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EDITORIAL

CYTOLOGICAL SPECIMENS IN THE MOLECULAR ERA OF METASTATIC MELANOMA: FROM DIAGNOSTIC ALTERNATIVE TO PRECISION TOOL

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With the increasing adoption of targeted therapies and immunotherapies, molecular profiling, particularly the assessment of BRAF, NRAS, and KIT mutations, has become integral to therapeutic decision-making in metastatic melanoma.

Current ESMO and ASCO guidelines do not provide explicit, stand-alone recommendations on the use of cytology for the diagnosis of suspected melanoma metastases, although it is widely acknowledged in clinical practice as a minimally invasive diagnostic tool. Both societies emphasize the need for adequate tissue sampling to confirm metastatic disease and to enable comprehensive biomarker testing. In particular, ASCO highlights the importance of obtaining histologic material – preferably through core needle biopsy – when molecular profiling is required to guide systemic therapy. Nevertheless, in selected clinical scenarios, we underline that essential molecular testing, especially for BRAF V600E, may be reliably performed on cytological material and, when positive, can be sufficient to promptly initiate targeted therapy (1, 2).

Indeed, Fine-Needle Aspiration Cytology (FNAC) is tolerated well in these environments and often gives diagnostic biomaterial. Therefore, cytological samples should satisfy the morphological and molecular factors to be clinically applicable (3). Cytological samples, despite being characterized by low cellularity, variable fixation, and the potential for nucleic acid degradation, have been shown to perform as well as histologically determined samples in detecting major molecular alterations when proper pre-analytical and analytical steps are taken (4, 5). The recent technology of NGS and techniques for higher-resolution DNA/RNA extraction have paved the way for cytological samples as a gold standard for molecular diagnosis, thus underscoring that cytopathology has played a key role for precision oncology (6-8). Examples of cytological samples applicable to molecular analysis include direct smear, cell block, needle rinses, and liquid-based methods. Air-dried and alcohol-fixed direct smears provide high-quality nucleic acids and have the advantage of selecting tissues rich in tumor via microdissection (3). Cell blocks (CB), derived from residual material, resemble formalin-fixed paraffin-embedded (FFPE) tissue, thus enabling their compatibility with immunocytochemistry (ICC) and many other ancillary assays (3). Recent improvements in cell block (CB) preparation (*e.g.*, introduction of CytoMatrix) can provide better preservation of cells and yields of nucleic acids (6), allowing to preserve excellent cytomorphological integrity while promoting molecular suitability. To maximize the yield from small tumoral cells samples, the proper disposition of cytologic biomaterial for both smears and ICC and molecular tests is cru-

cial (3). Tumor cellularity impacts sensitivity to mutation detection greatly. In particular, a greater than 20% tumor fraction is often essential for reproducible polymerase chain reaction (PCR) or NGS-based assays (7). Fixation appears to be more important; indeed, alcohol-fixed or air-dried smears retain nucleic acids more effectively than formalin-fixed cytological biomaterial, but formalin-fixed cytological biomaterial is required for ICC assay (8). The inclusion of the needle rinses is a pragmatic means of obtaining additional tumor material for molecular tests without compromising the quality of cytological confirmation (9). It has been previously established in some studies that cytological and histological samples are highly congruent for BRAF mutation testing. Both Sanger sequencing and real-time PCR have > 95% concordance when detecting BRAF V600E in paired samples (5, 10). For example, mutation-specific immunocytochemistry for the detection of BRAF V600E with the VE1 clone antibody is being used for rapid, low-cost and inexpensive assays used in cell block section (10). These tests seem particularly useful when swift therapeutic decision-making is required or when molecular laboratory access is limited (10). Likewise, NGS to cytological samples has been successfully performed (7, 8), with superimposable results applied to those derived from FFPE tissue. In the case of molecular melanoma, therefore, these results indicate that in managed by validated workflow cytology is also sufficient and usually best (3). Still, some problems persist, despite this progress. The primary limiting factor is low tumor cellularity or tumor cells are distributed heterogeneously, which results in false negative diagnosis (3). The inhibition of DNA extraction and ICC analysis and subsequent adjustments, such as bleaching or image processing, may occur by melanin pigment (3). However, cytopathology has its advantages in relation to histopathology. Indeed, FNACs permit repeat minimally invasive sampling of metastatic sites. Cytological sampling reduces complications due to adverse effects and is generally well tolerated with the appropriate diagnostic and predictive quality (3). Integration of digital cytology with AI-based analysis can also tie cytomorphologic patterns to molecular profiles to predict mutational status and treatment response directly from the process of scanning slides (3). Cytology is thus rapidly developing into a powerful and real-time tool of precision oncology in a practical way (3). For optimal optimization of these for cytological samples for molecular applications, close collaborations within our interdisciplinary work are necessary. Close discussions between cytopathologists, oncologists, and molecular laboratories allow for the most efficient triage and selecting test in a laboratory setting (3, 10). It also means that our laboratories will find that their own laboratories can provide timely and appropriate sample selection (3, 10). Specifically, reflex testing protocols could have been established to auto-submission for BRAF or NGS analysis of cytology-confirmed melanoma metastases, substantially reducing turnaround times. To make such predictive evaluations available (*e.g.*, PDL1 detection) (10, 11), laboratories should define standard adequacy thresholds, enact protocols for non-destructive nucleic acid extraction, and establish storage practices that maintain biomaterial integrity. The reporting should be open to the public, accounting for the type of sample used, an estimated tumor fraction, and analytic sensitivity, highlighting the credibility and interpretive limitations of the analysis for clinicians (3, 10).

The major limitation of cytology in metastatic melanoma lies in predictive immunohistochemistry, particularly for PD-L1 detection, due to the very low clinically significant cut-off. While PD-1/PD-L1 testing is now standard, emerging immune-checkpoint biomarkers such as LAG-3 and TIGIT are under investigation with promising early results, but their evaluation often requires preserved tissue architecture, making cytology alone generally insufficient. Current ESMO and ASCO guidelines recommend adequate tissue sampling – typically via core needle biopsy – for histologic confirmation and molecular testing, although essential biomarkers like BRAF V600 can, in selected cases, be reliably assessed on cytological material to guide targeted therapy (1). In addition, BRAF testing on cytological specimens is a rapid and minimally invasive strategy for therapeutic stratification; however, most routine assays are primarily designed to detect canonical p.V600E mutations. Consequently, non-canonical class II/III variants or rare alterations may be missed, despite their potential clinical relevance and eligibility for targeted-therapy trials. In selected clinical contexts, negative cytology-based results should be confirmed with a second level testing, such as NGS, or alternatively because of lack of a significant number of neoplastic cells the core biopsy is needed (12).

In conclusion, cytology represents a valuable and pragmatic alternative to conventional biopsy with histological examination, offering minimal invasiveness, rapid turnaround time, and the possibility of repeated sampling. Nonetheless, its limitations

include reduced architectural assessment and, critically, limited tumor cellularity, which may constrain broad molecular profiling (*e.g.*, NGS) and the application of predictive immunohistochemistry. This is particularly relevant for PD-L1 assessment, whose evaluation may be challenging on scant material and is of specific clinical interest in metastatic melanoma. Beyond diagnosis, cytology remains pivotal in the assessment of suspicious lymph node metastases for staging purposes, enabling timely therapeutic stratification and potential access to neoadjuvant immunotherapy strategies associated with significant pathological responses and improved outcomes in selected patients.

FUTURE PERSPECTIVES

The future of cytology in molecular diagnostics is promising. Ongoing advances in low-input sequencing technologies, multiplex PCR, and microfluidic DNA extraction methods are making cytology-based molecular testing increasingly feasible and reliable. Expanded gene panels using compact platforms, as well as RNA fusion detection, can now be successfully performed even on limited material, thereby broadening the genomic scope assessable on cytological specimens.

Beyond DNA analysis, transcriptomic and proteomic approaches are progressively being applied to cytological preparations, offering the potential to integrate cytomorphology with functional molecular data. Artificial intelligence tools may further strengthen this integration by predicting molecular alterations directly from digital images and optimizing sample selection for downstream assays (1-8).

In conclusion, cytological specimens have evolved from a purely diagnostic tool to an integral component of the molecular diagnostic armamentarium in metastatic melanoma. They reflect the principle of obtaining maximal clinically relevant information through minimally invasive procedures. Within a well-coordinated multidisciplinary framework, current evidence supports cytology as a robust and reproducible substrate for molecular testing. Continued progress will depend on harmonized workflows, standardized quality assurance protocols, and expert-driven consensus guidelines for cytological material in melanoma. The formal validation of cytology as a molecular diagnostic platform will enhance precision oncology and facilitate timely, patient-centered testing in routine clinical practice.

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REVIEW

BEYOND TARGETED THERAPY: ENVIRONMENTAL DETERMINANTS AND METABOLIC STRATEGIES IN CANCER PREVENTION AND TREATMENT

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ABSTRACT: Despite significant advances in cancer care, cancer remains the second leading cause of death in the USA. For a cost-effective initiative to decrease cancer incidence, we argue in this review that an understanding of modifiable environmental factors in cancer and risk mitigation strategies should come first, as a population health approach to cancer. Additionally, this review also motivates the development of broad spectrum metabolic approaches to cancer, which may be effective over a broad array of cancers, instead of current antineoplastic agents, which are targeted against a small subset of cancers. By using a preventative approach, as well as utilizing low cost and broad spectrum therapeutic agents, it may be possible to improve cancer outcomes without a significant increase in cost. This review provides a roadmap for environmental risk mitigation as well as adjunctive, broad spectrum therapeutics.

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Impact statement: This review assesses the feasibility of generalist approaches to cancer care, which may be helpful to a broad swathe of the population.

Key words: *Cancer; metabolic therapy; environmental factors; warburg effect; ketogenic diet.*

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INTRODUCTION

While the burden of preventable disease declines with advances in sanitation, public health, and economic development, cancer remains a major global challenge, currently accounting for about 0.55% of global GDP in expenditures (1). Cancer incidence is influenced by several factors, environmental factors are associated with cancer incidence and may explain some of the trends in cancer (2).

Most prosaically, mortality from other diseases is dropping, and so as lifespan increases, people are more prone to get cancer, as cancer's incidence rises with age(3). Early stage screening has also contributed to lowered cancer mortality (4, 5), and treatments for some specialized cancers have decreased the overall mortality burden of cancer (6).

While most cancers are due to mutations acquired over the lifespan, inherited genetic factors play a significant role in cancer development in a proportion of total cancers. It is estimated that between 5 and 10% of all cancers are associated with an inherited mutation (7). The wider prevalence of genetic testing may inform people of their cancer risk predisposition, which may provide actionable information in specific cases (8).

