

Annals of *Research* in *Oncology*

www.annals-research-oncology.com

PERSPECTIVE

DEVELOPMENT AND IMPLEMENTATION OF A MULTIDISCIPLINARY DIAGNOSTIC AND THERAPEUTIC CARE PATHWAY (PDTA) FOR PLEURAL MESOTHELIOMA: A METHODOLOGICAL FRAMEWORK FOR REGIONAL ONCOLOGY NETWORKS

REVIEW

RECALCITRANT CANCERS: WHERE ARE WE NOW?

REVIEW

FERTILITY PRESERVATION IN YOUNG WOMEN WITH BREAST CANCER: UPTAKE, DETERMINANTS OF ACCESS, AND LONG-TERM REPRODUCTIVE OUTCOMES: A REVIEW

OPINION PAPER

VITAL STAINING AND ARTIFICIAL INTELLIGENCE IN ORAL CANCER DETECTION: A HYBRID DIAGNOSTIC APPROACH FOR GLOBAL HEALTH EQUITY

EDITORS IN CHIEF

Antonio Giordano
Carmine Pinto

EXECUTIVE EDITOR

Francesca Pentimalli

SECTION EDITORS AND EDITORIAL BOARD

Francesco Agustoni
Luigi Alfano
Giovanni Baglio
Marcella Barbarino
Riccardo Caccialanza
Orazio Caffo
Jean Calleja Agius
Mariasosaria Campitiello
Claudio Cerchione
Alessandro Comandone
Salvatore Cortellino
Laura Cortesi
Anna Crispo
Romano Danesi
Gennaro Daniele
Vincenzo De Falco
Simona De Summa
Fernando De Vita
Massimo Di Maio
Nicola Fazio
Enrico Franceschi
Renato Franco
Paolo Giorgi Rossi
Giovanni Grignani
Gualberto Gussoni

Khamel Khalili
Prashant Kharkar
Prashant Kumar
Giovanna Liguori
Roberto Lucchini
Amalia Luce
Umberto Malapelle
Lucia Mangone
Sendurai Mani
Canio Martinelli
Andrea Morrione
Paolo Pedrazzoli
Giuseppe Procopio
Davide Ruggero
Daniele Santini
Pierluigi Scalia
Cristian Scatena
Diego Serraino
Simona Sperlongano
Gary Stein
Tiziano Tuccinardi
Marco Vanoni
Bruno Vincenzi
Stephen J. Williams
Haining Yang

Editors in Chief and Executive Editor

Antonio Giordano
Carmine Pinto
Francesca Pentimalli

Editorial Coordinator

Marco Malagutti

Publishing Editor

Jessica Guenzi
editorialoffice@annals-research-oncology.com

Sales

dircom@lswr.it
Ph. 0687776757

Edra Media S.r.l.

Viale Forlanini, 21
20134 Milano - Italy
Ph. 0039 (0)2-88184.1
Fax 0039 (0)2-88184.301
www.edizioniedra.it

*"Annals of Research in Oncology" registered at Tribunale di Milano
n. 63 on 24.06.2020*

© 2026 Annals of Research in Oncology - ARO.

Published by Edra Media S.r.l. All rights reserved.

To read our Privacy Policy please visit www.edraspa.it/privacy

I Table of contents

Development and implementation of a multidisciplinary diagnostic and therapeutic care pathway (PDTA) for pleural mesothelioma: a methodological framework for regional oncology networks	126
Maria Pagano, Federica Grosso, Laura Abate-Daga, Massimo Aglietta, Marinella Bertolotti, Vincenzo Adamo, Giuseppe Casablanca, Fabrizio Capuano, Sandro Pignata, Stefano Lovadina, Alessandra Guglielmi, Annamaria Catino, Giovanna Masala, Michele Maio, Sandra Cavone, Lucia Bisceglie, Erika Gervasi, Giamaica Ciardiello, Eleonora Borghi, Carmine Pinto	
Recalcitrant cancers: where are we now?	136
Patrizia Maiorano, Shendi Shani, Alberto Marino, Canio Martinelli, Salvatore Cortellino	
Fertility preservation in young women with breast cancer: uptake, determinants of access, and long-term reproductive outcomes: a review	161
Annamaria Pezzarossi, Isabella Bisceglia, Francesco Marinelli, Maria Barbara Braghiroli, Filippo Giovanardi, Fortunato Morabito, Lucia Mangone	
Vital staining and artificial intelligence in oral cancer detection: a hybrid diagnostic approach for global health equity	178
Abhijeet Satpathy, Rupsa Das, Satya Ranjan Misra	

PERSPECTIVE

DEVELOPMENT AND IMPLEMENTATION OF A MULTIDISCIPLINARY DIAGNOSTIC AND THERAPEUTIC CARE PATHWAY (PDTA) FOR PLEURAL MESOTHELIOMA: A METHODOLOGICAL FRAMEWORK FOR REGIONAL ONCOLOGY NETWORKS

Maria **Pagano**^{1,*}, Federica **Grosso**^{2,*}, Laura **Abate-Daga**³, Massimo **Aglietta**⁴,
Marinella **Bertolotti**⁵, Vincenzo **Adamo**⁶, Giuseppe **Casablanca**⁷, Fabrizio **Capuano**⁸,
Sandro **Pignata**⁹, Stefano **Lovadina**¹⁰, Alessandra **Guglielmi**¹¹, Annamaria **Catino**¹²,
Giovanna **Masala**¹³, Michele **Maio**¹⁴, Sandra **Cavone**¹⁵, Lucia **Bisceglie**¹⁶, Erika **Gervasi**¹,
Giamai **Ciardiello**¹, Eleonora **Borghi**¹, Carmine **Pinto**¹⁷

¹ Oncologia Medica, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy

² Director Mesothelioma, Melanoma and Rare Cancers Unit AOU "S.S. Antonio e Biagio e Cesare Arrigo", Alessandria, Italy; Director Mesothelioma Unit S Spirito Hospital Casale Monferrato, Alessandria, Italy; MET.I, Fondazione Mesothelioma Team Italy, Milan, Italy

³ TU.TO.R. Tumori Toracici Rari, Patient Advocacy, Milan, Italy; MET.I, Fondazione Mesothelioma Team Italy, Milan, Italy

⁴ Coordinatore Rete Oncologica Piemonte e Valle d'Aosta, Italy

⁵ Epidemiologist Research and Innovation Department (DAIRI), IRCCS Azienda Ospedaliero-Universitaria "S.S. Antonio e Biagio e Cesare Arrigo", Alessandria, Italy

⁶ Coordinatore Rete Oncologica Siciliana, Scientific Consultant Oncology Clinical Trial Center, Papardo Hospital, Messina, Italy

⁷ Department of Thoracic Surgery, SC AO Papardo Hospital, Messina, Italy

⁸ Coordinatore regionale FAVO Campania e Consigliere Esecutivo Nazionale, Italy

⁹ Department of Urology and Gynecology, Istituto Nazionale Tumori IRCCS Fondazione G Pascale, Naples, Italy

¹⁰ Alta Specialità Chirurgia Toracica Mininvasiva, SC Chirurgia Toracica ASUGI, Trieste, Italy

¹¹ Unit of Medical Oncology, University of Trieste, Trieste, Italy

¹² SSD Oncologia Toracica IRCCS Istituto Tumori "Giovanni Paolo II", Bari, Italy; MET.I, Fondazione Mesothelioma Team Italy, Milan, Italy

¹³ Institute for Cancer Research, Prevention and Clinical Network (ISPRO), Florence, Italy

¹⁴ University of Siena, Italy; Center for Immuno-Oncology, Department of Oncology, University Hospital of Siena, Siena, Italy

¹⁵ TU.TO.R. Tumori Toracici Rari, Patient Advocacy, Milan, Italy

¹⁶ AReSS Puglia, Italy

¹⁷ Oncologia Medica, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy; MET.I, Fondazione Mesothelioma Team Italy, Milan, Italy

* Correspondence to: ✉ pagano.maria@ausl.re.it; <https://orcid.org/0000-0001-7201-8697>;

✉ federica.grosso@ospedale.al.it; <https://orcid.org/0000-0002-6843-3369>

ABSTRACT: Pleural mesothelioma (PM) is a rare, aggressive tumor strongly linked to occupational asbestos exposure, with a complex presentation that often delays diagnosis. Effective management requires a structured, multidisciplinary approach integrating diagnostic, therapeutic, psychological, and supportive care across the healthcare system.

Conducted within the PNC-SINTESIproject – funded by the Italian Ministry of Health under the PNC/PNRR for environment-health surveillance in Sites of National Interest – this study analyzed diagnostic and therapeutic care pathways (PDTAs) for PM developed by eight Italian regions (Emilia-Romagna, Piemonte, Sicilia, Puglia, Toscana, Campania, Marche and Sardegna). Regional documents were compared across eleven key dimensions, including healthcare professionals involved, access criteria, hub-and-spoke center definitions, and process indicators, to identify shared components and propose a harmonized national model. The analysis identified a core multidisciplinary team – medical oncologist, radiation oncologist, thoracic surgeon, pulmonologist, radiologist, pathologist, and palliative care specialist – alongside a standardized hub-and-spoke framework and six key process indicators for quality monitoring. Early, concurrent integration of palliative care and psychological support emerged as critical, consistent with evidence that timely psychosocial involvement improves patients' and families' quality of life. Given the established asbestos link, the framework also recommends occupational disease notification and medico-legal counselling to protect patients' rights and support epidemiological surveillance. This reproducible model aims to reduce regional variability in PM care and may serve as a reference for international standardization.

Doi: 10.48286/aro.2026.131

Impact statement: A harmonized hub-and-spoke PDTA model for pleural mesothelioma may standardize multidisciplinary care and reduce interregional variability in Italy.

Key words: *Pleural mesothelioma; care pathway; multidisciplinary team; palliative care; hub-and-spoke model.*

Received: Jul 21, 2026/**Accepted:** Sept 04, 2026

Published: September 30, 2026

INTRODUCTION

Pleural mesothelioma (PM) is a rare and aggressive malignancy arising from the mesothelial cells of the pleura, strongly associated with previous occupational or environmental exposure to asbestos. The long latency between exposure and clinical onset, often spanning decades, means that PM incidence will remain substantial for years despite the progressive ban on asbestos in many countries (1-3). Its clinical presentation is insidious and non-specific, frequently mimicking other pleuropulmonary conditions, which delays diagnosis and results in many patients presenting at an advanced stage (4, 5). Adequate tissue sampling and specialized histopathological and molecular characterization are essential to confirm the diagnosis and histological subtype, both of which carry major prognostic and therapeutic weight (4).

The therapeutic landscape has moved from a period in which single-modality treatment was uniformly disappointing towards multimodal strategies combining surgery, systemic therapy and radiotherapy, with the more recent addition of immunotherapy (6, 7). This evolution, together with the need to correctly allocate patients to active treatment or supportive care based on age, performance status and physiological reserve, is why international guidelines consistently recommend that every patient with PM

be evaluated by a multidisciplinary team with specific expertise in mesothelioma (4, 5, 8). In Italy, the National Mesothelioma Registry (ReNaM) has systematically documented asbestos exposure and mesothelioma incidence, underscoring the continued public health relevance of the disease (3). The multidisciplinary approach is increasingly translated into clinical practice through diagnostic and therapeutic care pathways (PDTAs), which represent essential organizational tools for coordinating the management of patients with PM. By defining roles, responsibilities, organizational structures, and timelines across the entire care pathway, PDTAs promote multidisciplinary integration, ensure continuity of care between hospital and community settings, and support the delivery of timely, appropriate, and high-quality care. However, because PM-specific PDTAs have been developed independently by individual Italian regions, substantial heterogeneity may exist in their structure, content, and implementation, potentially leading to interregional variability in patient management.

Since 2024, the PNC SINTESI project has been underway in Italy. It is a national program involving 14 Italian regions, guided by Puglia region, funded by the Italian Ministry of Health to establish a permanent health surveillance system in the Italian National Priority Contaminated Sites (Siti di Interesse Nazionale, SINs), with the aim of reducing the health burden

associated with environmental pollution and social inequalities through targeted primary and secondary prevention strategies. Within this program, a specific objective focuses on PM and aims to define the minimum requirements that diagnostic, therapeutic, and care pathways (PDTAs) for mesothelioma should meet across all Italian regions.

The present study was conducted within this framework. We systematically reviewed and compared the regional PDTAs for PM currently available in Italy and used them as the basis for a structured discussion within the project working group. This process enabled the identification and critical appraisal of the key organizational and clinical domains that should be addressed by any mesothelioma PDTA, with the aim of defining a shared set of minimum requirements and proposing a harmonized, reproducible, and adaptable framework to support regional implementation, reduce interregional variability, and serve as a methodological reference for regional oncology networks.

A SURVEY-AND CONSENSUS-BASED COMPARISON ACROSS EIGHT ITALIAN REGIONS

The study was conducted between February 2025 and April 2026 within the PNC SINTESI project. The overall objective of this initiative was to define a shared set of minimum organizational and clinical standards that every regional PM PDTA should meet. Specifically, the proposed framework aims to reduce diagnostic and treatment waiting times through the definition of regional standards; standardize therapeutic pathways; improve patient information, communication, and shared decision-making; ensure access to rehabilitation and psychological support; promote the systematic assessment of occupational and environmental asbestos exposure; facilitate case reporting to the Regional Mesothelioma Registries (CORs) and occupational disease notification procedures; support the implementation of clinical and translational research programs; optimize and continuously monitor the quality of care through the definition of process and outcome indicators and the development of a structured data collection and analysis system; and establish a national repository to evaluate health impact, monitor adherence to quality standards, and support epidemiological, preventive, translational, and clinical research.

A dedicated working group was established, involving representatives from 8 of the 14 Italian regions participating in the project: Emilia-Romagna, Friuli Venezia Giulia, Marche, Piemonte, Puglia, Sardegna, Sicilia, and Toscana. Each participating region subsequently established a dedicated regional working group, in collaboration with its Regional Oncology Network, involving the professionals considered most relevant to the organization and delivery of malignant PM care within the regional health-care system.

As a first step, a structured questionnaire was developed and distributed to all participating regions to perform a comprehensive assessment of the regional organization of PM care. The survey collected information on the annual number of PM cases, the presence major asbestos-related industrial exposure sources, the availability of regional PM PDTA, designated referral centers, the availability of specialized services (medical oncology, pulmonology, thoracic surgery, radiation oncology, pathology, and nuclear medicine), supportive care resources (including palliative care and psychological support), access to clinical trials, and the main organizational and clinical challenges identified in each region.

For regions with a PM PDTA formally approved by the Regional Oncology Network, additional information was collected on the date of the most recent update, case notification procedures, entry points to the care pathway, the inclusion of diagnostic and therapeutic standards, and the use of process and outcome indicators for pathway monitoring and evaluation.

A nationwide survey was subsequently conducted to identify the existence of additional regional PM PDTAs in Italian regions not participating in the project, in order to ensure the most comprehensive assessment of the organizational models adopted across the country.

TOWARD A SHARED MODEL: THE BUILDING BLOCKS WE CONSIDER NON-NEGOTIABLE

Organizational and clinical domains of the care pathway

The comparative analysis of the available regional PM PDTAs enabled the identification of the main organizational and clinical domains of the care pathway. Based on these findings, a structured set of

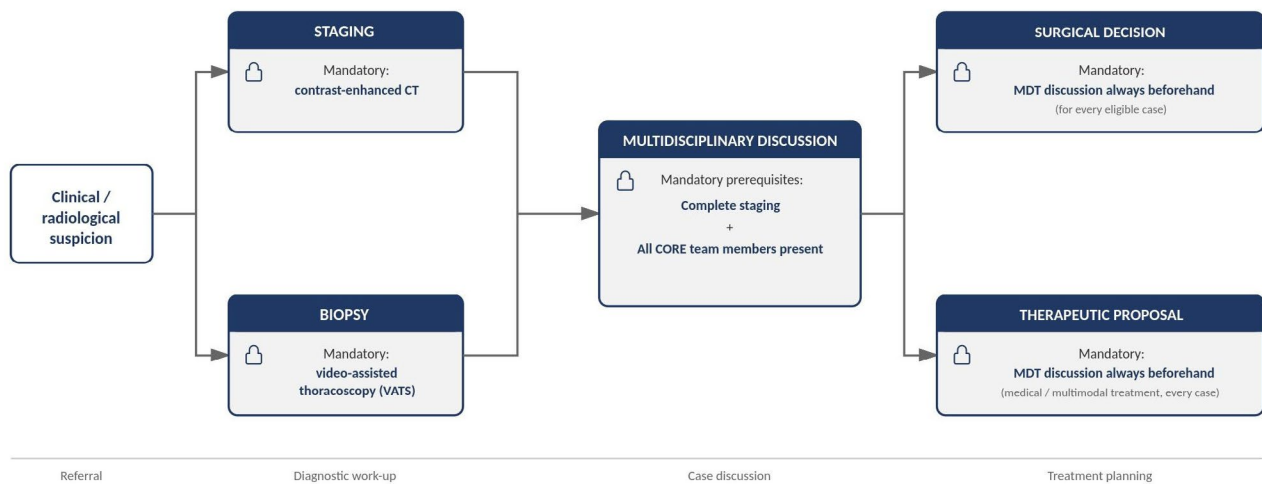


Figure 1. Mandatory sequential requirements (“gates”) within the pleural mesothelioma diagnostic-therapeutic pathway (PDTA). Clinical/radiological suspicion leads to two parallel, mandatory diagnostic requirements – staging by contrast-enhanced computed tomography (CT) and biopsy by video-assisted thoracoscopy (VATS) – both of which must be completed before multidisciplinary discussion. Multidisciplinary discussion additionally requires attendance of all core team members (CORE). Both the surgical decision and the therapeutic proposal are gated by mandatory prior multidisciplinary discussion, for every case.

CORE: core multidisciplinary team members (medical oncologist, thoracic surgeon, pulmonologist, radiologist, pathologist, radiation oncologist, palliative care specialist, nurse case manager); CT: computed tomography; MDT: multidisciplinary team; VATS: video-assisted thorascopic surgery.

questions was developed to guide the discussions within each regional working group. The questionnaire explored the following domains: governance of the regional PDTA; composition of the multidisciplinary team and involvement of additional specialists according to clinical needs; eligibility criteria and entry points to the care pathway; organization of the hub-and-spoke network, including criteria for the identification and roles of first-level (spoke) and second-level (hub) centers; access to clinical trials; and process indicators for monitoring pathway implementation. The responses from each regional working group were registered in an appropriate database and subsequently discussed during 4 plenary meetings of the regional working groups to reach consensus on the minimum organizational and clinical requirements for regional PM PDTAs. Finally, the results of the consensus process were synthesized into a shared reference document defining the key organizational and clinical elements that every regional PM PDTA should include.

Standardizing the route to multidisciplinary discussion

Among the eight participating regions, three had a PM PDTA formally approved by their Regional Oncology Network: Piemonte, Emilia-Romagna, and Sicilia.

A fourth regional PM PDTA, from Campania, was identified through the subsequent nationwide survey and was also included in the comparative analysis. Based on the findings of the initial structured questionnaire, the comparative analysis of the four available regional PM PDTAs, and the consensus reached on the structured questions, the following key elements of the care pathway were identified.

The definition of a regional PM PDTA should be entrusted to a multidisciplinary working group comprising, at a minimum, medical oncologists, thoracic surgeons, pulmonologists, radiologists, pathologists, radiation oncologists, nurse case managers, palliative care specialists, physiatrists/rehabilitation medicine specialists, occupational physicians, psychologists, dietitians or nutritionists, representatives of the Regional Mesothelioma Registry (COR/ReNaM), and accredited patient and former asbestos-exposed workers' associations.

The multidisciplinary team (MDT) responsible for defining the most appropriate pathway for each patient should consist of a core team, whose members should participate routinely in case discussions, and an extended team, whose members should be involved according to the patient's specific clinical needs (**Figure 1**). At a minimum, the core team should include medical oncologists, thoracic surgeons, pul-

monologists, radiologists, pathologists, radiation oncologists, palliative care specialists, and a nurse case manager. The extended team should include, as appropriate, nuclear medicine physicians, hospital pharmacists, physiatrists and physiotherapists, psychologists, clinical geneticists, molecular biologists, and dietitians or nutritionists. The nurse case manager should coordinate the patient's pathway, facilitate communication among team members, and ensure continuity of care throughout the diagnostic, therapeutic, and follow-up phases.

The main clinical criteria warranting referral to the PM PDTA include recurrent unilateral pleural effusion, pleural effusion in individuals with a history of asbestos exposure, pleural effusion associated with pleural thickening, and isolated pleural thickening. Each regional PM PDTA should clearly define the referral entry point to the pathway, identifying the healthcare professionals and healthcare facilities responsible for patient referral, as well as the procedures for patient enrolment and care coordination following entry into the pathway.

The timelines for every phase of the diagnostic pathway should define the recommended. These should include the initial diagnostic work-up following clinical and/or radiological suspicion, the staging pathway and the healthcare professionals responsible for its completion, the recommended biopsy procedures, and the pathological assessment, including specimen requirements, diagnostic criteria, immunohistochemical analyses, and classification according to the current WHO classification. The pathway should also specify the procedures for obtaining a second pathological opinion in diagnostically challenging cases or when the diagnosis is established in low-volume centers, together with predefined reporting timelines. In addition, mandatory and optional imaging investigations (e.g. contrast-enhanced CT, MRI, and 18F-FDG PET/CT) should be clearly identified, together with their indications within the diagnostic algorithm.

Each regional PM PDTA should adopt a hub-and-spoke organizational model, allowing the coordination of local care pathways with referral centers providing specialized expertise for complex cases. This organizational approach is in line with the principles of the European Reference Networks (ERNs) and may facilitate future integration within the Italian National Rare Cancer Network (Rete Nazionale Tumori Rari, RNTR). Where the Regional Oncology Network has not already formally identified referral centers, the PDTA should explicitly define the criteria

for the designation of first-level (spoke) and second-level (hub) centers, together with their respective roles and responsibilities. The criteria proposed by the working group and agreed upon through the consensus process are summarized below.

First-level (spoke) centers should include a dedicated multidisciplinary team for thoracic malignancies with access to thoracic surgery expertise and should ensure timely diagnostic work-up, staging, and delivery of standard treatments. Their responsibilities should include patient referral and enrolment, diagnostic investigations (including imaging and pathology), case notification to the Regional Mesothelioma Registry (COR/ReNaM), provision of standard systemic therapies, palliative care, rehabilitation, nutritional and psychosocial support, and integration with the regional network through telemedicine where available.

Second-level (hub) centers should provide all the functions of spoke centers and additionally guarantee access to highly specialized procedures and treatments. These should include invasive diagnostic procedures performed by specialized pulmonology, thoracic surgery, or interventional radiology teams; molecular pathology; pleurectomy/decortication (P/D), performed in centers with dedicated thoracic surgery units and appropriate expertise according to current evidence; multimodal radiotherapy; access to experimental therapies and clinical trials; and coordination of the multidisciplinary management of complex cases.

Each regional PDTA should identify the designated hub centers in agreement with the Regional Oncology Network. In regions where one or more hub functions are unavailable, formal interregional referral pathways should be established to ensure equitable access to specialized care.

In organizational terms, Level I (spoke) centers were responsible for diagnostic testing, pathological diagnosis, medico-legal requirements, reporting to the COR, imaging-based staging, psychosocial support, conventional therapies, palliative care, nutritional support, and physical rehabilitation. By contrast, Level II (hub) centers were responsible for invasive diagnostic procedures, molecular biology and advanced diagnostic testing, surgery requiring specialized expertise, radiotherapy within multimodal treatment strategies, and experimental therapies not available at Level I centers.

The patient management and all major therapeutic decisions must be discussed and agreed upon by the multidisciplinary team. Therapeutic strategies

should be defined according to histological subtype and disease stage, in accordance with the most recent national and international clinical guidelines, and should include specific recommendations for selected patient subgroups, such as frail and older patients, individuals with hereditary cancer predisposition. The PDTA should also provide clear recommendations for post-treatment follow-up, including the modalities and timing of clinical and instrumental surveillance. Simultaneous supportive care should be integrated throughout the entire patient journey, ensuring early involvement of palliative care specialists as members of the core multidisciplinary team, together with access to rehabilitation, psychological and nutritional support, specialized nursing care, and continuity of care between hospital and community services.

Whenever available, patients should be offered participation in clinical trials, with Regional Oncology Networks serving as the reference point for information and access to ongoing studies; coordination among regional networks and the development of a shared national platform for clinical trial referral should be encouraged.

A research infrastructure built into the pathway, not bolted on

We also think a PDTA should do more than organize clinical care: it should generate the data needed to improve it. Each regional PDTA should promote the prospective collection of standardized clinical data through a shared electronic dataset, including patient characteristics, treatment information, and survival outcomes, with harmonized data collection across regions and integration with national collabo-

rative initiatives such as MET.I (Mesothelioma Team Italy). In parallel, the PDTA should encourage the systematic collection of archival tissue and biological specimens and identify dedicated regional biobanks or referral centers responsible for their collection, processing, storage, and management according to standardized procedures. Clinical, pathological, and biological data should be prospectively linked through appropriate data set, thereby establishing a comprehensive infrastructure to support epidemiological, translational, and clinical research, as well as the development and validation of novel diagnostic and therapeutic strategies.

Measuring what matters: core process indicators

To enable the monitoring and evaluation of pathway performance, a core set of process indicators was identified and agreed upon by the working group. These indicators include: the proportion of newly diagnosed PM cases reported to the Regional Mesothelioma Registry (COR/ReNaM) (target: 100%); the proportion of patients undergoing diagnostic and/or staging thoracoscopic biopsy according to the recommendations of the multidisciplinary team (target: $\geq 90\%$); the proportion of patients receiving multidisciplinary evaluation within 30 days of histological diagnosis and disease staging (target: $\geq 90\%$); the proportion of patients starting medical treatment within 30 days of multidisciplinary discussion (target: 100%); the proportion of patients undergoing surgery following preoperative multidisciplinary assessment (target: 100%); and the proportion of patients referred to palliative care within the last six months of life (target: $\geq 90\%$) (**Table 1**).

Table 1. Core process indicators proposed for monitoring the regional pleural mesothelioma (PM) diagnostic and therapeutic care pathway (PDTA).

PATHWAY DOMAIN	PROCESS INDICATOR	TARGET
Case registration	Proportion of newly diagnosed PM cases reported to the Regional Mesothelioma Registry (COR/ReNaM)	100%
Diagnostic work-up	Proportion of patients undergoing diagnostic and/or staging thoracoscopic biopsy according to multidisciplinary team recommendations	$\geq 90\%$
Multidisciplinary evaluation	Proportion of patients receiving multidisciplinary evaluation within 30 days of histological diagnosis and disease staging	$\geq 90\%$
Medical treatment	Proportion of patients starting medical treatment within 30 days of multidisciplinary discussion	100%
Surgical treatment	Proportion of patients undergoing surgery following preoperative multidisciplinary assessment	100%
Palliative care	Proportion of patients referred to palliative care within the last six months of life	$\geq 90\%$

COR/ReNaM: Regional Mesothelioma Registry / National Mesothelioma Registry; MDT: multidisciplinary team; PM: pleural mesothelioma.

LIMITATIONS AND A PATH FORWARD

The present study should be interpreted in light of its methodological characteristics. Our proposal is based on the comparative analysis of four formally approved regional PM PDTAs and on a structured consensus process involving representatives from eight Italian regions. As such, it represents a consensus-based organizational framework derived from existing regional PDTAs rather than an evaluation of clinical outcomes or pathway effectiveness, and it does not assess the extent to which regional pathways are implemented in routine clinical practice. Furthermore, the proposed process indicators have not yet been prospectively validated and should therefore be considered an initial point for quality assessment, to be refined and validated through future implementation studies. Finally, not all Italian regions were represented, although the four regional PDTAs included in the comparative analysis cover geographically diverse areas of the country (North-West, North-East, Central, and Southern Italy), providing a broad overview of the organizational models currently adopted and supporting the development of a framework with potential national applicability. Despite these limitations, this study provides, to our knowledge, the first national consensus-based framework defining the minimum organizational and clinical requirements for regional PM diagnostic, therapeutic, and care pathways in Italy. By establishing a shared set of minimum standards, it offers a practical methodological reference to support the harmonization of regional care pathways, reduce unwarranted variability in patient management, and provide a foundation for future evaluation of pathway quality and outcomes.

Our comparative analysis of four Italian regional PDTAs for PM identified a shared core of essential multidisciplinary professionals, a consistent hub-and-spoke organizational architecture, and a set of six reproducible process indicators. These convergent elements provide the basis for a harmonized, regionally adaptable model of care for this rare and complex malignancy.

In this context, the PDTA represents a structured organizational framework for the diagnosis, treatment, and supportive care of patients with PM, developed on the basis of the best available evidence and designed to ensure coordinated, timely, and continuous care across hospital and community settings while taking into account the available human

and technical resources. Given the complexity of PM management, clearly defined responsibilities, referral pathways, and timelines are essential to improve coordination, appropriateness, and efficiency of care. Evidence on clinical pathways supports their role in improving professional practice, enhancing care coordination, and reducing unwarranted variability in healthcare delivery (9). Multidisciplinary management and effective integration between hospital- and community-based services represent the fundamental principles underpinning high-quality PM care.

Given the complexity of PM management and the need to ensure efficient use of healthcare resources, it is essential to distinguish between professionals who should routinely participate in the multidisciplinary management of all patients and those whose involvement should be tailored to specific clinical needs. Consistent with current international recommendations (4, 10), the core multidisciplinary team should include, at a minimum, a medical oncologist, thoracic surgeon, pulmonologist, radiologist, pathologist, radiation oncologist, palliative care specialist, and nurse case manager. Additional specialists, including nuclear medicine physicians, occupational physicians, rehabilitation specialists, psychologists, dietitians or nutritionists, clinical geneticists, molecular biologists, hospital pharmacists, and other organ-specific specialists, should be involved whenever clinically indicated. This organizational model allows multidisciplinary expertise to be integrated in a flexible and sustainable manner, improving coordination, facilitating timely decision-making, and promoting greater consistency in patient management across different healthcare settings.

The hub-and-spoke model represents a key component of regional PM PDTAs, balancing equitable access to care with the concentration of specialized expertise (11). For this model to be effective, Regional Oncology Networks should play a central role in formally identifying and accrediting Level I (spoke) and Level II (hub) centers, clearly defining their respective functions and responsibilities according to available expertise, case volume, and available facilities. This represents a crucial effort to ensure that patients are managed within an integrated regional network and have equitable access to optimal multidisciplinary care, regardless of where they first enter the healthcare system. Such an organization is particularly important in PM, where optimal outcomes depend on the strategy defined at diagnosis in experienced centers capable of delivering complex mul-

timodal management, and it may also facilitate the timely referral of eligible patients to clinical trials. Process indicators are practical tools for assessing the efficiency and effectiveness of pathway activities over time and for supporting continuous quality improvement. To be useful, these indicators should be reproducible, measurable, and adaptable across different healthcare settings. Their routine application enables the evaluation of pathway implementation, facilitates benchmarking across settings, and supports evidence-based refinement of clinical practice.

