies ranged from 10 to 100 Hz were optimized. The selection of optimum frequency is a key factor for improving the signal-to-noise ratio and facilitating the electrochemical responses which would generate accurate and reproducible results during target capture.

Electrochemical detection of miRNA-107

Based on the optimized experimental parameters, we carried out the electrochemical detection of miRNA-107 using paper-based SPEs. The working electrode area was modified by drop-casting AuNPs to enhance surface conductivity and probe immobilization. Following AuNP deposition, 20 µL of MB-labeled DNA probe was drop-cast onto the electrode. The modified electrodes were incubated in a humidity chamber for 1 hour to allow probe immobilization via Au-S bonding. After incubation, the electrodes were rinsed with DI water to remove unbound probe. Next, 20 µL of MCH was applied to the electrode surface. MCH acts as a blocking agent to minimize non-specific adsorption and improve probe orientation. The electrodes were again placed in the humidity chamber for 1 hour and 30 minutes. After incubation, the surface was washed thoroughly with DI water. For electrochemical analysis, the modified electrodes were connected to a PalmSens 4 portable potentiostat. Each SPE was exposed to 70 µL of blank PBS, pH 7.4 and allowed to stabilize for 30 minutes to establish a consistent electrochemical baseline. The detection mechanism relies on a “signal-off” electrochemical response. To quantify the analytical response, the percent-

age (%) signal change was calculated using the following equation (1):

$$\text{Signal change (\%)} = 100 \times \frac{(I_0 - I_t)}{I_0} \quad (1)$$

Where I_0 : initial current, and I_t: current recorded after the addition of miRNA-107.

RESULTS AND DISCUSSION

Optimization of experimental parameters

We optimized the experimental biosensor parameters like volume of AuNPs deposited on the electrode surface, the concentration of the MB-labeled DNA probe, and the frequency applied during SWV. The results of this optimization are presented in **Figure 1**.

Effect of AuNPs volume on signal response

As shown in **Figure 1A**, different volumes of AuNPs (2, 4, 8, and 10 µL) were optimized. The highest signal change was observed with 2 µL of AuNPs, suggesting that a low-volume deposition provides a uniform, conductive surface without excessive nanoparticle aggregation. Larger volumes resulted in a gradual decrease in signal response, likely due to the formation of a dense nanoparticle layer that could hinder probe immobilization, increase surface roughness, or impair electron transfer. Thus, 2 µL was selected as the optimal volume, balancing conductivity, probe accessibility, and hybridization efficiency.

Optimization of DNA probe concentration

To determine the optimal probe density on the electrode surface, anti-miRNA-107 DNA probes were immobilized at different concentrations (50, 100, 150, and 200 nM). As depicted in **Figure 1B**, the signal change increased with probe concentration up to 100 nM, beyond which the response declined. This behavior is attributed to the balance between sufficient surface coverage for target recognition and excessive probe crowding, which can induce steric hindrance and reduce hybridization efficiency. Additionally, higher probe densities can affect the orientation and electron transfer of the MB redox tag. Therefore, 100 nM was selected as the optimal probe concentration, offering maximum signal change with efficient target recognition and redox activity.

Influence of SWV frequency on detection sensitivity

The SWV frequency is a critical factor affecting both the resolution and sensitivity of redox-based detection. Frequencies ranging from 10 to 100 Hz were evaluated (**Figure 1C**), with the maximum signal change observed at 50 Hz. At lower frequencies, the system may not efficiently capture the rapid redox events of the MB label, leading to underdeveloped signals. Conversely, higher frequencies may compromise the sensitivity by limiting the time available for redox cycling, thus reducing the current response. Based on this, 50 Hz was determined to be the optimal setting, offering a high signal-to-noise ratio and well-resolved voltammetric peaks.