Diet is a significant factor in cancer incidence (9). Ultra processed food (UPF) consumption is associated with elevated cancer risk (10). There are multiple pathways by which ultra processed foods can contribute to cancer incidence (11), though broadly, these are high in calories (12) and unhealthy fats (13), have low nutrient content (14), and may contain additives including preservatives (15) and emulsifi-

ers (16). The increased palatability of UPFs makes it easier for people to overeat (17), which can contribute to metabolic conditions and obesity (18), increasing cancer risk (19).

Besides micronutrients, UPFs also are lower in phytonutrients (20), and regular consumption has negative impacts on the gut microbiota (21), as UPFs are typically lower in fiber (22). Micronutrients (23), phytonutrients (24), probiotics (25), and prebiotic fiber (26) demonstrate anticancer associations in epidemiological studies. Micronutrient consumption is associated with overall health (27), and can be important in the context of cancer. Dietary nutrient density per calorie is inversely associated with cancer risk (28).

Packaging (29) and additives represent another dimension in which UPFs differ from unprocessed foods. Additives, while not a significant source of nutrition, being present in small amounts, may have health effects, though any toxicity typically manifests at consumption levels far above what consumers would be exposed to. Producers include additives to alter the texture, appearance, material properties, or extend the shelf life of a food product. EU regulation defines 27 categories of food additives, including sweeteners, flavor enhancers, emulsifiers, colorants, preservatives, and stabilizers (30) (**Supplementary Table 1**). While it is difficult to characterize the toxicological profile of the wide variety of food additives (The Food and Feed Informa-

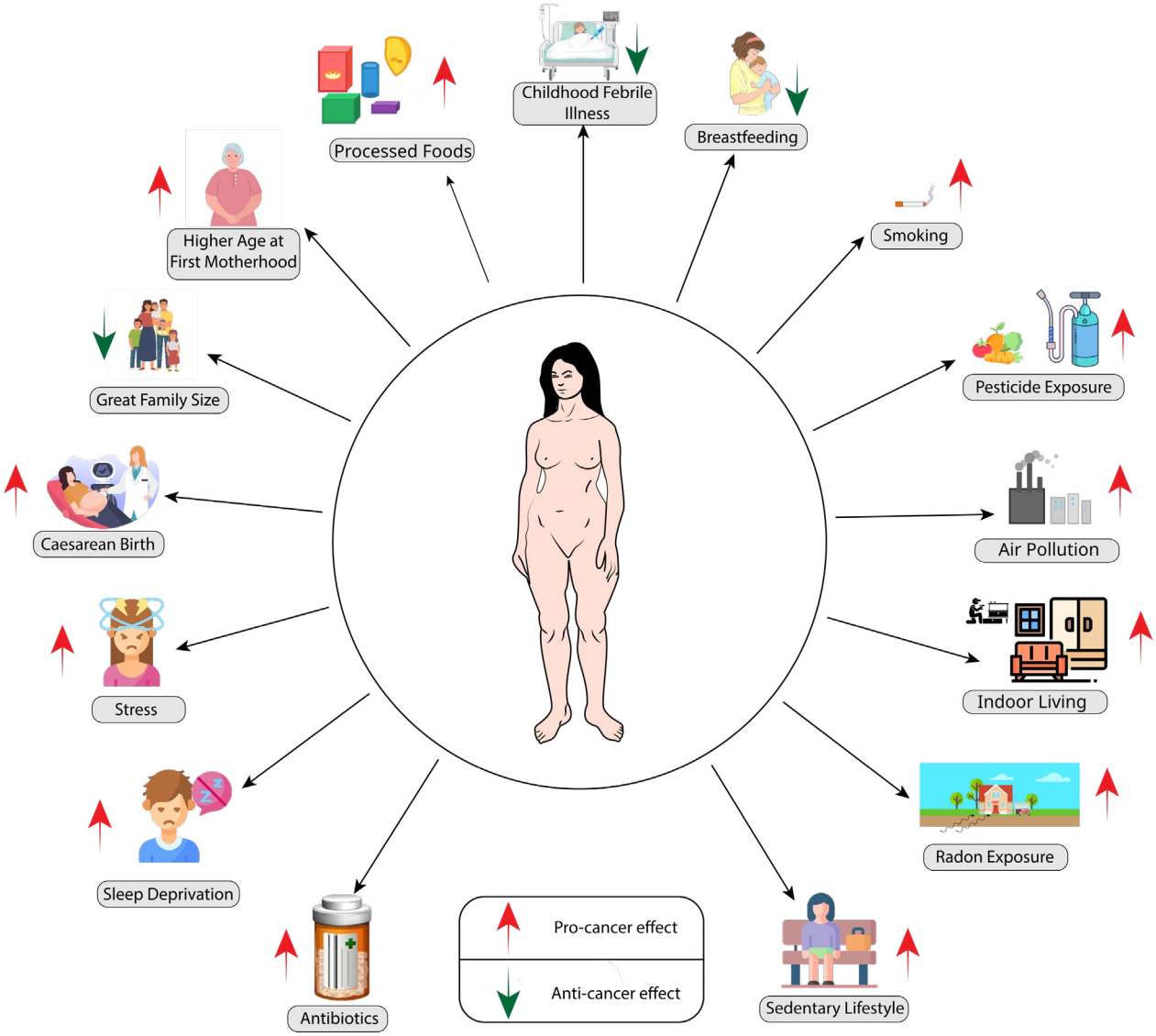

Figure 1. *Epidemiological factors and their impact on cancer incidence.*
Green downward arrows mean that the factor is associated with lower rates of cancer, whereas upward red arrows mean that the factor is associated with an increased risk of cancer.

tion Portal of the European Commission lists 412 unique food additives in its Version 5.6.0 Database, accessed February 11, 2026) (31) in the limited space of this review, it is important to note that some additives have been regulated or face restrictions owing to observed toxicity (32), sometimes in the form of carcinogenicity (33), albeit typically out of an abundance of caution.

Beyond ultra processed food, even whole foods contain pesticide residues and have lower nutrient densities than their counterparts of a few decades past, and are novel on the time scale of human evolution (34).

Beyond diet, environmental exposures may mediate cancer risk (35); these are outlined in **Supplementary Table 2** and **Figure 1** of this article.

Lifestyle factors

Physical activity, sleep, and stress

Modern humans spend most of their life indoors and increasingly live sedentary lives, as more people are employed in the information economy and spend most of their working day using a computer (36). While increasingly, standing desks and even treadmill desks are being used (37), still, the levels of physical activity have decreased. Physical activity has many anti-cancer effects, and exercise is associated with a lowered risk of cancer development (38).

Poor sleep quality and short sleep duration are another contributor to cancer and ill health in general (39). Devices and lights can disrupt sleep and artificial light at night (ALAN) can disrupt melatonin production and result in poorer sleep quality and duration (40).

Chronic stress is another contributor towards lower quality of life and may play a role in cancer progression (41). These factors are also interacting, as stress (42) and poor sleep (43) can induce cravings for sugary food.

Microbiome

A healthy and diverse gut microbiome is associated with a lower cancer risk, and perturbations are associated with the cancer phenotype (44). Several factors have altered human gut bacterial composition over millennia, including a lower exposure to microbes in the environment, greater hygiene, and the increased use of antibiotics and birthing via caesarean section (45).

The number of antibiotic prescriptions has declined in recent years (46, 47). Antibiotics necessarily depopulate the gut microbiome, and can lead to dysbiosis (48, 49). Among other conditions (50, 51), antibiotic use is associated with an increased rate of colorectal cancer (52) and other cancers (53).

Family size

Children born into larger families are less likely to develop allergies, which can serve as a proxy for gut health (54). Number of older siblings is negatively associated with risk of Hodgkin's Lymphoma (55), acute monocytic leukemia and childhood acute lymphoblastic leukemia (56). However, this benefit accrues to the younger siblings, and having four or more total siblings was associated with greater rates of multiple myeloma, acute monocytic leukemia and childhood acute lymphoblastic leukemia (56). Interestingly, number of siblings was positively associated with gastric cancer risk (57). Childhood febrile illness is also associated with a lower risk of cancer later in life (58).

Changing reproductive norms

When babies are born vaginally, they are coated with the vaginal fluid of the mother, which provides colonization for the baby's microbiome (59); without this initial influence, gut dysbiosis in the newborn can occur (60, 61). It is observed that children born via cesarean section have higher rates of autoimmune disorders (62), allergies (63, 64), respiratory diseases (65), and cancers (66-68).

Another reproductive factor, the increasing age at first birth (69), can have an impact on breast cancer risk, as women who are under 25 when their first baby is born have a 35% reduction in their breast cancer risk (70). The use of hormonal birth control is also associated with a higher risk of breast cancer (71), and hormonal birth control is ubiquitous, with one in four US women aged 15-44 using oral contraceptives in a survey between 2011 and 2013 (72).

Environmental exposures

Pesticides

Two developments mark distinct turning points in agricultural technology, the development of the Haber-Bosch process in the early 1910s for producing fertilizer, and the production of synthetic pesticides in industrial quantities, enabling agricultural production on a much larger scale. While some synthetic chemical pesticides were used in the 19th centuries, production and use reached an

inflection point in the 1940's (73). Before the widespread use of pesticides, much of agriculture would be considered organic by today's standards (74), as synthetic fertilizers and pesticides were not widely available.

These developments are important for two reasons, as synthetic fertilizers have contributed to soil micronutrient loss (75), and conventional farming practices can contribute to lower nutrient levels when compared to organically grown foods (76). Secondly, exposure to synthetic pesticides is carcinogenic in many cases (77-79). Other chemical applications may also have negative impacts on health, such as glyphosate used as a wheat desiccant and in the harvesting process (80).

Synthetic pesticide uses also saw another inflection point in the 1990's, with the development of roundup ready soybeans and corn, for use with the pesticide glyphosate (81). Glyphosate was first commercialized in 1974 and worldwide consumption has increased drastically from 56 thousand tons in 1994 to 825 thousand tons in 2014, an almost 15 fold increase (82). The genetic modification process involved inserting a gene for glyphosate resistance, found in a species of plants, into the genomes of soybeans, corn and cotton to enable glyphosate resistance (83). This allowed 'Roundup-Ready', or glyphosate resistant crops to tolerate glyphosate, and the surrounding weeds, lacking the resistance gene to glyphosate, would perish (84).

Unfortunately, glyphosate resistant weeds have emerged (85), and so other strategies are being employed, including using multiple pesticides in combination. With regards to the former approach, multi-pesticide resistant weeds have also emerged, and an arms race is in progress between weeds and pesticides (86).

Meta-analyses have found occupational exposure to glyphosate is associated with an increased risk of non-Hodgkin's Lymphoma (NHL) (87, 88), though this may be confounded by the co-presence of other pesticides (87) or socioeconomic factors (89).

Built environment

Materials used in the built environment have carcinogenic potential (90). As people spend the majority of their lives indoors, exposure to building materials is more significant, including natural radiation (91). Radon contamination from the natural environment is common in residential buildings, and high radon exposure is associated with a higher risk of lung cancer (92).