PM is a highly challenging malignancy to manage. Owing to its frequently advanced stage at diagnosis, treatment is curative in only a minority of patients and, in most cases, is primarily aimed at prolonging survival while maintaining disease control, alleviating symptoms, and preserving quality of life. Throughout the course of the disease, patients experience one of the highest symptom burdens among thoracic malignancies, with pain, dyspnea, fatigue, anorexia, and psychological distress often occurring simultaneously and significantly impairing daily functioning. These characteristics make PM an ideal setting for the early integration of palliative care alongside active therapies, according to the principles of simultaneous care. The World Health Organization defines palliative care as *"an approach that improves the quality of life of patients and their families facing the problems associated with life-threatening illness, through the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other physical, psychosocial, and spiritual problems"* (12). In patients with advanced cancer, early integration of palliative care has consistently been associated with less aggressive end-of-life care, better symptom control, improved quality of life, and greater patient and caregiver satisfaction (13, 14). A potential survival benefit has also been reported, most notably in patients with metastatic non-small-cell lung cancer (15). Although comparable prospective evidence is currently lacking in PM, the disease's clinical characteristics and substantial supportive care need provide a compelling rationale for integrating palliative care from the time of diagnosis.

Despite these considerations, palliative care remains underutilized in patients with PM and is frequently introduced only in the final stages of the disease. In a retrospective study, Baird *et al.* reported that although referrals to palliative care services had increased over time, more than half of patients

with PM still did not receive specialist palliative care despite their limited life expectancy (16). These findings highlight the persistent gap between evidence-based recommendations and clinical practice, emphasizing the need for structured multidisciplinary care pathways that facilitate the timely incorporation of palliative care throughout the disease trajectory.

Accordingly, in the proposed pathway, palliative care should be introduced early and delivered concurrently with disease-directed treatment as an integral component of comprehensive patient management rather than being reserved for end-of-life care. Early access to psychological support should likewise be ensured to address the significant emotional and psychosocial burden experienced by both patients and their caregivers. In addition, collaboration with patient and volunteer associations may further strengthen supportive care by helping identify unmet needs, facilitating communication between patients, families, and healthcare professionals, and promoting a more patient-centered approach throughout the care pathway. The active involvement of accredited patient associations should also be pursued in PM PDTAs. This recommendation is supported by the recent Italian State-Regions Agreement of 4 December 2024 on the participation of non-profit organizations in Regional Oncology Networks, which formally recognizes their role in organizational and clinical decision-making, the development and revision of oncology care pathways, patient advocacy, health promotion and research, and the evaluation of patient experience and healthcare quality.

This work demonstrates that defining a structured, multidisciplinary PDTA for PM, based on a clearly identified core multidisciplinary team, a well-defined hub-and-spoke model, mandatory multidisciplinary case discussion, shared process indicators, integrated clinical and biological data collection, and early integration of psychological and supportive/palliative care, is both feasible and necessary to promote equitable, high-quality care. Future efforts should focus on prospectively validating the proposed quality indicators, extending the consensus process to all Italian regions, and implementing a national infrastructure linking clinical, pathological, and biological data to support quality assessment, outcome evaluation, and epidemiological, translational, and clinical research. Although developed within the Italian healthcare system, the framework proposed in this study provides a practical reference for the harmonization of regional PM care pathways

and may represent a useful model for other countries seeking to reduce organizational variability in the management of this rare and complex disease. The framework may represent a useful reference for the development of the Italian National Rare Cancer Network (Rete Nazionale Tumori Rari, RNTR) and for the alignment of regional PM care pathways with the European Reference Networks (ERNs) model for rare and complex diseases.

COMPLIANCE WITH ETHICAL STANDARDS

Funding

This research received specify PNC/PNRR grant but the funding source did not have a role in study design, data collection/interpretation, or the decision to submit for publication.

Conflicts of interest

The authors declare no competing interests.

Availability of data and materials

The regional PDTA documents analyzed in this study are publicly available through the respective regional health authorities.

Authors' contributions

MP, FG: conceptualization, writing - original draft, writing - review & editing, supervision. Other authors: conceptualization. We acknowledge MET.I (Mesothelioma Team Italy) for its support.

Ethical approval

This study is based exclusively on the review and analysis of publicly available regional PDTA documents. No patients, animals, or personal data were involved. Ethics committee approval and informed consent were therefore not required.

Publications 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

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

REFERENCES

1. Lanphear BP, Buncher CR. Latent period for malignant mesothelioma of occupational origin. *J Occup Med*. 1992;34(7):718-721.
2. Robinson BWS, Lake RA. Advances in malignant mesothelioma. *N Engl J Med*. 2005;353(15):1591-1603. doi: 10.1056/NEJMra050152.
3. Marinaccio A, Binazzi A, Bonafede M, Corfiati M, Di Marzio D, Scarselli A, et al. Malignant mesothelioma due to non-occupational asbestos exposure from the Italian national surveillance system (ReNaM): epidemiology and public health issues. *Occup Environ Med*. 2015;72(9):648-655. doi:10.1136/oemed-2014-102297.
4. Popat S, Baas P, Faivre-Finn C, Girard N, Nicholson AG, Nowak AK, et al. Malignant pleural mesothelioma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Ann Oncol*. 2022;33(2):129-142. doi: 10.1016/j.annonc.2021.11.005.
5. Scherpereel A, Opitz I, Berghmans T, Psallidas I, Glatzer M, Rigau D, et al. ERS/ESTS/EACTS/ESTRO guidelines for the management of malignant pleural mesothelioma. *Eur Respir J*. 2020;55(6):1900953. doi: 10.1183/13993003.00953-2019.
6. De Bondt C, Psallidas I, Van Schil PEY, van Meerbeeck JP. Combined modality treatment in mesothelioma: a systematic literature review with treatment recommendations. *Transl Lung Cancer Res*. 2018;7(5):562-573. doi: 10.21037/tlcr.2018.10.02.
7. Su S. Mesothelioma: path to multimodality treatment. *Semin Thorac Cardiovasc Surg*. 2009;21(2):125-131. doi: 10.1053/j.semtcvs.2009.07.006.
8. Woolhouse I, Bishop L, Darlison L, De Fonseka D, Edey A, Edwards J, et al. British Thoracic Society Guideline for the investigation and management of malignant pleural mesothelioma. *Thorax*. 2018;73(Suppl 1):i1-i30. doi: 10.1136/thorax-jnl-2017-211321.
9. Rotter T, Kinsman L, James E, Machotta A, Gothe H, Willis J, et al. Clinical pathways: effects on professional practice, patient outcomes, length of stay and hospital costs. *Cochrane Database Syst Rev*. 2010;(3):CD006632. doi: 10.1002/14651858.CD006632.pub2.
10. Kindler HL, Ismaila N, Armato SG 3rd, Bueno R, Hesdorffer M, Jahan T, et al. Treatment of Malignant Pleural Mesothelioma. *Ann Oncol*. 2017;28(12):1811-1824. doi: 10.1093/annonc/mdx181.

- nant Pleural Mesothelioma: American Society of Clinical Oncology Clinical Practice Guideline. *J Clin Oncol.* 2018;36(13):1343-1373. doi: 10.1200/JCO.2017.76.6394.
11. Elrod JK, Fortenberry JL Jr. The hub-and-spoke organization design: an avenue for serving patients well. *BMC Health Serv Res.* 2017;17(Suppl 1):457. doi: 10.1186/s12913-017-2341-x.
 12. Sepúlveda C, Marlin A, Yoshida T, Ullrich A. Palliative care: the World Health Organization's global perspective. *J Pain Symptom Manage.* 2002;24(2):91-96. doi: 10.1016/s0885-3924(02)00440-2.
 13. Bakitas MA, Tosteson TD, Li Z, Lyons KD, Hull JG, Li Z, et al. Early versus delayed initiation of concurrent palliative oncology care: patient outcomes in the ENABLE III randomized controlled trial. *J Clin Oncol.* 2015;33(13):1438-1445. doi: 10.1200/JCO.2014.58.6362.
 14. Haun MW, Estel S, Rücker G, Friederich HC, Vilalobos M, Thomas M, et al. Early palliative care for adults with advanced cancer. *Cochrane Database Syst Rev.* 2017;6(6):CD011129. doi: 10.1002/14651858.CD011129.pub2.
 15. Temel JS, Greer JA, Muzikansky A, Gallagher ER, Admane S, Jackson VA, et al. Early palliative care for patients with metastatic non-small-cell lung cancer. *N Engl J Med.* 2010;363(8):733-742. doi: 10.1056/NEJMoa1000678.
 16. Baird A, Nasser A, Tanuseputro P, Webber C, Wheatley-Price P, Munro C. Involvement of palliative care in malignant pleural mesothelioma patients and associations with survival and end-of-life outcomes. *Curr Oncol.* 2024;31(2):1028-1034. doi: 10.3390/curroncol31020076.

REVIEW

RECALCITRANT CANCERS: WHERE ARE WE NOW?

Patrizia **Maiorano**¹, Shendi **Shani**², Alberto **Marino**³, Canio **Martinelli**², Salvatore **Cortellino**^{4, *}

¹ Department of Medical Biotechnology, University of Siena, Siena, Italy

² Sbarro Institute for Cancer Research and Molecular Medicine, College of Science and Technology, Temple University, Philadelphia (PA), U.S.A.

³ Clinical and Translational Oncology, Scuola Superiore Meridionale (SSM), Naples, Italy

⁴ SHRO Italia Foundation ETS, Turin, Italy

* Correspondence to: ✉ s.cortellino@ssmeridionale.it; <https://orcid.org/0000-0001-8331-3800>

ABSTRACT: Recalcitrant tumors pose a significant clinical challenge because of their biological complexity and resistance to conventional therapies. These tumors develop mechanisms to evade drug toxicity, including alterations in drug metabolism, enhanced DNA repair capabilities, and changes in the tumor microenvironment that support their survival. The lack of identifiable molecular targets in many recalcitrant cancers limits precision medicine approaches, necessitating the development of novel therapeutic strategies. Early diagnosis remains difficult because these tumors present with nonspecific symptoms or are detected at advanced stages when treatments are less effective, contributing to poor outcomes.

Emerging epidemiological studies have highlighted the role of lifestyle modifications in tumor onset and progression. Advances in molecular biology and genomics have enabled the identification of new biomarkers and therapeutic targets, leading to innovative approaches including immunotherapy, targeted therapy, and combination therapies to overcome resistance. This narrative review emphasizes integrated prevention strategies, including lifestyle interventions, summarizes progress in understanding recalcitrant tumor pathophysiology, and evaluates current and experimental treatments to improve survival rates and quality of life for patients.

Doi: 10.48286/aro.2026.130

Impact statement: This review investigates the primary factors that render recalcitrant tumors incurable and evaluates prospective strategies for enhancing early diagnosis, prevention, and the overcoming of therapeutic resistance.

Key words: *Recalcitrant tumor; nutrition; immuno-checkpoint inhibitor; replication stress response; tumor stiffness.*

Received: May 29, 2026/**Accepted:** Aug 24, 2026

Published: Sept 30, 2026

INTRODUCTION

Recalcitrant tumors constitute a highly aggressive and challenging category of neoplasms, characterized by their plasticity, which enables them to develop novel mechanisms and adopt phenotypes that confer resistance to conventional therapies. The overall 5-year survival rate for patients with recalcitrant tumors is less than 20% (1-5). Although these tumors account for approximately 20-25% of annual cancer diagnoses globally, they are responsible for nearly half of all cancer-related deaths (6). Pancreatic ductal adenocarcinoma (PDAC), small cell lung

cancer (SCLC), glioblastoma (GBM), hepatocellular carcinoma (HCC), cholangiocarcinoma (CCA), and esophageal cancers are classified as recalcitrant tumors and represent the most lethal tumor types. Despite originating in different organs and being driven by distinct genetic alterations, these neoplasms share several common characteristics: 1) a high risk associated with unhealthy lifestyles, such as smoking, alcohol consumption, and obesity; 2) nonspecific and silent symptoms; 3) a lack of markers and diagnostic techniques for early detection of the disease; and 4) an absence of specific molecular targets (**Figure 1**).

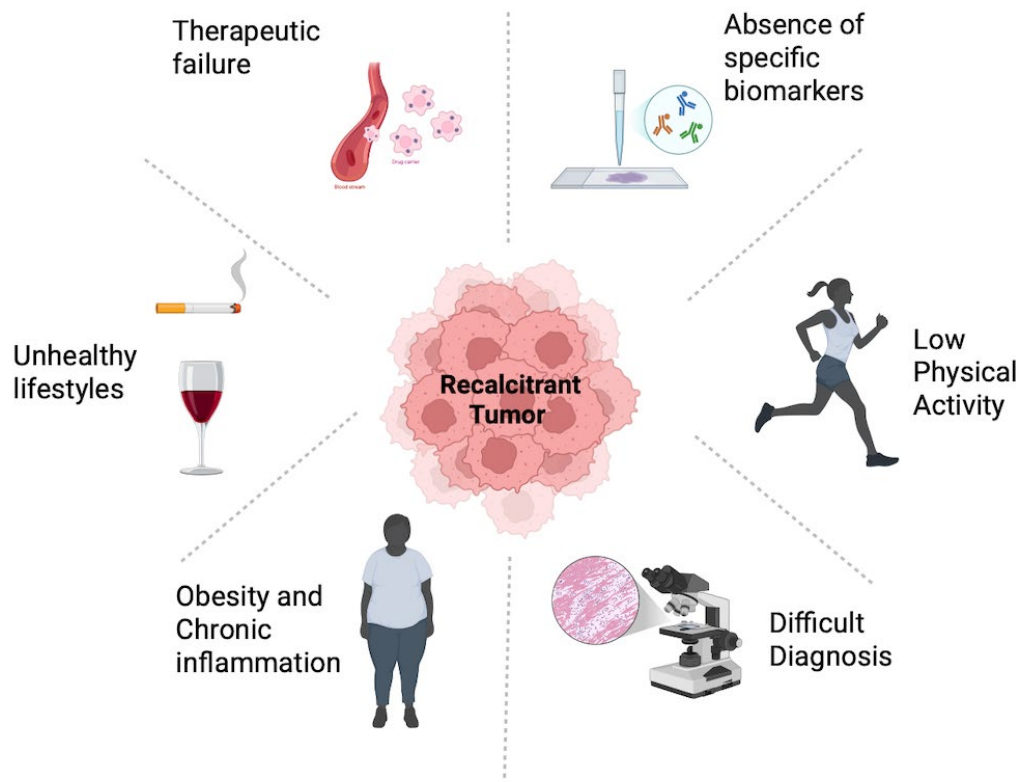


Figure 1. Risk factors driving the onset and progression of recalcitrant tumors. Schematic overview of the principal contributing factors.

LIFESTYLE AND RECALCITRANT CANCERS

Smoking and obesity are significant shared risk factors for various recalcitrant cancers because of their contribution to chronic inflammation, metabolic alteration, and DNA damage (7, 8). Obesity exacerbates cancer development and progression via several mechanisms. The rapid expansion of adipose tissue is not supported by adequate vascularization, leading to hypoxia, adipocyte death, and macrophage recruitment.

These immune cells release proinflammatory cytokines, establishing chronic inflammation, generating oxidative stress, and increasing the risk of DNA damage and oncogenic mutations (9, 10). Obesity-induced insulin resistance and hyperinsulinemia result in elevated IGF-1 levels and activation of the pro-oncogenic PI3K/Akt/mTOR signaling pathway, promoting the proliferation of transformed cells. Excess adipose tissue supports tumor growth by releasing fatty acids that serve as substrates for beta-oxidation, increase reactive oxygen species production,

and providing intermediate metabolites essential for proinflammatory factor synthesis while reducing precursors of anti-inflammatory factors. Additionally, obesity-induced metabolic changes lead to hormonal imbalances and altered adipokine synthesis, characterized by the overproduction of leptin, which promotes cell division and tumor survival, and a deficiency of adiponectin, a hormone with antitumor and anti-inflammatory properties, further exacerbating inflammation (11). Chronic inflammation, hormonal dysregulation, and lipid overload contribute to the expansion and maintenance of cancer stem cells (CSCs) (12).

Tobacco primarily contributes to cancer development by inducing loss-of-function mutations in tumor suppressor genes and gain-of-function mutations in oncogenes. These genes are crucial for regulating cell growth and maintaining genomic stability. These mutations compromise the mechanisms that regulate cellular proliferation, facilitating the initiation and progression of malignancy. Chemical compounds in tobacco smoke, such as polycyclic aromatic hydrocarbons and nitrosamines, form DNA

adducts that cause replication errors, increasing the likelihood of mutations in genes critical for cell cycle control and apoptosis (13).

Smoking and obesity are significant independent risk factors for PDAC (7, 8). Tobacco smoke promotes PDAC onset by inducing KRAS mutations and HDAC4 expression, thereby enhancing tumor invasion and metastasis (14). Visceral adipose tissue (VAT) and intrapancreatic fat deposition (IPFD) exceeding 10% are associated with PDAC as both risk prognostic factors, promoting cancer cell proliferation and immune evasion through chronic inflammation, cytokine release, extracellular vesicles (EVs), insulin resistance, and hyperinsulinemia, which drive pancreatic cancer initiation and progression (15-17). Both smoking and obesity often accelerate carcinogenesis, with the risk of pancreatic cancer in current smokers with a body mass index (BMI) ≥ 30 kg/m² being up to five-fold higher (18).

Smoking is the leading risk factor for SCLC, with 95-98% of cases occurring in current or former smokers, whereas never-smokers account for approximately 2% of cases (19, 20). In SCLC, tobacco carcinogens cause inactivating mutations in TP53 and RB1 (13, 21), whereas nicotine promotes progression and migration through activation of the CXCR4/CXCL12 pathway (22).

Obesity (BMI ≥ 30 kg/m²) and high-fat diets are linked to aggressive GBM, reduced survival, and higher disease risk, especially in patients with abdominal obesity (23, 24). Excess adipose tissue supplies substrates for membrane synthesis and ATP production via beta oxidation. Cholesterol-rich macrophages promote tumor growth and suppress local immunity (25). High-fat diets reduce brain hydrogen sulfide (H₂S) production, fostering tumor growth and enriching cancer stem cells (26). Additionally, upregulation of the fat mass and obesity-associated (FTO) gene, a brain-expressed demethylase, supports GBM progression and therapeutic resistance through metabolic reprogramming and m6A RNA/DNA demethylation (27).

Obese individuals have an 89% higher risk of HCC than those of normal weight. Adult weight gain leads to nonalcoholic steatohepatitis (NASH), characterized by remodeling of the liver microenvironment, which predisposes individuals to HCC. The accumulation of lipids, chronic inflammation mediated by interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α), and insulin resistance collectively promote HCC development by exacerbating oxidative stress and DNA damage (28). Additionally, smok-

ing increases the risk of HCC by 21-86%, with obesity further amplifying this effect (29).

Obesity doubles the risk of intrahepatic cholangiocarcinoma (ICC) and increases the risk of extrahepatic cholangiocarcinoma (ECC) by 1.3-fold (30). Smoking is a moderate risk factor, increasing the risk by 40-100% depending on the biliary site. Tobacco carcinogens, after hepatic metabolism and biliary excretion, damage bile duct epithelial cells, facilitate gallstone formation, and induce chronic inflammation of the biliary epithelium by modulating adipokine levels, specifically increasing leptin and decreasing adiponectin (31).

Obesity is the primary risk factor for esophageal adenocarcinoma and is linked to gastroesophageal reflux disease (GERD). Increased abdominal pressure promotes GERD, leading to Barrett's esophagus, a precancerous condition. Systemic inflammation and insulin dysregulation independently activate oncogenic pathways, contributing to carcinogenesis (32). Smoking increases the risk of squamous cell carcinoma and adenocarcinoma by inducing genetic mutations in the esophageal mucosa (33).

In contrast, physical activity (PA) lowers cancer risk in the general population and is incorporated into programs aimed at preventing second primary cancers. For patients undergoing tumor treatment, PA enhances the quality of life by reducing chemotherapy side effects, sarcopenia, and cancer recurrence, while improving fitness and providing emotional and psychological benefits (34). The mechanisms, optimal levels, and effective doses of PA in oncology remain unclear; however, exercise modulates key GBM progression pathways, including the RTK/PI3K/Akt/mTOR signaling, angiogenesis, and metabolic reprogramming pathways. Additionally, PA promotes cytotoxic T cell and natural killer (NK) cell infiltration while reducing immunosuppressive cells such as Tregs and MDSCs (35).

THE SILENT SYMPTOMATOLOGY OF RECALCITRANT CANCER

Recalcitrant tumors are often asymptomatic or present with nonspecific symptoms during their early stages, complicating early detection and diagnosis. These subtle and often vague signs are frequently misinterpreted as benign or transient conditions, leading to delays in patients seeking medical attention and, consequently, in diagnosis. The nonspecific nature of early symptoms results in many patients

remaining asymptomatic or experiencing mild discomfort that does not immediately raise concern, thereby allowing the disease to progress unnoticed. For instance, PDAC typically manifests with insidious symptoms that may develop intermittently over several years before a formal diagnosis is made. This delayed presentation is partly attributed to the pancreas's retroperitoneal anatomical location, which limits early symptom development, and the current lack of specific early biomarkers for PDAC, making screening and early detection particularly challenging (36). Similarly, SCLC is often called a "silent" disease because its early respiratory symptoms – such as coughing, wheezing, or chest discomfort – are nonspecific. These symptoms are easily attributed to common respiratory infections or chronic conditions, which contribute to delayed diagnosis and treatment initiation (37). GBM also frequently manifests with early-stage symptoms that are subtle and nonspecific, including mild headaches, fatigue, and cognitive alterations, which are often erroneously attributed to stress, general fatigue, or other benign neurological conditions. The brain's exceptional capacity to adapt and compensate for slowly progressive tumor growth can obscure these initial indicators, thereby further impeding prompt diagnosis (38). In the case of HCC and CCA, the early stages are typically asymptomatic or present with vague systemic symptoms such as fatigue, decreased appetite, or weight loss (39, 40). In esophageal cancers, initial symptoms such as intermittent dysphagia, heartburn, hoarseness, cough, and weight loss may be easily mistaken for benign gastrointestinal disorders, including GERD or esophageal irritation (41). Overall, the common thread among these malignancies is the predominance of silent or nonspecific clinical presentations during their early stages of development. This shared characteristic underscores the critical need for heightened clinical vigilance, improved screening strategies, and the development of more sensitive and specific biomarkers to facilitate early detection and improve patient outcomes.

THE CHALLENGE OF EARLY DIAGNOSIS

Approximately one-third of patients with recalcitrant tumors are diagnosed incidentally during emergency hospital admissions, often at an advanced stage due to the nonspecific nature of the symp-

toms. Early detection is especially difficult because of the lack of specific biomarkers and challenges in identifying precursor or premalignant lesions using routine clinical imaging.

High-grade pancreatic intraepithelial neoplasia (PanIN), an early precursor lesion, is mainly detected via histopathology, as conventional imaging and biomarkers such as CA19-9 lack specificity for early malignant changes (36). SCLC diagnosis relies on minimally invasive sampling, such as bronchoscopy or fine-needle aspiration, often yielding small specimens that are extensively necrotic, which may lead to misdiagnosis and erroneous identification as treatable neoplasms. Unlike non-small cell lung cancer, no early detection biomarkers exist for SCLC, and computed tomography (CT) scans may miss small lesions or fail to distinguish them from other conditions (37). Early GBM detection is challenging because of tumor infiltration into normal brain tissue and the limited permeability of contrast agents across the blood-brain barrier. Consequently, magnetic resonance imaging (MRI) cannot reliably differentiate between low-grade and atypical GBM forms. Molecular heterogeneity within GBM tumors complicates early detection, as no single biomarker reliably indicates initial-stage disease, hindering the identification of universal markers (38). Pre-HCC lesions under 10 mm are often undetectable by MRI or CT and may mimic regenerative nodules in cirrhotic livers. Biomarkers such as alpha-fetoprotein (AFP) show low sensitivity for detecting small HCC (40). Pre-neoplastic biliary lesions, such as biliary intraepithelial neoplasia (BillIN) and intraductal papillary neoplasms of the bile duct (IPNB), resemble inflammatory changes and are difficult to detect because of their location. Brush cytology during endoscopic retrograde cholangiopancreatography (ERCP) has low sensitivity (< 50%) because desmoplastic tumors yield limited amounts of tissue. Serum markers, such as CA19-9, lack specificity because their levels increase with benign biliary inflammation (39). Early esophageal tumors may be missed by conventional endoscopy, requiring advanced imaging such as Lugol's iodine chromoendoscopy for precancerous lesion identification (41) (**Table 1**). The potential of liquid biopsy combined with multiomic technologies offers a promising approach for early detection of tumors that evade traditional screening. Liquid biopsy analyzes tumor-derived components in bodily fluids, such as circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), exosomes, and EVs. While widely used in advanced cancer management,

Table 1. Major diagnostic challenges and current biomarker approaches across recalcitrant tumors.

TUMOR TYPE	MAIN DIAGNOSTIC CHALLENGES	LIMITATIONS OF CURRENT IMAGING OR SAMPLING	CURRENT BIOMARKERS OR DIAGNOSTIC ADJUNCTS	KEY UNRESOLVED NEEDS
Pancreatic ductal adenocarcinoma (PDAC)	Nonspecific and intermittent symptoms; retroperitoneal location; precursor PanIN lesions are predominantly microscopic.	Standard imaging does not reliably identify high-grade PanIN or very small early lesions.	CA19-9 has insufficient specificity to distinguish benign pancreatic disease from early malignancy and limited value for early detection.	Prospective validation in high-risk cohorts; discrimination from pancreatitis and other benign conditions; standardized pre-analytical and analytical workflows.
Small-cell lung cancer (SCLC)	Bronchoscopic or fine-needle specimens may be small or necrotic; histologic overlap with carcinoid tumors, basaloid squamous-cell carcinoma, and lymphoma may complicate classification.	CT may miss small early-stage lesions and may not reliably distinguish malignancy from other thoracic conditions.	No established biomarker is available for population-level early detection.	Validation in limited-stage disease; distinction from other lung neoplasms; reproducibility across cohorts and sequencing platforms.
Glioblastoma (GBM)	Infiltrative growth, marked intratumoral heterogeneity, and radiologic overlap with abscess, tumefactive demyelination, or stroke impede early recognition.	MRI is the standard modality but may not reliably distinguish early or atypical lesions; the blood-brain barrier limits contrast-agent effectiveness.	No universal circulating biomarker is established for early detection.	A sufficiently sensitive noninvasive assay; differentiation from benign neurologic conditions; tumor-specific validation of plasma <i>versus</i> cerebrospinal-fluid approaches.
Hepatocellular carcinoma (HCC)	Small dysplastic nodules in cirrhotic livers may resemble regenerative nodules and lack the vascular features of overt HCC.	MRI and CT frequently fail to detect nodules <10 mm; 20-30% may remain undetected. Biopsy of small lesions in cirrhotic liver is technically difficult.	Alpha-fetoprotein (AFP) has low sensitivity for small or early HCC.	Discrimination of early HCC from cirrhosis, dysplasia, and inflammation; clinically validated thresholds; longitudinal surveillance studies.
Cholangiocarcinoma (CCA)	BillIN and intraductal papillary neoplasms may resemble inflammatory biliary changes; deep-seated and desmoplastic lesions yield limited cellular material.	ERCP brush cytology has low sensitivity (<50%); MRI/MRCP may not reliably distinguish early intrahepatic CCA from benign lesions or metastases.	CA19-9 has limited sensitivity for early disease and may be elevated in benign biliary inflammation; no reliable blood-based screening test is established.	Reliable distinction between malignant and benign biliary strictures; validation in high-risk populations; standardization by anatomical subtype.
Esophageal cancer	Early lesions may be asymptomatic and subtle, delaying clinical recognition.	Conventional endoscopy may miss early lesions; enhanced endoscopic techniques are required for improved visualization of precancerous changes.	Non valid circulating biomarker is currently established for early detection.	Separate validation for squamous-cell carcinoma and adenocarcinoma; discrimination from inflammatory or premalignant conditions; sensitivity in stage I disease.

its early diagnostic application faces several biological and technical challenges. Early tumors release biomarkers at low levels, making it difficult to distinguish cancer-specific signals from those of normal cell-free DNA. Integrating molecular data – including mutation analysis, DNA methylation, early-stage proteins and enzymatic biomarkers, and lipid changes – may help overcome some of these limitations and detect molecular signals before tumors are visible on imaging (42, 43).

THERAPEUTIC FAILURE IN RECALCITRANT TUMORS

At the molecular level, these tumors exhibit alterations in MYC or RAS oncogenes. RAS mutations are present in PDAC tumors in 90-95% of cases and in CCA in 7-25% of cases (44, 45). In contrast, RAS is overexpressed either directly through genetic alterations or indirectly through the activation of other pathways, such as EGFR and MET, in HCC, GBM, and esophageal cancer (46). MYC is overexpressed in 42% of PDAC cases, 50% of SCLC cases, 30-70% of HCC cases, 60-80% of GBM, and 20-25% of esophageal cancer cases (47-51). Oncogene activation is often associated with mutations in tumor suppressor genes, including TP53 and CDKN2A. TP53 mutations occur in 75-80% of PDAC cases, 90% of SCLC cases, 20-50% of HCC cases, 30-35% of GBM cases, 10-50% of CCA cases, and 72-80% of esophageal cancer cases (52-57). Deletion or mutation of CDKN2a is observed in 60-95% of PDAC tumors, 60% of GBM cases, 6-30% of HCC cases, 15-30% of CCA cases, and 12-25% of esophageal cancers (58-62). Hyperactivation of MYC and RAS, coupled with the inactivation of TP53 and CDKN2A, confers stemness characteristics to cells and induces metabolic reprogramming, epigenetic remodeling, and genetic instability (**Figure 2**). This cellular transformation influences the tumor microenvironment by promoting phenotypic reprogramming through cytokines, growth factors, and cell-cell interactions, thereby establishing an immunosuppressive milieu that facilitates immune evasion.