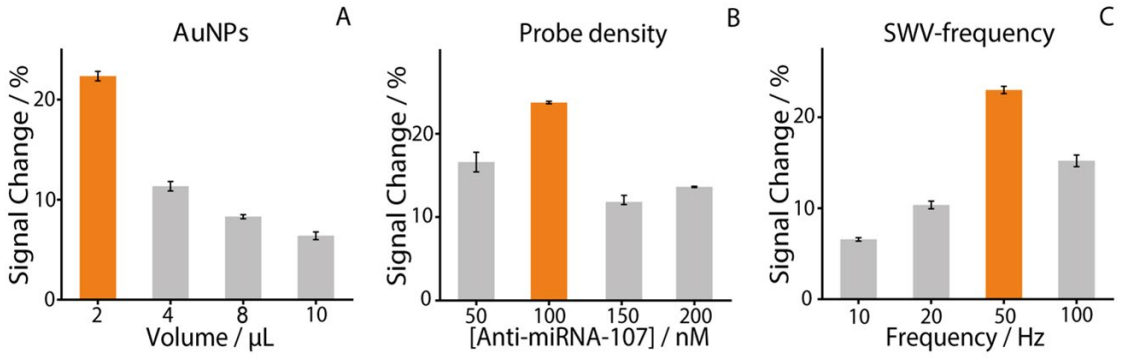


Figure 1. *Optimization of experimental parameters for miRNA-107 detection.*
***(A)** Effect of AuNPs volume (2-10 µL) on signal change. **Experimental conditions:** probe concentration = 100 nM; frequency = 50 Hz; Estart = 0 V, Eend = −0.6 V, Estep = 0.001 V, amplitude = 0.01 V, equilibrium time = 5 s; **(B)** Optimization of anti-miRNA-107 probe concentration (50–200 nM). **Experimental conditions:** AuNPs volume = 2 µL; frequency = 50 Hz; Estart = 0 V, Eend = −0.6 V, Estep = 0.001 V, amplitude = 0.01 V, equilibrium time = 5 s; **(C)** Optimization of SWV frequency (10-00 Hz). **Experimental conditions:** AuNPs volume = 2 µL; probe concentration = 100 nM; Estart = 0 V, Eend = −0.6 V, Estep = 0.001 V, amplitude = 0.01 V, equilibrium time = 5 s. All experiments were conducted in triplicate using 40 nM of miRNA-107 in PBS (pH 7.4), and results are reported as mean ± SD (n = 3).*

Analytical characterization of miRNA-107 detection in PBS and serum

The electrochemical biosensor developed for miRNA-107 detection demonstrated excellent analytical performance across a broad dynamic range in both PBS and serum. As shown in the calibration plots (**Figure 2**), the sensor response increased in a sigmoidal fashion with rising miRNA-107 concentrations, reflecting a characteristic binding curve indicative of efficient and specific hybridization between the surface-bound probe and the target sequence. Following the optimization of experimental parameters, the analytical performance of the developed electrochemical biosensor was systematically evaluated across a wide dynamic range of miRNA-107 concentrations upto 400 nM in both PBS and serum. As shown in **Figure 2**, the biosensor exhibited a characteristic sigmoidal response curve in both matrices, indicating a concentration-dependent decrease in current signal consistent with target-probe hybridization. The calibration data were well-fitted using a four-parameter Hill equation, demonstrating the cooperative nature of binding and supporting its use for quantitative analysis by equation (2).

$$f(x) = y_0 + \frac{a \cdot x^b}{c^b + x^b} \quad (2)$$

A limit of detection (LOD) of 1.8 nM was achieved, with a strong correlation (R^2 = 0.9909), confirming the reliability and linearity of the electrochemical response within the tested range. Notably, the biosensor retained robust performance in serum with LOD of 1.0 nM with an R^2 of 0.9860 and reproducibility in physiologically relevant conditions.

The ability of the biosensor to achieve lower detection limits for miRNA-107 in both buffer and serum matrices represents a substantial advancement in the field of electrochemical diagnostics. This level of sensitivity aligns with the clinical concentration range of circulating miRNAs, affirming the platform's suitability for early-stage disease detection. The sensor shows its robustness, selectivity, and practical translatability in serum. Importantly, this work validates a minimally invasive, cost-effective, and portable diagnostic approach that holds strong potential for deployment in POC cancer screening and longitudinal therapeutic monitoring, particularly in settings where centralized laboratory infrastructure is limited.

Selectivity study

To evaluate the specificity of the developed biosensor, its electrochemical response was measured against miRNA-107 and three non-target miRNAs (miRNA-4676, miRNA-218, and miRNA-21), all at the same concentration (40 nM) as shown in **Figure 3**. As shown in **Figure 3A**, in buffer conditions, miRNA-107 produced a distinct and pronounced signal suppression (~23%), reflecting efficient hybridization with the complementary probe. In contrast, the non-target sequences generated minimal signal changes (≤8%), indicating negligible non-specific binding or cross-reactivity. This selectivity profile was retained in serum (**Figure 3B**), where miRNA-107 still yielded a strong response (~24%), while the off-target miRNAs resulted in significantly lower signal shifts. Despite the increased complexity of the