Air pollution is associated with higher rates of lung cancer (93), and it is estimated that globally, air pollution is responsible for hundreds of thousands of lung cancer cases annually (94).

CHANGING PARADIGMS IN CANCER TREATMENT

Oncology has been characterized by an implicit somatic mutation theory of cancer, whereby mutations in precancerous cells result in the deactivation of the hallmarks of normal cells. The hallmarks of cancer cells have been described in an oft-cited review by Hanahan and Weinberg in 2000, where cancer has the following characteristics (95):

1. Sustaining proliferative signaling
2. Evading growth suppressors
3. Resisting cell death (apoptosis)
4. Enabling replicative immortality
5. Inducing angiogenesis
6. Activating invasion and metastasis

These hallmarks were amended in 2011 to add two additional general hallmarks: reprogramming of energy metabolism and evading immune destruction (96). Metabolic reprogramming refers to the switch from oxidative phosphorylation method of energy production to fermentation, even in the presence of oxygen, a phenomenon known as the Warburg effect (97). The metabolic switch is not unique to cancer, as it has been observed in rapidly dividing embryonic tissue.

This framework has played a role in the comprehension of how numerous tumors assist in quick proliferation and biosynthesis, though it must be critically understood that not all tumor types, stages, and microenvironments necessitate uniform metabolic dependencies (98, 99). Practically, tumors can be highly heterogeneous with regard to metabolism and may alternate between glycolytic and oxidative phosphorylation, as well as alter their state in response to oxygen and nutrient levels (97).

From a cellular energetics perspective, fermentation is an inefficient process, producing 2 ATP per molecule of glucose as opposed to the 38 ATP of the standard oxidative phosphorylation glycolytic pathway (100). Intense exertion can create transient hypoxia within the cell, and the cell can briefly switch to fermentation while lifting a heavy load or other intense exercises (101, 102).

This increased fermentation of glucose increases levels of lactic acid, a byproduct of fermentation, in the tumor microenvironment. This lowers the pH in the extracellular environment and further potentiates the evolution of the surrounding cells to be metastatic, to escape the acidic conditions (103). This increased acidity also decreases the structural integrity of the extracellular matrix holding the cells together, further contributing to metastasis (104).

Given that cancer cells preferentially consume glucose, several researchers have investigated metabolic approaches to differentially target cancer cells, without adversely impacting healthy cells. The metric used is typically the selectivity ration, or the ratio of the inhibitory concentration (to kill 50% of cells in a given sample) in cancer cells to that of normal cells (105).

Lower selectivity results in more toxicity for the patient, though drug selectivity can be improved via sensitizing approaches, which make the cell more vulnerable to a stressor, such as radiotherapy, reactive oxygen species, or chemotherapy. Meanwhile, tumor metabolism also should not be over-simplified into the idea that the deprivation of glucose will always effectively starve tumors and leave normal tissue unaffected, as cancers may evade glucose depletion (106, 107). Even though cells under normal conditions tend to have preserved metabolic flexibility, tumors themselves tend to develop and evolve in a nutrient-restricted microenvironment, including, but not limited to, comparatively glucose-poor regions (108). This leads to the tumor cells being able to develop compensatory survival mechanisms, including more intensive use of alternative substrates, adaptive stress programs including nutrient scavenging behavior (*e.g.*, macropinocytosis), cell recycling pathways (*e.g.*, autophagy) (109), and, under certain conditions, consumption by other cells (*e.g.*, cannibalism-like feeding) (110). As such, glucose restriction should be portrayed as a context-dependent intervention as opposed to a universally selective or universally cytotoxic intervention, and any assertion of extraordinarily high selectivity must be viewed with a degree of caution and must also be correlated to tumor type, microenvironmental conditions, and other stressors (111). Even normal cell functioning can be enhanced during low-glucose conditions (112).

Multidrug combinations have enabled oncologists to lower the doses of drugs in some circumstances, resulting in a better overall toxicity profile for the patient. While this approach lessens the likelihood of drug resistance (113, 114), multidrug resistance is a common occurrence, and cancers can re-emerge after treatment (115).

Drug resistance is a challenge in oncology, which can be addressed via multidrug cocktails. These combinations of anticancer agents attack the cancer cell by multiple pathways such that it is less likely to survive and develop resistance (113, 114). Additionally, reductions in tumor size must be treated with caution, as while some of the pathological impacts of the tumor may be ameliorated, cancer stem cells remain (116).

Besides selectivity, another consideration is how broadly can a therapeutic strategy be used. Though it is true that more aerobic glycolysis is widespread in most cancers (117), it has some significant exceptions and conditions when other metabolic programs outcompete aerobic glycolysis (118). Clinical trials underway are included in **Supplementary Table 3**. For the fourteen novel chemotherapeutic agents approved in 2024, ten are usable for less than 2% of cancers, based on target indication. Metabolic approaches, by comparison, may be applicable to a broader range of cancers, given the ubiquity of metabolic reprogramming in cancer cells (**Supplementary Table 4** and **Supplementary Figure 2**) (119). In practice, ketogenic diets may not be applicable to a most cancer cases, while most preclinical studies indicate an antitumor effect, 8% of preclinical studies of the ketogenic diet indicated a protumor effect (120).

TREATMENT USING METABOLIC APPROACHES

In the last ten years, there has been a surge of clinical research on metabolic therapies for cancer. Nevertheless, the general clinical evidence is still heterogeneous and is often limited by small sample sizes, inconsistent definitions of diets, divergence in adherence, and brief intervention periods. Although there are studies that document positive metabolic responses and indicators of clinical benefit in specific settings, oncology programs have not yet determined any consistent changes in tumor outcomes in various tumors, and encouraging results in a particular cancer type need to be validated in larger, sufficiently powered trials (121). Accounts of individual responses of remarkable difference among the patients following through with dietary regimes must therefore be viewed as hypothesis-generat-

ing as opposed to a conclusive indication of overall efficacy (122). Dietary interventions tend to be less expensive than most specific pharmacological treatments, and are commonly thought to have fewer acute toxic side effects, although this benefit must be balanced against unknown risks in the long term, and in active cancer therapy (123).

The metabolic interventions are usually characterized by high levels of carbohydrate restriction to achieve a ketosis condition, whereby the body starts to depend on ketones as the major energy source instead of glucose (124). Metabolic adaptation to ketosis occurs differently in different individuals depending on their initial dietary practices and clinical conditions. Fasting can also induce ketosis (125), and exogenous ketones, including beta-hydroxybutyrate, can also help hasten the process (126). Notably, achieving and maintaining therapeutic ketosis may be difficult in populations with oncology, specifically, during chemotherapy, radiotherapy, and periods of low intake.

At the early stage of adaptation, one could also have a lowered energy level, mainly because the glycolytic pathways are suppressed (127). This period of adaptation is not fixed, and occasionally, patients may drop the dietary regimen because of initial discomfort (128). Exogenous ketone use could help reduce the side effects of the initial phase, thereby promoting adherence (129). However, due to the nature of the majority of cancer trials, which evaluate ketogenic diets in rather short periods, the long-term metabolic, cardiovascular, renal, hepatic, and endocrine effects have not been well defined in cancer patients, and research gap for further research to close.

Given that 40-80% of cancer patients experience malnutrition (130), nutritional support should prioritize correction of documented deficiencies and maintenance of lean body mass under professional supervision (131). High-protein diets, albeit non-ketogenic, are effective at maintaining lean mass (132), while conflicting results show ketogenic diets alleviating (133) or worsening cachexia (134) in preclinical models. This variability in the clinical setting supports the necessity of close patient selection and monitoring, especially in patients with a loss of weight, frailty, sarcopenia, or oral intake restriction. In this context, the use of metabolism-directed supplements and repurposed drugs can be considered as supplements (135, 136).

Efficient decreasing of blood glucose is pertinent in the induction of ketosis and metabolic modulation techniques. It must be noted, though, that cancer metabolism is heterogeneous, and the therapeutic utility of glucose-lowering as an antitumor modality has not always been shown across different cancers in trials and has not been uniformly found useful in cancer therapy. Additionally, sustained carbohydrate restriction can pose certain risks, especially concerning oncology patients, such as the unknown long-term cardiovascular consequences and the lack of information regarding its renal, endocrine, and hepatic outcomes when used in the long term. Thus, any glucose-reducing plan must be introduced as investigational, preferably carried out under medical control and in a structured regimen (135). Drugs like berberine and re-purposed pharmaceutical metformin have shown effectiveness in decreasing blood glucose and enhancing glycemic control (137).

Repurposed drugs

Drug repurposing should be viewed within the context of drug development wherein 22% of drug development failures are due to drug toxicity (138), high attrition rates (>90%) hamper new drug development (139), with many failures happening in late stage preclinical or clinical testing, at a very high cost (140). In addition, the average antineoplastic agent takes 6.9 years to undergo clinical trials, and an extra 0.7 years for approval (141).

Given that the average cost of advancing drug to the market is ~$1.1 billion (142), early prediction of failure due to toxicity is vital. From 1990□2010, 133 drugs were pulled off the market due to safety reasons. The most notable examples were Vioxx from Merck & Co., Inc. (143) and Bextra from Pfizer Inc. (144) after cardiotoxicity was discovered (145). Furthermore, legal costs associated with Vioxx lawsuits reached almost $5 billion (146).

Identifying toxicity issues is an emerging market; the predictive toxicology market is growing at a compounded annual growth rate (CAGR) of 15% (2012) (147). This growth is based on the understanding that improved predictive toxicology tools may save millions of dollars in drug development costs (148). It should be noted that repurposed drugs largely obviate this issue of toxicity emerging later in development, as there is significant safety data available. Drug repurposing can be accelerated and optimized using computational prediction methods which are rapidly becoming highly reliable (149-152). This is typically followed by experimental and clinical validation of *in silico* data (153). Combinatorial chemistry

(154) and high throughput screening (HTS) quickly produce many therapeutic drug candidates, and *in silico* tools are becoming more accepted for rapidly selecting molecules for further development (155). The adoption of *in silico* methods allows for significant cost and time savings in both drug development and drug repurposing (156). The FDA has recently been discussing the development of computational toxicology platforms as part of safety assessment measures (157).

The advantages of drug repurposing are first, that it is an already available and known drug. As the estimated cost for new cancer drugs is on the order of hundreds of millions (often wrongly quoted as billions to justify exorbitant costs), significant cost savings can already be achieved by re-using already used drugs as opposed to bringing new drugs to market, which involves a long regulatory process and establishing manufacturing centers.