THE CHALLENGE OF UNDRUGGABLE PROTEINS

MYC inhibitors face significant clinical challenges due to the absence of a conventional binding site, ren-

dering them “undruggable” by standard small-molecule drugs. Unlike other enzymes, MYC does not possess a deep “pocket” that accommodates drug binding. Additionally, high-molecular-weight drugs have difficulty accessing the nucleus. Although numerous candidates have demonstrated promising *in vitro* binding affinities, they have failed clinically because of off-target effects and poor selectivity. Given that MYC regulates essential processes in healthy cells, such as proliferation and metabolism, its complete inhibition results in severe side effects (63).

The failure of RAS inhibitors in clinical trials is attributed to the complex biology of the protein, including the absence of binding pockets, high GTP/GDP affinity, and tumor adaptation through alternative pathways. While early farnesyltransferase inhibitors were unsuccessful due to isoform resistance, contemporary KRAS G12C inhibitors face challenges with resistance mechanisms, such as mutations, bypass pathway activation, and tumor rewiring, necessitating combination therapies for durable responses. Because RAS signaling is required for proliferation and survival in normal cells, complete inhibition can damage healthy tissues. Efforts to target downstream RAS signals have often failed due to side effects in normal tissues and feedback mechanisms that require intolerable doses (64). Pharmaceutical research has advanced with the development of next-generation anti-RAS agents, such as daraxonrasib, which significantly improves outcomes in patients with pancreatic cancer. In the phase 3 RASolute 302 trial, daraxonrasib approximately doubled median overall survival rate (65). Daraxonrasib inhibits RAS in its active, GTP-bound state, overcoming the limitations of earlier inhibitors that targeted the inactive [RAS(OFF)] form. Daraxonrasib functions as a pan-RAS inhibitor, effectively targeting a broad spectrum of activating mutations beyond the KRAS G12C specificity of previous drugs. Moreover, it forms a complex with cyclophilin A, which binds RAS with high affinity, resulting in sustained protein inactivation (65).

Owing to the challenges in directly targeting RAS and MYC, research has shifted toward developing molecules that modulate the downstream pathways affected by these oncogenes and tumor suppressors (TP53, CDKN2A), including cell cycle regulation, genomic stability, DNA damage response, epigenetic remodeling, metabolism, immune modulation, and extracellular matrix composition.

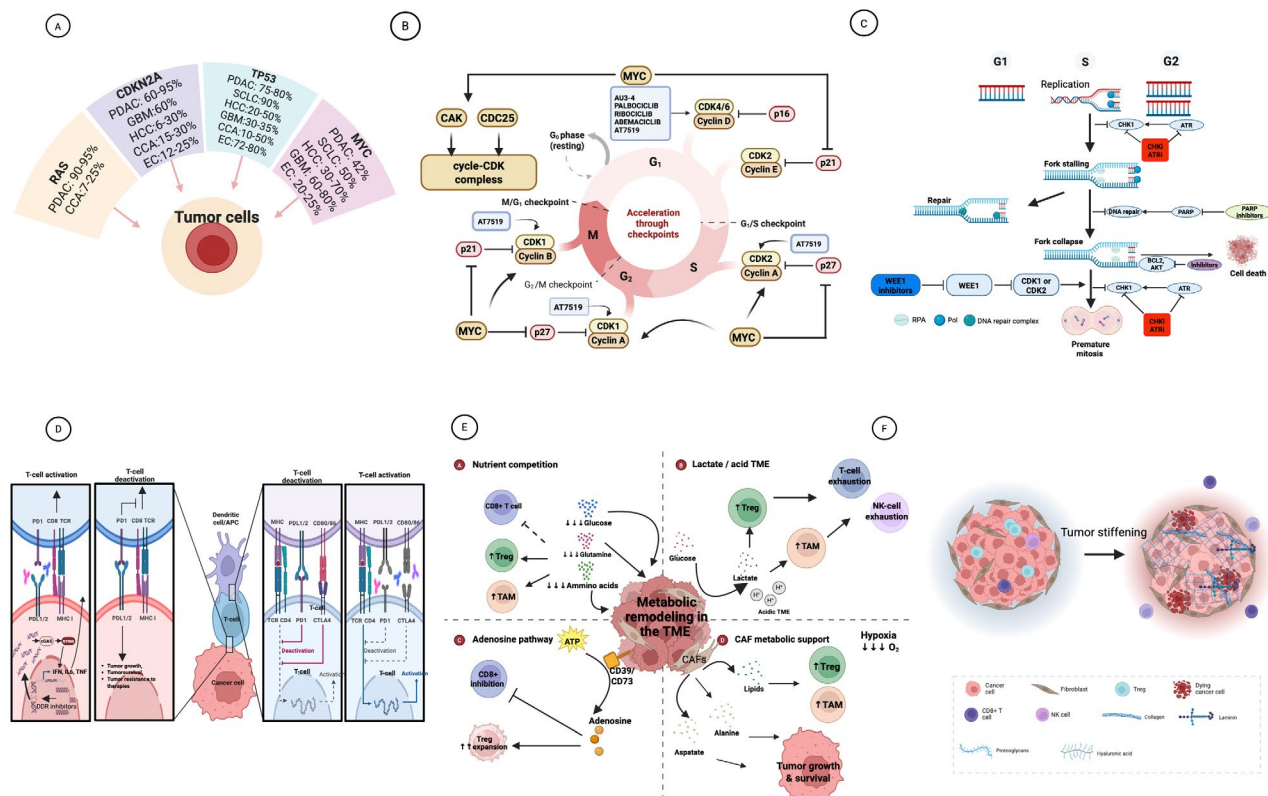


Figure 2. Molecular and microenvironmental alteration in recalcitrant tumors and their potential therapeutic targeting.

(A) Mutations in oncogenes and tumor suppressor genes are prevalent in recalcitrant tumors. **(B)** CDK inhibitors restrict cell-cycle progression in recalcitrant tumors. **(C)** Recalcitrant tumors enhance DDR expression to prevent replication fork stalling and promote tumor cell proliferation. **(D)** Recalcitrant tumors evade immune recognition by recruiting immunosuppressive cells, which, in collaboration with cancer cells, induce exhaustion in effector CD8 T cells through the expression of immune checkpoint proteins. Inhibiting the DDR pathway with inhibitors targeting WEE1, ATM, and ATR can enhance the efficacy of immune checkpoint inhibitors by stalling replication fork and activating the cGAS-STING-mediated interferon pathway through the release of cytosolic DNA. **(E)** Cancer cells remodel the tumor microenvironment and foster an immunosuppressive, tumor-supportive environment by employing the following strategies: a) nutrient deprivation leading to exhaustion of effector T cells and natural killer cells; b) increasing glycolytic metabolism and lactate production, which induces immunosuppressive M2 TAM polarization and Treg differentiation and proliferation; c) CD39 convert ATP/ADP to AMP, whereas CD37 convert AMP to adenosine, promoting Treg expansion and T cell exhaustion; d) reprogramming cancer-associated fibroblasts (CAFs) to release amino acids (alanine and aspartate) to support tumor growth in a hypoxic environment, provide lipid-derived energy substrates, and promote Treg and TAM expansion. **(F)** Recalcitrant tumors reprogram CAFs to synthesize and release more extracellular matrix and lysyl oxidase (LOX), which crosslinks collagen and elastin, creating a stiff barrier that impedes lymphocyte infiltration into the tumor.

LIMITATIONS OF CONVENTIONAL THERAPIES IN RECALCITRANT TUMORS

CDK inhibitors

The ineffectiveness of Cyclin-Dependent Kinase (CDK) inhibitors, particularly CDK4/6 inhibitors such as palbociclib, ribociclib, and abemaciclib, in the treatment of resistant tumors poses a substantial challenge in the field of oncology. The limited efficacy of CDK inhibitors is largely attributable to mechanisms of intrinsic and acquired resistance.

Clinical trials of CDK4/6 inhibitors, such as palbociclib, in resistant PDAC have yielded limited success as stand-alone therapies. This limitation is attributed to the rapid emergence of adaptive resistance mechanisms. PDAC cells bypass CDK4/6 inhibition by elevating cyclin D1 and E levels and activating alternative pathways, notably the PI3K/mTOR and MEK pathways. Clinical trials have revealed that although palbociclib inhibits tumor proliferation, it simultaneously enhances metastasis and invasion by inducing epithelial-to-mesenchymal transition (EMT). Furthermore, trials with nonspecific CDK inhibitors, including CDK9 inhibitors, flavopiri-

dol, and dinaciclib, have shown unacceptable toxicity and poor efficacy (66).

In SCLC, CDK inhibitors have yielded mixed results, with earlier generations proving ineffective in addressing relapsed or resistant cases. Initial “pan-CDK” inhibitors, such as flavopiridol and roscovitine, failed in clinical trials due to toxicity and insufficient specificity. Although trilaciclib has demonstrated efficacy in mitigating chemotherapy-induced myelosuppression, its effectiveness as an anticancer agent is constrained because its function is to protect healthy bone marrow rather than to target resistant tumors. Recent CDK9 inhibitors, such as KB-0742, have shown preclinical potential by targeting transcriptional mechanisms, offering an approach to overcome resistance beyond cell cycle regulation (67).

The use of CDK inhibitors in GBM treatment poses a significant therapeutic challenge. Although alterations in the CDK4/6-Rb axis occur in 80% of cases, outcomes remain suboptimal because of limited BBB penetration and drug resistance. Current efforts are focused on molecules with superior pharmacokinetic profiles and combination therapies to overcome resistance. Zotiraciclib (TG02), an oral multi-CDK inhibitor targeting CDK9 and reducing the expression or levels of c-MYC and MCL-1, is being assessed in phase Ib trials (NCT03224104) alongside temozolomide or radiotherapy, following limited efficacy as a monotherapy in recurrent GBM (PFS-6 of 6.7%). Abemaciclib, known for its superior BBB permeability, is being evaluated in clinical trials for patients exhibiting alterations in the Rb pathway. AU3-14, a novel CDK4/6 inhibitor with high brain penetration, delays resistance to temozolomide (TMZ) in preclinical models. AT7519, a selective inhibitor of CDK1, 2, 4, 6, and 9, induces apoptosis, pyroptosis, and cell cycle arrest in human GBM cells, as demonstrated in preclinical studies (68-70).

Clinical trials assessing the efficacy of CDK inhibitors as standalone treatments for advanced HCC have encountered notable obstacles. Despite promising results in preclinical studies, clinical trials of palbociclib have faced challenges owing to the emergence of compensatory pathways and resistance mechanisms. Notably, trials NCT04162301 and NCT02255461 were either discontinued or demonstrated limited efficacy when CDK4/6 inhibitors were used as monotherapies, as their cytostatic action was insufficient to combat aggressive tumors. Resistance arises from the activation of alternative pathways, such as PI3K/mTOR and MAPK, enabling tumors to

bypass CDK4/6 inhibitor-induced cell cycle arrest (71) (**Table 2**).

In cases of recalcitrant CCA, the efficacy of single-agent CDK4/6 inhibitors, such as palbociclib, is limited in both clinical and preclinical settings. These inhibitors have been observed to upregulate cIAP1/2 (Cellular Inhibitor of Apoptosis Proteins) through NF- κ B signaling pathways, thereby protecting tumor cells against apoptosis. Although these inhibitors induce temporary G0/G1 cell cycle arrest, tumors frequently circumvent this blockade, resulting in disease progression and the development of treatment resistance (72, 73) (**Table 2**).

Clinical trials investigating the use of CDK4/6 inhibitors as monotherapy for refractory esophageal cancer have demonstrated suboptimal outcomes. A significant number of upper gastrointestinal carcinomas exhibit intrinsic resistance attributed to cyclin E overexpression, which facilitates cell cycle progression despite CDK4/6 inhibition. Due to the limited clinical efficacy observed, plans to extend phase II trials for agents such as ribociclib in this context were discontinued (74) (**Table 2**).

Replication stress response (RSR) inhibitors

Replication stress (RS), resulting from the stalling or collapse of replication forks, is a hallmark of therapy-resistant cancers, including SCLC and pancreatic cancer. These tumors rely on the replication stress response (RSR), mediated by the ATR-CHK1 axis, to mitigate RS, prevent mitotic entry, and promote survival. Oncogene-driven RS, caused by MYC amplification or loss of TP53 and RB1, exacerbates replication stress, increasing the risk of fork collapse into lethal double-strand breaks (DSBs). Therapeutic inhibition of RSR components ATR, CHK1, and WEE1 induces replication catastrophe, which is a promising strategy for selectively targeting these malignancies (75). RSR inhibitors targeting ATR, WEE1, and CHK1 have emerged as promising therapeutic agents for PDAC, a cancer characterized by replication stress, KRAS mutations, and genomic instability (76-79). However, clinical trials have faced setbacks due to acquired drug resistance. Early trials combining CHK1 or ATR inhibitors with chemotherapy frequently resulted in dose-limiting toxicity (80-82). Consequently, the research focus is shifting toward combining these inhibitors with ICIs, as RSR defects can induce cytosolic DNA accumulation, thereby activating immune responses via the cGAS-STING pathway (83). Ongoing studies, including trial NCT06015659, are evaluating the WEE1 inhibitor azenosertib in combina-

tion with gemcitabine, alongside the identification of biomarkers that predict therapeutic response (84) (**Table 2**).

ATR inhibitors, such as berzosertib, show potential in treating SCLC because of the cancer's dependence on DNA repair mechanisms for rapid cell proliferation. Nevertheless, clinical trials addressing the recalcitrant nature of SCLC face significant challenges, including limited therapeutic responses, toxicity, and the development of resistance. Notably, clinical trials targeting RSR in SCLC have faced setbacks, as exemplified by the termination of a Phase II trial for berzosertib (M6620/VX-970) (85, 86). Furthermore, the ineffectiveness of prexasertib in treating SCLC was corroborated by the Phase II study NCT02735980 (87). Despite preclinical evidence suggesting that SCLC is an optimal target for these interventions, the clinical outcomes have been disappointing (**Table 2**).

GBM resistance to standard therapies is driven by glioma stem cells (GSCs) experiencing replication stress from transcription-replication collisions in long neural genes, which activate the ATR-CHK1 pathway, stabilizing replication forks and preventing genomic instability, enabling tumor survival against chemotherapy and radiotherapy (88). Targeting the RSR pathway is a promising approach to overcome drug resistance, with ATR inhibitors (AZD6738, M6620) being evaluated to sensitize GBM to radiotherapy and PARP inhibitors (89, 90). A Phase I trial is assessing the CHK1/CHK2 inhibitor triapine with radiotherapy in recurrent GBM (NCT06860594). Additionally, the antisense oligonucleotide TUG1-DDS, which targets the long noncoding RNA (lncRNA) Taurine Upregulated Gene 1 (TUG1), induces R-loop accumulation and DNA damage, suppressing tumor growth preclinically and supporting future clinical development (91) (**Table 2**).

Table 2. Selected clinical trials and therapeutic strategies in recalcitrant tumors.

TUMOR TYPE	THERAPEUTIC STRATEGY	AGENT(S) / REGIMEN	TRIAL IDENTIFIER	PHASE	MAIN MESSAGE (REVISED)
PDAC	CDK inhibition	Palbociclib	Not specified	Not specified	CDK4/6 inhibition showed limited clinical activity as monotherapy, consistent with rapid adaptive bypass signaling and lack of durable disease control in PDAC.
PDAC	RSR inhibition	Azenosertib + gemcitabine	NCT06015659	Phase II	This trial reflects a biomarker-oriented strategy to exploit replication stress in second-line advanced PDAC, but efficacy remains under investigation.
PDAC	ICI + DDR/RSR targeting	PARP inhibitor + PD-1/PD-L1 blockade	NCT04548752; NCT04493060; NCT03330405	Not consistently specified	These studies test whether DDR-targeted therapy can increase tumor immunogenicity and sensitize selected PDAC subsets, particularly BRCA-mutated or platinum-sensitive disease, to immunotherapy.
PDAC	TME/adenosine axis	Quemliclustat + etrumadenant + SBRT-based neoadjuvant therapy	NCT06048484 (AIRPanc)	Phase II	This study evaluates whether adenosine-pathway blockade plus radiotherapy and immunotherapy can remodel the immunosuppressive PDAC microenvironment in surgically eligible disease.

(Continued on next page)

(Continued from previous page)

TUMOR TYPE	THERAPEUTIC STRATEGY	AGENT(S) / REGIMEN	TRIAL IDENTIFIER	PHASE	MAIN MESSAGE (REVISED)
PDAC	TME/CXCL12 axis	NOX-A12 (olaptesed pegol) + pembrolizumab	NCT03168139	Phase I/II	This trial investigates stromal chemokine blockade as a strategy to improve immune-cell trafficking and overcome CAF-mediated immune exclusion.
PDAC	Autophagy inhibition	Hydroxychloroquine + gemcitabine/nab-paclitaxel	NCT01978184	Phase II	This randomized trial tested autophagy inhibition as a metabolic vulnerability in resectable PDAC; clinical benefit appears modest and insufficient to establish HCQ as an effective standard strategy.
SCLC	CDK/DDR interface	Trilaciclib	Not an antitumor efficacy trial in this context	Approved supportive-care use	Trilaciclib should not be framed as an active antitumor strategy; its clinical value in SCLC is myelopreservation during chemotherapy rather than direct tumor control.
SCLC	RSR inhibition	Prexasertib (LY2606368)	NCT02735980	Phase II	Prexasertib monotherapy showed limited efficacy in extensive-stage SCLC despite strong biological rationale, underscoring the gap between replication-stress dependency and clinical benefit.
SCLC	DDR inhibition	Berzosertib + topotecan	DDRiver SCLC 250	Phase II	The combination represents an attempt to potentiate topotecan through ATR inhibition in platinum-resistant SCLC, but available evidence remains preliminary and does not yet support practice change.
SCLC	ICI + PARP inhibition	Olaparib + durvalumab	MEDIOLA	Phase I/II	The regimen was tolerable but did not achieve the desired level of antitumor activity, highlighting the need for biomarker-guided combination strategies in relapsed SCLC.
SCLC	CHK1 inhibition + immunotherapy	Prexasertib + anti-PD-L1	NCT03495323	Early-phase/clinical exploration	This program is hypothesis-generating and should be described as translation of strong preclinical synergy into early clinical testing, not as evidence of established efficacy.
GBM	Multi-CDK inhibition	Zotiraciclib (TG02)	NCT03224104	Phase Ib / early clinical program	Zotiraciclib-based regimens illustrate continued interest in transcriptional CDK targeting in GBM, but clinical activity to date remains limited and investigational.

(Continued on next page)

(Continued from previous page)

TUMOR TYPE	THERAPEUTIC STRATEGY	AGENT(S) / REGIMEN	TRIAL IDENTIFIER	PHASE	MAIN MESSAGE (REVISED)
GBM	RSR inhibition	Triapine + radiotherapy	NCT06860594	Phase I	This first-in-human combination explores whether checkpoint kinase inhibition can radiosensitize recurrent glioblastoma while remaining tolerable in the CNS setting.
GBM	PARP inhibition	Talazoparib + chemotherapy (TAC-GReD)	NCT04740190	Early-phase trial	This trial targets DDR-deficient recurrent high-grade glioma, testing whether PARP inhibition can exploit molecularly defined DNA-repair vulnerability.
GBM	ICI	Nivolumab-based programs	Not specified	Phase III	Large randomized trials of checkpoint blockade in GBM failed to demonstrate meaningful survival improvement, reinforcing the immune-cold biology of this disease and the need for rational combinations.
HCC	CDK4/6 inhibition	Palbociclib	NCT04162301; NCT02255461	Early-phase	Clinical development of CDK4/6 inhibition in advanced HCC has been constrained by limited efficacy and pathway compensation, indicating that cytostatic monotherapy is unlikely to be sufficient.
HCC	RSR inhibition	WEE1/CHK1 pathway-targeted regimens	NCT04158336	Ongoing clinical trial	This trial exemplifies the strategy of leveraging high replication stress in HCC, but outcome data remain immature and should be presented as investigational.
CCA/BTC	RSR inhibition	ATR/CHK1/WEE1/ PARP-targeted programs	NCT04298021; NCT03878095; NCT02576444	Early-phase trials	These studies collectively reflect an exploratory DDR-targeting approach in biliary tract malignancies, especially in genomically selected or treatment-refractory settings.
CCA/BTC	PARP inhibition	Olaparib	NCT04042831	Phase II	The manuscript should correct the registry number to NCT04042831; this phase II study evaluates olaparib in metastatic biliary tract cancer with DNA-repair alterations.
CCA/BTC	ICI + chemotherapy	Durvalumab + gemcitabine/ cisplatin	Not specified	Phase III	TOPAZ-1 established chemo-immunotherapy as a clinically meaningful standard in advanced biliary tract cancer, although absolute survival gains remain modest and resistance remains common.

(Continued on next page)

TUMOR TYPE	THERAPEUTIC STRATEGY	AGENT(S) / REGIMEN	TRIAL IDENTIFIER	PHASE	MAIN MESSAGE (REVISED)
CCA/BTC	ICI-based combination	Nivolumab + gemcitabine/ cisplatin or nivolumab + ipilimumab	Not specified	Randomized Phase II	BitT-01 supports continued evaluation of immune-based combinations in untreated advanced biliary cancer, but its results should be interpreted as signal-seeking rather than definitive practice-changing evidence.
Esophageal cancer	CDK4/6 inhibition	Palbociclib	Phase II trial in advanced esophageal or gastric cancer	Phase II	Palbociclib monotherapy showed limited efficacy in upper gastrointestinal cancers, consistent with intrinsic cell-cycle bypass mechanisms such as cyclin E upregulation.
Esophageal cancer	ICI	Nivolumab vs chemotherapy	ATTRACTION-3	Phase III	ATTRACTION-3 demonstrated an overall survival advantage for nivolumab over chemotherapy in previously treated advanced esophageal squamous cell carcinoma, validating immune checkpoint blockade in a subset of patients.
Esophageal cancer	ICI	Pembrolizumab vs chemotherapy	KEYNOTE-181	Phase III	KEYNOTE-181 showed that benefit from pembrolizumab is concentrated in biomarker-enriched populations, supporting patient selection rather than indiscriminate monotherapy use.

PDAC: pancreatic ductal adenocarcinoma; SCLC: small-cell lung cancer; GBM: glioblastoma; HCC: hepatocellular carcinoma; CCA/BTC: cholangiocarcinoma/biliary tract cancer; RSR: replication stress response; DDR: DNA damage response; ICI: immune checkpoint inhibitor; TME: tumor microenvironment.

Tumors exhibiting a “High Replication Stress” profile, such as HCC, are associated with poorer survival outcomes but demonstrate increased sensitivity to therapies targeting the ATR-CHK1-WEE1 pathway (92-94). Agents such as ceralasertib (AZD6738) and elimusertib (BAY1895344) are currently being evaluated in early phase clinical trials for advanced solid tumors, including those resistant to standard treatments (95, 96). Preclinical studies have shown that ATR inhibition in high-risk HCC suppresses tumor progression and modulates antitumor immune response (92). Inhibitors of WEE1 and CHK1 induce mitotic catastrophe in cancer cells, further supporting their therapeutic potential. Ongoing trials (NCT04158336) are investigating these agents as monotherapies or

in combination with chemotherapy and immune checkpoint inhibitors to exploit high replication stress (97, 98). Additionally, research is progressing toward integrating RSR inhibitors with radiotherapy or immunotherapy to reverse the immunosuppressive tumor microenvironment characteristics of resistant HCC.

In CCA, ongoing clinical trials are investigating inhibitors of RSR proteins, including ATR, CHK1, WEE1, and PARP as potential strategies (NCT04298021, NCT03878095, NCT02576444) (99). Of particular interest, the PARP inhibitor olaparib is currently under evaluation in Phase II clinical trials (NCT04042831) for metastatic biliary tract cancer characterized by mutations in DNA repair genes (**Table 2**).

Gastroesophageal adenocarcinomas with high chromosomal instability and TP53 mutations in 72-90% of cases are particularly sensitive to CHK1 inhibitors (100). Clinical trials have indicated that the combination of these inhibitors with chemotherapy enhances therapeutic efficacy. The ATR inhibitor VX-970 (berzosertib) sensitizes esophageal cancer cells to chemotherapy and radiotherapy in preclinical models (101). Other ATR inhibitors, including ceralasertib and elimusertib (BAY 1895344), are under clinical evaluation for advanced solid tumors with DNA damage response defects (102, 103) (**Table 2**).

Immune checkpoint inhibitors

Many recalcitrant tumors exhibit an immunologically “cold” phenotype with low T-cell infiltration and abundant immunosuppressive cells, facilitating immune evasion and resistance to immunotherapy. Evaluating immune checkpoints remains crucial for developing combination therapies to convert cold tumors into “hot” tumors.

PDAC is characterized by a dense stroma, known as a desmoplastic reaction, which prevents immune cells from accessing the cancer cells. The tumor microenvironment (TME) contains immunosuppressive cells, including myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs), and regulatory T cells (Tregs), which inhibit cytotoxic T cell infiltration. Dense stroma causes poor vascularization, creating a hypoxic and acidic environment that kills or exhausts effector T cells (104). PDACs have a low-to-intermediate mutational burden, resulting in fewer neoantigens and limited immune recognition (105). Consequently, ICIs show minimal efficacy in resistant PDAC and are FDA-approved only for patients with microsatellite instability-high (MSI-H) or deficient mismatch repair (dMMR) (~0.5-1%) (106). Meta-analyses have indicated that combining ICIs with chemotherapy provides modest benefits over chemotherapy alone (107). Clinical trials are evaluating ICI and RSR inhibitor combinations in resistant PDAC, focusing on PARP and PD-1/PD-L1 inhibitors in BRCA-mutated or platinum-sensitive cases (NCT04548752, NCT04493060, and NCT03330405). The Phase II POLAR study assessed ATR inhibitors to counteract PARP inhibitor resistance linked to ATR/CHK1 pathway hyperactivation (108) (**Table 2**). Despite a high tumor mutational burden from smoking-induced DNA damage, SCLC exhibits an immune-cold microenvironment with low immune infiltration, enabling immune evasion (109). Nonetheless, ICIs have improved treatment efficacy, with

the Phase III IMpower133 (Atezolizumab) and CASPIAN (Durvalumab) trials demonstrating the efficacy of ICI-chemotherapy combinations in extensive-stage SCLC (110). The MEDIOLA trial of olaparib plus durvalumab in relapsed SCLC showed tolerability but failed to meet primary efficacy endpoints, underscoring the need for biomarker-based patient selection (111). Prexasertib (Chk1 inhibitor) combined with anti-PD-L1 induced significant tumor regression in SCLC mouse models, achieving complete regression in up to 60% of cases, prompting early phase clinical evaluation (NCT03495323) (112) (**Table 2**). GBM is an immunologically cold tumor characterized by low T cell infiltration and an immunosuppressive microenvironment driven by M2-type TAMs and Tregs. Its low mutational burden contributes to poor ICI monotherapy outcomes, as shown in the CheckMate 143, 498, and 548 trials (113-116). Additionally, the blood-brain barrier restricts the delivery of therapeutic antibodies, further limiting the engagement of the immune system. Current approaches focus on combination therapies, neoadjuvant use, and novel checkpoint targets. RSR defects are emerging biomarkers for predicting responses. Preclinical studies are assessing whether ATR inhibitors can prevent radiation-induced CD8+ T-cell depletion and target glioma stem cells with high replication stress (117, 118). Clinical trials are evaluating PARP inhibitors, such as talazoparib, combined with chemotherapy in high-grade gliomas (NCT04740190) (119) (**Table 2**). Recalcitrant HCC is an immunologically cold tumor characterized by poor T cell infiltration, high immunosuppression, and limited responses to ICIs despite arising in a chronically inflamed liver. Tumors exploit liver immune tolerance by accumulating M2-type TAMs, Tregs, and CAFs, which hinder immune cell infiltration (120). Many HCCs have few neoantigens, rendering them “invisible” to immune recognition (121). ICIs have changed HCC treatment, particularly in advanced cases that fail conventional therapies. Although atezolizumab plus bevacizumab is the first-line treatment for advanced HCC, ongoing trials are exploring combination and neoadjuvant therapies to overcome drug resistance (122). Clinical trials are evaluating RSR inhibitors in patients with advanced HCC. Patients with HBV-associated HCC show increased sensitivity to DNA damage, making DNA damage repair (DDR) inhibitors suitable for immunotherapy in this subpopulation (123). RSR inhibitors, including ATR inhibitors ceralasertib and berzosertib, combined with anti-PD-1/PD-L1, show promise in enhancing T cell activation in preclinical

models (102). Prexasertib and adavosertib (WEE1 inhibitor) have been evaluated in refractory solid tumors, showing potential benefits in Phase I trials (124, 125) in HCC with genomic signatures of chromosomal instability (CIN).

CCA is an immunologically cold tumor characterized by poor T cell infiltration, an immunosuppressive microenvironment, desmoplasia, and low antigenicity, leading to resistance to standard immunotherapy (126). In relapsed or advanced CCA, immune checkpoint inhibitors (durvalumab, nivolumab, and pembrolizumab) modestly improve overall survival compared to gemcitabine/cisplatin chemotherapy (1-2 months) (127-130). Combining ICIs with RSR inhibitors, including PARP inhibitors (olaparib) and ATR inhibitors (camonsertib), is under clinical investigation to enhance efficacy by targeting DNA repair mechanisms, increasing tumor mutational burden, and stimulating antitumor immune pathways (NCI-2020-05323, BIL-PPP) (131). While ICI monotherapy shows limited response rates (5-20%), combination strategies may improve outcomes in resistant CCA (**Table 2**).

Esophageal cancers are immunologically cold, characterized by an immunosuppressive microenvironment with Tregs and MDSCs and loss of MHC-I expression, enabling immune evasion (132). ICIs have improved treatment outcomes, with monotherapy and combination regimens (ATTRACTION-3, KEYNOTE-181) extending survival, although response rates remain 10-20% (133, 134). The combination of ICIs with RSR inhibitors is being investigated to enhance tumor immunogenicity and improve durable responses in resistant esophageal cancer (**Table 2**).

Remodeling of TME metabolism

Remodeling TME metabolism is essential for overcoming resistance in refractory tumors by reversing immunosuppression and inhibiting adaptive survival mechanisms under hypoxic and nutrient-deprived conditions. Tumor cells preferentially consume glucose, glutamine, and amino acids, thereby limiting the nutrients required for immune-cell activation and effector function. Lactic acid accumulation acidifies the TME, impairing the cytotoxic functions of NK cells and T lymphocytes. Extracellular ATP released during cellular stress is enzymatically converted to immunosuppressive adenosine via the action of CD39 and CD73. Under hypoxic conditions, refractory tumors depend on enhanced lipid biosynthesis and cholesterol accumulation to

evade immune surveillance and sustain their proliferation (135).