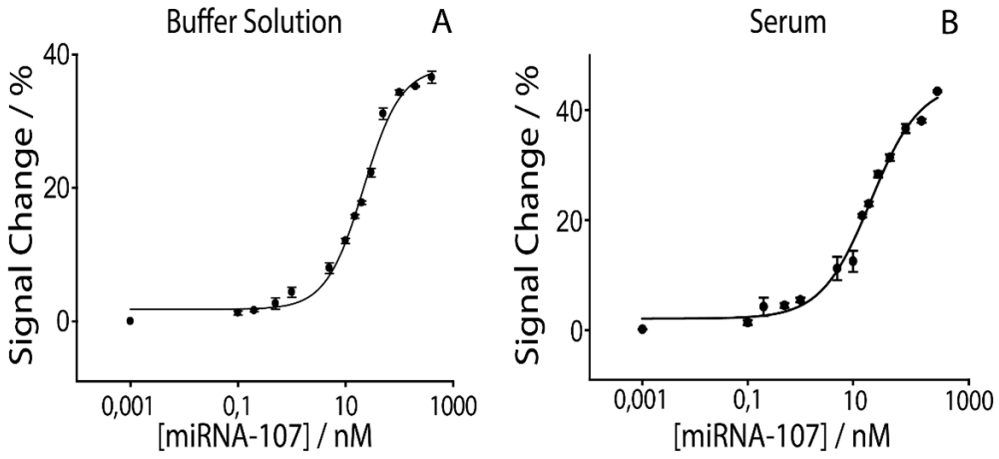


Figure 2. *Analytical characterization of the miRNA-107 biosensor in* ***(A)*** *buffer;* ***(B)*** *serum. Signal change (%) was measured in a concentration upto 400 nM. The data were fitted using a four-parameter Hill equation and sigmoid behavior was observed.* ***Experimental conditions:*** *AuNPs volume = 2 µL; probe concentration= 100 nM; Estart = 0 V, Eend = −0.6 V, Estep = 0.001 V, amplitude = 0.01 V, equilibrium time = 5 s, SWV frequency= 50 Hz. All the experiments were conducted in triplicate and results are expressed as mean ± standard deviation (n = 3).*

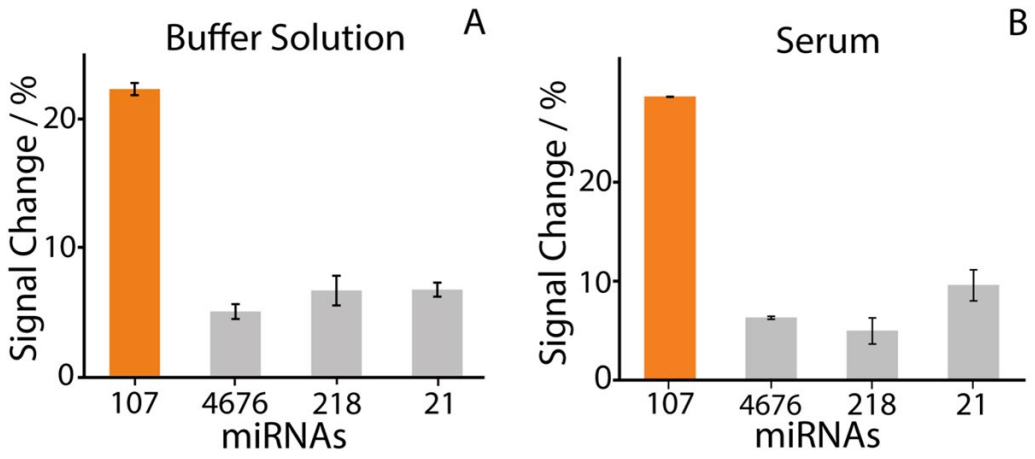


Figure 3. *Selectivity study of the miRNA-107 biosensor in* ***(A)*** *buffer solution;* ***(B)*** *serum using non-target miRNAs (miRNA-4676, miRNA-218, and miRNA-21) at 40 nM concentration.* ***Experimental conditions:*** *AuNPs volume = 2 µL; probe concentration= 100 nM; Estart = 0 V, Eend = −0.6 V, Estep = 0.001 V, amplitude = 0.01 V, equilibrium time = 5 s, SWV frequency= 50 Hz. All the experiments were conducted in triplicate and results are expressed as mean ± standard deviation (n = 3).*

serum matrix-which contains a wide array of electroactive species, proteins, and nucleases-the sensor maintained high target discrimination. The performance in serum validates the robustness of the probe design, surface chemistry, and blocking strategy used during sensor fabrication.