Repurposed drugs also have a known safety profile, which can aid in the cost-benefit analysis to use these drugs. While pre-licensing trials are performed for new cancer drugs, often they exhibit significant toxicity (158). While in many instances this tradeoff can be reasonable, still, a well-known side-effect profile is preferred. A safety signal takes a median duration 11.5 months to manifest and be detected after approval, and the median time to action following signal detection is another 21 months (159). Therefore, it takes almost 3 years for regulatory action to respond to a safety signal, in which time people continue to be harmed by the drug. Additional time also allows for tailoring of treatment, and 21% of evaluable new molecular entities (NMEs) approved between 1980 and 2000 had their dosages adjusted for safety (160).

Lastly, repurposed drugs are often generic and cheap drugs, lacking the patent protection extended to many NMEs (161). Generic drugs are almost universally cheaper than patented medicines (162, 163), and this can be a benefit to consumers. One counter-strategy by the industry is to develop 'me-too' drugs (164), which are almost identical molecular entities to a previously developed one, developed for the purpose of selling at patented medicine rates, which re higher than generic medications rates.

Overall, drug repurposing is a promising strategy not just for cancer, but for many diseases, especially emerging diseases where there is need for a swift response. Repurposed drugs, along with free informational sharing amongst practitioners, is a viable way to respond to future emerging health issues.

Important considerations

Using drugs in new contexts carries risks, though risk is in principle minimized through the existing safety record of the drug. The potential safety concerns associated with the use of repurposed medications, particularly when administered at higher doses or for new indications, are important to acknowledge. One of the primary advantages of repurposing established medications is the availability of an extensive safety record, often informed by both clinical studies and real-world use. In some cases, accidental overdoses have provided valuable insights into the safety margins of these drugs. For example, Chiew *et al.* describe a case in which a 55-year-old woman ingested 132 grams of extended-release metformin (165), far exceeding the typical therapeutic dose, and subsequently developed severe lactic acidosis.

While the established safety data for repurposed drugs can help mitigate some risks, the potential for unexpected toxicities remains, especially when novel drug combinations are employed. It is important to note, however, that this risk is generally lower than with entirely new chemical entities, as repurposed drugs benefit from a well-characterized safety profile, including known drug interactions and adverse effects at various dosages. Even clinical trials, which evaluate drugs in the intended patient population, may fail to detect certain safety signals if they are underpowered, do not assess relevant clinical outcomes, or if adverse effects are subtle or emerge only after prolonged exposure. These limitations can contribute to the identification of safety concerns only after a drug is approved and marketed. Notably, approximately 7% of approved drugs in the United States were withdrawn from the market due to safety issues between 1980 and 2009 (166).

For most repurposed drugs, the proposed dosages are within established ranges, allowing researchers to reference existing safety data for individual agents. However, the greatest risk of unforeseen adverse events arises from drug-drug interactions, particularly when novel combinations are used. While software tools exist to predict potential toxicities from drug-drug interactions, these approaches are not infallible (167). To mitigate these risks, a cautious, stepwise approach is recommended. This would involve initiating treatment with repurposed drugs at low doses, either as single agents or in combinations with well-established safety profiles, and gradually escalating

doses or introducing additional agents as appropriate. Such a strategy aims to minimize the likelihood of adverse side effects while maximizing patient safety.

INTEGRATIVE ONCOLOGY: THE FUTURE OF CANCER CARE?

The market for complementary and alternative medicine (CAM) comprised some 3% of total US healthcare spending in 2008 (168). Data from 2012 show US consumer expenditures on complementary healthcare approaches of $30.2 billion (169), compared to the $2.8 trillion in total healthcare spending that same year (170), or roughly 1%. One factor acting against CAM adoption is many expenses are not eligible for reimbursement by insurance providers (171), so more of the costs are borne out of pocket.

Despite comparatively low adoption in the US healthcare ecosystem, CAM is adopted widely in Asian countries (172), and integrative oncology enjoys institutional support in other countries (173, 174). US patients are open to integrative oncology, as evidenced by a majority of US cancer patients using some form of CAM during their treatment (175). CAM adoption in oncology appears largely patient driven, as an Australian survey showed a majority of cancer patients using some form of CAM, with 90% of respondents saying that doctors should consider learning more about CAM (176).

With regards to cancer treatment, CAM focuses on several areas: 1) prevention of initial cancer or recurrence, 2) use for an anticancer effect, typically alongside other treatments, or 3) to alleviate side effects of cancer or cancer treatment (177). Importantly, the National Cancer Institute has recommend against taking supplements, particularly antioxidant supplements, during cancer therapy (178), as these have been postulated to interfere with chemotherapy or radiotherapy (179). This guideline is supported by a recent study showing lower survival in individuals taking dietary antioxidant supplements compared to individuals not taking antioxidant supplements (180). Some natural products show preliminary evidence for their efficacy, but insufficient to make a recommendation.

However, despite the limited evidence for some modalities, practitioners may adopt unproven methodologies which may put patients at unnecessary risk, especially if they refuse conventional treatment (181, 182). While the use of unproven modalities carries definite harms, it should not prevent the adoption of evidence-based CAM. Currently, CAM is only recommended for addressing effects of cancer or cancer treatment, and not for treating cancer itself. Major cancer centers, such as MD Anderson and Memorial Sloan Kettering Cancer Center, have adopted interventions such as mindfulness, yoga and acupuncture (183), though these modalities focus on psychological symptoms.

A joint guideline document published in conjunction with the American Society of Clinical Oncology (ASCO) and the Society for Integrative Oncology (SIO) provides a strong recommendation for mindfulness based therapies as well as Qigong to alleviate fatigue during cancer treatment, and a conditional recommendation for American Ginseng (184). For anxiety and depression, the ASCO and SIO provide a strong recommendation for mindfulness based interventions, and moderate recommendations for yoga, hypnosis, and music therapy (184). For pain during cancer, manual therapies, including acupuncture, yoga, reflexology and massage received recommendations from the ASCO and SIO, along wide guided imagery with progressive muscle relaxation and hypnosis (185). A 2017 ASCO and SIO guideline on integrative oncology in breast cancer does not recommend or examine agents or modalities which would affect cancer recurrence or survival due to a lack of randomized control trial evaluating these endpoints (186). The focus of subsequent CIO guidelines for the use of adjunctive cancer agents has not included therapeutics focused on improving cancer recurrence or survival, as opposed to managing other symptoms (184).

Despite a lack of current evidence, several integrative therapies may improve cancer treatment through an anti-cancer effect (177), such as intravenous vitamin C (187) and mushroom extracts (188, 189), though the evidence does not rise to the level of clinical recommendation. While numerous agents, such as curcumin (190), show promise in *in vitro* experiments, these effects often do not translate to the clinic, due to bioavailability or differences in metabolism or effective concentration in both *in vitro* (191) and *in vivo* studies (192).

There is a cultural divide between patients interested in CAM and their doctors. Nearly half of US CAM users do not inform their doctors (193). Most physicians have a desire to increase their knowledge of CAM, though lack of education and the short time

spent with patients is a barrier to wider CAM adoption (194). Nurses are also more likely than doctors to be open to CAM (195, 196).

Interestingly, a survey in Pakistan showed greater skepticism towards the efficacy of CAM in the general student population than those students studying pharmacy (197). This finding, where those with more professional experience demonstrated greater openness to CAM, is echoed by a survey in Germany, which found that internists were more skeptical of CAM than family physicians with more experience (198). Still, recalcitrance on the part of conventional doctors and lack of understanding hampers CAM adoption (196, 199).

Considering these challenges to adoption, it is impendent upon advocates of integrative oncology to provide educational opportunities for conventional physicians, and to also demonstrate rigorously the benefits of integrative oncology. Communication between integrative oncologists and conventional physicians and oncologists will be the linchpin of wider adoption, and a compelling case can be made based on treatment efficacy, cost-efficacy, ease of treatment (able to perform in outpatient setting) and quality of life.

Industry strategies to influence the public presentation of science are well documented (200, 201). Cancer-center spending on advertising increased over 3-fold between 2005 ($54 million) and 2014 ($173 million) (202). Compared to the numerous oncology drugs available, only approved two botanical drugs are available on the market as of 2020 (203). Interest in CAM appears to be increasing (204), with popular books receiving accolades as well as wide readership (205-208). One downside of the popularization of CAM treatments is the greater level of non-professional advice circulating, which can be ameliorated by professional and accredited CAM consultation (209). Training CIM practitioners to be able to work with conventional oncologists is important for the success of the program (210).

Real-world examples

Israel has adopted integrative oncology at some of its cancer centers, and there are currently 10 active oncology complementary and integrative medicine (CIM) programs (173). The use of CAM in the urban Jewish population doubled from 6% in 1993 to 12% in 2007 (211). Beyond uptake, a study at the oncology service of the Lin Medical Center in Haifa Israel showed statistically significant increases in individuals' well-being, appetite, anxiety, depression, nausea and fatigue (212) when they participated in an integrative oncology program. Other studies have demonstrated decreased use of medications to manage cancer therapy side effects in an integrative oncology setting (213).

Beyond institutional integrative oncology, several practitioners used ketogenic diets in addition to standard of care (214). Increased awareness has improved the accessibility of metabolic treatments (215), even for those with dietary restrictions, such as vegans (216). While rigorous clinical trials are limited (217-219), clinicaltrials.gov lists 53 studies using ketogenic diets as an intervention for various types of cancers* (**Supplementary Table 2**) Recent meta-analyses have found that ketogenic diets in cancer may improve mental health (220), and while clinical data on treatment efficacy is limited, several published studies show improvements in survival rates (221-224), though expert meta-analysis evaluates the evidence for anti-cancer effects as weak to moderate (225). These trials are necessary to evaluate the efficacy and establish best practices for using ketogenic diets in cancer care, and should take place before recommendation as part of cancer care. Fortunately, active work is underway to rigorously assess ketogenic approaches to cancers (226).

FUTURE OUTLOOK

Trends in the incidences of specific cancers have been varying, and treatment remains a challenge for cancers. Currently, a paradigm shift is occurring in the metabolic understanding of cancer, which can potentially provide non-invasive interventions for prevention and treatment (both primary and adjuvant) of cancer. The metabolic paradigm in cancer treatment potentially allows for broad spectrum therapeutics against a common hallmark of cancer, a penchant for the fermentation of glucose in the presence of oxygen (97).

Combined with repurposed drugs, there is significant potential for a substantial decrease in the costs associated with cancer treatment (213), as well as improved treatment outcomes and quality of life (227).

Search terms "ketogenic diet" in "Interventions" and "cancer" in "Disease" fields. Search reveals 66 results, 14 results not relevant, either not cancer related or do not use a ketogenic diet. Final studies are shown in* ***Supplementary Table 1**.*

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Authors' contributions

MH: conceptualization, literature search, writing – original draft, visualization, writing – review & editing. PM: conceptualization, critical revision of the manuscript, supervision, writing – original draft, writing – review & editing. JV: writing – review & editing. JT: conceptualization, methodology, writing – review & editing, supervision, project administration.