PDAC cells induce adjacent CAFs to undergo glycolysis, producing metabolites such as lactate and alanine that support tumor growth. This metabolic reprogramming generates a highly acidic and hypoxic TME that promotes aggressive tumor phenotypes. Fibroblast Activation Protein (FAP)-expressing CAFs secrete CXCL12, thereby inhibiting T cell infiltration, while inflammatory CAFs (iCAFs) release proinflammatory factors that facilitate immune evasion and tumor progression (136-138). Several clinical trials have investigated therapies targeting the TME. Study NCT06048484 evaluates stereotactic body radiation therapy (SBRT) combined with quemli-clustat, a CD73 inhibitor, and etrumadenant, an A2A/B receptor antagonist, aiming to block the adenosine pathway exploited by tumors for immune suppression (139). A Phase I/II trial (NCT03168139) assessed pembrolizumab in combination with NOX-A12, a CXCL12 inhibitor, targeting chemokine secretion from FAP-positive CAFs to impede tumor progression (140). Another trial focused on inhibiting the CSF1R/FAK pathway to reprogram MDSCs and TAMs, thereby enhancing T cell-mediated tumor eradication (141). PDAC also depends on macropinocytosis and autophagy for survival under nutrient deprivation, making these pathways potential therapeutic targets (142). Although hydroxychloroquine (HCQ) is the only autophagy inhibitor clinically tested in PDAC (NCT01978184), tolerated doses may paradoxically enhance macropinocytosis, thereby limiting its efficacy (143) (**Table 2**). Novel macropinocytosis inhibitors, including ivermectin, tyrphostin A9, and pyrimethamine, are currently under preclinical investigation in combination with autophagy blockers (144). Additional preclinical studies are exploring nutrient restriction strategies, such as vitamin B6 depletion, to inhibit PDAC growth (145). Despite promising preclinical results, translation to human trials remains challenging because of stromal heterogeneity.

SCLC features a hypoxic environment that drives its drug resistance. Hypoxia induces glycolysis, with CAFs activating FGF/FGFR signaling to enhance glycolysis and lactate production in non-neuroendocrine SCLC cells. Targeting monocarboxylate transporters (MCTs) reduce TME acidification and immune suppression. FGFR inhibitors are being investigated to block FGF-mediated metabolic and phenotypic tumor progression (146). SCLC cells have reduced levels of n-3 fatty acids in their phospholipids com-

pared to healthy tissues, contributing to a pro-inflammatory TME that promotes tumor progression. CAF-driven lipid metabolism reprogramming creates a fat-rich, immunosuppressive niche that supports tumor survival under hypoxia (147). Antigen-presenting CAFs (apCAF) sequester CD8⁺ T cells or induce their differentiation into Tregs, impairing cytotoxic responses (148). Inhibition of phospholipase D1 (PLD1), which generates pro-tumor phosphatidic acid, reduces lung metastasis and sensitizes chemotherapy-resistant tumors in preclinical models (149).

The GBM TME is an adaptive ecosystem in which CAFs drive therapeutic resistance by producing metabolites, such as lactate and pyruvate, that promote tumor growth. CAFs and GBM-associated mesenchymal stem cells (GA-MSCs) enhance GSC stemness and supply amino acids, such as glutamine and aspartate, which are vital for tumor biosynthesis under hypoxia (150). Glutaminase inhibitors (e.g., CB-839) show limited efficacy because of poor blood-brain barrier penetration (151, 152). Monocarboxylate transporter 4 (MCT4) inhibitors offer a promising strategy for blocking lactate export from CAFs and disrupting tumor-stroma metabolic crosstalk (153). Emerging therapies aim to reprogram CAFs from pro-tumorigenic to tumor-suppressive phenotypes, rather than eliminating them, to minimize potential harm to healthy tissues (154).

HCC and CCA exhibit an immunosuppressive TME driven by nutrient competition, lactate accumulation, and hypoxia. CAFs promote metabolic crosstalk that supports tumor progression and immune evasion by inducing the Warburg effect and producing lactate and metabolites that enhance chemoresistance and suppress immunity (155). Metabolic reprogramming of CAFs generates a hypoxic, hypoglycemic, and acidic TME that impairs CD8⁺ T cell functions. CAFs increase fatty acid synthase (FASN) expression and secrete lipids to fuel HCC cells while supplying glutamine for proliferation and glutathione synthesis to reduce oxidative stress (156). Preclinical targeting of CAF metabolism reduces tumor burden, normalizes vasculature, and enhances CD8⁺ T cell infiltration by inhibiting CAF cytokines, fatty acid synthesis, and CD36-mediated lipid uptake (156).

Esophageal cancer cells exhibit high glucose uptake, depleting energy sources for effector T cells and producing lactate, which acidifies the TME, inducing T cell exhaustion and M2 macrophage polarization. CAFs enhance lipogenesis and secrete lipids, such as lysophosphatidylcholine, supporting tumor

metabolism via PI3K/AKT pathway activation. The metabolic exchange of aspartate and glutamate between tumor cells and CAFs sustains proliferation and redox balance under nutrient scarcity (157, 158). Targeting CAF metabolism with phosphodiesterase 5 (PDE5) inhibitors, such as sildenafil, restricts fibroblast transdifferentiation, sensitizing tumors to chemotherapy (159). Inhibitors of FASN and lipid transporter CD36 are also under investigation to disrupt CAF-mediated metabolic support (160, 161).

Physical activity is a viable strategy for modulating the TME in patients with cancer. Tumors resistant to ICIs are often characterized by low infiltration of antitumor immune cells and an immunosuppressive TME. Exercise enhances immune cell circulation and improves immunosurveillance. Recent studies have indicated that physical activity facilitates the mobilization and redistribution of immune cells into the TME, thereby augmenting antitumor immune responses and potentially improving the efficacy of ICIs (162).

Softening of the extracellular matrix

Extracellular matrix (ECM) remodeling is a critical factor in the progression of recalcitrant tumors, particularly solid malignancies such as PDAC and GBM. The dense ECM serves as a physical barrier, impeding drug delivery and immune cell infiltration while simultaneously promoting tumor survival and metastasis (163). Current clinical trials are focused on “normalizing” the ECM to overcome chemoresistance and enhance immunotherapy efficacy. One approach involves the degradation of barrier components using enzymes such as pegvorhialuronidase alfa (PEGPH20) to degrade hyaluronic acid or collagen, thereby reducing tumor interstitial pressure and facilitating drug delivery (164). Another strategy targets the inhibition of remodeling processes, with drugs such as pamrevlumab, an antibody targeting connective tissue growth factor (CTGF) was evaluated in Phase III trials for unresectable pancreatic cancer. The available clinical data did not demonstrate the expected benefits, suggesting that the projected therapeutic benefits were not fully achieved (165). Additionally, the modulation of matrix stiffness is being investigated through the inhibition of lysyl oxidase (LOX) to prevent collagen cross-linking, thus reducing the stiffness that supports tumor stem cell survival and metastasis (166). Despite historically disappointing outcomes, new generations of integrin inhibitors are being developed to block

the interaction between the extracellular matrix and surface receptors, thereby disrupting tumor survival signaling (167, 168).

CONCLUSIONS

Recalcitrant tumors represent a major challenge in oncology because of their resistance to conventional therapies, necessitating a comprehensive multidisciplinary approach to improve patient outcomes. Preventive strategies remain essential, with lifestyle modifications – such as abstaining from alcohol and tobacco, maintaining a balanced diet, and engaging in regular physical activity – serving as foundational measures to reduce cancer risk. Early detection is equally critical, and advancements in liquid biopsy combined with multiomic analyses offer promising avenues for identifying novel biomarkers that enable diagnosis at stages when therapeutic interventions are more effective. This potential for early intervention could significantly improve clinical outcomes by facilitating personalized treatment strategies tailored to the tumor's molecular profile.

Looking ahead, the future of oncology is centered on innovative therapeutic modalities targeting the TME and its pro-tumorigenic functions. Emerging pharmacological strategies, including proteolysis-targeting chimera (PROTAC) platforms, chimeric antigen receptor T cells (CAR T cells) engineered to recognize tumor-specific antigens, and immune modulation therapies, are anticipated to transform cancer treatment. These interventions aim to inhibit tumor progression and reprogram the TME to enhance antitumor immunity, thereby improving the efficacy of current therapies and patient survival outcomes. Until these advancements become widely accessible, maintaining a healthy lifestyle remains an accessible and effective strategy for cancer-risk reductions and overall health promotion.

COMPLIANCE WITH ETHICAL STANDARDS

Funding

None.

Conflicts of interest

The authors declare no competing interests.

Availability of data and materials

No datasets were generated during the current study.

Authors' contributions

All authors contributed equally to the conceptualization and writing (original draft and review & editing) of the manuscript.

Ethical approval

Human studies and subjects

N/A.

Animal studies

N/A.

Publication ethics

Plagiarism

The article provides a comprehensive review of the latest studies in the field, with accurate citations. This manuscript does not contain plagiarism.

Data falsification and fabrication

The manuscript is original and was prepared by the authors.

REFERENCES

1. Banales JM, Rodrigues PM, Affò S, Andersen JB, Aspichueta P, Boulter L, et al. Cholangiocarcinoma 2026: status quo, unmet needs and priorities. *Nat Rev Gastroenterol Hepatol.* 2026;23(1):65-96. doi:10.1038/s41575-025-01153-w.
2. Chen S, Shi Y, Lu Z, Wang M, Cong L, Yang B, et al. Esophageal Carcinosarcoma: Analysis of Clinical Features and Prognosis of 24 Cases and a Literature Review. *Cancer Control.* 2021;28:10732748211004886. doi:10.1177/10732748211004886.
3. Kim SY, Park HS, Chiang AC. Small Cell Lung Cancer: A Review. *JAMA.* 2025;333(21):1906. doi:10.1001/jama.2025.0560.
4. Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, et al. Cancer treatment and survivorship statistics, 2016. *CA A Cancer J Clinicians.* 2016;66(4):271-89. doi:10.3322/caac.21349.
5. Tan AC, Ashley DM, López GY, Malinzak M, Friedman HS, Khasraw M. Management of glioblastoma: State of the art and future directions.

- CA A Cancer J Clinicians. 2020;70(4):299-312. doi:10.3322/caac.21613.
6. Siegel RL, Kratzer TB, Giaquinto AN, Sung H, Jemal A. Cancer statistics, 2025. *CA A Cancer J Clinicians*. 2025;75(1):10-45. doi:10.3322/caac.21871.
 7. Hermann PC, Sancho P, Cañamero M, Martinelli P, Madriles F, Michl P, et al. Nicotine Promotes Initiation and Progression of KRAS-Induced Pancreatic Cancer via Gata6-Dependent Dedifferentiation of Acinar Cells in Mice. *Gastroenterology*. 2014;147(5):1119-1133.e4. doi:10.1053/j.gastro.2014.08.002.
 8. Klein AP. Pancreatic cancer epidemiology: understanding the role of lifestyle and inherited risk factors. *Nat Rev Gastroenterol Hepatol*. 2021;18(7):493-502. doi:10.1038/s41575-021-00457-x.
 9. Rakib A, Mamun MAA, Mandal M, Sinha P, Singh UP. Obesity-cancer axis crosstalk: Molecular insights and therapeutic approaches. *Acta Pharm Sin B*. 2025;15(6):2930-44. doi:10.1016/j.apsb.2025.04.029.
 10. Turner L, Wanasinghe AI, Brunori P, Santosa S. Is Adipose Tissue Inflammation the Culprit of Obesity-Associated Comorbidities? *Obesity Rev*. 2025;26(11):e13956. doi:10.1111/obr.13956.
 11. Shen S, Brown KA, Green AK, Iyengar NM. Obesity and Cancer: A Translational Science Review. *JAMA*. 2026;335(15):1341. doi:10.1001/jama.2026.1114.
 12. Khaledian B, Thibes L, Shimono Y. Adipocyte regulation of cancer stem cells. *Cancer Sci*. 2023;114(11):4134-44. doi:10.1111/cas.15940.
 13. Adler N, Bahcheli AT, Cheng KCL, Al-Zahrani KN, Slobodyanyuk M, Pellegrina D, et al. Mutational processes of tobacco smoking and APOBEC activity generate protein-truncating mutations in cancer genomes. *Sci Adv*. 2023;9(44):eadh3083. doi:10.1126/sciadv.adh3083.
 14. Yang J, Chheda C, Lim A, Hauptschein D, Zayou L, Tang J, et al. HDAC4 Mediates Smoking-Induced Pancreatic Cancer Metastasis. *Pancreas*. 2022;51(2):190-5. doi:10.1097/MPA.0000000000001998.
 15. Zhang AMY, Xia YH, Lin JSH, Chu KH, Wang WCK, Ruiter TJJ, et al. Hyperinsulinemia acts via acinar insulin receptors to initiate pancreatic cancer by increasing digestive enzyme production and inflammation. *Cell Metab*. 2023;35(12):2119-2135.e5. doi:10.1016/j.cmet.2023.10.003.
 16. Xue C, Zhao S, Zhou Y, Chen Z, Liu J, Deng S, et al. Extracellular vesicles from obese visceral adipose promote pancreatic cancer development and resistance to immune checkpoint blockade therapy. *Cell Metab*. 2025;37(12):2381-2401.e9. doi:10.1016/j.cmet.2025.10.015.
 17. Yamazaki H, Streicher SA, Wu L, Fukuhara S, Wagner R, Heni M, et al. Evidence for a causal link between intra-pancreatic fat deposition and pancreatic cancer: A prospective cohort and Mendelian randomization study. *Cell Rep Med*. 2024;5(2):101391. doi:10.1016/j.xcrm.2023.101391.
 18. Larsson SC, Permert J, Håkansson N, Näslund I, Bergkvist L, Wolk A. Overall obesity, abdominal adiposity, diabetes and cigarette smoking in relation to the risk of pancreatic cancer in two Swedish population-based cohorts. *Br J Cancer*. 2005;93(11):1310-5. doi:10.1038/sj.bjc.6602868.
 19. Torres-Durán M, Curiel-García MT, Ruano-Ravina A, Provencio M, Parente-Lamelas I, Hernández-Hernández J, et al. Small-cell lung cancer in never-smokers. *ESMO Open*. 2021;6(2):100059. doi:10.1016/j.esmoop.2021.100059.
 20. Tseng JS, Chiang CJ, Chen KC, Zheng ZR, Yang TY, Lee WC, et al. Association of Smoking With Patient Characteristics and Outcomes in Small Cell Lung Carcinoma, 2011-2018. *JAMA Netw Open*. 2022;5(3):e224830. doi:10.1001/jamanetworkopen.2022.4830.
 21. Pfeifer GP, Denissenko MF, Olivier M, Tretyakova N, Hecht SS, Hainaut P. Tobacco smoke carcinogens, DNA damage and p53 mutations in smoking-associated cancers. *Oncogene*. 2002;21(48):7435-51. doi:10.1038/sj.onc.1205803.
 22. Martínez-García E, Irigoyen M, González-Moreno Ó, Corrales L, Teixeira Á, Salvo E, et al. Repetitive Nicotine Exposure Leads to a More Malignant and Metastasis-Prone Phenotype of SCLC: A Molecular Insight into the Importance of Quitting Smoking during Treatment. *Toxicol Sci*. 2010;116(2):467-76. doi:10.1093/toxsci/kfq138.
 23. Weller J, Schäfer N, Schaub C, Potthoff AL, Steinbach JP, Schlegel U, et al. Prognostic impact of obesity in newly-diagnosed glioblastoma: a secondary analysis of CeTeG/NOA-09 and GLARIUS. *J Neurooncol*. 2022;159(1):95-101. doi:10.1007/s11060-022-04046-z.
 24. Kim YK, Song J. Metabolic imbalance and brain tumors: The interlinking metabolic pathways and therapeutic actions of antidiabetic drugs. *Pharmacol Res*. 2025;215:107719. doi:10.1016/j.phrs.2025.107719.
 25. Kloosterman DJ, Erban J, Boon M, Farber M, Handgraaf SM, Ando-Kuri M, et al. Macrophage-mediated myelin recycling fuels brain

- cancer malignancy. *Cell*. 2024;187(19):5336-5356.e30. doi:10.1016/j.cell.2024.07.030.
26. Silver DJ, Roversi GA, Bithi N, Wang SZ, Troike KM, Neumann CKA, et al. Severe consequences of a high-lipid diet include hydrogen sulfide dysfunction and enhanced aggression in glioblastoma. *J Clin Invest*. 2021;131(17):e138276. doi:10.1172/JCI138276.
 27. Huff S, Tiwari SK, Gonzalez GM, Wang Y, Rana TM. m⁶ A-RNA Demethylase FTO Inhibitors Impair Self-Renewal in Glioblastoma Stem Cells. *ACS Chem Biol*. 2021;16(2):324-33. doi:10.1021/acscchembio.0c00841.
 28. Llovet JM, Willoughby CE, Singal AG, Greten TF, Heikenwälder M, El-Serag HB, et al. Nonalcoholic steatohepatitis-related hepatocellular carcinoma: pathogenesis and treatment. *Nat Rev Gastroenterol Hepatol*. 2023;20(8):487-503. doi:10.1038/s41575-023-00754-7.
 29. Petrick JL, Campbell PT, Koshiol J, Thistle JE, Andreotti G, Beane-Freeman LE, et al. Tobacco, alcohol use and risk of hepatocellular carcinoma and intrahepatic cholangiocarcinoma: The Liver Cancer Pooling Project. *Br J Cancer*. 2018;118(7):1005-12. doi:10.1038/s41416-018-0007-z.
 30. Yugawa K, Itoh S, Iseda N, Kurihara T, Kitamura Y, Toshima T, et al. Obesity is a risk factor for intrahepatic cholangiocarcinoma progression associated with alterations of metabolic activity and immune status. *Sci Rep*. 2021;11(1):5845. doi:10.1038/s41598-021-85186-6.
 31. McGee EE, Jackson SS, Petrick JL, Van Dyke AL, Adami HO, Albanes D, et al. Smoking, Alcohol, and Biliary Tract Cancer Risk: A Pooling Project of 26 Prospective Studies. *JNCI*. 2019;111(12):1263-78. doi:10.1093/jnci/djz103.
 32. Schlottmann F, Dreifuss NH, Patti MG. Obesity and esophageal cancer: GERD, Barrett's esophagus, and molecular carcinogenic pathways. *Expert Rev Gastroenterol Hepatol*. 2020;14(6):425-33. doi:10.1080/17474124.2020.1764348.
 33. Huang Y, You L, Xie W, Ning L, Lang J. Smoking and risk of cholangiocarcinoma: a systematic review and meta-analysis. *Oncotarget*. 2017;8(59):100570-81. doi:10.18632/oncotarget.20141.
 34. Albini A, La Vecchia C, Magnoni F, Garrone O, Morelli D, Janssens JPh, et al. Physical activity and exercise health benefits: cancer prevention, interception, and survival. *Eur J Cancer Prev*. 2025;34(1):24-39. doi:10.1097/CEJ.0000000000000898.
 35. Xie L, Wang F. Physical activity and glioblastoma: a paradigm shift in neuro-oncology therapy. *Front Oncol*. 2025;15:1638060. doi:10.3389/fonc.2025.1638060.
 36. Stoffel EM, Brand RE, Goggins M. Pancreatic Cancer: Changing Epidemiology and New Approaches to Risk Assessment, Early Detection, and Prevention. *Gastroenterology*. 2023;164(5):752-65. doi:10.1053/j.gastro.2023.02.012.
 37. Rudin CM, Brambilla E, Faivre-Finn C, Sage J. Small-cell lung cancer. *Nat Rev Dis Primers*. 2021;7(1):3. doi:10.1038/s41572-020-00235-0.
 38. Weller M, Van Den Bent M, Preusser M, Le Rhun E, Tonn JC, Minniti G, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. *Nat Rev Clin Oncol*. 2021;18(3):170-86. doi:10.1038/s41571-020-00447-z.
 39. Banales JM, Cardinale V, Carpino G, Marzioni M, Andersen JB, Invernizzi P, et al. Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). *Nat Rev Gastroenterol Hepatol*. 2016;13(5):261-80. doi:10.1038/nrgastro.2016.51.
 40. Singal AG, Kanwal F, Llovet JM. Global trends in hepatocellular carcinoma epidemiology: implications for screening, prevention and therapy. *Nat Rev Clin Oncol*. 2023;20(12):864-84. doi:10.1038/s41571-023-00825-3.
 41. Schweigert M, Dubecz A, Stein HJ. Oesophageal cancer - an overview. *Nat Rev Gastroenterol Hepatol*. 2013;10(4):230-44. doi:10.1038/nrgastro.2012.236.
 42. De Falco A, Grisolia P, Giuffrida R, Iannarone C, Graziano C, Scrima M, et al. Knowledge-informed multimodal cfDNA analysis improves sensitivity and generalization in cancer detection. *Cancer Biol*. 2025;2025.10.20.683167. doi:10.1101/2025.10.20.683167.
 43. Martinelli C, Ercoli A, Vizzielli G, Burk SR, Cuomo M, Satasiya V, et al. Liquid biopsy in gynecological cancers: a translational framework from molecular insights to precision oncology and clinical practice. *J Exp Clin Cancer Res*. 2025;44(1):140. doi:10.1186/s13046-025-03371-1.
 44. Testa U, Pelosi E, Castelli G. Cholangiocarcinoma: Molecular Abnormalities and Cells of Origin. *Technol Cancer Res Treat*. 2023;22:15330338221128689. doi:10.1177/15330338221128689.
 45. Varghese AM, Perry MA, Chou JF, Nandakumar S, Muldoon D, Erakky A, et al. Clinicogenomic landscape of pancreatic adenocarcinoma identifies

- KRAS mutant dosage as prognostic of overall survival. *Nat Med*. 2025;31(2):466-77. doi:10.1038/s41591-024-03362-3.
46. Fang X, Cai Y, Zhao Z, Yang S, Li Z, Peng X, et al. Mechanisms of HRAS regulation of liver hepatocellular carcinoma for prognosis prediction. *BMC Cancer*. 2025;25(1):797. doi:10.1186/s12885-025-14131-x.
 47. Dhanasekaran R, Hansen AS, Park J, Lemaitre L, Lai I, Adeniji N, et al. MYC Overexpression Drives Immune Evasion in Hepatocellular Carcinoma That Is Reversible through Restoration of Proinflammatory Macrophages. *Cancer Research*. 2023;83(4):626-40. doi:10.1158/0008-5472.CAN-22-0232.
 48. Ireland AS, Micinski AM, Kastner DW, Guo B, Wait SJ, Spainhower KB, et al. MYC Drives Temporal Evolution of Small Cell Lung Cancer Subtypes by Reprogramming Neuroendocrine Fate. *Cancer Cell*. 2020;38(1):60-78.e12. doi:10.1016/j.ccell.2020.05.001.
 49. Lian Y, Niu X, Cai H, Yang X, Ma H, Ma S, et al. Clinicopathological significance of c-MYC in esophageal squamous cell carcinoma. *Tumour Biol*. 2017;39(7):101042831771580. doi:10.1177/1010428317715804.
 50. Sodir NM, Kortlever RM, Barthet VJA, Campos T, Pellegrinet L, Kupczak S, et al. MYC Instructs and Maintains Pancreatic Adenocarcinoma Phenotype. *Cancer Discov*. 2020;10(4):588-607. doi:10.1158/2159-8290.CD-19-0435.
 51. Tateishi K, Iafrate AJ, Ho Q, Curry WT, Batchelor TT, Flaherty KT, et al. Myc-Driven Glycolysis Is a Therapeutic Target in Glioblastoma. *Clin Cancer Res*. 2016;22(17):4452-65. doi:10.1158/1078-0432.CCR-15-2274.
 52. Batheja N, Suriawinata A, Saxena R, Ionescu G, Schwartz M, Thung SN. Expression of p53 and PCNA in Cholangiocarcinoma and Primary Sclerosing Cholangitis. *Modern Pathol*. 2000;13(12):1265-8. doi:10.1038/modpathol.3880231.
 53. Chen T, Ashwood LM, Kondrashova O, Strasser A, Kelly G, Sutherland KD. Breathing new insights into the role of mutant p53 in lung cancer. *Oncogene*. 2025;44(3):115-29. doi:10.1038/s41388-024-03219-6.
 54. Cho SY, Park C, Na D, Han JY, Lee J, Park OK, et al. High prevalence of TP53 mutations is associated with poor survival and an EMT signature in gliosarcoma patients. *Exp Mol Med*. 2017;49(4):e317-e317. doi:10.1038/emm.2017.9.
 55. Maddalena M, Mallel G, Nataraj NB, Shreiber-Shaked M, Hassin O, Mukherjee S, et al. TP53 missense mutations in PDAC are associated with enhanced fibrosis and an immunosuppressive microenvironment. *Proc Natl Acad Sci USA*. 2021;118(23):e2025631118. doi:10.1073/pnas.2025631118.
 56. Murai K, Dentro S, Ong SH, Sood R, Fernandez-Antoran D, Herms A, et al. p53 mutation in normal esophagus promotes multiple stages of carcinogenesis but is constrained by clonal competition. *Nat Commun*. 2022;13(1):6206. doi:10.1038/s41467-022-33945-y.
 57. Villanueva A, Hoshida Y. Depicting the role of TP53 in hepatocellular carcinoma progression. *J Hepatol*. 2011;55(3):724-5. doi:10.1016/j.jhep.2011.03.018.
 58. Farshidfar F, Zheng S, Gingras MC, Newton Y, Shih J, Robertson AG, et al. Integrative Genomic Analysis of Cholangiocarcinoma Identifies Distinct IDH-Mutant Molecular Profiles. *Cell Rep*. 2017;19(13):2878-80. doi:10.1016/j.celrep.2017.06.008.
 59. Ganguli P, Basanta CC, Acha-Sagredo A, Misetic H, Armero M, Mendez A, et al. Context-dependent effects of CDKN2A and other 9p21 gene losses during the evolution of esophageal cancer. *Nat Cancer*. 2025;6(1):158-74. doi:10.1038/s43018-024-00876-0.
 60. He S, Zhang Y, Xu J, Liang X. Systematic analysis identifies CDKN2A as a prognostic biomarker for hepatocellular carcinoma. *Discov Onc*. 2025;16(1):1657. doi:10.1007/s12672-025-03496-x.
 61. McWilliams RR, Wieben ED, Rabe KG, Pedersen KS, Wu Y, Sicotte H, et al. Prevalence of CDKN2A mutations in pancreatic cancer patients: implications for genetic counseling. *Eur J Hum Genet*. 2011;19(4):472-8. doi:10.1038/ejhg.2010.198.
 62. Zhuang D, Han T, Guo D, Kong R, Chen S, Dong Y, et al. Prevalence and characteristics analysis of CDKN2A/B deletion in glioma. *JCO*. 2023;41(16_suppl):e14026-e14026. doi:10.1200/JCO.2023.41.16_suppl.e14026.
 63. Duffy MJ, Tang M, Crown J. MYC as a Target for Cancer Treatment: from Undruggable to Druggable? *Targ Oncol*. 2025;20(5):791-801. doi:10.1007/s11523-025-01169-x.
 64. Parikh K, Banna G, Liu SV, Friedlaender A, Desai A, Subbiah V, et al. Drugging KRAS: current perspectives and state-of-art review. *J Hematol Oncol*. 2022;15(1):152. doi:10.1186/s13045-022-01375-4.

65. Wolpin BM, Park W, Garrido-Laguna I, Spira A, Starodub A, Sommerhalder D, et al. Darax-onrasib in Previously Treated Advanced RAS -Mutated Pancreatic Cancer. *N Engl J Med.* 2026;394(18):1790-802. doi:10.1056/NEJ-Moa2505783.
66. Knudsen ES, Kumarasamy V, Ruiz A, Sivinski J, Chung S, Grant A, et al. Cell cycle plasticity driven by MTOR signaling: integral resistance to CDK4/6 inhibition in patient-derived models of pancreatic cancer. *Oncogene.* 2019;38(18):3355-70. doi:10.1038/s41388-018-0650-0.
67. Zhang M, Zhang L, Hei R, Li X, Cai H, Wu X, et al. CDK inhibitors in cancer therapy, an overview of recent development. *Am J Cancer Res.* 2021;11(5):1913-35.
68. Juric V, Murphy B. Cyclin-dependent kinase inhibitors in brain cancer: current state and future directions. *Cancer Drug Resist.* 2020;3(1):48-62. doi:10.20517/cdr.2019.105.
69. Le Rhun E, Gorlia T, Furtner J, Felsberg J, Jongen J, Maurage CA, et al. Zoliraciclib (TG02) for newly diagnosed glioblastoma in the elderly or for recurrent glioblastoma: The EORTC 1608 STEAM trial. *JCO.* 2023;41(16_suppl):2054-2054. doi:10.1200/JCO.2023.41.16_suppl.2054.
70. Zhao W, Zhang L, Zhang Y, Jiang Z, Lu H, Xie Y, et al. The CDK inhibitor AT7519 inhibits human glioblastoma cell growth by inducing apoptosis, pyroptosis and cell cycle arrest. *Cell Death Dis.* 2023;14(1):11. doi:10.1038/s41419-022-05528-8.
71. Zevenrijn LJ, Looze EJ, Thavaneswaran S, Van Berge Henegouwen JM, Simes RJ, Hoes LR, et al. Limited clinical activity of palbociclib and ribociclib monotherapy in advanced cancers with cyclin D-CDK4 /6 pathway alterations in the Dutch DRUP and Australian MoST trials. *Int J Cancer.* 2023;153(7):1413-22. doi:10.1002/ijc.34649.
72. Supramote O, Prasoporn S, Aroonpruksakul S, Ponvilawan B, Makjaroen J, Suntiparpluacha M, et al. The Acquired Vulnerability Caused by CDK4/6 Inhibition Promotes Drug Synergism Between Oxaliplatin and Palbociclib in Cholangiocarcinoma. *Front Oncol.* 2022;12:877194. doi:10.3389/fonc.2022.877194.
73. Taebprakhon C, Sorin S, Khawkhiaiw K, Phoomak C, Seubwai W, Wongkham S, et al. Pro-Tumorigenic Roles of Cyclin Dependent Kinase 2 and its Associated Cyclins in Cholangiocarcinoma Progression under High Glucose Condition. *Arch Med Res.* 2026;57(3):103383. doi:10.1016/j.arc-med.2026.103383.
74. Karasic TB, O'Hara MH, Teitelbaum UR, Damjanov N, Giantonio BJ, d'Entremont TS, et al. Phase II Trial of Palbociclib in Patients with Advanced Esophageal or Gastric Cancer. *Oncologist.* 2020;25(12):e1864-8. doi:10.1634/theoncologist.2020-0681.
75. da Costa AABA, Chowdhury D, Shapiro GI, D'Andrea AD, Konstantinopoulos PA. Targeting replication stress in cancer therapy. *Nat Rev Drug Discov.* 2023;22(1):38-58. doi:10.1038/s41573-022-00558-5.
76. Engelke CG, Parsels LA, Qian Y, Zhang Q, Karnak D, Robertson JR, et al. Sensitization of Pancreatic Cancer to Chemoradiation by the Chk1 Inhibitor MK8776. *Clin Cancer Res.* 2013;19(16):4412-21. doi:10.1158/1078-0432.CCR-12-3748.
77. Fu Y, Kang X, Li W, Wang Z, Luo W, Yang B, et al. The low-dose CHK1 inhibitor prexasertib triggers VDAC1 dephosphorylation to activate mtDNA-STING signaling and synergize immunotherapy. *Cell Rep.* 2025;44(5):115605. doi:10.1016/j.celrep.2025.115605.
78. Lal S, Zarei M, Chand SN, Dylgieri E, Mambelli-Lisboa NC, Pishvaian MJ, et al. WEE1 inhibition in pancreatic cancer cells is dependent on DNA repair status in a context dependent manner. *Sci Rep.* 2016;6(1):33323. doi:10.1038/srep33323.
79. Morimoto Y, Takada K, Nakano A, Takeuchi O, Watanabe K, Hirohara M, et al. Combination of S-1 and the oral ATR inhibitor ceralasertib is effective against pancreatic cancer cells. *Cancer Chemother Pharmacol.* 2024;94(6):763-74. doi:10.1007/s00280-024-04716-x.
80. Huffman BM, Feng H, Parmar K, Wang J, Kapner KS, Kochupurakkal B, et al. A Phase I Expansion Cohort Study Evaluating the Safety and Efficacy of the CHK1 Inhibitor LY2880070 with Low-dose Gemcitabine in Patients with Metastatic Pancreatic Adenocarcinoma. *Clin Cancer Res.* 2023;29(24):5047-56. doi:10.1158/1078-0432.CCR-23-2005.
81. Italiano A, Infante JR, Shapiro GI, Moore KN, LoRusso PM, Hamilton E, et al. Phase I study of the checkpoint kinase 1 inhibitor GDC-0575 in combination with gemcitabine in patients with refractory solid tumors. *Annals Oncol.* 2018;29(5):1304-11. doi:10.1093/annonc/mdy076.
82. Thomas A, Redon CE, Sciuto L, Padiernos E, Ji J, Lee MJ, et al. Phase I Study of ATR Inhibitor M6620 in Combination With Topotecan in Patients With Advanced Solid Tumors. *JCO.* 2018;36(16):1594-602. doi:10.1200/JCO.2017.76.6915.