This signifies the ability of the biosensor to differentiate miRNA-107 from closely related sequences underscores the reliability of the probe design and its potential clinical utility. From a translational perspective, such high selectivity ensures diagnostic accuracy by minimizing false positives-an essential requirement for any biosensor intended for use in early cancer screening, treatment monitoring, or recurrence prediction. The fact that this level of performance is maintained in complex biological matrices like serum further reinforces the sensor's suitability for **POC** and **real-world clinical applications,** especially in minimally invasive blood-based diagnostics.

CONCLUSIONS

The research introduces a major advancement in liquid biopsy diagnostics. The diagnostic system consists of a portable electrochemical biosensor. The sensor identifies miRNA 107 which serves as a crucial biomarker for prostate cancer progression and aggressiveness. The biosensor utilized AuNPs modified SPEs. These electrodes were immobilized with an MB labeled DNA probe. The SWV technique served as the detection method. The detection system operated through a signal off mechanism. The design shows both sensitivity and selectivity. It is also easy to use. These features make it appropriate for clinical use and POC applications.

Critical parameters like AuNPs volume, probe concentration, and SWV frequency were systematically optimized to maximize hybridization efficiency and signal resolution. The biosensor exhibited a sigmoidal response curve, achieving detection limit of 1.8 nM in PBS and 1.0 nM in serum. The probe also showed high specificity for miRNA-107, even in complex biological samples, confirming its accuracy.

The biosensor provides practical advantages together with its analytical merits which make it suitable for POC deployment. It works at low-cost while being simple to operate and its production materials can be scaled up for large-scale manufacturing.

Further, the research will evaluate both the shelf-life duration and its resistance to environmental factors to enable wider field applications. The future development of this technology requires multiplexed detection capabilities and clinical specimen validation to achieve routine use in cancer screening and monitoring. Testing with real patient samples will confirm its performance. This study offers a simple, non-invasive tool for cancer diagnosis. The research demonstrates how this accessible non-invasive platform can help with early diagnosis and personalized care in both centralized and resource limited healthcare settings.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflicts of interests

The authors declare no competing interests.

Data availability

All data generated or analyzed during this study are included in this article.

Author's contributions

Conceptualization: SC and SS; writing-original draft: SS and AG; figures: SS and AG; reviewing: SC and MDL; funding acquisition: SC, MDL, AG and SS.
All authors reviewed the manuscript and prepared for submission.

Ethical approval

All solutions were commercially available. Real samples were not used.

Publication ethics

Plagiarism

Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

Data falsification and fabrication

All the data corresponds to the real**.**

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ORIGINAL ARTICLE

NO SMOKING IN THE WORKPLACE: IMPLEMENTING TOBACCO CONTROL STRATEGIES IN A LARGE HEALTHCARE ORGANIZATION IN NORTHERN ITALY

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ABSTRACT: cigarette smoking remains a major public health issue, contributing to numerous oncological, cardiovascular, and neurological diseases. Despite extensive scientific evidence demonstrating the harmful effects of tobacco use and increasing regulatory restrictions at local and national levels, smoking prevalence remains high.
This study describes the actions implemented by a large healthcare organization in Northern Italy to reduce smoking among employees and the general public. Over two years, a multidisciplinary working group introduced various training, awareness, and enforcement, including public campaigns, social media initiatives, health promotion events, and smoking cessation support. A key innovation was the integration of smoking bans and enforcement strategies not only within hospitals but across all healthcare facilities, such as vaccination centers and diagnostic units. Additionally, standardized smoking cessation messages were incorporated into hospital discharge summaries and outpatient reports, and enforcement officers were trained to apply sanctions to individuals violating smoking regulations. These interventions have led to increased awareness, broad public engagement, and reported reductions in smoking from 34 to 20% in our province. Although primary prevention outcomes are difficult to measure, a comprehensive approach involving healthcare professionals, patients, and the community may contribute to long-term behavioral change and a healthier environment.

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Impact statement: cigarette smoking remains a major public health problem. The Local Health Authority of Reggio Emilia has launched a massive campaign to reduce smoking habits among its employees.