Publications ethics

Plagiarism

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

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SUPPLEMENTARY MATERIALS

Supplementary Table 1. *Classes of food additives under European Union regulation.*

NO.	FUNCTIONAL CLASS	DEFINITION (1)
1	Sweeteners	Substances used to impart a sweet taste to foods or in table-top sweeteners
2	Colours	Substances which add or restore colour in a food, including natural constituents and preparations from foods obtained by physical/chemical extraction
3	Preservatives	Substances which prolong shelf-life by protecting against deterioration caused by micro-organisms and/or pathogenic micro-organism growth
4	Antioxidants	Substances which prolong shelf-life by protecting against oxidation (*e.g.*, fat rancidity, colour changes)
5	Carriers	Substances used to dissolve, dilute, disperse or physically modify additives, flavourings, enzymes, or nutrients without altering their function
6	Acids	Substances which increase acidity and/or impart a sour taste
7	Acidity regulators	Substances which alter or control the acidity or alkalinity of a foodstuff
8	Anti-caking agents	Substances which reduce the tendency of particles to adhere to one another
9	Anti-foaming agents	Substances which prevent or reduce foaming
10	Bulking agents	Substances which contribute to volume without contributing significantly to available energy value
11	Emulsifiers	Substances which form or maintain a homogenous mixture of immiscible phases (*e.g.*, oil and water)
12	Emulsifying salts	Substances which convert cheese proteins into dispersed form for homogenous fat distribution
13	Firming agents	Substances which maintain fruit/vegetable firmness or interact with gelling agents to produce/strengthen gels
14	Flavour enhancers	Substances which enhance existing taste and/or odour
15	Foaming agents	Substances which form a homogenous dispersion of gas in a liquid or solid foodstuff
16	Gelling agents	Substances which give texture through gel formation
17	Glazing agents	Substances which impart a shiny appearance or protective coating to external surfaces (includes lubricants)
18	Humectants	Substances which prevent drying out or promote powder dissolution in aqueous media
19	Modified starches	Substances from chemically treated edible starches (may include physical/ enzymatic treatment, acid/alkali thinning, or bleaching)
20	Packaging gases	Gases other than air introduced into a container before, during, or after placing a foodstuff
21	Propellants	Gases other than air which expel a foodstuff from a container
22	Raising agents	Substances which liberate gas to increase dough/batter volume
23	Sequestrants	Substances which form chemical complexes with metallic ions
24	Stabilisers	Substances which make it possible to maintain the physico-chemical state of a foodstuff; stabilisers include substances which enable the maintenance of a homogenous dispersion of two or more immiscible substances in a foodstuff, substances which stabilise, retain or intensify colour of a foodstuff and substances which increase the binding capacity of the food, including the formation of cross-links between proteins enabling the binding of food pieces into re-constituted food
25	Thickeners	Substances which increase the viscosity of a foodstuff
26	Flour treatment agents	Substances, other than emulsifiers, which are added to flour or dough to improve its baking quality
27	Contrast enhancers	Substances which, when applied to the external surface of fruit or vegetables following depigmentation of predefined parts (*e.g.*, by laser treatment), help to distinguish these parts from the remaining surface by imparting colour following interaction with certain components of the epidermis

Supplementary Table 2. *Environmental exposures associated with cancer risk, and the trend in exposure levels/rates.*

FACTOR	TREND	IMPACT ON CANCER IN ISOLATION	EXPOSURE LEVEL
Smoking	Global decrease of 28% for men and 38% for women between 1990 and 2019 (7)	RR = 46 for small cell lung cancer (SCLC) for male current smokers compared to men who have never smoked. RR = 22 for SCLC for female current smokers compared to women who have never smoked (8)	11.5% of US adults smoke (2021) (9)
Pesticide exposure-occupational	Increase in pesticide use 7% between 1996 and 2011(10)	Non-Hodgkin's Lymphoma associated with glyphosate exposure: RR = 1.3 (11) RR = 2.02 (12)	2.4 million farm workers in USA (2013) (13). 0.6% of USA farming acreage is organic*
Pesticide exposure -food	Glyphosate tonnage grew by 17% on average annually between 1990 and 2014 (16)	Organic food consumption associated with a decreased risk (RR = 0.79) of non-Hodgkin Lymphoma (17)	On average 1.0kg per hectare of farmland applied in USA (16) 59% of corn and soy samples test positive for glyphosate and glufosinate residues (18)
Beauty products	Global annual growth rate of 4.5% over the last 20 years (19) Decrease of 13% in North America from 1998 to 2007 (14)	Breast cancer hazard ratio 1.15 for frequent white female users of beauty products relative to infrequent users (15)	85% of adolescent girls use body products on a daily basis (16)
Fire retardants in furniture	Production of chlorinated organophosphate flame retardants increases from 14,000 tons per year (mid-1980's) to 38,000 tons per year (2012) (17)	Flame retardants decabromodiphenyl ether and tris(2-chloroethyl) phosphate associated with greater risk (RR = 2.3) of papillary thyroid cancer (18)	Ubiquitous in furniture owing to flame-retardant requirements of furniture (19), (20)
Radon exposure	Should be stable, Radon's source primarily geological (21)	Every 100 Bq/m^3 increase in Radon concentrated estimated to increase relative risk for lung cancer by 8-16% (22)	Second biggest cause of non-occupational lung cancer behind smoking (22)
Antibiotics	Drop in recent years in USA. 5% decrease in number of prescriptions between 2011 and 2016 (23). 25% drop between 2016 and 2020 (23), (24) Global increase from 9.8 defined daily doses (DDD) per 1000 per day in 2000 to 14.3 DDD per 1000 per day in 2018 (25).	RR = 1.37 between lowest and highest exposure group for cancer (26)	In USA, 613 antibiotic prescriptions per 1000 people in 2020 (24)
EMF exposure	Increasing (27)	Increased RR = 2.0 for childhood leukemia for exposures of ≥0.4 μT compared to <0.1 μT (28)	Ubiquitous
Sedentary lifestyle	Increase in 39% in rates of meeting physical activity guidelines between 1998 and 2013 (29) Declines in active transport among children and adolescents (30)	Combined healthy lifestyle reduced risk of cancer (RR = 0.29 compared to those reporting no physical exercise or positive health behaviors) (31)	2/3 of adults do not meet physical activity guidelines (150min per week of moderate to vigorous physical activity) (29)
Sleep deprivation	Relatively stable sleep duration in adults (32), (33), but decreases in sleep quality (34)	Increased risk of colorectal cancer (RR = 1.08) and lung cancer (RR = 1.11) in poor sleep category (35)	More than 1/3 of US adults sleep fewer than 7 hours per night (2014) (36)

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FACTOR	TREND	IMPACT ON CANCER IN ISOLATION	EXPOSURE LEVEL
Stress	Work stress has been on the rise in Europe (37)	Association between work stress and risk of colorectal (RR = 1.36), lung (RR = 1.24) and esophageal (RR = 2.12) cancers (38)	71% of employees typically feel tense or stressed out during the workday (2019) (39)
Caesarean birth	Increase in rate of caesarean section from 30% in 2003 to 37% in 2010(40)	Increased rate of childhood kidney cancer (RR = 1.25) (41)	Approximately one-third of North American births in 2010 (42)
Family size	Decrease from 3.33 in 1960 to 2.50 in 2022 (43)	Hodgkin's Lymphoma risk lower for increased number of older siblings: RR = 0.72 for three or more older siblings compared to none (44) RR = 0.41 for five or more older siblings compared to none (45) Acute monocytic leukemia RR = 0.35 for three or more older siblings compared to none (45) Acute lymphoblastic leukemia RR = 0.69 for three or more older siblings compared to none (45)	Average family size of 2.50 in 2022 (43)
Mother's age at first birth	Increasing (46)	RR~1/3 for women giving birth before age 18 compared to those giving birth after 35 (47)	Average age in USA is 27.1 years (2020) (48)
Febrile illness	No trend in presentation rates to emergency department (49)	Lower rates on non-breast cancers for adults experiencing childhood febrile illness (50)	2.8 million children <2 years with fever present to emergency departments annually in USA (49)
Hormonal birth control	In the UK, hormonal birth control prescription proportion dropped 45% between 2000 and 2018 (51). Between 1995 and 2010, approximately 82% of sexually experienced women use the pill, staying relatively constant (52)	RR = 1.20 for breast cancer for users compared to non-users (53)	one in four US women aged 15-44 using oral contraceptives (2013) (54)
Breastfeeding (mother)	Increase in proportion of mothers breastfeeding from 75% in 2010(55) to 81.1% in 2016 (56)	Decrease in 2% breast cancer risk for every 5 months breastfeeding (57). Decreased risk of premenopausal breast cancer (RR = 0.88) (58) RR = 0.76 for invasive epithelial ovarian cancer (59)	81.1% of mothers breastfeed at birth (2016) (56)

**Total certified organic acres operated 5.5 million (2019) (14). Total land in farms 897.4 million acres (2019) (15).*

Supplementary Table 3. *Clinical trials for ketogenic diets in the treatment of cancer.*