83. Lee S gyeong, Kim J, Jeong E, Myung K. DNA damage response inhibitors in cancer therapy: Mechanisms, clinical development, and combination strategies. *DNA Repair*. 2025;153:103887. doi:10.1016/j.dnarep.2025.103887.
84. Huffman B, Horick NK, Bird A, Gibson C, Brais LK, Graham C, et al. Targeting replication stress in pancreatic cancer: A phase II trial investigating azenosertib in combination with gemcitabine in second-line advanced pancreatic cancer. *JCO*. 2024;42(16_suppl):TPS4211-TPS4211. doi:10.1200/JCO.2024.42.16_suppl.TPS4211.
85. Navarro A, Thomas A, Cheng Y, Chen Y, Reguart N, Yoshida T, et al. A phase II, multicenter, open-label, single-arm study of berzosertib plus topotecan in patients with relapsed platinum-resistant small-cell lung cancer (DDRiver SCLC 250). *ESMO Open*. 2026;11(1):105918. doi:10.1016/j.esmoop.2025.105918.
86. Schultz CW, Zhang Y, Elmeskini R, Zimmermann A, Fu H, Murai Y, et al. ATR inhibition augments the efficacy of lurbinectedin in small-cell lung cancer. *EMBO Mol Med*. 2023;15(8):e17313. doi:10.15252/emmm.202217313.
87. Byers LA, Navarro A, Schaefer E, Johnson M, Özgüroğlu M, Han JY, et al. A Phase II Trial of Prexasertib (LY2606368) in Patients With Extensive-Stage Small-Cell Lung Cancer. *Clin Lung Cancer*. 2021;22(6):531-40. doi:10.1016/j.clcc.2021.04.005.
88. Carruthers RD, Ahmed SU, Ramachandran S, Strathdee K, Kurian KM, Hedley A, et al. Replication Stress Drives Constitutive Activation of the DNA Damage Response and Radioresistance in Glioblastoma Stem-like Cells. *Cancer Res*. 2018;78(17):5060-71. doi:10.1158/0008-5472.CAN-18-0569.
89. Dillon MT, Barker HE, Pedersen M, Hafsi H, Bhide SA, Newbold KL, et al. Radiosensitization by the ATR Inhibitor AZD6738 through Generation of Acentric Micronuclei. *Mol Cancer Ther*. 2017;16(1):25-34. doi:10.1158/1535-7163.MCT-16-0239.
90. Gorainow N, Sander F, Picard D, Frölich MC, Eul K, Etemadi Afshar S, et al. Berzosertib enhances the sensitivity of pediatric diffuse midline glioma H3K27-altered cells to radiotherapy. *Cell Death Dis*. 2026;17(1):331. doi: 10.1038/s41419-026-08567-7.
91. Deguchi S, Ohka F, Shiba Y, Yamaguchi J, Sato A, Shinjo K, et al. Investigator-initiated phase I trial of an oligonucleotide therapeutic targeting long noncoding RNA TUG 1 for recurrent glioblastoma. *BMC Cancer*. 2025;25(1):251. doi:10.1186/s12885-025-13623-0.
92. Sheng H, Huang Y, Xiao Y, Zhu Z, Shen M, Zhou P, et al. ATR inhibitor AZD6738 enhances the antitumor activity of radiotherapy and immune checkpoint inhibitors by potentiating the tumor immune microenvironment in hepatocellular carcinoma. *J Immunother Cancer*. 2020;8(1):e000340. doi:10.1136/jitc-2019-000340.
93. Guo Y, Wang J, Benedict B, Yang C, Van Gemert F, Ma X, et al. Targeting CDC7 potentiates ATR-CHK1 signaling inhibition through induction of DNA replication stress in liver cancer. *Genome Med*. 2021;13(1):166. doi:10.1186/s13073-021-00981-0.
94. Meng X, Gao JZ, Gomendoza SMT, Li JW, Yang S. Recent Advances of WEE1 Inhibitors and Statins in Cancers With p53 Mutations. *Front Med*. 2021;8:737951. doi:10.3389/fmed.2021.737951.
95. Kim R, Kwon M, An M, Kim ST, Smith SA, Loembé AB, et al. Phase II study of ceralasertib (AZD6738) in combination with durvalumab in patients with advanced/metastatic melanoma who have failed prior anti-PD-1 therapy. *Annals Oncol*. 2022;33(2):193-203. doi:10.1016/j.annonc.2021.10.009.
96. Kim ST, Smith SA, Mortimer P, Loembé AB, Cho H, Kim KM, et al. Phase I Study of Ceralasertib (AZD6738), a Novel DNA Damage Repair Agent, in Combination with Weekly Paclitaxel in Refractory Cancer. *Clin Cancer Res*. 2021;27(17):4700-9. doi:10.1158/1078-0432.CCR-21-0251.
97. Kong A, Mehanna H. WEE1 Inhibitor: Clinical Development. *Curr Oncol Rep*. 2021;23(9):107. doi:10.1007/s11912-021-01098-8.
98. Ma J, Liu W, Li J, Kim D, Kim S, Levy A, et al. Azenosertib Is a Potent and Selective WEE1 Kinase Inhibitor with Broad Antitumor Activity Across a Range of Solid Tumors. *Mol Cancer Ther*. 2025;24(8):1171-85. doi:10.1158/1535-7163.MCT-24-1194.
99. Ngoi NYL, Pham MM, Tan DSP, Yap TA. Targeting the replication stress response through synthetic lethal strategies in cancer medicine. *Trends in Cancer*. 2021;7(10):930-57. doi:10.1016/j.tre-can.2021.06.002.
100. Sahgal P, Patil DT, Sztupinszki ZM, Tisza V, Spisak S, Huffman B, et al. Replicative stress in gastroesophageal adenocarcinoma is associated with chromosomal instability and sensitivity to DNA damage response inhibitors. *Cancer Biol*; 2023. doi:10.1101/2023.03.27.534412.

101. Leszczynska KB, Dobrynin G, Leslie RE, Ient J, Boumelha AJ, Senra JM, et al. Preclinical testing of an ATR inhibitor demonstrates improved response to standard therapies for esophageal cancer. *Radiother Oncol.* 2016;121(2):232-8. doi:10.1016/j.radonc.2016.10.023.
102. Hardaker EL, Sanseviero E, Karmokar A, Taylor D, Milo M, Michaloglou C, et al. The ATR inhibitor ceralasertib potentiates cancer checkpoint immunotherapy by regulating the tumor microenvironment. *Nat Commun.* 2024;15(1):1700. doi:10.1038/s41467-024-45996-4.
103. Kuboki Y, Matsubara N, Nakajima H, Fujisawa T, Koyama T, Sato J, et al. Ceralasertib, an ATR kinase inhibitor, as monotherapy in Japanese patients with advanced solid malignancies: Results from a phase 1 study. *Invest New Drugs.* 2025;43(6):1235-46. doi:10.1007/s10637-025-01592-x.
104. Ju Y, Xu D, Liao M miao, Sun Y, Bao W dai, Yao F, et al. Barriers and opportunities in pancreatic cancer immunotherapy. *NPJ Precis Onc.* 2024;8(1):199. doi:10.1038/s41698-024-00681-z.
105. Singh G, Kutcher D, Lally R, Rai V. Targeting Neoantigens in Pancreatic Ductal Adenocarcinoma. *Cancers.* 2024;16(11):2101. doi:10.3390/cancers16112101.
106. Quintanilha JCF, Storandt MH, Graf RP, Li G, Keller R, Lin DI, et al. Tumor Mutational Burden in Real-World Patients With Pancreatic Cancer: Genomic Alterations and Predictive Value for Immune Checkpoint Inhibitor Effectiveness. *JCO Precision Oncology.* 2023;(7):e2300092. doi:10.1200/PO.23.00092.
107. Sohrabi H, Ghavam SM, Azadnajafabad S, Rezaei N. Efficacy and Safety of Combination Therapy With Immune Checkpoint Inhibitors and Chemotherapy With Gemcitabine and Nab-Paclitaxel in Pancreatic Cancer: A Systematic Review. *Cancer Med.* 2026;15(2):e71637. doi:10.1002/cam4.71637.
108. Park W, O'Connor C, Chou J, Hilmi M, Tarcan Z, Schwartz C, et al. Pembrolizumab and Olaparib (POLAR) Maintenance Therapy in Metastatic Pancreatic Cancer With or Without Homologous Repair Deficiency: A Biomarker Selected Phase II Trial. *Res Sq [Preprint].* 2025;rs.3.rs-7334701. doi: 10.21203/rs.3.rs-7334701/v1.
109. Mahadevan NR, Knelson EH, Wolff JO, Vajdi A, Saigí M, Campisi M, et al. Intrinsic Immunogenicity of Small Cell Lung Carcinoma Revealed by Its Cellular Plasticity. *Cancer Discov.* 2021;11(8):1952-69. doi:10.1158/2159-8290.CD-20-0913.
110. Horn L, Mansfield AS, Szczesna A, Havel L, Krzakowski M, Hochmair MJ, et al. First-Line Atezolizumab plus Chemotherapy in Extensive-Stage Small-Cell Lung Cancer. *N Engl J Med.* 2018;379(23):2220-9. doi:10.1056/NEJMoa1809064.
111. Krebs MG, Delord JP, Jeffry Evans TR, De Jonge M, Kim SW, Meurer M, et al. Olaparib and durvalumab in patients with relapsed small cell lung cancer (MEDIOLA): An open-label, multicenter, phase 1/2, basket study. *Lung Cancer.* 2023;180:107216. doi:10.1016/j.lungcan.2023.107216.
112. Sen T, Rodriguez BL, Chen L, Corte CMD, Morikawa N, Fujimoto J, et al. Targeting DNA Damage Response Promotes Antitumor Immunity through STING-Mediated T-cell Activation in Small Cell Lung Cancer. *Cancer Discov.* 2019;9(5):646-61. doi:10.1158/2159-8290.CD-18-1020.
113. Reardon DA, Brandes AA, Omuro A, Mulholland P, Lim M, Wick A, et al. Effect of Nivolumab vs Bevacizumab in Patients With Recurrent Glioblastoma: The CheckMate 143 Phase 3 Randomized Clinical Trial. *JAMA Oncol.* 2020;6(7):1003. doi:10.1001/jamaoncol.2020.1024.
114. Lim M, Weller M, Idbaih A, Steinbach J, Finocchiaro G, Raval RR, et al. Phase III trial of chemoradiotherapy with temozolomide plus nivolumab or placebo for newly diagnosed glioblastoma with methylated MGMT promoter. *Neuro Oncol.* 2022;24(11):1935-49. doi:10.1093/neuonc/noac116.
115. Omuro A, Brandes AA, Carpentier AF, Idbaih A, Reardon DA, Cloughesy T, et al. Radiotherapy combined with nivolumab or temozolomide for newly diagnosed glioblastoma with unmethylated MGMT promoter: An international randomized phase III trial. *Neuro Oncol.* 2023;25(1):123-34. doi:10.1093/neuonc/noac099.
116. Tomaszewski W, Sanchez-Perez L, Gajewski TF, Sampson JH. Brain Tumor Microenvironment and Host State: Implications for Immunotherapy. *Clin Cancer Res.* 2019;25(14):4202-10. doi:10.1158/1078-0432.CCR-18-1627.
117. Lozinski M, Bowden NA, Graves MC, Fay M, Day BW, Stringer BW, et al. ATR inhibition using gartisertib enhances cell death and synergises with temozolomide and radiation in patient-derived glioblastoma cell lines. *Oncotarget.* 2024;15(1):1-18. doi:10.18632/oncotarget.28551.

118. Vendetti FP, Karukonda P, Clump DA, Teo T, Lalonde R, Nugent K, et al. ATR kinase inhibitor AZD6738 potentiates CD8+ T cell-dependent antitumor activity following radiation. *J Clin Invest.* 2018;128(9):3926-40. doi:10.1172/JCI96519.
119. Lecoultre M, Woo PYM, Leung G, Chow SWJ, Ho J, Hong JH, et al. The TAC-GReD trial: The combination of talazoparib and carboplatin in DNA damage repair deficient recurrent high-grade glioma. *JCO.* 2024;42(16_suppl):2047-2047. doi:10.1200/JCO.2024.42.16_suppl.2047.
120. Seyhan D, Allaire M, Fu Y, Conti F, Wang XW, Gao B, et al. Immune microenvironment in hepatocellular carcinoma: from pathogenesis to immunotherapy. *Cell Mol Immunol.* 2025;22(10):1132-58. doi:10.1038/s41423-025-01308-4.
121. Wong M, Kim JT, Cox B, Larson BK, Kim S, Waters KM, et al. Evaluation of tumor mutational burden in small early hepatocellular carcinoma and progressed hepatocellular carcinoma. *Hepat Oncol.* 2021;8(4):HEP39. doi:10.2217/hep-2020-0034.
122. Zanuso V, Pirozzi A, Balsano R, Pressiani T, Rimassa L. Safety and Efficacy of Atezolizumab and Bevacizumab Combination as a First Line Treatment of Advanced Hepatocellular Carcinoma. *J Hepatocell Carcinoma.* 2023;10:1689-708. doi:10.2147/JHC.S347932.
123. Ma J, Tang D, Cui G, Zhang X, Wang X, Li Y, et al. The molecular characteristics of DNA damage and repair related to P53 mutation for predicting the recurrence and immunotherapy response in hepatocellular carcinoma. *Sci Rep.* 2025;15(1):14939. doi:10.1038/s41598-025-99853-5.
124. Bauer TM, Moore KN, Rader JS, Simpkins F, Mita AC, Beck JT, et al. A Phase Ib Study Assessing the Safety, Tolerability, and Efficacy of the First-in-Class Wee1 Inhibitor Adavosertib (AZD1775) as Monotherapy in Patients with Advanced Solid Tumors. *Targ Oncol.* 2023;18(4):517-30. doi:10.1007/s11523-023-00965-7.
125. Fu S, Yao S, Yuan Y, Previs RA, Elias AD, Carvajal RD, et al. Multicenter Phase II Trial of the WEE1 Inhibitor Adavosertib in Refractory Solid Tumors Harboring CCNE1 Amplification. *JCO.* 2023;41(9):1725-34. doi:10.1200/JCO.22.00830.
126. Zhang G, Li J, Li G, Zhang J, Yang Z, Yang L, et al. Strategies for treating the cold tumors of cholangiocarcinoma: core concepts and future directions. *Clin Exp Med.* 2024;24(1):193. doi:10.1007/s10238-024-01460-7.
127. Oh DY, He AR, Qin S, Chen LT, Okusaka T, Kim JW, et al. Durvalumab plus chemotherapy in advanced biliary tract cancer: 3-year overall survival update from the phase III TOPAZ-1 study. *J Hepatol.* 2025;83(5):1092-101. doi:10.1016/j.jhep.2025.05.003.
128. Sahai V, Griffith KA, Beg MS, Shaib WL, Mahalingam D, Zhen DB, et al. A randomized phase 2 trial of nivolumab, gemcitabine, and cisplatin or nivolumab and ipilimumab in previously untreated advanced biliary cancer: BIL-T-01. *Cancer.* 2022;128(19):3523-30. doi:10.1002/cnrc.34394.
129. Siwek M, Chmiel P, Grabowska H, Grąt M, Kraj L. Second-Line Treatment in cholangiocarcinoma - Current State of the Art and Future Perspectives. *Curr Treat Options in Oncol.* 2025;26(12):1061-77. doi:10.1007/s11864-025-01365-x.
130. Wang BC, Kuang BH, Lin GH, Fu C. Durvalumab and pembrolizumab in advanced biliary tract cancer: a reconstructed patient-level mimic head-to-head comparative analysis. *Front Immunol.* 2024;15:1497415. doi:10.3389/fimmu.2024.1497415.
131. Yap TA, Fontana E, Lee EK, Spigel DR, Højgaard M, Lheureux S, et al. Camonsertib in DNA damage response-deficient advanced solid tumors: phase 1 trial results. *Nat Med.* 2023;29(6):1400-11. doi:10.1038/s41591-023-02399-0.
132. Lin EW, Karakasheva TA, Hicks PD, Bass AJ, Rustgi AK. The tumor microenvironment in esophageal cancer. *Oncogene.* 2016;35(41):5337-49. doi:10.1038/onc.2016.34.
133. Kato K, Cho BC, Takahashi M, Okada M, Lin CY, Chin K, et al. Nivolumab versus chemotherapy in patients with advanced oesophageal squamous cell carcinoma refractory or intolerant to previous chemotherapy (ATTRACTION-3): a multicentre, randomised, open-label, phase 3 trial. *Lancet Oncol.* 2019;20(11):1506-17. doi:10.1016/S1470-2045(19)30626-6.
134. Kojima T, Shah MA, Muro K, Francois E, Adenis A, Hsu CH, et al. Randomized Phase III KEY-NOTE-181 Study of Pembrolizumab Versus Chemotherapy in Advanced Esophageal Cancer. *JCO.* 2020;38(35):4138-48. doi:10.1200/JCO.20.01888.
135. Li S, Zhang Y, Tong H, Sun H, Liao H, Li Q, et al. Metabolic regulation of immunity in the tumor microenvironment. *Cell Rep.* 2025;44(11):116463. doi:10.1016/j.celrep.2025.116463.
136. Li X, Zhou J, Wang X, Li C, Ma Z, Wan Q, et al. Pancreatic cancer and fibrosis: Targeting met-

- abolic reprogramming and crosstalk of cancer-associated fibroblasts in the tumor microenvironment. *Front Immunol.* 2023;14:1152312. doi:10.3389/fimmu.2023.1152312.
137. Papavassiliou KA, Papavassiliou AG. Hunger for fat: Metabolic crosstalk with lipid-rich CAFs fuels pancreatic cancer. *Cell Metabolism.* 2024;36(6):1172-4. doi:10.1016/j.cmet.2024.05.007.
 138. Shan T, Chen S, Chen X, Lin WR, Li W, Ma J, et al. Cancer-associated fibroblasts enhance pancreatic cancer cell invasion by remodeling the metabolic conversion mechanism. *Oncol Rep.* 2017;37(4):1971-9. doi:10.3892/or.2017.5479.
 139. Manji GA, Labadie BW, Jamison JK, Wu L, Wang S, Pellicciotta I, et al. AIRPanc: A phase 2, open-label, multicenter, randomized study evaluating neoadjuvant therapy targeting the adenosine immunosuppressive pathway in combination with immune checkpoint blockade and radiation therapy in patients with advanced pancreatic ductal adenocarcinoma who are candidates for surgical resection. *JCO.* 2024;42(3_suppl):TPS714-TPS714. doi:10.1200/JCO.2024.42.3_suppl.TPS714.
 140. Suarez-Carmona M, Williams A, Schreiber J, Hohmann N, Pruefer U, Krauss J, et al. Combined inhibition of CXCL12 and PD-1 in MSS colorectal and pancreatic cancer: modulation of the microenvironment and clinical effects. *J Immunother Cancer.* 2021;9(10):e002505. doi:10.1136/jitc-2021-002505.
 141. Saung MT, Zheng L. Adding combination immunotherapy consisting of cancer vaccine, anti-PD-1 and anti-CSF1R antibodies to gemcitabine improves anti-tumor efficacy in murine model of pancreatic ductal adenocarcinoma. *Ann Pancreat Cancer.* 2019;2:21-21. doi:10.21037/apc.2019.11.01.
 142. Su H, Yang F, Fu R, Li X, French R, Mose E, et al. Cancer cells escape autophagy inhibition via NRF2-induced macropinocytosis. *Cancer Cell.* 2021;39(5):678-693.e11. doi:10.1016/j.ccell.2021.02.016.
 143. Zeh HJ, Bahary N, Boone BA, Singhi AD, Miller-Ocuin JL, Normolle DP, et al. A Randomized Phase II Preoperative Study of Autophagy Inhibition with High-Dose Hydroxychloroquine and Gemcitabine/Nab-Paclitaxel in Pancreatic Cancer Patients. *Clin Cancer Res.* 2020;26(13):3126-34. doi:10.1158/1078-0432.CCR-19-4042.
 144. Brambillasca S, Cera MR, Andronache A, Dey SK, Fagá G, Fancelli D, et al. Novel selective inhibitors of macropinocytosis-dependent growth in pancreatic ductal carcinoma. *Biomed Pharmacother.* 2024;177:116991. doi:10.1016/j.biopha.2024.116991.
 145. He C, Mehla K. Abstract C050: Vitamin B6 restriction impairs pancreatic cancer progression. *Cancer Res.* 2024;84(17_Supplement_2):C050-C050. doi:10.1158/1538-7445.PANCREATIC24-C050.
 146. Lu Y, Li H, Zhao P, Wang X, Shao W, Liu Y, et al. Crosstalk between cancer-associated fibroblasts and non-neuroendocrine tumor cells in small cell lung cancer involves in glycolysis and antigen-presenting features. *Mol Med.* 2024;30(1):274. doi:10.1186/s10020-024-01051-y.
 147. Paunovic M, Stojanovic A, Pokimica B, Martacic JD, Cvetkovic Z, Ivanovic N, et al. Metabolic Reprogramming of Phospholipid Fatty Acids as a Signature of Lung Cancer Type. *Cancers.* 2024;16(19):3320. doi:10.3390/cancers16193320.
 148. Mathieson L, Koppensteiner L, Dorward DA, O'Connor RA, Akram AR. Cancer-associated fibroblasts expressing fibroblast activation protein and podoplanin in non-small cell lung cancer predict poor clinical outcome. *Br J Cancer.* 2024;130(11):1758-69. doi:10.1038/s41416-024-02671-1.
 149. Tang L, Ge Y, Li Y, Chen X, Liu K, Dong Z, et al. Phospholipase D: emerging therapeutic targets in signaling, metabolism, and immune-oncology. *Cell Commun Signal.* 2025;24(1):42. doi:10.1186/s12964-025-02596-z.
 150. Galbo PM, Madsen AT, Liu Y, Peng M, Wei Y, Ciesielski MJ, et al. Functional Contribution and Clinical Implication of Cancer-Associated Fibroblasts in Glioblastoma. *Clin Cancer Res.* 2024;30(4):865-76. doi:10.1158/1078-0432.CCR-23-0493.
 151. Gross MI, Demo SD, Dennison JB, Chen L, Chernov-Rogan T, Goyal B, et al. Antitumor Activity of the Glutaminase Inhibitor CB-839 in Triple-Negative Breast Cancer. *Mol Cancer Ther.* 2014;13(4):890-901. doi:10.1158/1535-7163.MCT-13-0870.
 152. Koch K, Hartmann R, Tsiampali J, Uhlmann C, Nickel AC, He X, et al. A comparative pharmacometabolomic study of glutaminase inhibitors in glioma stem-like cells confirms biological effectiveness but reveals differences in target-specificity. *Cell Death Discov.* 2020;6(1):20. doi:10.1038/s41420-020-0258-3.
 153. Behera MM, Purkait S, Ghosh A, Sable MN, Sahu RN, Chhabra G. The Monocarboxylate Transporters MCT1 and MCT4 Are Highly Expressed in Gli-

- blastoma and Crucially Implicated in the Pathobiology. *Neuropathology*. 2025;45(4):e70006. doi:10.1111/neup.70006.
154. Yamazaki M, Ishimoto T. Targeting Cancer-Associated Fibroblasts: Eliminate or Reprogram? *Cancer Sci*. 2025;116(3):613-21. doi:10.1111/cas.16443.
 155. Jia W, Liang S, Cheng B, Ling C. The Role of Cancer-Associated Fibroblasts in Hepatocellular Carcinoma and the Value of Traditional Chinese Medicine Treatment. *Front Oncol*. 2021;11:763519. doi:10.3389/fonc.2021.763519.
 156. Shi X, Zhu W, Zhang J, Fan C, Zhang J. Cancer-associated fibroblasts in hepatocellular carcinoma: origins, heterogeneity, and therapeutic implications. *Front Immunol*. 2025;16:1620075. doi:10.3389/fimmu.2025.1620075.
 157. Wang Z, Sun X, Li Z, Yu H, Li W, Xu Y. Metabolic reprogramming in esophageal squamous cell carcinoma. *Front Pharmacol*. 2024;15:1423629. doi:10.3389/fphar.2024.1423629.
 158. Zhang C, Cheng W, Yang T, Fang H, Zhang R. Lactate secreted by esophageal cancer cells induces M2 macrophage polarization via the AKT / ERK pathway. *Thorac Cancer*. 2023;14(22):2139-48. doi:10.1111/1759-7714.14998.
 159. Sharpe BP, Hayden A, Manousopoulou A, Cowie A, Walker RC, Harrington J, et al. Phosphodiesterase type 5 inhibitors enhance chemotherapy in preclinical models of esophageal adenocarcinoma by targeting cancer-associated fibroblasts. *Cell Rep Med*. 2022;3(6):100541. doi:10.1016/j.xcrm.2022.100541.
 160. Wang H, Liu F, Wu X, Zhu G, Tang Z, Qu W, et al. Cancer-associated fibroblasts contributed to hepatocellular carcinoma recurrence and metastasis via CD36-mediated fatty-acid metabolic reprogramming. *Exp Cell Res*. 2024;435(2):113947. doi:10.1016/j.yexcr.2024.113947.
 161. Zhou Y, Niu C, Li Y, Gao B, Zheng J, Guo X, et al. Fatty acid synthase expression and esophageal cancer. *Mol Biol Rep*. 2012;39(10):9733-9. doi:10.1007/s11033-012-1838-y.
 162. Brummer C, Pukrop T, Wiskemann J, Bruss C, Ugele I, Renner K. Can Exercise Enhance the Efficacy of Checkpoint Inhibition by Modulating Anti-Tumor Immunity? *Cancers (Basel)*. 2023;15(18):4668. doi:10.3390/cancers15184668.
 163. Mancini A, Gentile MT, Pentimalli F, Cortellino S, Grieco M, Giordano A. Multiple aspects of matrix stiffness in cancer progression. *Front Oncol*. 2024;14:1406644. doi:10.3389/fonc.2024.1406644.
 164. Van Cutsem E, Tempero MA, Sigal D, Oh DY, Fazio N, Macarulla T, et al. Randomized Phase III Trial of Pegvorhialuronidase Alfa With Nab-Paclitaxel Plus Gemcitabine for Patients With Hyaluronan-High Metastatic Pancreatic Adenocarcinoma. *JCO*. 2020;38(27):3185-94. doi:10.1200/JCO.20.00590.
 165. Picozzi VJ, Varghese AM, Oberstein PE, Hidalgo M, Wolpin BM, Lim KH, et al. Pamrevlumab plus nab-paclitaxel/gemcitabine (Pam + GA) as first- and second-line therapy in metastatic pancreatic cancer (mPDAC): Results from Precision Promise (PrP) Bayesian platform trial. *JCO*. 2025;43(4_suppl):673-673. doi:10.1200/JCO.2025.43.4_suppl.673.
 166. Chitty JL, Yam M, Perryman L, Parker AL, Skhinas JN, Setargew YFI, et al. A first-in-class pan-lysyl oxidase inhibitor impairs stromal remodeling and enhances gemcitabine response and survival in pancreatic cancer. *Nat Cancer*. 2023;4(9):1326-44. doi:10.1038/s43018-023-00614-y.
 167. Jung DY, Go A, Chun Y, Lee BS, Kweon S, Han H, et al. Abstract A127: A novel Integrin beta-6 targeted antibody-drug conjugate featuring a topoisomerase I inhibitor, PBX-004 with potent antitumor activity in multiple preclinical solid tumor models. *Mol Cancer Ther*. 2025;24(10_Supplement):A127-A127. doi:10.1158/1535-7163.TARG-25-A127.
 168. Reck M, Lu S, O'Byrne KJ, Barrios C, Pavlov D, Tay F, et al. Frontline sigvotatug vedotin plus pembrolizumab vs pembrolizumab for non-small cell lung cancer with PD-L1 tumor proportion score $\geq 50\%$: phase III study design. *Future Oncol*. 2025;21(30):3891-901. doi:10.1080/14796694.2025.2596228.