Key words: *tobacco control; smoking cessation; healthcare policy; workplace smoking ban; public health intervention.*

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INTRODUCTION

Tobacco smoke exposure is a well-established risk factor for morbidity and mortality. According to the World Health Organization (WHO) (1), exposure to tobacco smoke is causally linked to various diseases, disabilities, and premature death. Passive smoking has been identified as a significant risk factor for respiratory diseases (2, 3) and for malignancies (4), including but not limited to lung cancer (5), with occupational exposure further contributing to these risks (6, 7).

Despite the known health hazards associated with second hand smoke, data on recent trends in passive smoking exposure remain limited. Various preventive measures, particularly smoking bans in public places, have been implemented across several European countries, potentially influencing these trends (8-10). Evidence suggests that stringent anti-smoking have contributed to a reduction in biomarkers of tobacco exposure, such as cotinine levels in urine (11) and saliva (12). However, while such improvements have been observed in occupational settings, they appear less pronounced in lower socioeconomic groups (13). A study conducted in China (14) reported that, despite restrictions on smoking in bars and restaurants, ambient nicotine concentrations remained elevated. Similarly, a multi-country European study showed that passive smoking prevalence was higher in domestic environments (ranging from 13% to 40%), compared to workplaces (3% to 32%) (15).

Although workplace smoking bans have led to reduced cotinine levels in children from disadvantaged backgrounds (16), there is concern that these measures may inadvertently shift smoking behavior from workplaces to homes (17). Notably, exposure to second hand smoke in occupational settings has significantly declined over the past decade, decreasing from 31.9% of employed individuals in 1990-1995, to 17.5% in 1998-2003, and further to 2.5% in 2010-2014. Male gender and lower educational attainments have been identified as key risk factors for workplace exposure to passive smoking (18).

In Italy, data from the Progressi delle Aziende Sanitarie per la Salute in Italia (PASSI) study indicates that approximately 24% of the population smokes (19), a prevalence comparable to the European average. Northern European countries report the lowest smoking rates, with Sweden at 8%, the Netherlands at 11%; and Denmark at 14% (20). Additionally, alternative nicotine products are gaining traction, with 3% of the Danish population using electronic cigarettes and approximately 1% smoking heated tobacco products (21), while in Italy, the prevalence of heated tobacco product use is estimated at 2.4% (22). Second hand smoke, as defined by the WHO (23), includes both sidestream smoke from the burning end of tobacco products and mainstream smoke exhaled by smokers. Exposure to second-hand smoke has been strongly associated with an increased risk of lung cancer and cardiovascular disease (24, 25). Importantly, the WHO states that no safe level of exposure to passive smoking exists (26), and even outdoor exposure can reach significant levels, depending on environmental factors such as wind and proximity to active smokers (27).

This study aims to describe some initiatives undertaken to eliminate tobacco smoking in all healthcare settings, including hospital and community health facilities, within a province of northern Italy.

MATERIALS AND METHODS

The study examined all the initiatives implemented following the establishment of a dedicated working group aimed at identifying and addressing areas for intervention to reduce or eliminate tobacco smoking in the workplace. The multidisciplinary team overseeing these interventions comprises pulmonologists, oncologists, cardiologists, biologists, psychologists, hygienists, nurses, and other health professionals. The initiatives undertaken by the Local Health Authority of Reggio Emilia have been supported by the Lega italiana per la Lotta ai Tumori, a major patient association. Through this collaboration, the organization manages one of Italy's most important centers for primary cancer prevention, known as Luoghi di Prevenzione (28).

The activities carried out in 2023-2024 encompassed a range of measures, including training courses, awareness campaigns, modifications to signage, legislation changes, conferences, and media outreach through television and newspaper interviews. The Local Health Authority of Reggio Emilia is responsible for healthcare provision across the province of Reggio Emilia, situated in the central Emilia-Romagna region, one of the most industrialized areas of Italy, with a resident population of 528,401 inhabitants and is organized into six districts, each with its hospital. Notably, the Arcispedale Santa Maria Nuova-IRCCS in the Reggio Emilia district is a recognized center of excellence for advanced technologies and innovative