NCT NUMBER	STUDY TITLE
NCT06896552	Single-Center Trial on Ketogenic Diet and Immunotherapy in Advanced Cancer This Study Evaluates the Safety and Effects of a Ketogenic Diet (KD) Combined With Immunotherapy in Adults With Advanced Melanoma, cSCC, or RCC
NCT06391099	Ketogenic Dietary Intervention to Improve Response to Immunotherapy in Patients With Metastatic Melanoma and Metastatic Kidney Cancer
NCT06106139	Ketogenic Diet Improves Thrombocytopenia in Cancer Patients
NCT06046755	Nutritional Intervention-induced Weight Loss During the Oncological Treatment of Obesity-related Breast Cancer
NCT05938322	Ketogenic Diet Compliance in Patients Affected by Locally Advanced Rectal Cancer Patients Who Undergo to Radiotherapy
NCT05708716	Diet and Cognitive Training in Hematologic Cancer Survivors
NCT05708352	A Phase 2 Study of the Ketogenic Diet *vs* Standard Anti-cancer Diet Guidance for Patients With Glioblastoma in Combination With Standard-of-care Treatment
NCT05564949	A Ketogenic Diet as a Complementary Treatment on Patients With High-grade Gliomas and Brain Metastases
NCT05428852	Keto-Brain:Investigating the Use of Ketogenic Diets in Brain Metastases
NCT05373381	The KetoGlioma (Ketogenic Glioma) Study
NCT05234502	Effects of Ketogenic Diet in Overweight and Obese Women With Breast Cancer
NCT05183204	Paxalisib With a High Fat, Low Carb Diet and Metformin for Glioblastoma
NCT05119010	A Pilot Study Evaluating a Ketogenic Diet Concomitant to Nivolumab and Ipilimumab in Patients With Metastatic Renal Cell Carcinoma
NCT05090358	Preventing High Blood Sugar in People Being Treated for Metastatic Breast Cancer
NCT04750941	Study of Copanlisib and Ketogenic Diet
NCT04730869	Metabolic Therapy Program In Conjunction With Standard Treatment For Glioblastoma
NCT04691960	A Pilot Study of Ketogenic Diet and Metformin in Glioblastoma: Feasibility and Metabolic Imaging
NCT04631445	Study Evaluating the Ketogenic Diet in Patients With Metastatic Pancreatic Cancer
NCT04469296	Diet Modification in pAtients With Luminal Early Breast Cancer Candidate for Primary Surgery
NCT04461938	Characterization of Metabolic Changes in the Glioma Tumor Tissue Induced by Transient Fasting (ERGO3)
NCT04316520	Ketogenic Diet for Patients Receiving Treatment for Metastatic Renal Cell Carcinoma
NCT04231734	Ketogenic Diet in Patients With Untreated Low Tumor Burden Mantle Cell Lymphoma
NCT03962647	A 2-Week Ketogenic Diet in Combination With Letrozole to Modulate PI3K Signaling in ER+ Breast Cancer
NCT03955068	Strict Classic Ketogenic Diet as a Therapy for Recurrent or Progressive and Refractory Brain Tumors in Children
NCT03679260	Carbohydrate Restricted Diet Intervention for Men on Prostate Cancer Active Surveillance
NCT03591861	Therapeutic Targeting of Sex Differences in Pediatric Brain Tumor Glycolysis
NCT03535701	Ketogenic Diet and Chemotherapy in Affecting Recurrence in Patients With Stage IV Breast Cancer
NCT03451799	Ketogenic Diet in Combination With Standard-of-care Radiation and Temozolomide for Patients With Glioblastoma
NCT03328858	Ketogenic Diet in Children With Malignant or Recurrent/Refractory Brain Tumor
NCT03285152	A Study of Ketogenic Diet in Newly Diagnosed Overweight or Obese Endometrial Cancer Patients
NCT03278249	Feasibility Study of Modified Atkins Ketogenic Diet in the Treatment of Newly Diagnosed Malignant Glioma
NCT03194516	Ketogenic Diet and Prostate Cancer Surveillance Pilot
NCT03171506	Targeted Disruption to Cancer Metabolism and Growth Through Dietary Macronutrient Modification
NCT03160599	Restricted Calorie Ketogenic Diet as a Treatment in Malignant Tumors
NCT03075514	Ketogenic Diets as an Adjuvant Therapy in Glioblastoma
NCT02983942	Ketogenic Diet Adjunctive to HD-MTX Chemotherapy for Primary Central Nervous System Lymphoma

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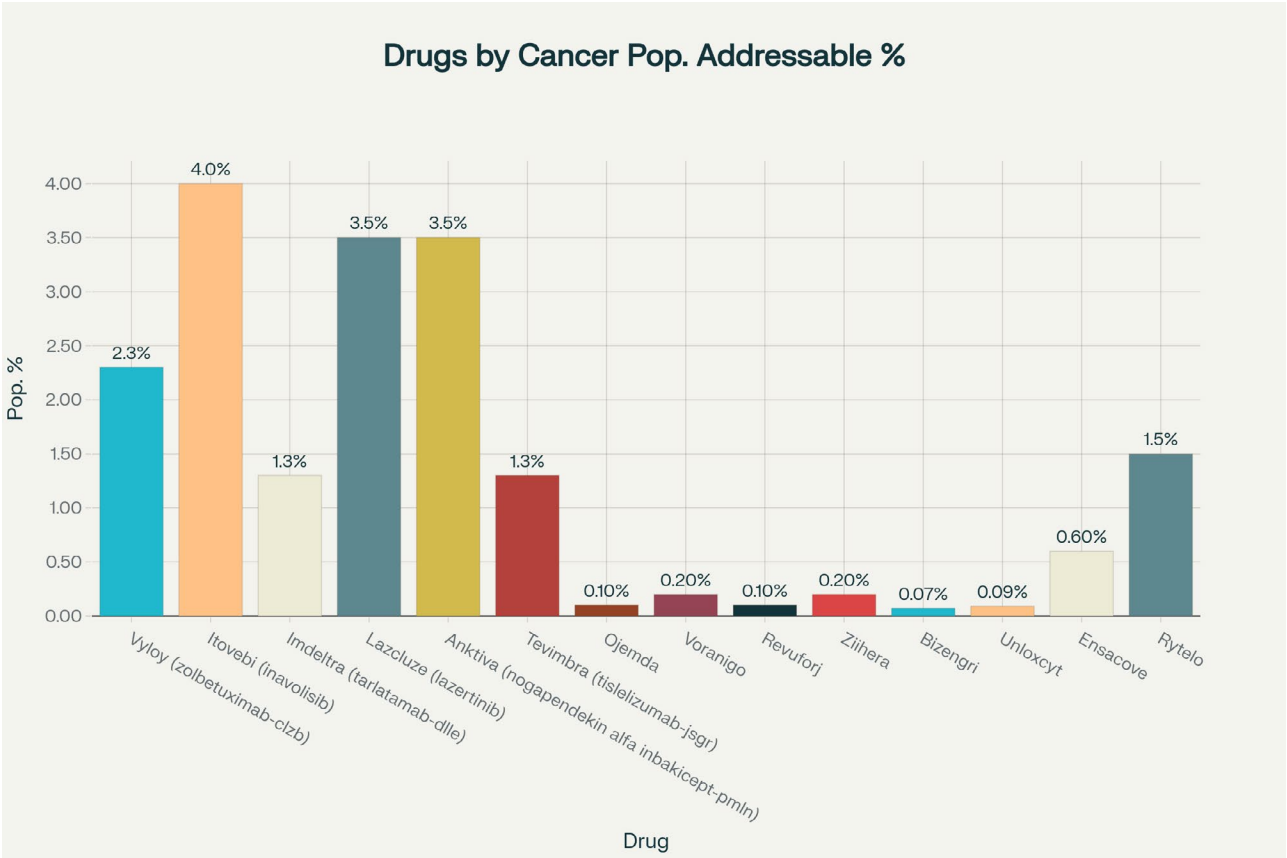
NCT NUMBER	STUDY TITLE
NCT02964806	Development and Clinical Validation of Ketogen-based Therapeutic Diet for Pancreaticobiliary Cancer Patients
NCT02939378	Ketogenic Diet Adjunctive to Salvage Chemotherapy for Recurrent Glioblastoma:a Pilot Study
NCT02516501	Impact of a Ketogenic Diet Intervention During Radiotherapy on Body Composition
NCT02302235	Ketogenic Diet Treatment Adjunctive to Radiation and Chemotherapy in Glioblastoma Multiforme: a Pilot Study
NCT02286167	Glioma Modified Atkins-based Diet in Patients With Glioblastoma
NCT02092753	Ketogenic Or LOGI Diet In a Breast Cancer Rehabilitation Intervention (KOLIBRI)
NCT02046187	Ketogenic Diet With Radiation and Chemotherapy for Newly Diagnosed Glioblastoma
NCT01975766	Ketogenic Diet Phase 1 for Head & Neck Cancer
NCT01865162	Ketogenic Diet as Adjunctive Treatment in Refractory/End-stage Glioblastoma Multiforme: a Pilot Study
NCT01754350	Calorie-restricted, Ketogenic Diet and Transient Fasting During Reirradiation for Patients With Recurrent Glioblastoma
NCT01716468	Ketogenic Diet in Advanced Cancer
NCT01535911	Pilot Study of a Metabolic Nutritional Therapy for the Management of Primary Brain Tumors
NCT01419587	Ketogenic Diet With Chemoradiation for Lung Cancer (KETOLUNG)
NCT01419483	Ketogenic Diet With Concurrent Chemoradiation for Pancreatic Cancer
NCT01092247	The Effect of Ketogenic Diet on Malignant Tumors- Recurrence and Progress
NCT00575146	Ketogenic Diet for Recurrent Glioblastoma



Supplementary Figure 2. *Drugs by cancer population.*

Supplementary Table 4. *Addressable population for new anticancer agents approved in 2024. For new anticancer agents approved in 2024 (60), based on their indication, the addressable population as a percentage of cancer patients is calculated.*

TREATMENT (GENERIC NAME)	INDICATION	ADDRESSABLE POPULATION (% OF CANCER CASES)
Lumisight (pegulicianine)	Imaging agent for detection in breast cancer	N/A
Vyloy (zolbetuximab-clzb)	Gastric, gastroesophageal junction cancers (CLDN18.2+)	~6% of cancers are gastric cancers (61) 38% of those with gastric cancers are claudin (CLDN18.2) positive (62) Vyloy is applicable to ~2.3% of cancers
Itovebi (inavolisib)	HR+, HER2-, PIK3CA-mutated advanced/ metastatic breast cancer	Breast cancer is ~15% of total cancers (63) HR+/HER2- are ~70% of female breast cancer (64) PIK3CA mutation in ~40% of HR+ breast cancers (65) Itovebi is applicable for ~4% of cancers
Imdeltra (tarlatamab-dlle)	Extensive-stage small cell lung cancer	Lung cancer is ~11% of cancers (66) SCLC ~15% of lung cancers (67) ES-SCLC is 2/3 of SCLC cases (68) Imdeltra is applicable for ~1.3% of cancers
Lazcluze (lazertinib)	EGFR-mut. non-small cell lung cancer	11% of cancers are lung cancer (66) ~85% of lung cancers are NSCLC (69) 32.3% of all NSCLC cases are EGFR-mutated (70) Lazcluze is applicable to 3.5% of cancers
Anktiva (nogapendekin alfa inbakicept-pmln)	BCG-unresponsive non–muscle-invasive bladder cancer	Lung cancer ~13% of all cancers NSCLS ~85% of lung cancers (67) EGFR mutations in 32% of NSCLC patients (70) Anktiva is applicable to 3.5% of total cancers
Tevimbra (tislelizumab-jsgr)	Esophageal Squamous Cell Carcinoma (ESCC) Gastric or Gastroesophageal Junction Adenocarcinoma (G/GEJ)	Gastric cancers ~1.5% of total cancer cases in the USA (71) GEJ cancers are 33% of all gastric cancers (72) Esophageal cancers are 1% of cancers in the USA (71) ECSC are 80% of all esophageal cancers (73) Tevimbra is applicable for ~1.3% of all cancers
Ojemda	Pediatric low-grade glioma	Childhood cancers ~1–2% of all cancers (WHO) (74). Brain & CNS tumors ~20–25% of childhood cancers (ACS) (75). pLGG is 33% of pediatric brain tumors (ABTA) (76). Ojemda applies to ~0.1% of cancers.
Voranigo	IDH-mutant glioma (astrocytoma/ oligodendroglioma)	Brain & CNS tumors 1.6% of cancers (77). ~70–90% of Grade 2 gliomas are IDH mutant (78), (79). Voranigo applies to ~0.2% of cancers.
Revuforj	Acute leukemia with KMT2A translocation	Leukemias account for ~2–3% of cancers (80). ~50–55% are acute leukemias (81). ~10% KMT2A rearrangements (82). Revuforj applies to ~0.1% of cancers.
Ziihera	HER2-positive biliary tract cancer	BTC ~1% of cancers (83, 84). ~5–20% HER2-positive cases (85). Ziihera applies to ~0.2% of cancers.
Bizengri	NSCLC and pancreatic adenocarcinoma with NRG1 fusion	Lung 12.4% (77). Pancreas 2.6% of cancers (77). NRG1 fusions <1% in these (86), (87). Bizengri applies to ~0.07% of cancers.
Unloxcyt	Cutaneous squamous cell carcinoma	Together, skin cancers (MSC + NMSC) make up ~8% of all global cancers (83), (88). CSCC constitutes 20-50-% of all skin cancers (89), (90). 1–4% progress to advanced/metastatic (91). Unloxcyt applies to ~ 0.02–0.16% of cancers.
Ensacove	ALK-positive NSCLC	Lung cancers account for 12.4 % of all cancers (77). ALK rearrangement occurs in 5-6% of NSCLC (92). Ensacove applies to ~0.6% of cancers.
Rytelo	Myelodysplastic syndromes	MDS1–2% of all cancers (93). Rytelo applies to 1-2% of cancers.