REVIEW

FERTILITY PRESERVATION IN YOUNG WOMEN WITH BREAST CANCER: UPTAKE, DETERMINANTS OF ACCESS, AND LONG-TERM REPRODUCTIVE OUTCOMES: A REVIEW

Annamaria Pezzarossi ¹, Isabella Bisceglia ^{1,*}, Francesco Marinelli ¹, Maria Barbara Braghiroli ¹, Filippo Giovanardi ², Fortunato Morabito ³, Lucia Mangone ¹

¹ Epidemiology Unit, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy

² Medical Oncology Unit, Azienda USL-IRCCS di Reggio Emilia, Reggio Emilia, Italy

³ "Vittorio Lodini per la Ricerca in Chirurgia" di Reggio Emilia Association, Reggio Emilia, Italy

* Correspondence to: ✉ isabella.bisceglia@ausl.re.it; <https://orcid.org/0000-0002-9225-781X>

ABSTRACT: This narrative review addresses how evidence on treatment-related fertility risk, fertility-preservation strategies, inequities in access, and post-treatment reproductive outcomes can be integrated into individualized and equitable care for young women with breast cancer. Its objective is not to restate existing guideline recommendations, but to identify where their implementation fails in routine practice and to synthesize clinically actionable strategies across the care continuum, from diagnosis to survivorship. Fertility preservation (FP) has emerged as a critical component of comprehensive care for young women diagnosed with breast cancer. Advances in reproductive technologies, including oocyte and embryo cryopreservation, alongside growing evidence on ovarian protection strategies and prospective trials such as the POSITIVE (Pregnancy Outcome and Safety of Interrupting Therapy for Women with Endocrine-Responsive Breast Cancer) study, have expanded both counseling and reproductive options for survivors. Observational data and meta-analyses consistently demonstrate that pregnancy after breast cancer is generally safe, including in hormone receptor-positive disease and among BRCA mutation carriers, without increasing recurrence risk when appropriately timed. Nevertheless, substantial disparities persist in FP access, influenced by socioeconomic status, race/ethnicity, insurance coverage, and geographic location. System-level interventions – such as automatic referrals to oncofertility specialists, dedicated FP navigation programs, and mandated or publicly funded coverage – have improved uptake and patient satisfaction. Psychosocial interventions and structured decision aids further reduce decisional conflict and regret, supporting informed reproductive choices. Emerging challenges include understanding the impact of newer oncologic therapies, such as PARP (Poly (ADP-ribose) polymerase) inhibitors, immune checkpoint inhibitors, and antibody-drug conjugates, on fertility potential. By bringing together evidence that is usually dispersed across oncology, reproductive medicine, health-services research, and survivorship studies, this review highlights practical actions for clinicians: early risk-based counselling, prompt referral, multidisciplinary coordination, navigation support, and attention to financial and geographic barriers. Long-term survivorship registries capturing ovarian reserve, FP utilization, pregnancy outcomes, child health, and psychosocial metrics are essential for evaluating real-world effectiveness and equity. Integrating FP into routine breast cancer care requires a dual strategy: implementation of evidence-based clinical pathways and system-level support, combined with research to refine guidance as therapies evolve. This approach has the potential to safeguard reproductive potential, reduce psychosocial burden, and enhance quality of life for young breast cancer survivors.

Doi: 10.48286/aro.2026.129

Impact statement: This review highlights clinically actionable strategies for timely fertility counselling and preservation in young women with breast cancer, addressing treatment-related risks, access disparities, and reproductive outcomes to improve survivorship care.

Key words: *Fertility preservation; breast cancer; oncofertility; reproductive outcomes; health disparities.*

Received: Apr 20, 2026/**Accepted:** Jul 27, 2026

Published: Sept 30, 2026

INTRODUCTION

Breast cancer diagnosed during the reproductive years represents both a clinical and psychosocial challenge. While age cutoffs vary, many studies and guidelines define “young” as ≤ 40 years (some use ≤ 45) because fertility considerations, hormonal milieu, and life plans differ substantially from older patients. In many high-income settings, breast cancer before age 40 accounts for a small but clinically important minority of all cases, generally estimated at approximately 5% (1, 2). In contrast, data from certain low- and middle-income countries report substantially higher proportions – for instance, up to 10-20% of breast cancers in women under 40 (2). This higher proportional burden partly reflects the younger underlying population age structure in many low- and middle-income countries, in addition to differences in incidence patterns, reproductive risk factors, and access to early detection; proportional comparisons should therefore not be interpreted as direct evidence of a uniformly higher age-specific risk. Survivorship improvements make reproductive questions central to care planning; fertility preservation (FP) counseling at diagnosis is now standard-of-care in most oncology guidelines (3, 4). Young patients often face a compressed timeline:

diagnosis → need for rapid treatment initiation → complex fertility decisions that require specialist input.

The cancer treatment itself (chemotherapy, ovarian suppression, certain targeted agents) can reduce ovarian reserve and increase the risk of premature ovarian insufficiency (POI). Beyond biological risk, disparities in awareness, referral, and access lead to unequal uptake of preserving options across socioeconomic strata – an issue of reproductive justice (4, 5). Although international guidelines consistently recommend timely discussion of infertility risk and referral for FP, they necessarily provide broad recommendations and do not fully resolve how treatment-related risk, patient preferences, access barriers, and subsequent reproductive plans should be integrated in routine breast cancer care. The evidence is dispersed across oncology, reproductive medicine, health-services research, and survivorship studies, often using different populations, outcomes, and definitions. Consequently, clinicians may know that FP should be discussed, yet still lack an integrated framework for identifying where implementation fails, which patients are most likely to encounter barriers, and how counsel-

ing should extend beyond gamete cryopreservation to pregnancy planning and long-term reproductive health. This review therefore addresses the following question: how can current evidence on gonadotoxic risk, fertility-preservation strategies, inequities in access, and reproductive outcomes after breast cancer be integrated to support equitable, individualized clinical decision-making in young women? Our objective is to provide a clinically oriented synthesis of the care continuum – from diagnosis and treatment planning to survivorship – and to identify actionable system-level interventions and priority evidence gaps.

METHODS

This article was designed as a narrative review supported by a structured literature search. The aim was to address a broad, clinically integrative question spanning treatment-related gonadotoxicity, fertility-preservation interventions, health-care access, social determinants, pregnancy after breast cancer, and long-term survivorship outcomes. Because these domains include heterogeneous study designs, populations, exposures, outcome definitions, and health-system contexts, a systematic review focused on a single, prespecified outcome was not considered suitable. Rather than estimate a pooled effect, we aimed to interpret how evidence across these domains can inform individualized counselling and equitable clinical pathways.

We searched PubMed/MEDLINE and Embase for English-language articles published up to March 2026 using combinations of the terms “breast neoplasms”, “fertility preservation”, “oncofertility”, “premature ovarian insufficiency”, “pregnancy after breast cancer”, and “socioeconomic factors”. We used the search to identify influential and contemporary evidence, prioritising clinical practice guidelines, prospective cohorts, registry-based studies, clinical trials, systematic reviews, and studies reporting fertility-related outcomes in women diagnosed at ≤ 40 -45 years. Reference lists of key studies and guidelines were also reviewed. This was not a systematic review: we did not conduct duplicate screening, a formal risk-of-bias assessment, PRISMA-based study selection, or quantitative pooling. The resulting evidence was organised into a structured qualitative synthesis around four clinically connected domains: treatment-related fertility risk, available preservation strategies, determinants of access and uptake, and reproductive outcomes during survivorship.

FERTILITY RISK ASSOCIATED WITH BREAST CANCER THERAPY

Advances in systemic therapy have markedly improved survival outcomes for young women with breast cancer, yet many of these same treatments pose a threat to reproductive function. The degree of gonadal toxicity varies widely by drug class, cumulative dose, patient age, and baseline ovarian reserve, but the potential for POI or long-term infertility remains a central survivorship concern.

Chemotherapy and ovarian failure

Contemporary anthracycline-cyclophosphamide regimens, with or without taxanes, are integral to early breast cancer therapy but confer a measurable risk of chemotherapy-induced ovarian dysfunction and POI. Although individual studies vary in methodology and outcome definitions, the collective evidence from prospective trials and meta-analyses confirms that the majority of premenopausal women experience transient ovarian failure during treatment, and that a substantial subset develop persistent dysfunction thereafter. In a large pooled prospective analysis of 696 women aged ≤ 45 years treated with anthracycline/taxane-based chemotherapy, Furlanetto *et al.* reported that 85.1% met criteria for chemotherapy-induced ovarian failure at the end of treatment. Among those with longitudinal follow-up, most subsequently regained premenopausal hormone levels, but a clinically relevant minority had persistent ovarian dysfunction at 24 months (6). These findings underscore the distinction between transient treatment-related amenorrhea and long-term POI. Complementary evidence from biochemical studies underscores the extent of gonadal damage: a systematic review and meta-analysis by Romito *et al.* documented consistent and clinically meaningful declines in anti-Müllerian hormone (AMH) following standard breast cancer chemotherapy in women under 40, reflecting substantial depletion of ovarian reserve (7). In clinical practice, AMH is a widely used biomarker of ovarian reserve and a robust predictor of ovarian response to stimulation for oocyte or embryo cryopreservation, but AMH alone is a limited predictor of natural fertility and time to spontaneous conception. At the population level, a comprehensive meta-analysis including 68 studies and more than 26,000 patients estimated that approximately 64% of women experienced chemotherapy-induced amenorrhea at the reported follow-up time points, with substantial heterogeneity in defini-

tions and timing across studies (8). This figure therefore reflects a high overall burden of treatment-related amenorrhea rather than a precise estimate of permanent POI.

These findings collectively indicate that while most younger women eventually recover ovarian function, anthracycline- and taxane-based regimens carry a clinically relevant long-term risk of ovarian insufficiency, particularly in those approaching the late reproductive years. Consistent data from AMH-based longitudinal analyses further reinforce that the risk of sustained ovarian dysfunction rises sharply with age and cumulative cyclophosphamide exposure. Although women under 35 years often retain some degree of ovarian reserve, they nevertheless experience pronounced AMH declines, signifying subclinical gonadotoxicity and reduced reproductive potential. These observations highlight the importance of integrating structured FP counseling into treatment planning for all premenopausal patients, irrespective of apparent low-risk age or regimen intensity.

Endocrine therapy

In hormone receptor-positive (HR+) breast cancer, adjuvant endocrine therapy remains fundamental, typically extending over 5-10 years. Standard approaches include tamoxifen alone or aromatase inhibitors combined with ovarian function suppression (OFS), depending on menopausal status and recurrence risk. Although these drugs are not directly cytotoxic to ovarian follicles, their prolonged use substantially delays childbearing, creating a unique challenge for young women with reproductive aspirations. The central dilemma is balancing oncologic safety against fertility goals. Long-term endocrine therapy effectively reduces recurrence risk but overlaps with a woman's most fertile years, often compelling difficult decisions regarding pregnancy timing and the risks associated with therapy interruption or deferral (9). The landmark POSITIVE trial (Pregnancy Outcome and Safety of Interrupting Therapy for Women with Endocrine-Responsive Breast Cancer) directly addressed this issue. The study enrolled premenopausal women with HR+ early breast cancer who had completed 18-30 months of adjuvant endocrine therapy and desired pregnancy. Participants temporarily paused treatment for up to two years to attempt conception. The results demonstrated no excess short-term recurrence risk compared with historical controls and favorable pregnancy outcomes among participants who interrupted therapy under close monitoring (10).

Table 1. Fertility-related impact of systemic therapies used in young women with breast cancer.

THERAPY CLASS	MECHANISM AND CLINICAL CONTEXT	EVIDENCE OF FERTILITY IMPACT	MAIN MODIFIERS OF RISK OR CLINICAL CONSIDERATIONS	KEY REFERENCES
Chemotherapy: anthracycline-cyclophosphamide regimens with or without taxanes	Cytotoxic therapy damages growing and resting ovarian follicles and remains a major component of treatment for early breast cancer.	High risk of transient amenorrhea and clinically relevant risk of persistent ovarian dysfunction or premature ovarian insufficiency. In prospective studies, ovarian failure was common at the end of treatment, while a smaller proportion had persistent dysfunction during follow-up. Chemotherapy is also associated with substantial declines in anti-Müllerian hormone, reflecting reduced ovarian reserve.	Older age, especially >40 years; cumulative cyclophosphamide dose; baseline ovarian reserve; and concomitant ovarian suppression. Resumption of menstruation does not necessarily indicate full recovery of fertility potential.	[6-8]
Endocrine therapy: tamoxifen with or without ovarian function suppression; aromatase inhibitors plus ovarian function suppression	Endocrine therapy is not directly cytotoxic to ovarian follicles but may delay pregnancy because treatment is usually prescribed for 5-10 years.	The principal fertility-related effect is postponement of childbearing during the years of highest natural fertility. In selected women with hormone receptor-positive early breast cancer, temporary interruption of endocrine therapy after 18-30 months to attempt pregnancy did not increase short-term recurrence risk.	Duration of planned endocrine therapy; residual recurrence risk; age and ovarian reserve; reproductive plans; and the feasibility of a monitored treatment interruption.	[9-11]
HER2-directed monoclonal antibodies: trastuzumab and pertuzumab	Targeted anti-HER2 therapies are commonly administered with chemotherapy in HER2-positive breast cancer.	HER2-directed monoclonal antibodies alone are not considered directly gonadotoxic, and there is no established evidence that they independently cause premature ovarian insufficiency.	Fertility risk is primarily determined by accompanying chemotherapy, patient age, and baseline ovarian reserve. Pregnancy should be avoided during treatment because of fetal risk.	[13]
Antibody-drug conjugates: trastuzumab emtansine and trastuzumab deruxtecan	HER2-targeted antibodies conjugated to cytotoxic payloads.	Human data on ovarian toxicity are limited. The cytotoxic payload creates a plausible risk of off-target ovarian injury; therefore, fertility risk should be considered uncertain rather than absent.	Cumulative exposure; concurrent or previous chemotherapy; patient age; and treatment setting. Fertility preservation counselling should be offered before treatment whenever feasible.	[13,14]
PARP inhibitors: olaparib and talazoparib	DNA-repair inhibitors used mainly in germline BRCA-mutated breast cancer.	Preclinical studies indicate embryotoxicity and teratogenicity. Their effect on human ovarian reserve and long-term fertility remains uncertain.	Duration of treatment; previous exposure to chemotherapy; BRCA status; and planned pregnancy. Effective contraception is required during treatment and for the recommended period after treatment discontinuation.	[14,15]
Immune checkpoint inhibitors: pembrolizumab and atezolizumab	Anti-PD-1/PD-L1 therapies increasingly used in triple-negative and high-risk early breast cancer.	Definitive clinical data on ovarian reserve, fertility, pregnancy, and offspring outcomes are lacking. Potential effects include immune-mediated ovarian inflammation and disruption of reproductive immune tolerance, but current evidence remains limited.	Pre-existing autoimmune disease; duration of exposure; concomitant chemotherapy; endocrine immune-related adverse events; and the need for long-term reproductive monitoring.	[16]

AMH: anti-Müllerian hormone; HER2: human epidermal growth factor receptor 2; PARP: poly(ADP-ribose) polymerase; PD-1: programmed cell death protein 1; PD-L1: programmed death-ligand 1.

These findings have reshaped clinical counseling, providing evidence-based reassurance that temporary interruption of endocrine therapy can be feasible and oncologically safe in carefully selected patients. Recent expert consensus and guideline updates emphasize a multidisciplinary approach – encompassing oncology, reproductive endocrinology, and psycho-oncology – to ensure that fertility goals are addressed without compromising disease control (11, 9). This collaborative framework enhances reproductive autonomy while maintaining adherence to oncologic principles, marking a major step toward patient-centered survivorship care in HR+ disease.

Targeted and novel agents

The rapid evolution of targeted and immune-based therapies in breast cancer has introduced new uncertainties about their reproductive safety. HER2-directed monoclonal antibodies such as trastuzumab and pertuzumab, integral to HER2-positive regimens, are not classically gonadotoxic and have not been associated with POI (12). However, newer antibody-drug conjugates (ADCs), including trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan, deliver cytotoxic payloads that may induce off-target ovarian injury, though clinical data remain limited (13, 14). Similarly, poly (ADP-ribose) polymerase (PARP) inhibitors such as olaparib and talazoparib – now standard for germline *BRCA*-mutated early and metastatic disease – show embryotoxic and teratogenic effects in preclinical models. Their effect on ovarian reserve in humans remains uncertain, but animal studies suggest potential sublethal follicular injury, warranting strict contraception during and after therapy (13, 14). Immune checkpoint inhibitors, including pembrolizumab and atezolizumab, increasingly used in triple-negative and high-risk early breast cancer, may influence fertility indirectly through immune-mediated ovarian inflammation or interference with placental immune tolerance. Current evidence is limited to preclinical findings and isolated clinical reports, but systematic reproductive monitoring in ongoing trials is warranted (14, 15). Despite the widespread adoption of these agents, fertility-related outcomes remain poorly captured in pivotal clinical studies. Consequently, major societies such as ASCO (4), ESMO (16), and ESHRE (17) underscore the need to discuss FP with all women of reproductive age before initiating treatments with uncertain gonadotoxic profiles. Until robust human data are

available, a precautionary approach should prevail, ensuring that oocyte or embryo cryopreservation is offered whenever feasible, especially for those receiving experimental or high-risk regimens. This gap between oncologic innovation and reproductive survivorship highlights the urgency of incorporating fertility and pregnancy outcomes as pre-defined endpoints in future clinical trials. Doing so will align precision oncology with reproductive health equity for young women with breast cancer (18, 19).

Table 1 summarizes the variable impact of breast cancer therapies on fertility, highlighting chemotherapy as the main cause of ovarian damage, the timing challenges posed by endocrine therapy, and the uncertain gonadotoxicity of newer targeted and immune agents. Early, individualized fertility counseling remains essential.

FP IN YOUNG BREAST CANCER PATIENTS: CURRENT TECHNIQUES, IMPLEMENTATION, AND UPTAKE

FP has become a fundamental component of comprehensive cancer care for reproductive-age women, reflecting both the increasing survival of young patients and the recognition of reproductive health as a key determinant of long-term quality of life. Current international guidelines from ASCO, ESMO, and ESHRE unanimously recommend that all women of reproductive age who are candidates for gonadotoxic therapy receive prompt counselling on fertility risks and timely referral to FP services (16, 17, 20).

Established FP techniques

Among established strategies, embryo cryopreservation remains the gold standard for women with a partner or those willing to use donor sperm. The process involves controlled ovarian stimulation (COS), oocyte retrieval, *in vitro* fertilization, and vitrification of embryos, achieving the highest cumulative live-birth rates (CLBRs) when performed at younger ages and with more embryos preserved (21, 22). The introduction of random-start COS protocols has revolutionized practice, allowing ovarian stimulation at any point in the menstrual cycle and enabling FP even in patients requiring urgent chemotherapy initiation (23). For women without a partner or those preferring to avoid embryo creation, oocyte cryopreservation represents an equally

effective and ethically acceptable alternative. Population-based data confirm that live-birth rates following oocyte vitrification now approximate those of embryo cryopreservation, especially when FP is undertaken before age 35 (24, 25). The technical reliability and efficiency of modern vitrification have consolidated oocyte cryopreservation as a cornerstone of oncofertility practice. Ovarian tissue cryopreservation (OTC) occupies a unique role for patients unable to undergo ovarian stimulation or for prepubertal girls. The procedure entails laparoscopic retrieval and storage of ovarian cortical tissue, followed by reimplantation after cancer therapy. More than 200 live births have been reported worldwide, demonstrating the capacity of OTC to restore both endocrine function and fertility (26). Nonetheless, the potential for malignant cell reintroduction – particularly in hematologic malignancies – remains a concern, mandating strict patient selection and rigorous tissue screening. Gonadotropin-releasing hormone agonists (GnRHa) during chemotherapy offer a non-invasive adjunctive measure to reduce ovarian toxicity. An individual patient-level meta-analysis confirmed that GnRHa co-treatment significantly decreases the risk of chemotherapy-induced POI and increases post-treatment pregnancies (27, 28). Although GnRHa do not preserve gametes, they serve as a valuable option when cryopreservation is not feasible. Additional surgical approaches, such as ovarian transposition prior to pelvic irradiation, can safeguard ovarian function by repositioning the ovaries outside the radiation field (29). Emerging experimental techniques, including *in vitro* follicle maturation and artificial ovary bioengineering, are under investigation and hold promise for expanding FP options in high-risk or time-constrained scenarios.

Ethical and legal considerations

Ethical and legal frameworks governing FP vary substantially across countries, necessitating explicit informed consent for embryo creation, storage, and potential disposition (30). In Italy, for example, regulatory changes since the enactment of Law 40/2004 have progressively expanded the permissibility of assisted reproduction techniques, reflecting evolving societal and clinical perspectives (31).

Counselling and uptake in clinical practice

Despite clear guideline recommendations, real-world uptake of fertility counselling and preservation remains limited. Across large observational

and registry-based cohorts, reported fertility counselling rates among eligible young breast cancer patients have ranged from approximately 40% to 70%, depending on country, insurance coverage, institutional resources, and the presence of dedicated oncofertility pathways. In these same settings, uptake of fertility-preservation strategies has generally remained substantially lower, often around 10-30% of eligible patients. These figures illustrate a persistent unmet need but should not be interpreted as universal benchmarks, given the marked heterogeneity across healthcare systems and study populations (32-34). Barriers include inadequate referral systems, lack of interdisciplinary coordination, time constraints, and unequal regional availability of FP services. Within this context, the women who proceed with active FP – most commonly oocyte or embryo cryopreservation – tend to be younger, nulliparous, and of higher socioeconomic status or with private insurance coverage, while financial and logistical barriers continue to deter participation, particularly in settings without public reimbursement (16, 35). Encouragingly, recent years have witnessed gradual improvements in both counselling and FP uptake, reflecting wider awareness, dissemination of international guidelines, and technological advances such as vitrification and random-start COS. Nonetheless, persistent inequities related to socioeconomic status, geography, and ethnicity remain. Overcoming these disparities will require system-level strategies including standardized referral protocols, integration of fertility specialists within oncology teams, and policy frameworks ensuring equitable access. In conclusion, FP is now recognized as an essential element of multidisciplinary care for young breast cancer patients. Embryo and oocyte cryopreservation remain the most effective and widely accessible techniques, while OTC and GnRHa co-treatment extend the spectrum of options. Although significant progress has been achieved, consistent implementation and equitable access remain challenges. Embedding fertility counselling and preservation into standard oncologic pathways is crucial to ensure that all women facing breast cancer can make informed, autonomous reproductive decisions.

Figure 1 illustrates current FP techniques, ethical frameworks, and real-world uptake among young breast cancer patients, emphasizing the gap between guideline recommendations and clinical implementation.

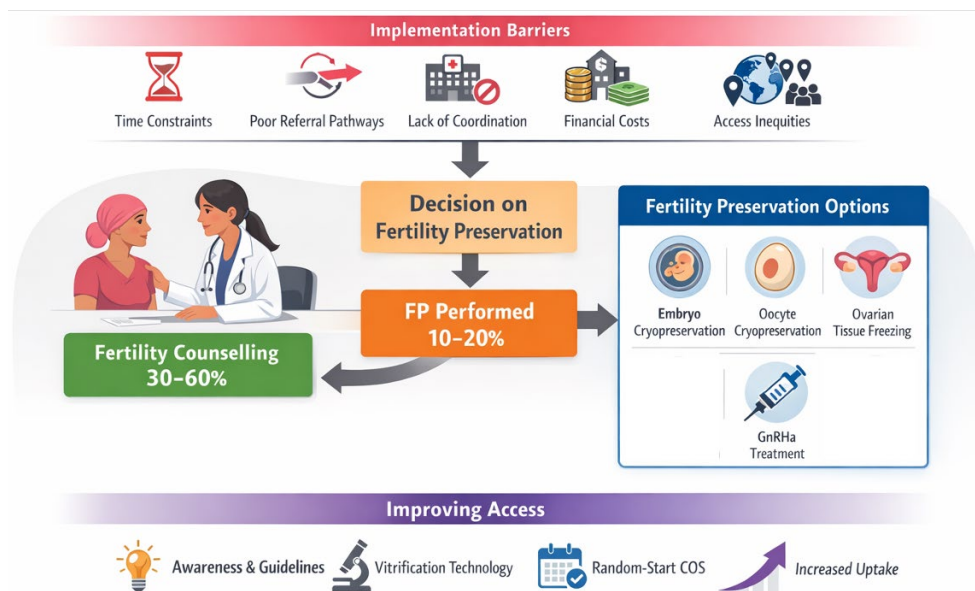


Figure 1. Fertility preservation in young breast cancer patients: counselling, uptake, and implementation barriers.

This schematic illustrates the clinical pathway from diagnosis of breast cancer in reproductive-age women to FP decision-making. Although international guidelines recommend fertility counselling for all eligible patients, only 40-70% of women receive counselling in routine practice. Among those counselled, approximately 10-20% proceed with active FP, highlighting a substantial gap between recommendations and real-world uptake. Available FP strategies include embryo cryopreservation, oocyte cryopreservation, ovarian tissue cryopreservation, and ovarian suppression with GnRHa during chemotherapy. Multiple barriers contribute to limited implementation, including time constraints before treatment initiation, inadequate referral pathways, limited interdisciplinary coordination, financial costs, and geographic disparities in access to fertility services. At the same time, increasing awareness, wider dissemination of international guidelines, and technological advances such as vitrification and random-start controlled ovarian stimulation (COS) are progressively improving access and uptake of FP among young women with breast cancer.

DETERMINANTS OF DECISION-MAKING

FP represents a critical yet unevenly accessible component of oncofertility care, where persistent disparities across racial, socioeconomic, and systemic lines profoundly shape reproductive outcomes for young women with cancer. Understanding why some women receive fertility counseling and pursue cryopreservation while others do not require a multidimensional perspective encompassing patient, provider, and system-level determinants. Evidence consistently shows that socioeconomic status, education, race and ethnicity, geographic access, physician attitudes, and psychosocial characteristics jointly influence FP decision-making in oncology.

Inequities in FP access

The landmark study by Letourneau *et al.* (36) highlighted these disparities, showing that minority women and those with lower socioeconomic status were substantially less likely to receive fertility counseling or pursue FP. Among 1,041 reproductive-age women diagnosed with cancer, White and

privately insured patients were disproportionately represented among those who received counseling and underwent oocyte or embryo cryopreservation. Even after controlling for age, parity, and cancer type, racial and socioeconomic factors remained independently predictive of FP uptake, indicating systemic inequities embedded in oncology care delivery. Building on these findings, Tierney *et al.* (5) demonstrated that disparities begin even earlier, at the level of disease incidence. Their population-based analysis revealed that young Black and Hispanic women had higher incidence rates of fertility-impacting malignancies, yet were less likely to receive counseling or preservation services. These inequities are compounded by broader social determinants of health, including insurance gaps, environmental risk exposures, and geographic barriers to specialty care. Despite increasing awareness and dissemination of ASCO and ESMO FP guidelines, access to FP services remains uneven across patient populations. Recent evidence indicates that referral and uptake of FP interventions are frequently suboptimal, with marked disparities linked to race,

socioeconomic status, and geography. Turner *et al.* reported persistent gaps in oncofertility care in the United States, highlighting that guideline dissemination alone has not resolved inequities in counseling or service utilization (37). Structural barriers further compound these disparities: Peipert *et al.* showed through geospatial analysis that distance to fertility centers and limited regional availability of reproductive specialists disproportionately affect women in rural or economically disadvantaged areas (38). Similarly, Coker Appiah *et al.* identified ongoing unmet needs among pediatric, adolescent, and young adult female patients, emphasizing the role of provider awareness and institutional resources alongside sociodemographic factors in shaping access (39). Population-based analyses corroborate these findings, demonstrating that minority and low-income women are less likely to receive timely referrals to reproductive specialists, with persistent barriers including limited provider knowledge, inconsistent insurance coverage, and cultural or communication gaps between patients and oncology teams (40). A recent narrative review also highlighted structural inequities such as cost barriers, differential clinic availability, and underrepresentation in clinical research as contributors to ongoing racial disparities in both FP and assisted reproductive technology (ART) utilization (41). Together, these data underscore that translating FP guidelines into equitable clinical practice remains incomplete and call for coordinated policy initiatives, insurance reform, and culturally tailored counseling strategies to ensure all women with cancer can access fertility-preserving care.

LONG-TERM REPRODUCTIVE OUTCOMES ACROSS SOCIOECONOMIC STRATA

Most studies of social determinants in oncofertility have focused on counselling, referral, and FP uptake, whereas fewer have reported long-term reproductive outcomes stratified by socioeconomic position. Existing data, although limited, suggest that social gradients in access to FP may extend to subsequent pregnancies and live births. For example, registry-based cohorts and insurance-claims analyses have shown that women with private or comprehensive insurance are more likely to undergo FP and to achieve post-treatment pregnancies than those with public or no insurance, even after accounting for

age and tumor characteristics (39, 40, 42). Similarly, studies from racially and ethnically diverse health systems indicate lower cumulative live-birth rates and higher rates of treatment-related amenorrhea among Black and Hispanic survivors compared with White survivors, patterns that likely reflect intersecting effects of structural racism, differential access to specialist care, and financial barriers (5, 36, 37). At the same time, robust data on child health outcomes and on ovarian-reserve trajectories by socioeconomic strata remain scarce. Closing these evidence gaps – through prospective survivorship registries that capture both social determinants and reproductive endpoints – should be considered a priority for future research.

SOCIOECONOMIC STATUS AND INSURANCE COVERAGE

Socioeconomic status and insurance coverage are among the strongest predictors of FP utilization. Multiple studies show that higher income, private insurance, and residence in states with FP benefit mandates correlate with increased FP uptake. Sauerbrun-Cutler *et al.* (43) reported that expanding insurance mandates and employer-based fertility benefits significantly improved utilization. Similarly, Flores Ortega *et al.* (44) demonstrated that cost remains the predominant barrier in systems reliant on out-of-pocket payment, while Peipert *et al.* (38) showed that FP benefit mandates led to measurable increases in service utilization. Collectively, these data affirm that financial policy reform can materially reduce inequities in FP access.

Education and health literacy

Educational attainment and health literacy strongly influence understanding of infertility risks and subsequent decisions regarding FP. Women with higher levels of education are more likely to appreciate the gonadotoxic potential of cancer therapy and to seek timely counselling. A systematic review of interventions to improve FP decision-making among young women with cancer found that structured counselling, decision aids, and educational tools can enhance patient knowledge and reduce decisional conflict, particularly mitigating disparities linked to limited health literacy (45, 46). Similarly, evidence from decision aid trials in breast cancer patients demonstrated that these tools increase knowledge, confidence, and satisfaction with fertility-related decisions,

with the greatest benefit observed among women with higher baseline health literacy (47, 48). However, persistent gaps remain among socioeconomically disadvantaged groups, emphasizing the need for tailored communication strategies.