oncology care models. The Local Health Authority of Reggio Emilia is one of the largest employers in the province, with 7,296 employees as of December 31, 2022. The workforce is predominantly female (76%), with males comprising 24%. Healthcare professionals constitute the majority (72%) of the workforce, while the remaining employees hold technical-administrative roles. The organization plays a critical role in public health by providing services across the spectrum of cancer prevention, including primary prevention (28), secondary prevention through oncological screening programs (29), and tertiary prevention via an extensive regional oncology network (30).
In Italy the first smoking regulations were introduced in 1975, prohibiting smoking in specific indoor spaces, including hospital wards, school classrooms, waiting rooms, closed rooms used for public meetings, cinemas, and ballrooms (31). Subsequent legislation in 1995 extended the smoking ban to additional public service areas (32). However, these early measures had limited impact on smoking behaviors, as they did not impose comprehensive restrictions on smoking in all enclosed spaces.
A significant milestone was reached in 2003 when Italy enacted legislation prohibiting smoking in all public and private workplaces, commercial establishments, restaurants, recreational venues, gyms, and sports centres. The only exceptions of premises reserved for smokers and strictly private areas (civil homes) to this regulation were designated smoking areas and private residences (33).

RESULTS

Actions implemented in 2023 (Table 1)

In March 2023, a *formal resolution* was adopted to establish a dedicated working group consisting of 23 healthcare professionals. The group convenes every two months to coordinate and implement tobacco control interventions.
In May, coinciding with *World No Tobacco Day*, the Local Health Authority of Reggio Emilia organized the event *Only Life, No Smoke* in the city's largest square that aimed to raise awareness regarding the health consequences of tobacco use.
In addition, a *Smoke-Free Walk* was conducted with the participation of 30 people, and testimonies from healthcare professionals and citizens were featured on local television and newspapers. Simultaneously, information points were set up at the entrance of the six hospitals, there was an information point on the Smoking Cessation Centers, where healthcare personnel and peer-educator students involved in the *Smoke-Free Schools* project distributed informational materials and promotional items.
In September, during *Head and Neck Cancer Prevention Week*, an outreach event took place in the city square engaging six specialists from the Otolaryngology Department of the Local Health Authority of Reggio Emilia. That same month, the working group participated in the *Health Festival*, an event attracting approximately 1,000 attendees with activities

Table 1. *Type of interventions performed by the Hospital and Smoke-Free Territory Working Group in 2023.*

MONTH	INTERVENTION	ACTION	RESULTS
March	*Resolution Adoption*	Establishment of the Working Group	23 professionals involved
	All-Life No Smoking Day	Pulmonologists and Cardiologists in the Square	79 spirometry tests performed; information on the dangers of smoking provided; 500 informational flyers distributed
	Smoke-Free walk	10 health workers leading the initiative	Over 30 citizens participated in a 5 km walk
	Smoke-free schools Initiative	Health personnel stationed at the hospital entrance	Distribution of informational leaflets and promotional materials to support smoking cessation
September	*Head and Neck Cancer Prevention Week*	6 professionals involved	200 citizens received consultations and underwent rhinofibrolaryngoscopy
	Health Festival	Public engagement in health-promoting activities	Approximately 1,000 citizens participated in workshops on cooking, wellness, movement, and other activities
October	*Cardiovascular Prevention Initiative – Keep Your Heart in Shape*	Cardiologists, sports doctors, prevention specialists	200 citizens screened through blood tests; 260 citizens screened for atrial fibrillation; 500 apples distributed along with health maps promoting healthy lifestyles

included health promotion initiatives such as cooking and wellness workshops.
In October, as part of a regional initiative for cardiovascular disease prevention titled *Keep Your Heart in Shape* a mobile clinic was set up where cardiologists conducted blood screening tests. Additionally, sports medicine specialists, dieticians, public health professionals, and Smoking Cessation Center staff provided education on cardiovascular prevention, emphasizing physical activity, healthy eating, and smoking cessation strategies.

Actions implemented in 2024 (Table 2)

In February, an updated *Sanctioning Protocol* was introduced for individuals violating smoking prohibitions within Local Health Authority premises.
In May, the event Week of *Only Life, No Smoke* was organized, featuring a public event involving 12 healthcare professionals.
A seminar was held titled "Fighting Smoking and Digital Smoking" for health workers, educators, and the general public was also planned (**Figure 1**).
In August, a standardized message in medical records was inserted into the outpatient specialist reports of the departments, encouraging smoking cessation. In September, during *Head and Neck Cancer Prevention Week*, a city event was held with the participation of six ENT specialists from the Local Health Authority of Reggio Emilia, and over 200 citizens. The working group also participated in the *Health Festival* for a second consecutive year. Approximately 1200 participants engaged in activities, including children's games, animal-assisted therapy, cooking workshops, yoga, gymnastics sessions, and health promotion initiatives.
In October, a knowledge questionnaire assessing awareness and habits related to traditional and electronic cigarette use was developed and was sent to 7000 employees.
In December, two specialized *Training Courses* were conducted for enforcement officers and educators. Over the past decade, our healthcare organization has implemented numerous anti-smoking campaigns aimed at reducing tobacco consumption among both employees and the general population. While the impact of such initiatives is not immediately quantifiable, a progressive increase in the utilization of our Anti-Smoking Center has been observed with the number of individuals seeking support rising from 134 in 2022 to 215 in 2024. Concurrently, a decline in smoking prevalence has been documented within the region, with the proportion of smokers decreasing from 30% to 24% in the Emilia-Romagna region, and from 34% to 20% in the province of Reggio Emilia, for the period 2008-2023.