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REVIEW

ARTIFICIAL INTELLIGENCE IN HISTOLOGICAL PROFILING OF HEPATOCELLULAR CARCINOMA: STATE OF THE ART

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ABSTRACT: Hepatocellular cancer (HCC) is the fifth most common malignancy and the fourth leading cause of cancer-related deaths worldwide. Liver histology plays a crucial role in the biological profiling of HCC, informs cancer prognosis, and supports personalized treatment strategies. Over the past decade, machine learning, deep learning algorithms, and convolutional neural networks have emerged as powerful tools for the histological and molecular assessment of malignancies. Machine learning and deep learning algorithms applied to whole slide digital images (WSIs) of liver cancers have demonstrated significant accuracy in distinguishing non-cancer *versus* cancer liver tissue and histologically subtyping HCC phenotypes associated with different clinical outcomes. Generative Artificial Intelligence models applied to WSIs obtained from hematoxylin and eosin-stained (H&E) histology specimens have shown promising results in delivering crucial insights into the genetic HCC disarrangement, potentially providing the biological rationale for molecular-targeted therapeutic strategies. This review highlights the diagnostic advances in computational histology for primary liver cancer. The manuscript focuses on the state of the art in AI-based histotyping and molecular profiling for HCC. A critical evaluation of their current performance is essential for inspiring the clinical research priorities and promoting the safe employment of AI models in managing HCC patients.

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Impact statement: Histology-based AI applied to H&E whole-slide images improves HCC diagnosis, phenotyping, molecular inference and prognostication, strengthening risk stratification and personalized care, and accelerating integration of computational pathology into routine clinical workflows.

Key words: *Artificial intelligence; liver cancer; hepatocellular carcinoma; deep learning; machine learning.*

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INTRODUCTION

In adult populations, hepatocellular carcinoma (HCC) is the fifth most common liver malignancy and the fourth most prevalent cancer-related death worldwide (1, 2). Liver cirrhosis is the cancerization field for HCC, with varying etiologies in different epidemiological settings. The etiological impact of transmissible (HBV or HCV) and non-transmissible (toxic agents, autoimmunity) agents differs significantly according to the considered populations (3).

Assessing liver malignancies and delivering patient-tailored treatments involves combining clinical evaluation, imaging, histology, and molecular profiling (4). Artificial intelligence (AI) has emerged as an extraordinary opportunity in the clinical management of malignancies in the last decade. Gigantic datasets may collect and connect multiparametric information (histology, genomic, proteomic, and metabolomic data), resulting in a hyper-human integration of different analytic methods and ultimately shaping the patient management strategy (5-8). A schematic overview of this multimodal workflow and its potential clinical outputs is provided in **Figure 1**. Within this multimodal framework, liquid biopsy offers a minimally invasive, longitudinal source of biomarkers that can complement tissue-based AI for risk stratification and disease monitoring.

Machine learning (ML) supervised and unsupervised models, Deep algorithms (DL) and Convolutional Neural Networks (CNN) have been developed and tested for their effectiveness in enhancing human-based diagnostics performance (9).

In histologically profiling HCC (diagnosis and grading), generative artificial intelligence (GAI) performs close to that of trained pathologists, and deep learning (DL) models have shown promising potential in predicting HCC-associated genetic disarrangements that can be exploited for targeted therapies (5, 10, 11). GAI models based on whole slide images (WSIs) obtained from hematoxylin and eosin-stained (H&E) "traditional" histological slides may effectively assist pathologists in HCC diagnosis, molecular profiling, prognostication, and assessment of therapy efficacy (9). As already seen in diagnostic radiology, AI implementation in diagnostic pathology irreversibly modifies the landscape of pathology competencies and operational workflow.

This review critically analyzes the diagnostic performance of GAI algorithms applied to whole-slide digital images (WSIs) obtained from H&E-stained histological specimens of cancer liver lesions.

This is a narrative review based on a targeted search of peer-reviewed literature primarily in PubMed/MEDLINE, including studies published up to May 2025. Search terms combined disease and methodology keywords (*e.g.*, HCC/liver tumors, digital pathology/whole-slide images, machine learning/deep learning, generative AI, microvascular invasion, molecular prediction, recurrence/survival), and reference lists of key articles were screened for additional records. We prioritized original studies and high-quality reviews with clear methodology and clinical endpoints relevant to histology-based AI in liver tumors, excluding radiology-only papers and reports lacking sufficient technical detail.

THE GLOSSARY IN COMPUTATIONAL HISTOLOGY

Machine learning (ML) algorithms include supervised and unsupervised training procedures. Supervised ML models involve using labeled (*i.e.*, categorized) data sets to train algorithms to classify or categorize data. This can be applied to optimize diagnostic procedures or predict clinical outcomes in clinical settings (9).

Unsupervised training uses unlabeled (*i.e.*, non-pre-categorized) data sets to reveal potential associations or patterns of variables within the data set. This "unlabeled" approach may uncover clustering or associations of variables, which may require interpretation and validation trials for clinical plausibility (9).

Deep learning (DL) is a subset of ML models mimicking human neuroanatomy. DL technology can learn complex representations, and its algorithms include artificial neural networks (synonym: Convolutional neural network (CNN)). CNN can automatically learn multiple levels of features based on a network of interconnected computing units organized in layers (12, 13).

AI ALGORITHMS CURRENTLY TESTED IN HCC HISTOLOGICAL ASSESSMENT

Different AI algorithms have been employed in the computational histology of liver tumors.

The ChOWDER (Cooperative Workspace DrivER) system is web-scalable and does not require specialized software, hardware, or expert annotations. It oper-

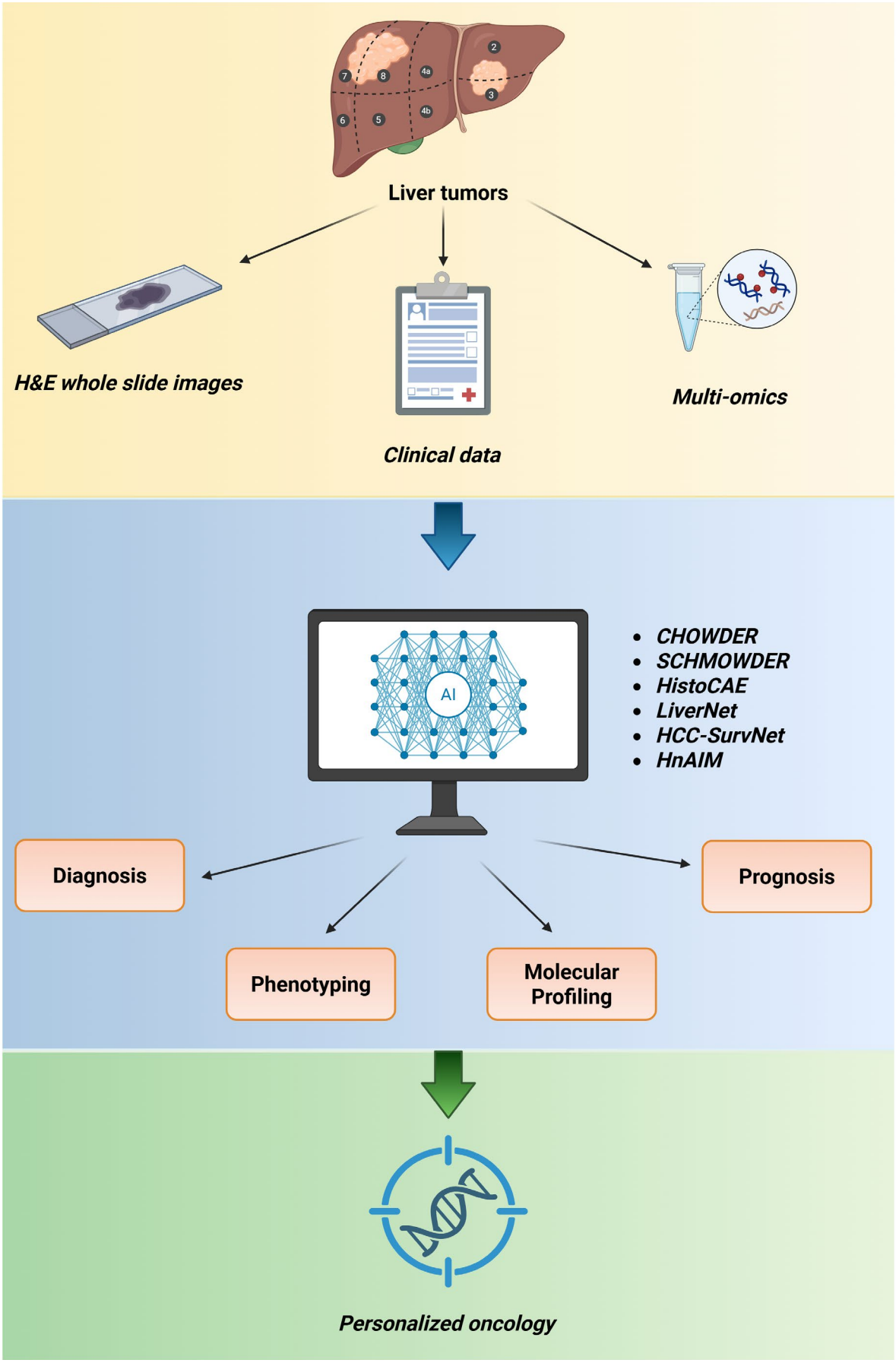

Figure 1. *Overview of an AI-enabled multimodal workflow for histological profiling of liver tumors.*
H&E whole-slide images, clinical data, and multi-omics features can be integrated to develop models supporting diagnosis, phenotyping, molecular profiling, and prognosis, ultimately enabling personalized oncology.

ates using multiple display devices, allowing a web browser to create a single large pixel space. Additionally, the ChWODER algorithm greatly enhances prognostic predictions based on HCC histology (14). The DL SCHMOWDER model has been used to predict patient survival by analyzing whole slide images (WSIs) of HCC. This algorithm calculates a prognostic risk score based on the histological cancer phenotype. Unlike the ChOWDER algorithm, SCHMOWDER requires human input to annotate the neoplastic areas that may be linked to the highest cancer aggressiveness levels, enhancing its prognostic accuracy (14). HistoCAE is a DL model developed for the segmentation of HCC in whole slide images. The model segments viable tumor areas, capturing fine spatial details missed by traditional histology (15).