Race and ethnicity

Racial and ethnic disparities in FP counselling and utilization are consistently documented across oncology care. Minority women – including Black and Hispanic/Latina patients – are less likely to receive FP counselling or to undergo fertility-preserving procedures, even when adjusting for income, insurance status and other sociodemographic factors. A New York City cohort study by Voigt *et al.* found that, among 107 patients who underwent medically indicated FP between 2017 and 2018, only 3 patients (2.8%) identified as Black and 6 (5.6%) as Hispanic. In the subgroup with available comparator data, these observed numbers were substantially lower than those expected from the racial and ethnic distribution of reproductive-age women with cancer in New York City: 3 observed *versus* 15 expected Black patients and 6 observed *versus* 15 expected Hispanic patients ($p < 0.001$) (42).

More broadly, Turner *et al.* analyzed national oncofertility data and reported significantly lower referral and utilization rates for minority women, implicating structural and cultural barriers, language differences and implicit clinician biases as critical drivers of inequity (37). A narrative review by Gadson, Sauerbrun-Cutler and Eaton highlighted the persistent gap in FP access among minority populations and emphasized that culturally tailored education, interpreter services and systematic monitoring of referral pathways are essential to ensure equitable utilization (41).

Geographic access

Geographic location exerts a profound influence on access to FP. Women treated at rural, community, or regional oncology centers often face delayed or absent referral to oncofertility specialists, limiting timely access to FP counselling and services. A systematic review by Pathak *et al.* found that clinics with limited local reproductive-specialty resources and fragmented referral pathways were associated with markedly lower FP uptake among eligible patients (49). In parallel, a national geospatial analysis by Peipert *et al.* showed that 3.63 million female individuals aged 15-44 years – corresponding to 5.70% of the reproductive-age female population in the

continental United States – lived outside a two-hour travel radius of an oncofertility center, particularly in states lacking FP insurance mandates, highlighting structural geographic inequities (38).

Interventions such as tele-medicine linkage to fertility specialists, standardized referral algorithms embedded in oncology workflow, and travel-support programs have shown promise in mitigating these geographic disparities.

Physician-related factors

Physician engagement and institutional support play a critical role in FP access. Oncologists vary substantially in how frequently, how early, and how comprehensively they address fertility risks with reproductive-age patients. In their systematic review, Deshpande *et al.* found that many women who were clearly eligible for FP never received counselling, underscoring failure at the provider- or system-level rather than solely patient choice (50). Provider knowledge, comfort with fertility discussions, competing workflow demands, and lack of standardized referral pathways all influence the likelihood of FP-counselling and referral. The use of dedicated fertility navigators – specialized coordinators embedded within oncology or oncofertility clinics – has emerged as a promising strategy. In a qualitative evaluation at an academic center, van den Berg *et al.* found that fertility navigators improved patient satisfaction, offered consistent information and emotional support, and strengthened collaboration between oncology and fertility teams (51). Centers that integrate navigators, standardized electronic-health-record prompts, and routine fertility referral-checklists report higher rates of timely FP discussion and uptake. However, enhancing physician-related factors requires institutional commitment to fertility pathways: mandatory referral triggers at diagnosis, training modules on oncofertility for oncology teams, and inter-disciplinary coordination with reproductive specialists. Only when counselling and referral become system-standard rather than optional will FP access approach equity across age, race, and socioeconomic strata.

Psychosocial determinants

Psychosocial factors play a significant role in fertility-preservation decision-making. Emotional distress at diagnosis, the perceived urgency to initiate oncologic treatment, relationship status, and partner support all influence whether a woman chooses to pursue FP. Time pressure, information overload, and

uncertainty often lead cancer patients to defer fertility-preservation decisions (52). Yuen *et al.* reported that patients who received oncofertility counselling were younger, with a median age of 35 compared with 38 years for those not counselled, and were more likely to be nulliparous, 45 percent *versus* 18.5 percent (32). A systematic review of fertility-preservation patient decision aids demonstrated that structured tools significantly increased fertility-preservation knowledge and reduced decisional conflict (53). Together, these findings emphasize the need to integrate psychosocial assessment and support into fertility-preservation counselling to facilitate informed, value-congruent choices.

Figure 2 provides a clear, visually intuitive summary of the complex interplay of patient, provider, and system determinants affecting FP decisions.

PREGNANCY AFTER BREAST CANCER

Pregnancy represents an important and often complex consideration for young breast-cancer survivors. Advances in oncologic care and fertility-preservation strategies mean that an increasing number of women diagnosed with breast cancer in reproductive years are contemplating conception after treatment. Clinicians and patients alike confront key questions about the safety of pregnancy, optimal timing, reproductive outcomes, and breastfeeding – all within the context of tumor biology, prior treatment exposures, ovarian reserve, and personal reproductive goals. This section synthesizes current evidence and guidelines to support informed decision-making in this population.

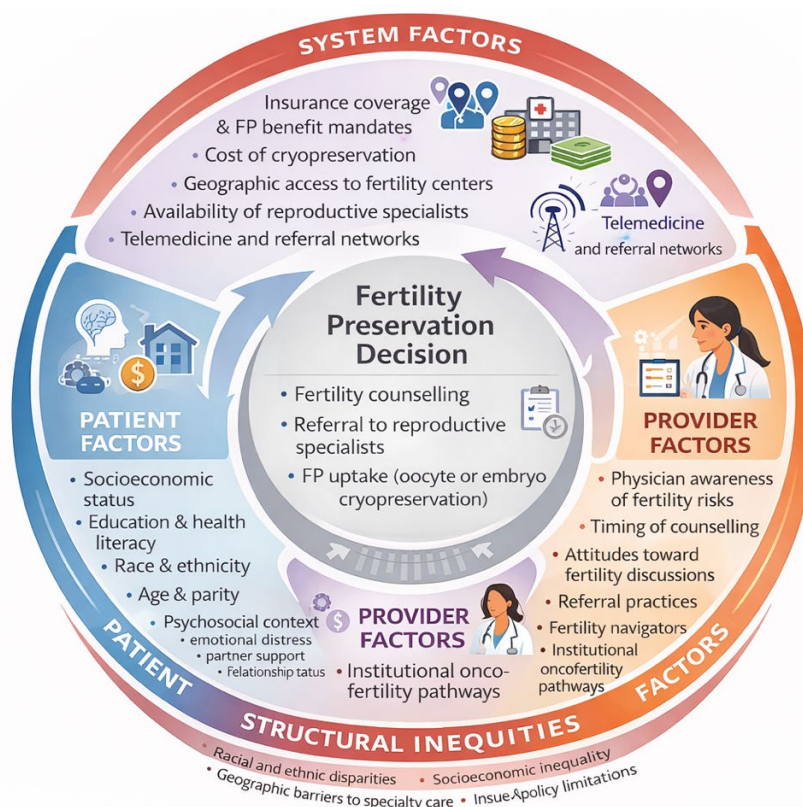


Figure 2. Multilevel determinants of fertility preservation decision-making in young women with cancer.

FP utilization is shaped by the interaction of patient-, provider-, and system-level determinants. Patient factors include socioeconomic status, education and health literacy, race and ethnicity, reproductive history, and psychosocial context. Provider determinants encompass clinician awareness of fertility risks, timing of counselling, referral practices, and the availability of fertility navigators or institutional oncofertility pathways. System-level influences include insurance coverage, cost barriers, geographic access to fertility centers, and the availability of reproductive specialists. These determinants operate within a broader context of structural inequities – including racial, socioeconomic, and geographic disparities – which collectively influence whether young women with cancer receive timely fertility counselling and ultimately pursue FP interventions.

Safety of pregnancy

The preponderance of observational evidence – from meta-analyses, registries, and narrative reviews – indicates that pregnancy after breast cancer does not increase the risk of recurrence; indeed, in many studies, survivors who become pregnant demonstrate similar or even apparently improved survival outcomes compared with those who do not, although these findings are likely influenced by the selection of women who remain recurrence-free and well enough to conceive. For example, in the narrative review by Carneiro *et al.*, the authors concluded that “the best published evidence suggests pregnancy after breast cancer does not increase the risk of disease recurrence, thus pregnancy should not be forbidden once treatment is completed” (54). Large systematic reviews further support this: Nolan *et al.* found no detrimental effect of pregnancy on Disease-Free Survival (DFS) or Overall Survival (OS) in patients with hormone-receptor (HR)-positive disease (55) and Arecco *et al.*, in a meta-analysis of young women with HR-positive early breast cancer, reported no adverse effect of pregnancy on DFS (HR 0.96, 95%CI 0.79-1.16) and an apparently improved OS (HR 0.46, 95%CI 0.24-0.87) (56); however, the latter is likely driven by strong selection of women who survived, remained recurrence-free, and subsequently pursued pregnancy (the “healthy mother effect”) rather than a causal protective effect of pregnancy.

More recently, a large cohort study of 4,732 women with germline BRCA1/2 pathogenic variants diagnosed with invasive breast cancer before age 40 found, among the 659 who became pregnant, an adjusted hazard ratio for DFS of 0.99 (95%CI 0.81-1.20) compared to those who did not become pregnant – indicating no increased risk in this high-risk genetic subgroup (57).

Taken together, these data provide robust reassurance that, in selected survivors (especially those in remission, with favorable tumor characteristics, and preserved fertility potential), pregnancy can be pursued safely. Some meta-analytic data even suggest superior survival outcomes in women who become pregnant (for example, pooled HR for DFS ~0.66; 95%CI 0.49-0.89) (3, 58). However, caution remains warranted due to potential selection bias (“healthy mother effect”), shorter follow-up in many studies, and the predominance of lower-risk patients in published cohorts.

Timing of pregnancy after breast cancer: evidence-based recommendations

Historically, clinicians advised delaying pregnancy for at least 2 years after treatment, coinciding with the

period of highest risk for recurrence. Emerging evidence now supports the possibility of earlier conception in carefully selected women, particularly when tumor characteristics, stage, and patient preferences are favorable. This question has been most rigorously evaluated in the POSITIVE trial, in which temporary interruption of endocrine therapy after 18-30 months to allow pregnancy did not increase short-term recurrence risk in carefully selected women with HR-positive disease (10) (see Endocrine Therapy section). Counseling should be individualized, taking into account tumor biology, stage, duration of endocrine therapy, fertility potential, and patient preferences. This evidence underscores the importance of early, multidisciplinary oncofertility consultation to guide timing, optimize outcomes, and support informed reproductive decisions.

Fertility, pregnancy, and postpartum outcomes

Pregnancy after breast cancer, whether achieved spontaneously or via FP strategies, is generally feasible, and many survivors successfully deliver healthy infants. Pregnancy rates in survivors remain lower than in age-matched general populations, but ART using cryopreserved gametes yields satisfactory conception and live birth rates. Slight increases in preterm birth or low birthweight have been reported in some series, yet overall neonatal outcomes are reassuring (59). Breastfeeding is generally safe and feasible, with no evidence of increased recurrence risk, though prior surgery or radiotherapy may limit lactation capacity. Importantly, breastfeeding preserves benefits for maternal-infant bonding, and structured postpartum support is recommended to optimize both maternal and neonatal outcomes (54). However, practical ability to breastfeed may be limited by prior breast surgery (e.g., mastectomy or extensive lumpectomy), radiotherapy involving the breast and axilla, or adjuvant systemic therapy. Clinicians should support survivors’ interest in breastfeeding with realistic counselling about potential anatomical/physiologic limitations.

Long-term reproductive and psychosocial outcomes

Long-term reproductive health remains a central concern for young breast cancer survivors. Anti-Müllerian hormone (AMH) is a widely used biomarker of ovarian reserve in clinical practice, and chemotherapy-related declines in AMH – particularly in older premenopausal women – underline the risk of sus-

tained ovarian dysfunction (see Chemotherapy and ovarian failure) (7, 60). Serial AMH monitoring may assist clinicians in counselling survivors about residual fertility potential and in planning the timing of conception attempts, although AMH alone is a limited predictor of natural fertility and time to spontaneous conception.

Among survivors of breast cancer who did not use cryopreserved gametes, spontaneous conception rates remain substantially lower than in age-matched controls. For example, one prospective cohort found that among women who returned to ovarian function after chemotherapy, ~65% attempted conception and about half achieved pregnancy through intercourse alone (61). In the oncofertility series more broadly, the rates of spontaneous pregnancy appear to cluster roughly in the range of ~10%-30% depending on age at diagnosis, treatment type, and duration of follow-up. Patients who banked oocytes or embryos before therapy demonstrate higher live-birth or pregnancy success rates, particularly when cryopreservation occurred at younger ages. Nevertheless, even when menstrual function resumes post-treatment, a diminished ovarian reserve may restrict the long-term fertility window. Fertility concerns in cancer survivors carry major psychosocial consequences. Pre-treatment fertility-preservation counselling has been associated with lower decisional regret, improved quality of life, and better satisfaction with fertility outcomes (50, 62). While direct data on structured decision-aid tools and peer-support programs for oncofertility remain relatively limited, emerging evidence supports their role in reducing decisional conflict and regret in reproductive-aged cancer survivors (62). Although much of the published work has focused on the safety and outcomes of pregnancy after breast cancer, the longer-term reproductive lifespan of survivors – including time to menopause, the residual fertility window beyond the first post-treatment conception, and the psychosocial sequelae across years of survivorship – remains under-investigated and deserves dedicated prospective research.

CONCLUSIONS

FP is an essential component of comprehensive care for young women with breast cancer. Advances over the past decade – including optimized oocyte and embryo cryopreservation, accumulation of meta-analytic evidence on ovarian-protection strategies, and

prospective trials such as the POSITIVE trial – have significantly expanded counseling and reproductive options (63). Despite these advances, substantial inequities persist, driven by socioeconomic status, race/ethnicity, insurance coverage, and geography, which limit equitable access to FP (64). System-level interventions can help bridge these gaps: automatic referrals to onco-fertility specialists, dedicated FP navigation programs, and mandated or publicly funded coverage for medically indicated FP have demonstrated effectiveness in improving uptake and patient satisfaction (65). Long-term survivorship registries capturing harmonized data on FP procedures, ovarian reserve markers, pregnancy attempts and outcomes, child health, and psychosocial metrics are crucial for evaluating real-world effectiveness and disparities, with international collaboration maximizing statistical power for subgroup analyses such as BRCA mutation carriers (66). Emerging evidence indicates that BRCA pathogenic variants may independently reduce ovarian reserve, emphasizing the need for tailored counselling and early FP in this population (67). Furthermore, the fertility effects of newer oncologic agents – including PARP inhibitors, immune-checkpoint inhibitors, antibody-drug conjugates, and other targeted therapies – remain largely uncharacterized, highlighting an urgent research priority for trials enrolling premenopausal women to include fertility endpoints and biologic sampling (4). Psychosocial interventions and structured decision aids are increasingly recognized as critical to reduce decisional regret, improve quality of life, and address anxiety and distress related to fertility concerns (53). Finally, health-economic research incorporating quality-of-life outcomes and the societal value of parenthood can inform policy and coverage decisions, ensuring equitable access to FP (68).

This review has several limitations. First, it is a narrative rather than a systematic review, and we may therefore have missed some relevant studies despite a broad search strategy. Second, most of the available evidence comes from high-income countries with well-resourced oncology and fertility services, limiting the generalizability of our conclusions to low- and middle-income settings. Third, long-term data on ovarian reserve, live-birth rates, and child health after FP remain sparse, particularly when stratified by socioeconomic status, race/ethnicity, or insurance coverage. Fourth, evidence on the gonadotoxicity and reproductive safety of newer agents – such as PARP inhibitors, antibody-drug conjugates,

and immune checkpoint inhibitors – is still evolving. Finally, pregnancy-after-cancer cohorts are inherently affected by selection bias, as only women who have survived, remained recurrence-free, and actively pursued pregnancy are captured, complicating causal inference regarding the impact of pregnancy on prognosis.

In conclusion, integrating FP into routine breast-cancer care requires a dual approach: implementing evidence-based clinical pathways and system-level support while pursuing targeted research to refine guidance as oncologic therapies evolve. Such an approach promises to safeguard reproductive potential, mitigate psychosocial burden, and enhance long-term quality of life for young breast-cancer survivors.

COMPLIANCE WITH ETHICAL STANDARDS

Funding

This study was partially supported by the Italian Ministry of Health - Ricerca Corrente Annual Program 2026.

Conflicts of interest

The authors declare no competing interests.

Availability of data and materials

The data presented in this study are available on request from the corresponding author.

Authors' contributions

AP: conceptualization, investigation, writing – original draft, visualization, supervision. IB: writing – review & editing, visualization, investigation, supervision. FMa: investigation, supervision. MBB: supervision. FMo: investigation, visualization. LM: conceptualization, writing – original draft, investigation, supervision.

Publication ethics

Plagiarism

This is a review article, and all original studies are cited as appropriate. Figures were generated with the help of AI.

Data falsification and fabrication

The contents of the article are original, and any overlaps with other articles are by the authors themselves and appropriately cited.

REFERENCES

1. Anders CK, Johnson R, Litton J, Phillips M, Bleyer A. Breast cancer before age 40 years. *Semin Oncol.* 2009;36(3):237-49. doi: 10.1053/j.seminoncol.2009.03.001.
2. Zhu JW, Charkhchi P, Adekunle S, Akbari MR. What Is Known about Breast Cancer in Young Women? *Cancers (Basel).* 2023;15(6):1917. doi: 10.3390/cancers15061917.
3. Lambertini M, Blondeaux E, Bruzzone M, Perachino M, Anderson RA, de Azambuja E, et al. Pregnancy After Breast Cancer: A Systematic Review and Meta-Analysis. *J Clin Oncol.* 2021;39(29):3293-3305. doi: 10.1200/JCO.21.00535.
4. Su HI, Lacchetti C, Letourneau J, Partridge AH, Qamar R, Quinn GP, et al. Fertility Preservation in People With Cancer: ASCO Guideline Update. *J Clin Oncol.* 2025;43(12):1488-1515. doi: 10.1200/JCO-24-02782.
5. Tierney KI, Therrien J, Ellwood S, Graves L. Inequalities in Fertility-Impacting Cancer Incidence Among Young Populations in the United States. *Cancer Med.* 2025;14(7):e70797. doi: 10.1002/cam4.70797.
6. Furlanetto J, Marmé F, Seiler S, Thode C, Untch M, Schmatloch S, et al. Chemotherapy-induced ovarian failure in young women with early breast cancer: Prospective analysis of four randomised neoadjuvant/adjuvant breast cancer trials. *Eur J Cancer.* 2021;152:193-203. doi: 10.1016/j.ejca.2021.04.038.
7. Romito A, Bove S, Romito I, Zace D, Raimondo I, Fragomeni SM, et al. Ovarian Reserve after Chemotherapy in Breast Cancer: A Systematic Review and Meta-Analysis. *J Pers Med.* 2021;11(8):704. doi: 10.3390/jpm11080704.
8. Wang Y, Li Y, Liang J, Zhang N, Yang Q. Chemotherapy-Induced Amenorrhea and Its Prognostic Significance in Premenopausal Women With Breast Cancer: An Updated Meta-Analysis. *Front Oncol.* 2022;12:859974. doi: 10.3389/fonc.2022.859974.
9. Natsuhara KH, Chien AJ. Impact of Systemic Therapy on Fertility in Women with Early-Stage Breast Cancer. *Curr Breast Cancer Rep.* 2024;16(1):61-68. doi: 10.1007/s12609-023-00516-z.
10. Partridge AH, Niman SM, Ruggeri M, Peccatori FA, Azim HA Jr, Colleoni M, et al. Interrupting Endocrine Therapy to Attempt Pregnancy after Breast Cancer. *N Engl J Med.* 2023;388(18):1645-1656. doi: 10.1056/NEJMoa2212856.

11. Arecco L, Borea R, Magaton IM, Janković K, Mariamizde E, Stana M, et al. Current practices in oncofertility counseling: updated evidence on fertility preservation and post-treatment pregnancies in young women affected by early breast cancer. *Expert Rev Anticancer Ther.* 2024;24(9):803-817. doi: 10.1080/14737140.2024.2372337.
12. Helgadottir H, Matikas A, Fernebro J, Frödin JE, Ekman S, Rodriguez-Wallberg KA. Fertility and reproductive concerns related to the new generation of cancer drugs and the clinical implication for young individuals undergoing treatments for solid tumors. *Eur J Cancer.* 2024;202:114010. doi: 10.1016/j.ejca.2024.114010.
13. Echavarria, Isabel; Jerez, Yolanda; Bringas, Marianela; López-Tarruella, Sara; Martin, Miguel. Ovarian Toxicity Linked to Early-stage Breast Cancer Treatment. *J Young Women Breast Can Health.* 2024;1(1&2):6-12.
14. Zagouri F, Dimopoulos MA, Andrikopoulou A. PARP inhibitors during conception and pregnancy in breast cancer. *Crit Rev Oncol Hematol.* 2025;210:104696. doi: 10.1016/j.critrevonc.2025.104696.
15. Shabir I, Foster C. Immune Checkpoint Inhibitors Impact Fertility: A Review. *Rev Recent Clin Trials.* 2025;20(3):185-190. doi: 10.2174/0115748871340496250130054721.
16. Lambertini M, Peccatori FA, Demeestere I, Amant F, Wyns C, Stukenborg JB, et al. Fertility preservation and post-treatment pregnancies in post-pubertal cancer patients: ESMO Clinical Practice Guidelines. *Ann Oncol.* 2020;31(12):1664-1678. doi: 10.1016/j.annonc.2020.09.006.
17. ESHRE Guideline Group on Female Fertility Preservation; Anderson RA, Amant F, Braat D, D'Angelo A, Chuva de Sousa Lopes SM, Demeestere I, Dwek S, Frith L, Lambertini M, Maslin C, Moura-Ramos M, Nogueira D, Rodriguez-Wallberg K, Vermeulen N. ESHRE guideline: female fertility preservation. *Hum Reprod Open.* 2020;2020(4):hoaa052. doi: 10.1093/hropen/hoaa052.
18. Ranjith S, Dave A. Filling the Gaps in Oncofertility Care by Addressing Challenges Faced by Patients and Providers. *Cureus.* 2023;15(12):e50219. doi: 10.7759/cureus.50219.
19. De Paola L, Napoletano G, Gullo G, Circosta F, Montanari Vergallo G, Marinelli S. The era of increasing cancer survivorship: Trends in fertility preservation, medico-legal implications, and ethical challenges. *Open Med (Wars).* 2025;20(1):20251144. doi: 10.1515/med-2025-1144.
20. Oktay K, Harvey BE, Partridge AH, Quinn GP, Reinecke J, Taylor HS, et al. Fertility Preservation in Patients With Cancer: ASCO Clinical Practice Guideline Update. *J Clin Oncol.* 2018;36(19):1994-2001. doi: 10.1200/JCO.2018.78.1914.
21. Porcu E, Cipriani L, Dirodi M, De Iaco P, Perrone AM, Zinzani PL, et al. Successful Pregnancies, Births, and Children Development Following Oocyte Cryostorage in Female Cancer Patients During 25 Years of Fertility Preservation. *Cancers (Basel).* 2022;14(6):1429. doi: 10.3390/cancers14061429.
22. Fraison E, Huberlant S, Labrune E, Cavalieri M, Montagut M, Brugnon F, et al. Live birth rate after female fertility preservation for cancer or haematopoietic stem cell transplantation: a systematic review and meta-analysis of the three main techniques; embryo, oocyte and ovarian tissue cryopreservation. *Hum Reprod.* 2023;38(3):489-502. doi: 10.1093/humrep/deac249.
23. Cakmak H, Rosen MP. Random-start ovarian stimulation in patients with cancer. *Curr Opin Obstet Gynecol.* 2015;27(3):215-21. doi: 10.1097/GCO.0000000000000180.
24. Ter Welle-Butalid ME, Derhaag JG, van Bree BE, Vriens IJH, Goddijn M, Balkenende EME, et al. Outcomes of female fertility preservation with cryopreservation of oocytes or embryos in the Netherlands: a population-based study. *Hum Reprod.* 2024;39(12):2693-2701. doi: 10.1093/humrep/deae243.
25. Kirshenbaum M, Stern C, Wale P, Lantsberg D, Gook D, Stevens J, et al. Outcomes of oocyte cryopreservation: an analysis based on age and treatment indications. *Hum Reprod.* 2025;40(10):1990-1997. doi: 10.1093/humrep/deaf147.
26. Fabbri R, Vicenti R, Magnani V, Paradisi R, Lima M, De Meis L, Rossi S, Raimondo D, Casadio P, Venturoli S, Maffi M, Seracchioli R. Ovarian tissue cryopreservation and transplantation: 20 years experience in Bologna University. *Front Endocrinol (Lausanne).* 2022;13:1035109. doi: 10.3389/fendo.2022.1035109.
27. Lambertini M, Moore HCF, Leonard RCF, Loibl S, Munster P, Bruzzone M, et al. Gonadotropin-Releasing Hormone Agonists During Chemotherapy for Preservation of Ovarian Function and Fertility in Premenopausal Patients With Early Breast Cancer: A Systematic Review and Meta-Analysis of Individual Patient-Level Data.

- J Clin Oncol. 2018;36(19):1981-1990. doi: 10.1200/JCO.2018.78.0858.
28. Massarotti C, Stigliani S, Gazzo I, Lambertini M, Anserini P. Long-acting gonadotropin-releasing hormone agonist trigger in fertility preservation cycles before chemotherapy. *ESMO Open*. 2023;8(4):101597. doi: 10.1016/j.esmoop.2023.101597.
 29. Turkgeldi L, Cutner A, Turkgeldi E, Al Chami A, Cassoni A, Macdonald N, et al. Laparoscopic Ovarian Transposition and Ovariopexy for Fertility Preservation in Patients Treated with Pelvic Radiotherapy with or without Chemotherapy. *Facts Views Vis Obgyn*. 2019;11(3):235-242.
 30. Khorshid A, Alvero R. Consenting and ethical considerations in embryo cryopreservation. *Curr Opin Obstet Gynecol*. 2020;32(5):380-384. doi: 10.1097/GCO.0000000000000653.
 31. Vergallo GM, Marinelli S, Napoletano G, De Paola L, Treglia M, Zaami S, et al. Since the Enactment of Italian Law No. 40/2004 on Medically Assisted Procreation: How It Has Changed and How It Could Change. *Int J Environ Res Public Health*. 2025;22(2):296. doi: 10.3390/ijerph22020296.
 32. Yuen S, McCool K, Bharucha M, Narloch K, Zhu J, Yong H. Evaluating Trends in Fertility Counseling among Reproductive-Age Patients with Breast Cancer. *Ann Surg Oncol*. 2025;32(11):8425-8431. doi: 10.1245/s10434-025-17857-x.
 33. Ruggeri M, Pagan E, Bagnardi V, Bianco N, Gallerani E, Buser K, et al. Fertility concerns, preservation strategies and quality of life in young women with breast cancer: Baseline results from an ongoing prospective cohort study in selected European Centers. *Breast*. 2019;47:85-92. doi: 10.1016/j.breast.2019.07.001.
 34. Arecco L, Perachino M, Damassi A, Latocca MM, Soldato D, Vallome G, et al. Burning Questions in the Oncofertility Counseling of Young Breast Cancer Patients. *Breast Cancer (Auckl)*. 2020;14:1178223420954179. doi: 10.1177/1178223420954179.
 35. Zaami S, Melcarne R, Patrone R, Gullo G, Negro F, Napoletano G, et al. Oncofertility and Reproductive Counseling in Patients with Breast Cancer: A Retrospective Study. *J Clin Med*. 2022;11(5):1311. doi: 10.3390/jcm11051311.
 36. Letourneau JM, Smith JF, Ebbel EE, Craig A, Katz PP, Cedars MI, et al. Racial, socioeconomic, and demographic disparities in access to fertility preservation in young women diagnosed with cancer. *Cancer*. 2012;118(18):4579-88. doi: 10.1002/cncr.26649.
 37. Turner KA, Spurlin EE, Jimenez PT. Disparities in Female Oncofertility Care in the United States: More Questions Than Answers. *Life (Basel)*. 2023;13(7):1547. doi: 10.3390/life13071547.
 38. Peipert BJ, Potapragada NR, Lantos PM, Harris BS, Reinecke J, Goldman KN. A Geospatial Analysis of Disparities in Access to Oncofertility Services. *JAMA Oncol*. 2023;9(10):1364-1370. doi: 10.1001/jamaoncol.2023.2780.
 39. Coker Appiah L, Fei YF, Olsen M, Lindheim SR, Puccetti DM. Disparities in Female Pediatric, Adolescent and Young Adult Oncofertility: A Needs Assessment. *Cancers (Basel)*. 2021;13(21):5419. doi: 10.3390/cancers13215419.
 40. Meernik C, Engel SM, Wardell A, Baggett CD, Gupta P, Rodriguez-Ormaza N, et al. Disparities in fertility preservation use among adolescent and young adult women with cancer. *J Cancer Surviv*. 2023;17(5):1435-1444. doi: 10.1007/s11764-022-01187-y.
 41. Gadson AK, MT, Eaton JL. Racial Disparities in Fertility Care: A Narrative Review of Challenges in the Utilization of Fertility Preservation and ART in Minority Populations. *J Clin Med*. 2024;13(4):1060. doi: 10.3390/jcm13041060.
 42. Voigt PE, Blakemore JK, McCulloh D, Fino ME. Equal opportunity for all? An analysis of race and ethnicity in fertility preservation in New York City. *J Assist Reprod Genet*. 2020;37(12):3095-3102. doi: 10.1007/s10815-020-01980-7.
 43. Sauerbrun-Cutler MT, Rollo A, Gadson A, Eaton JL. The Status of Fertility Preservation (FP) Insurance Mandates and Their Impact on Utilization and Access to Care. *J Clin Med*. 2024;13(4):1072. doi: 10.3390/jcm13041072.
 44. Flores Ortega RE, Yoeun SW, Mesina O, Kaiser BN, McMenamin SB, Su HI. Assessment of Health Insurance Benefit Mandates for Fertility Preservation Among 11 US States. *JAMA Health Forum*. 2021;2(12):e214309. doi: 10.1001/jamahealthforum.2021.4309.
 45. Peate M, Meiser B, Hickey M, Friedlander M. The fertility-related concerns, needs and preferences of younger women with breast cancer: a systematic review. *Breast Cancer Res Treat*. 2009;116(2):215-23. doi: 10.1007/s10549-009-0401-6.
 46. Muscat DM, Smith J, Mac O, Cadet T, Giguere A, Houston AJ, et al. Addressing Health Literacy in Patient Decision Aids: An Update from