Table 2. *Type of interventions performed by the Hospital and Smoke-Free Territory Working Group in 2024.*

MONTH	INTERVENTION	ACTION	RESULTS
February	*Updated Sanctions Report*	Review and discussion within the Working Group	Implementation of revised sanctioning measures for smoking violations in AUSL premises
May	*Week of Only Life, No smoke*	Public awareness events in city squares	Engagement of journalists and local television networks in tobacco control initiatives
	Seminar on Tobacco Control	*Fighting Smoking and Digital Smoking*	120 healthcare professionals and citizens participated
August	*Standardized Message in Medical reports*	Inclusion of a smoking cessation advisory statement	The wording "If you smoke and want help to quit smoking, you can call 0522320655 to request an appointment at the anti-smoking centre nearest to your residence. access is free" appears on discharge letters
September	*Head and Neck Cancer Prevention Week*	Public screening event in the city square	7 healthcare professionals were involved; approximately 200 ENT consultations and videoendoscopic examinations performed
	Health Festival	Public health event at Parco San Lazzaro (USL of Reggio Emilia	Approximately 1,200 citizens participated in activities, promoting physical exercise, healthy eating, and smoking cessation
October	*Employee Survey on Smoking Habits*	Distribution of a questionnaire to 7,200 employees	Approximately 1,600 responses received to date
December	*Training Courses for Inspection Agents*	Educational sessions on tobacco control regulations	7 instructors and 72 students participated

Figure 1. *Reggio Emilia. Initiatives of the working group with citizens in the main square of the city.*

A key strength of these interventions is their potential long-term impact on tobacco-related diseases. Notably, data from the Reggio Emilia Cancer Registry indicate a reduction in the incidence of lung cancer among women under the age of 50 (**Figure 2**), suggesting that sustained awareness campaigns and prevention strategies may be contributing to this decline.

DISCUSSION

The primary objective of this study was to describe the implementation of smoking reduction initiatives across healthcare settings, both hospital-based and community-based locally in a province of Northern Italy. During 2024 there were numerous events aimed at both the public and Local Health Authority professionals. In particular, four public events which saw the participation of thousands of citizens. Furthermore, an absolute novelty was introduced for patients and users of healthcare facilities, one of the first ever in Italy, which is a message in discharge letters in specialist reports with the words: "If you want to stop smoking, contact this centre". For employees, however, an absolute novelty was the updating of a protocol that contains sanctions for people who smoke in non-dedicated areas. A course was held for inspectors which saw the participation of 70 employees who were given information on the damage to health caused by smoking. Therefore, the first objective must be awareness-raising and then sanctions. Another absolute novelty is that a questionnaire was sent to the 7,200 employees, the answers to which are being evaluated.

Previous studies have demonstrated that an increasing number of hospitals worldwide have adopted comprehensive smoke-free policies, prohibiting smoking both indoors and outdoors (34). However, international research has shown that, despite these regulations, studies in hospitals with smoking behaviour persists among visitors, staff, and patients in outdoor areas (35-37). Nonetheless, institutional

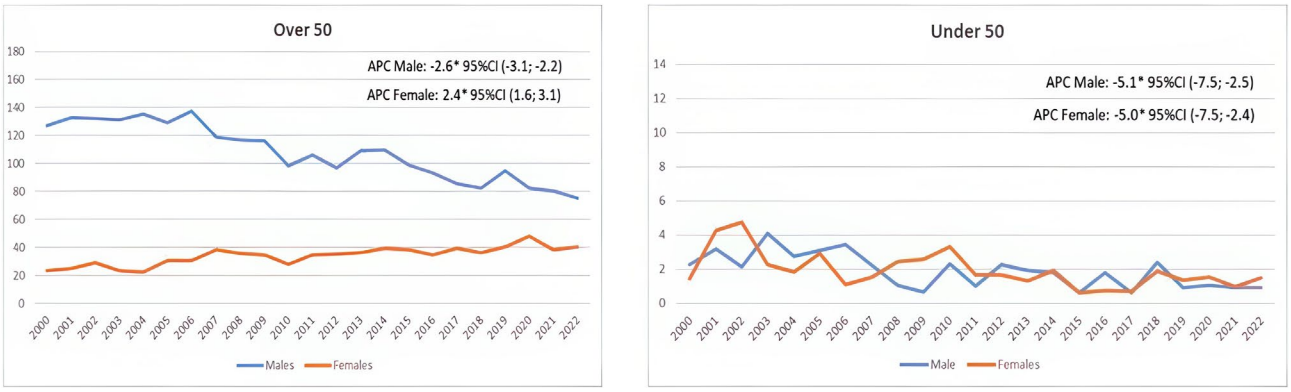

Figure 2. *Reggio Emilia Cancer Registry. Years 2000-2023. Lung cancer incidence by sex and age group (over 50 and under 50).*

policies contribute to an overall reduction in smoking prevalence and can catalyze broader tobacco control efforts. Given their position as trusted health institutions, hospitals play a major role in tobacco control, and healthcare professionals are uniquely positioned to lead by example (38, 39). An intriguing perspective comes from a study conducted among medical residents in the United States, which highlights a more nuanced approach to smoking cessation interventions. While physicians are expected to actively discourage smoking, the study suggests that a more comprehensive strategy - one that considers individual patient circumstances and incorporates harm reduction principles - may be more effective in supporting long-term smoking cessation (40). Historically, smoking was widely accepted in hospital settings, with physicians and nurses smoking in staff rooms and even cigarettes being sold in hospital shops (41). It was not until 1992 that a nationwide smoking ban in hospitals was implemented in the United States (42).

Today, one of the strongest arguments for banning smoking in outdoor hospital areas is to minimize public nuisance, including littering, fire hazards, and exposure to behaviors perceived as inappropriate in a healthcare setting (43). However, some public health experts, such as Simon Chapman, have cautioned against conflating community preferences with evidence-based public health priorities, arguing that tobacco control policies should be driven by rigorous scientific evidence rather than societal norms alone (44).

The impact of smoking bans in hospital settings remains a subject of ongoing debate, with some studies reporting mixed results (45). Nevertheless, hospitals hold a unique responsibility to establish and normalize smoke-free environments reinforcing their role as institutions that prioritize health and well-being (46). A persistent challenge is that, despite comprehensive smoking bans, individuals often find secluded areas, beyond designated non-smoking zones where they continue to smoke (47).

While strict enforcement of smoking bans is necessary to uphold hospital policies, a more patient-centered approach is required to effectively address nicotine dependence. Hospitals should aim to balance regulatory compliance with harm reduction strategies ensuring that nicotine withdrawal is managed appropriately while providing evidence-based smoking cessation support. This perspective aligns with principles derived from anthropology and harm reduction philosophy, which prioritize minimizing the negative consequences of substance while respecting individual autonomy (48, 49).

CONCLUSIONS

This work aims to share with other professionals an initiative undertaken in a large healthcare company in Northern Italy. The goal is to raise awareness among healthcare professionals about the risks of smoking while also strictly prohibiting smoking in healthcare environments. Despite previous failed attempts in recent years, it is important to persist this effort by engaging healthcare professionals, as well as citizens, patient associations, and stakeholders who can contribute through concrete actions.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflicts of interests

The authors declare no conflict of interest.

Availability of data and materials

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to ethical and privacy issues; re-quests for data must be approved by the Ethics Committee after the presentation of a study pro-tocol.

Authors' contributions

Conceptualization, investigation, writing-original draft, visualization, supervision, AA; visualization, supervision CG; conceptualization, investigation, writing-original draft MT; supervision CB; supervision NF; supervision ML; supervision EM; supervision AN; supervision ER; supervision AG; supervision CS; writing-review and editing supervision IB; conceptualization, writing-original draft, investigation, supervision, LM. All authors have read and agreed to the published version of the manuscript.

Informed Consent Statement

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Authors declare no potentially overlapping publications with the content of this manuscript and all original studies are cited as appropriate.

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All the data corresponds to the real.

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