- the International Patient Decision Aid Standards. *Med Decis Making*. 2021;41(7):848-869. doi: 10.1177/0272989X211011101.
47. Peate M, Meiser B, Cheah BC, Saunders C, Butow P, Thewes B, et al. Making hard choices easier: a prospective, multicentre study to assess the efficacy of a fertility-related decision aid in young women with early-stage breast cancer. *Br J Cancer*. 2012;106(6):1053-61. doi: 10.1038/bjc.2012.61.
 48. Stacey D, Légaré F, Lewis K, Barry MJ, Bennett CL, Eden KB, et al. Decision aids for people facing health treatment or screening decisions. *Cochrane Database Syst Rev*. 2017;4(4):CD001431. doi: 10.1002/14651858.CD001431.pub5.
 49. Pathak S, Vadaparampil ST, Sutter ME, Rice WS, McBride CM. Evaluating fertility preservation interventions for alignment with ASCO Guidelines for reproductive aged women undergoing cancer treatment: a systematic review. *Support Care Cancer*. 2023;31(12):689. doi: 10.1007/s00520-023-08133-3.
 50. Deshpande NA, Braun IM, Meyer FL. Impact of fertility preservation counseling and treatment on psychological outcomes among women with cancer: A systematic review. *Cancer*. 2015;121(22):3938-47. doi: 10.1002/cncr.29637.
 51. van den Berg M, Nadesapillai S, Braat DDM, Hermens RPMG, Beerendonk CCM. Fertility navigators in female oncofertility care in an academic medical center: a qualitative evaluation. *Support Care Cancer*. 2020;28(12):5733-5741. doi: 10.1007/s00520-020-05412-1.
 52. Silva C, Almeida-Santos AT, Melo C, Rama ACR. Decision on Fertility Preservation in Cancer Patients: Development of Information Materials for Healthcare Professionals. *J Adolesc Young Adult Oncol*. 2017;6(2):353-357. doi: 10.1089/jayao.2016.0064.
 53. Wang Y, Anazodo A, Logan S. Systematic review of fertility preservation patient decision aids for cancer patients. *Psychooncology*. 2019;28(3):459-467. doi: 10.1002/pon.4961.
 54. Carneiro MM, Cota AM, Amaral MC, Pedrosa ML, Martins BO, Furtado MH, et al. Motherhood after breast cancer: can we balance fertility preservation and cancer treatment? A narrative review of the literature. *JBRA Assist Reprod*. 2018;22(3):244-252. doi: 10.5935/1518-0557.20180032.
 55. Nolan K, Boland MR, Hill ADK. Pregnancy After Breast Cancer - Prognostic Safety and Pregnancy Outcomes According to Oestrogen Receptor Status: A Systematic Review. *J Breast Cancer*. 2022;25(6):443-453. doi: 10.4048/jbc.2022.25.e45.
 56. Arecco L, Blondeaux E, Bruzzzone M, Latocca MM, Mariamidze E, Begijanashvili S, et al. Safety of pregnancy after breast cancer in young women with hormone receptor-positive disease: a systematic review and meta-analysis. *ESMO Open*. 2023;8(6):102031. doi: 10.1016/j.esmoop.2023.102031.
 57. Lambertini M, Blondeaux E, Agostinetti E, Hamy AS, Kim HJ, Di Meglio A, et al. Pregnancy After Breast Cancer in Young BRCA Carriers: An International Hospital-Based Cohort Study. *JAMA*. 2024;331(1):49-59. doi: 10.1001/jama.2023.25463.
 58. Mordenfeld Kozlovsky N, Partridge AH, Sella T. Pregnancy after breast cancer: latest evidence and practical considerations. *Ther Adv Med Oncol*. 2025;17:17588359251346648. doi: 10.1177/17588359251346648.
 59. Maiorano MFP, Cormio G, Loizzi V, Maiorano BA, D'Oronzo S, Silvestris E. Tamoxifen and Fertility in Women with Breast Cancer: A Systematic Review on Reproductive Outcomes and Oncological Safety of Treatment Interruption. *Int J Mol Sci*. 2025;26(8):3787. doi: 10.3390/ijms26083787.
 60. Zhou B, Kwan B, Desai MJ, Nalawade V, Ruddy KJ, Nathan PC, et al. Long-term antimüllerian hormone patterns differ by cancer treatment exposures in young breast cancer survivors. *Fertil Steril*. 2022;117(5):1047-1056. doi: 10.1016/j.fertnstert.2022.01.016.
 61. Abel MK, Wald K, Sinha N, Letourneau JM, Simbulan R, Mok-Lin E, et al. Conception after chemotherapy: post-chemotherapy method of conception and pregnancy outcomes in breast cancer patients. *J Assist Reprod Genet*. 2021;38(7):1755-1765. doi: 10.1007/s10815-021-02133-0.
 62. Ehrbar V, Germeyer A, Nawroth F, Dangel A, Findeklee S, Urech C, et al. Long-term effectiveness of an online decision aid for female cancer patients regarding fertility preservation: Knowledge, attitude, and decisional regret. *Acta Obstet Gynecol Scand*. 2021;100(6):1132-1139. doi: 10.1111/aogs.14108.
 63. de Pedro M, Otero B, Martín B. Fertility preservation and breast cancer: a review. *Ecancer-medicalscience*. 2015;9:503. doi: 10.3332/ecancer.2015.503.
 64. Hong YH, Park C, Paik H, Lee KH, Lee JR, Han W, et al. Fertility Preservation in Young Women With Breast Cancer: A Review. *J Breast Cancer*. 2023;26(3):221-242. doi: 10.4048/jbc.2023.26.e28.

65. Campbell AG, Hillemeier M. Fertility counseling information adequacy as a moderator of regret among adolescent and young adult breast cancer survivors. *Support Care Cancer*. 2021;29(5):2689-2697. doi: 10.1007/s00520-020-05771-9.
66. Qin N, Wan Z, Kang Y, Luo Y, Duan Y, Xie J, et al. Effectiveness and feasibility of online fertility preservation decision aids for young female patients with cancer: a systematic review protocol. *BMJ Open*. 2023;13(3):e070294. doi: 10.1136/bmjopen-2022-070294.
67. Boutas I, Kontogeorgi A, Koufopoulos N, Dimas DT, Sitara K, Kalantaridou SN, et al. Breast Cancer and Fertility Preservation in Young Female Patients: A Systematic Review of the Literature. *Clin Pract*. 2023;13(6):1413-1426. doi: 10.3390/clinpract13060127.
68. Razeti MG, Spinaci S, Spagnolo F, Massarotti C, Lambertini M. How I perform fertility preservation in breast cancer patients. *ESMO Open*. 2021;6(3):100112. doi: 10.1016/j.esmoop.2021.100112.

OPINION PAPER

VITAL STAINING AND ARTIFICIAL INTELLIGENCE IN ORAL CANCER DETECTION: A HYBRID DIAGNOSTIC APPROACH FOR GLOBAL HEALTH EQUITY

Abhijeet Satpathy, Rupsa Das, Satya Ranjan Misra *

Department of Oral Medicine and Radiology, Institute of Dental Sciences, Siksha 'O' Anusandhan University, Bhubaneswar, Odisha, India

* Correspondence to: ✉ satyamisra@soa.ac.in; <https://orcid.org/0000-0002-1994-543X>

ABSTRACT: Oral squamous cell carcinoma (OSCC) remains a major public health challenge, particularly in low- and middle-income countries (LMICs), where early detection infrastructure is limited. Vital staining is affordable and chairside-simple but is undermined by operator variability and modest specificity. Artificial intelligence (AI)-assisted image analysis offers reproducibility and scale but is constrained by technological and logistical barriers in resource-poor settings. Our position in this Opinion is straightforward: neither modality alone will close the diagnostic gap in rural India, and withholding a low-cost triage layer from village populations while urban centers benefit from AI-assisted histopathology is inequity by omission. We argue for a hybrid workflow in which frontline workers apply a standardized dye, capture the stained lesion on a smartphone, and route the image through a cloud-hosted deep-learning classifier to a specialist hub. We defend this position with a pragmatic implementation pathway and a four-step, milestone-driven research agenda covering image repositories, prospective accuracy trials, cost-and-equity metrics, and regulatory-sandbox validation.

Doi: 10.48286/aro.2026.132

Impact statement: This Opinion proposes a pragmatic hybrid approach combining vital staining and artificial intelligence to strengthen early oral cancer triage, enhance diagnostic access, and decrease inequities in resource-limited settings.

Key words: *Artificial intelligence; early diagnosis; hybrid diagnostic model; oral squamous cell carcinoma; oral potentially malignant disorders; vital staining.*

Received: Jun 09, 2026/**Accepted:** Sept 18, 2026

Published: Sept 30, 2026

INTRODUCTION

Consider Kamala, a 52-year-old woman in rural Odisha who has chewed areca-nut-tobacco quids for two decades. A frontline health worker visiting her hamlet notices a white patch on her buccal mucosa, applies toluidine blue, photographs the stained lesion on a smartphone, and uploads it to a cloud-hosted classifier that flags it as high-risk within seconds. Within a week, an oral-medicine specialist at the district hub reviews the image and Kamala is scheduled for biopsy. This is not a technology story. It is an equity story: a triage layer that costs pennies

per image, delivered by workers who already visit her village, is either offered to Kamala or it is not. Oral squamous cell carcinoma (OSCC) is one of the most prevalent malignancies globally and disproportionately burdens South Asia. India recorded 143,759 new lip and oral cavity cancer cases and 79,979 deaths in 2022 – roughly a third of the global burden – with men bearing an age-standardized rate of 15.6 *versus* 5.0 per 100,000 in women (1, 2). That burden is overwhelmingly attributable to modifiable behavioral exposures: smokeless tobacco, areca-nut chewing, bidi smoking and alcohol together account for the great majority of Indian cases (3).

Human papillomavirus (HPV), while carcinogenic at the oropharynx, has neither a strong nor consistent causal association with cancer at the oral cavity subsite in Indian populations (4), which is why India's National Programme for Prevention and Control of Non-Communicable Diseases (NP-NCD, formerly NPCDCS) targets tobacco cessation and opportunistic oral, breast and cervical screening rather than viral prophylaxis; HPV vaccination sits under the Universal Immunisation Programme and is being introduced in phases for cervical cancer prevention (5). The implication for policy is unambiguous: any early-detection strategy for OSCC in India must be built around behavioral risk factors and the visible mucosal lesions they produce, and it must reach the villages in which those exposures occur.

The persistent challenges in early diagnosis stem from limited infrastructure, insufficient trained personnel, and lack of population-wide screening implementation. Even where NP-NCD has been rolled out, rural and underserved populations often lack access to timely screening, specialist consultation, and definitive diagnosis. What is needed is a diagnostic strategy that is cost-effective, scalable, and adaptable to varying health system capacities – one that meets this gap directly with a hybrid workflow combining a low-cost dye-based screen with an AI-assisted classification layer capable of running on a smartphone.

THE EXISTING LANDSCAPE OF OSCC DETECTION

Vital staining methods –Toluidine Blue, Methylene Blue, Lugol's Iodine – have long been used as adjuncts to clinical examination in OSCC detection. Their appeal in LMICs rests on affordability, simplicity and chairside use. They suffer, however, from operator

variability and reduced specificity, generating false positives and, less often, false negatives (6).

AI-assisted image analysis offers the reverse profile: reproducibility and scale when supported by adequate infrastructure. Convolutional neural networks and related deep-learning architectures have demonstrated strong classification performance for oral potentially malignant disorders (OPMDs) and OSCC on clinical, histological and radiographic images (7). Most such systems, however, are confined to academic or urban settings with the technological readiness to support them, while in LMICs – where the majority of OSCC deaths occur – the digital divide continues to hinder frontline deployment. A context-appropriate pairing of the two, rather than a choice between them, is the pragmatic path forward.

A TIERED GLOBAL OVERVIEW: DIAGNOSTIC CHOICES BY HEALTH SYSTEM STRENGTH

Diagnostic choices in OSCC do not diverge across settings by accident; they track health-system capacity in ways that matter for how any new tool is deployed. In high-resource tertiary centers, workflows are anchored by biopsy with histopathology, adjunct optical technologies and, increasingly, AI-assisted histopathology or radiology, in which specificity is prioritized over cost and where a false positive is absorbed as an additional pathology review. In middle-resource settings – urban and semi-urban public hospitals in India among them – clinicians rely on visual and adjunctive examination, opportunistic screening within NP-NCD, and referral pathways whose bottleneck is specialist availability rather than the screening step itself. In resource-limited village and outreach settings, the dominant modality remains visual inspection by non-specialist staff,

Table 1. *The diagnostic capabilities of countries with maximum oral cancer burden.*

COUNTRY TYPE	EXAMPLES (TOP 10 BURDEN)	DIAGNOSTIC PREFERENCE AND RATIONALE
Developed, robust healthcare systems	Japan, France	AI is increasingly adopted due to solid digital infrastructure and insurance-backed health systems.
Developed but inequitable	USA	Feasible AI use but hindered by high costs and disparities in healthcare access.
Rapidly developing	China, Brazil	Hybrid models are emerging with expanding digital literacy and infrastructure.
Resource-limited/developing	India, Bangladesh, Indonesia	Vital staining remains primary due to cost-effectiveness; AI integration is slowly emerging.

supplemented in some programs by vital staining and, where connectivity permits, by smartphone photography. **Table 1** summarizes this landscape. Two features of this comparison bear directly on the position we develop below. First, the diagnostic tools chosen at each tier reflect what is affordable and delegable, not what is most accurate; where health workers rather than clinicians perform the screen, sensitivity and low training burden matter more than headline specificity. Second, the transition between tiers – village screen to district hub to tertiary confirmation – is where preventable delay accumulates in Indian OSCC and where a triage layer, if it is standardized and digitally linked, does the most work per rupee spent. It follows that the design question is not whether to introduce AI, but where in the tiered pathway to place it so that the modality complements rather than displaces the human screen at the village end (8).

VITAL STAINING: STRENGTHS, LIMITATIONS, AND CURRENT EVIDENCE

Standardizing the pre-application decontamination, dye concentration and rinsing sequence, and documenting deviations at the time of image capture, may improve consistency in field-based staining (9, 10). Its utility as a low-cost triage tool in areas lacking biopsy facilities remains uncontested. Clinicians commonly use it to delineate biopsy sites, track lesion progression, or screen large populations during outreach programs. It is safe, requires minimal training, and yields immediate visual feedback – qualities that make it ideal for field-level application. Limitations persist: dysplastic changes may go unstained if the lesion is keratinized.

ARTIFICIAL INTELLIGENCE IN OSCC DETECTION: A NEW FRONTIER

AI applications in dentistry are rapidly evolving. Deep-learning algorithms trained on large image datasets have achieved sensitivity approaching 100% and specificity above 90%, in histopathologic and clinical imaging tasks (7, 11). A 2024 systematic review and meta-analysis by Li and colleagues reported a notably high diagnostic odds ratio for AI-assisted clinical photography, suggesting transformative

potential in early oral cancer detection (11). That pooled estimate, however, carries substantial caveats: recent meta-analyses report between-study heterogeneity of $I^2 > 98\%$ for both sensitivity and specificity, and the majority of primary studies are rated high or unclear risk of bias for patient selection under QUADAS-2, largely because of retrospective designs, convenience sampling and lack of external validation (12). Headline accuracy figures therefore reflect model performance under favorable conditions, not real-world screening performance in an unselected village population.

Given more than 6.5 billion smartphone users globally, even rural clinics can potentially implement AI-assisted imaging with modest resources. Two published pilots make this concrete. Birur and colleagues deployed a smartphone-based oral cancer screening application with frontline health workers across four Indian states from 2010 to 2018, screening 42,754 individuals in door-to-door, workplace and village programs and identifying 5,406 OPMDs, of which 516 were biopsy-confirmed at remote locations (13). In their initial targeted cohort, however, only 61% of images captured by frontline workers were deemed interpretable – a stark reminder that image quality, not model architecture, is the rate-limiting step. Uthoff and colleagues developed a dual-modality (autofluorescence plus white-light) smartphone probe and evaluated it in 190 patients from Indian oral-oncology clinics; the CNN classifier trained on data from 99 patients achieved point-of-care performance suitable for remote diagnosis but again required careful attention to acquisition geometry and lighting (14). These pilots demonstrate that smartphone-AI screening in rural India is feasible; they also show that interpretability rates and calibration drift, not headline AUCs, are the metrics that determine field utility.

AI performance in this domain depends on image quality and standardization, algorithm training across diverse populations, and clinician interpretation and validation. Challenges such as poor lighting, lesion retraction, anatomical complexity and image resolution all affect diagnostic accuracy (15-17), and models developed in homogeneous populations may generalize poorly across ethnic groups or lesion types. **Table 2** compares vital staining and AI across principle, cost, training, sensitivity, specificity and scalability; neither tool is sufficient alone, but synergizing both can improve diagnostic accuracy in both resource-rich and resource-poor environments.

Table 2. Comparison between vital staining and artificial intelligence as adjunct diagnostic tools.

PARAMETER	VITAL STAINING	ARTIFICIAL INTELLIGENCE (AI)
Principle	Dye retention in dysplastic epithelial cells	Pattern recognition from image datasets
Techniques	Toluidine Blue, Lugol's Iodine	CNNs, supervised/unsupervised learning
Application	Chairside adjunct to clinical exam	Image analysis, prediction models
Cost	Very low	High initially, cost reduces with scale
Invasiveness	Non-invasive	Non-invasive
Training needed	Moderate (clinical judgment)	High (AI model development, validation)
Turnaround Time	Immediate	Fast post-deployment
Sensitivity and specificity	Variable across studies; dependent on dataset, imaging modality and validation setting	
Accessibility	High in LMICs	Limited by infrastructure in LMICs
Use in mass screening	Highly feasible	Growing feasibility with mobile AI
Suitability in LMICs	Very high	Currently limited
Scalability	Proven	Uncertain, but promising

Our proposal differs from earlier hybrid concepts in a specific way. Most published integrations treat toluidine-blue uptake as a categorical input to a decision rule (positive stain $\rightarrow$ biopsy) or feed unstained clinical photographs into a CNN. We propose that the stained image itself – the spatial pattern, intensity gradient and border characteristics of dye retention – becomes the CNN input. This preserves the biological signal the dye captures (nucleic-acid-rich dysplastic epithelium) while adding the pattern-recognition capacity of deep learning to distinguish genuine dysplastic uptake from candidal, inflammatory or traumatic false-positive uptake.

CHALLENGES AND THE NEED FOR CLINICAL OVERSIGHT

AI systems face validation, ethical and legal barriers in clinical deployment. Misclassification mistaking benign lesions for malignancies, or *vice versa*, could have severe implications. AI must therefore be integrated as a decision-support tool, not a replacement for clinician judgment. Oral medicine specialists play a pivotal role, from standardized image acquisition to interpreting ambiguous outcomes; centralized repositories and biobanks can improve AI training, and protocols for intraoral photography (lighting, retraction, resolution) can standardize diagnostic inputs (15-18).

Beyond model validation, three governance issues warrant attention. First, data privacy: intraoral images

uploaded from village camps carry facial and identifying features and must be handled under India's Digital Personal Data Protection Act, 2023, with explicit consent for research reuse. Second, maintenance and calibration: classifier drift over time and across handset generations is well documented in real-world medical AI and mandates a scheduled re-validation cadence with locally acquired hold-out data. Third, training and accountability: frontline workers require a defined competency threshold before they upload images, and the clinician of record – usually the oral-medicine specialist at the hub – retains diagnostic responsibility for every flagged case, with the AI output treated as advisory. These governance elements are not optional add-ons; they are the difference between a research pilot and a system that survives public-sector procurement.

IMPLEMENTATION STRATEGY

A decision-tree protocol (**Figure 1**) can be employed in primary care settings. Where the frontline worker observes no dye retention, the patient is placed under routine monitoring, with a biopsy triggered only where clinical suspicion overrides the negative stain. Where dye is retained, a smartphone image of the stained lesion is captured under standardized conditions (fixed working distance, retracted field, ring-lit or flash-lit white balance) and uploaded to the AI classifier. Cases stratified as low-risk return to observation with a follow-up interval of eight

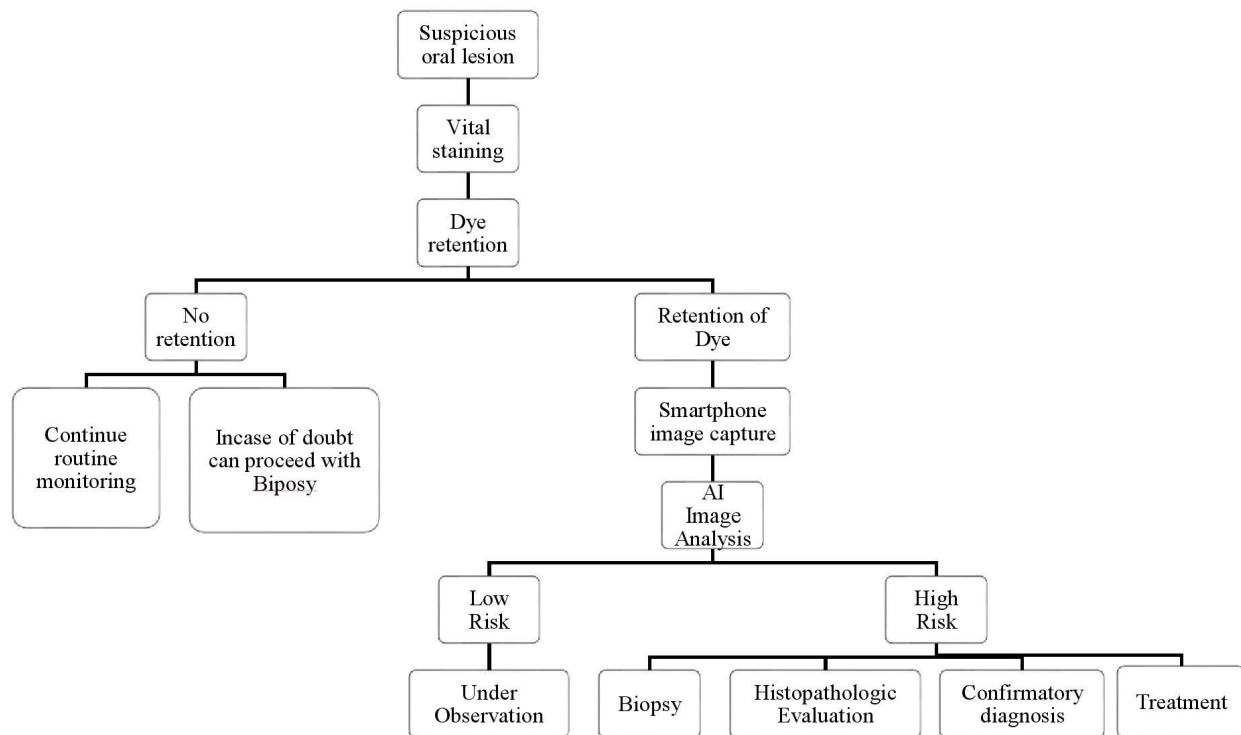


Figure 1. A flow chart describing the workflow of a hybrid model using vital staining integrating artificial intelligence for oral cancer diagnosis.

to twelve weeks; cases stratified as high-risk are referred through the tele-network to the specialist hub for biopsy, histopathology and definitive management.

FUTURE DIRECTIONS: A HYBRID, CONTEXTUALIZED APPROACH

The equity claim opened this Opinion; it also determines what research is worth doing next. If a smartphone classifier that costs pennies per image can shift even 10% of Indian OSCC diagnoses from stage IV to stage II, the mortality reduction dwarfs any conceivable investment in additional specialist infrastructure. That is the arithmetic. What follows from it is a hybrid workflow in which vital staining is the first-line screen at primary health centers and community camps, AI-based imaging is the second-line support that guides referral and remote consultation, and centralized diagnostic hubs, staffed by specialists, deliver review and intervention. Research over the next 24-36 months should follow four sequential, measurable milestones rather than the diffuse partnerships-and-repositories language of ear-

lier commentaries. In months 0-6, curate a population-representative image repository of at least 10,000 paired white-light and toluidine-blue-stained lesion images from at least four Indian states, with structured metadata (age, sex, tobacco and areca history, lesion site, histopathology ground truth, staining protocol used) following FAIR data-sharing standards. In months 6-18, conduct prospective, pre-registered diagnostic-accuracy trials enrolling at least 2,000 community-screened participants in a multi-center STARD-compliant study, with pre-specified classifier operating threshold, primary sensitivity and specificity endpoints, and subgroup analyses by state, sex and lesion site. In months 12-30, embed cost-effectiveness and equity metrics: incremental cost per OPMD detected, per stage-shift achieved and per disability-adjusted life-year averted, with equity reported as the ratio of rural-to-urban uptake, the reduction in the proportion of OSCC diagnosed at stage III/IV, and the time from screen-positive to biopsy result. In months 24-36, work with the Central Drugs Standard Control Organization and state health authorities to pilot the classifier as a software-as-a-medical-device under a phased regulatory sandbox, with periodic external audits. None of

this is speculative; each step has an owner, a time-frame and a measurable output.

CONCLUSIONS

No single diagnostic strategy will close the OSCC gap in India. Vital staining is indispensable for its simplicity and cost; AI adds precision and reproducibility. Together they can bridge the distance between advanced urban hospitals and resource-deprived rural clinics. The next step is not another position paper, but a coordinated four-step research program – image repository, prospective diagnostic-accuracy trial, cost-and-equity metrics, and regulatory sandbox – that converts the hybrid concept into evidence a district hospital can act on.

COMPLIANCE WITH ETHICAL STANDARDS

Funding

None.

Conflicts of interest

The authors declare no competing interests.

Availability of data and materials

N/A.

Authors' contributions

AS: conceptualization. RS, SRM: writing – original draft, writing, review & editing.

Publications ethics

Plagiarism

We hereby declare that this manuscript has not been published or submitted for publication elsewhere. All appropriate references have been cited wherever required. This manuscript does not contain plagiarism.

Data falsification and fabrication

The authors declare that no data fabrication, falsification, or manipulation has been carried out in the preparation of this work. Generative AI was used only for language editing and formatting checks; all scientific content, interpretation, and references were drafted, verified and approved by the authors.

REFERENCES

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2024;74(3):229-263. doi: 10.3322/caac.21834.
2. Francis DL, Reddy SSP. Oral cancer burden and mortality in India: a GLOBOCAN 2022 analysis with regional comparison. *ecancermedicalsecience.* 2026;20:2162. doi: 10.3332/ecancer.2026.2162.
3. Gupta B, Johnson NW, Kumar N. Global Epidemiology of Head and Neck Cancers: A Continuing Challenge. *Oncology.* 2016;91(1):13-23. doi: 10.1159/000446117.
4. Bouvard V, Nethan ST, Singh D, Warnakulasuriya S, Mehrotra R, Chaturvedi AK, et al. IARC perspective on oral cancer prevention. *N Engl J Med.* 2022;387(21):1999-2005. doi: 10.1056/NEJMs2210097.
5. Ministry of Health and Family Welfare, Government of India. Operational framework: management of common cancers under the National Programme for Prevention and Control of Non-Communicable Diseases (NP-NCD). New Delhi: MoHFW; 2023.
6. Walsh T, Macey R, Kerr AR, Lingen MW, Ogden GR, Warnakulasuriya S. Diagnostic tests for oral cancer and potentially malignant disorders in patients presenting with clinically evident lesions. *Cochrane Database Syst Rev.* 2021;7(7):CD010276. doi: 10.1002/14651858.CD010276.pub3.
7. Vijayalakshmi R, Selvaraj P, Alagarsamy R, Rajendran S, Suresh Babu K. Artificial intelligence in the early detection of oral cancer. *Oral Oncol.* 2022;126:105751.
8. Misra SR, Das R. Early discovery, early recovery: the principle behind successful oral squamous cell carcinoma treatment! *Oral Oncol.* 2023;145:106511.
9. Chaudhry A, Manjunath M, Ashwatappa D, Krishna S, Krishna AG. Comparison of chemiluminescence and toluidine blue in the diagnosis of dysplasia in leukoplakia: a cross-sectional study. *J Invest Clin Dent.* 2016;7(2):132-40. doi: 10.1111/jicd.12141.
10. Nagi R, Reddy-Kantharaj YB, Rakesh N, Janardhan-Reddy S, Sahu S. Efficacy of light based detection systems for early detection of oral cancer and oral potentially malignant disorders: Sys-

- tematic review. *Med Oral Patol Oral Cir Bucal*. 2016;21(4):e447-55. doi: 10.4317/medoral.21104.
11. Li J, Kot WY, McGrath CP, Chan BWA, Ho JWK, Zheng LW. Diagnostic accuracy of artificial intelligence assisted clinical imaging in the detection of oral potentially malignant disorders and oral cancer: a systematic review and meta-analysis. *Int J Surg*. 2024;110(8):5034-5046. doi: 10.1097/JS9.0000000000001469.
 12. Sahoo RK, Sahoo KC, Dash GC, Kumar G, Baliarsingh SK, Panda B, et al. Diagnostic performance of artificial intelligence in detecting oral potentially malignant disorders and oral cancer using medical diagnostic imaging: a systematic review and meta-analysis. *Front Oral Health*. 2024;5:1494867. doi: 10.3389/froh.2024.1494867.
 13. Birur NP, Patrick S, Bajaj S, Raghavan S, Suresh A, Sunny SP, et al. A Novel Mobile Health Approach to Early Diagnosis of Oral Cancer. *J Contemp Dent Pract*. 2018;19(9):1122-1128.
 14. Uthoff RD, Song B, Sunny S, Patrick S, Suresh A, Kolar T, et al. Point-of-care, smartphone-based, dual-modality, dual-view, oral cancer screening device with neural network classification for low-resource communities. *PLoS One*. 2018;13(12):e0207493. doi: 10.1371/journal.pone.0207493.
 15. Misra SR, Das R. Artificial intelligence in oral oncology: overcoming the challenges in diagnosis! *Oral Oncol*. 2022;135:106187.
 16. Das R, Misra SR. A proposal to establish a biorepository/biobank for research in oral oncology. *Oral Oncol*. 2022;134:106136.
 17. Misra SR, Das R. Central oral medicine repository: a proposal to speed up the diagnostic process for oral potentially malignant disorders and oral carcinoma! *Oral Oncol*. 2022;132:105998.
 18. Misra SR, Das R. Tele-screening for early detection of oral cancer during the COVID-19 pandemic era: diagnostic pitfalls and potential misinterpretations! *Oral Oncol*. 2022;128:105845.