Based on histological images (WSIs) of HHC samples, the MVI-DL (Microvascular Invasion-Deep Learning) algorithm focuses on detecting HCC microvascular invasion (16). This model has been successfully applied to the preoperative prognostic assessment of HCC patients (16).

The first-generation LiverNet deep learning model was developed to diagnose different subtypes of HCC based on H&E-stained whole slide images (WSIs) of tumor samples (17). In its original version, LiverNet classified HCC into three categories: low, intermediate, and high. This classification system helped pathologists make AI-assisted diagnoses of HCC. The improved second generation of the model (*i.e.*, LiverNet2) demonstrated a more than 97% diagnostic accuracy (18).

A recent, sophisticated deep convolutional neural network analyzes HCC histological images by capturing cellular modifications discarded by traditional assessment and effectively distinguishing high-grade HCC from cirrhosis (19).

A new deep learning model accurately predicts portal hypertension in HCC patients based on combined clinical variables and imaging, enabling timely therapeutic interventions (20).

AI IN HCC PHENOTYPING: WSIS ON H&E-STAINED HISTOLOGICAL SPECIMENS

Several studies have explored the performance of computational pathology (so-called "artificial" histology (21) in the biological profiling of different malignancies. These experimental experiences have provided essential insights into the diagnostic power of ML, DL, and CNN algorithms applied to H&E-stained, Whole Slide Digital Images (WSIs-H&E) obtained from "traditional" histological slides (22). These findings have consistently shown GAI's potential to expand histology-based information on cancer's phenotyping and clinical outcomes (23, 24). The AI algorithms extend the biological information on HCC far beyond the "traditional histology," accurately classifying cancer histology, identifying molecular imbalances, and predicting cancer outcome (25, 26).

In 2017, an early study by Li and coauthors focused on AI-based nuclear grading of HCC. By joining multiple fully connected CNNs with a learning machine (MFC-CNN-ELM), a supervised algorithm achieved an overall grading accuracy of 0.811 ± 0.029 (27).

Lin and coauthors combined multiphoton microscopy with deep-learning algorithms. Convolutional neural networks combined with a pre-trained model (VGG-16) for image classification resulted in an accuracy of the HCC-grading over 90%. These findings documented the successful combination of multiphoton microscopy with deep learning models in realizing label-free, automated diagnostic assessment, potentially exploitable in clinical contexts (28).

In 2020, Kiani and coauthors specifically addressed the accuracy of AI models in the differential diagnosis of hepatocellular *versus* intrahepatic cholangiocellular cancers (7). Based on H&E-stained WSIs, the authors evaluated the diagnostic performance of a trained AI model *versus* 11 pathologists with variable levels of expertise. On a validation set (26 WSIs), the model achieved an accuracy of 0.885 *versus* 0.842 obtained from an independent test set of 80 WSIs. While no change in the mean accuracy of the 11 pathologists was detected ($p = 0.184$, OR = 1.281), it significantly improved the accuracy ($p = 0.045$, OR = 1.499) of a subset of nine pathologists considered to have "well-defined" experience levels.

A Chinese study by Liao *et al*. applied an AI-based diagnostic classifier (HCC *versus* non-cancer tissue) trained on 31 H&E-stained WSIs of HCC available from the Cancer Genome Atlas and tissue microarray images from a Chinese hospital (29). The diagnostic model successfully assessed HCC images in internal and external validation sets, with areas under the receiver operating characteristic curves (ROC curves) of 0.988 and 0.886, respectively. Moreover, the model consistently differentiated cancer from non-cancer tissue and discriminated long- from short-survival patients (see below).

More recently, a multicenter Chinese study achieved significant diagnostic consistency in the phenotypic

assessment of nodular liver lesions applying an original ensemble of DL models (hepatocellular-nodular AI model: HnAIM) based on the integration of three different AI algorithms (ResNet50, InceptionV3, and Xception) (30). The trial involved surgical and biopsy tissue specimens representative of the full spectrum of nodular liver lesions, including focal nodular hyperplasia, cirrhosis, dysplastic nodules (high- and low-grade), hepatocellular adenoma, and H. The ROC curves and the AUC values on the testing database for models of Resnet50, Inception V3, Xception, and HnAIM demonstrated that the Xception and the HnAIM models performed the best, with AUC values of 0.9991. Moreover, the ensemble HnAIM model performed excellently in the external data set with an AUC value higher than 93%.

In a recent German study, the computational profiling of liver tumors achieved satisfactory prediction values in discriminating among histological classes (tile accuracy of 89%; case accuracy of 94%) and significantly distinguished liver metastasis from benign lesions at the case level (31).

The results obtained from both supervised and unsupervised models demonstrate the promising potential of (experimental) computational histology in distinguishing between cancer and non-cancer liver tissue. Additionally, they provide consistent information on cancer histotypes classification and grading, ultimately validating their potential to align with the diagnostic priorities in clinical practice (**Table 1**).

AI IN HCC PROGNOSIS AND PREDICTION OF TREATMENT OUTCOMES

Deep learning models, particularly CNN, have been extensively tested in liver cancer prognostication. Several studies focus on AI-driven imaging analyses, including CT scans, MRIs, and MRI radiomics (32-35). In some studies, CNN algorithms performed significantly well in prognostic prediction based on multiparametric data sets, including histology, among other clinical sources of information.

In 2020, a French study focused on the prognosis of HCC patients undergoing curative surgical resection. By applying the SCHMOWDER and CHOWDER DL algorithms, Saillard and colleagues obtained a "refined prediction" of their prognostic c-indices for both, with SCHMOWDER showing better performance due to expert pathologists' intervention in annotating neoplastic areas. Based on these results, the authors have emphasized the benefit resulting from pathologist-machine interactions in developing deep learning algorithms (14).

In 2021, two Japanese studies applied machine learning (36) and deep learning (37) algorithms to predict the risk of HCC recurrence after surgical treatment. In its conclusions, the study conducted by Saito and colleagues interpreted the "limitations" in the obtained prognostic estimates as stemming from the low number of cases considered (36). The deep learning model by Yamashita and coworkers obtained more satisfactory results (37). The risk score obtained by applying their original HCC-SurvNet DL achieved significant concordance indices (0.724 and 0.683) on the internal (0.724) and external (0.683) test cohorts, and both of them exceeded the prognostic performance of the "traditional" human-based TNM staging.

In 2021, a study by Jie-Yi Shi and coauthors applied a weakly supervised DL algorithm to explore the prognostic impact of HCC phenotypes computationally assessed on histological WSIs (38). The AI-based tumor risk score emerged as an independent prognostic factor with a predictive value superior to the clinical staging. Sinusoidal capillarization, macro-nucleoli, nucleus-to-cytoplasm ratio, and infiltrating inflammatory cells were identified as the main histological variables underlying the computational score of risk.

A recent 2025 study by Yixin Li and coauthors evaluated a deep learning model designed to predict the recurrence of HCC following surgical treatment (39). The model achieved an area under the receiver operating characteristic (AUROC) of 0.818 and 0.811 for predicting recurrence at 1 and 2 years, respectively, with external validation scores of 0.713 and 0.707. Additionally, the model effectively identified patients eligible for Sorafenib adjuvant therapy, enhancing its prognostic value by significantly recognizing candidates for targeted adjuvant treatment. Taken together, these histology-based deep learning models illustrate how AI can refine postsurgical risk stratification, recurrence prediction and treatment selection in HCC patients (**Table 2**).

HCC MOLECULAR PROFILING VIA AI ON H&E-STAINED WSIS

The AI models applied to liver cancer have been tested in their ability to provide information on

Table 1. *Histology-based and related AI models for diagnostic and phenotypic assessment of liver tumors.*

STUDY	AI MODEL / APPROACH	ENDPOINT	INPUT DATA	KEY FINDINGS
Li *et al*., 2017 (27)	Joint multiple fully connected CNNs + extreme learning machine (MFC-CNN-ELM)	Automatic nuclear grading of HCC	H&E-stained histological images of HCC nuclei	Overall grading accuracy 0.811 ± 0.029 for HCC nuclear grading.
Lin *et al*., 2019 (28)	CNN (VGG-16) applied to label-free multiphoton microscopy	Automated differentiation / grading of HCC	Label-free multiphoton microscopy images of liver tissue	High diagnostic accuracy (>90%) for HCC differentiation; demonstrates feasibility of label-free automated histological assessment.
Kiani *et al*., 2020 (7)	Deep-learning assistant for differential diagnosis	Differential diagnosis between HCC and intrahepatic cholangiocarcinoma	H&E WSIs; stand-alone AI *vs* pathologists *vs* AI-assisted pathologists	Model accuracy 0.885 on validation set and 0.842 on independent test; AI assistance significantly improved accuracy in a subset of pathologists with "well-defined" experience (OR ≈ 1.5; p = 0.045).
Liao *et al*., 2020 (29)	CNN-based diagnostic classifier	Classification of HCC *vs* non-cancer liver tissue and survival stratification	H&E WSIs from TCGA and an external Chinese cohort	AUC 0.988 (internal) and 0.886 (external) for HCC *vs* non-cancer; also discriminated long- *vs* short-survival patients.
Cheng *et al*., 2022 (HnAIM) (30)	HnAIM ensemble (ResNet50, InceptionV3, Xception)	Phenotypic classification of nodular liver lesions (*e.g.*, FNH, cirrhosis, dysplastic nodules, HCA, HCC)	Surgical and biopsy H&E WSIs of nodular liver lesions	Best models (Xception and HnAIM) reached AUC up to 0.9991 on the test set; ensemble HnAIM maintained AUC >0.93 on external data.
Kriegsmann *et al*., 2023 (31)	DL-based computational profiling of liver tumors	Classification of liver tumor histotypes; distinction of metastases *vs* benign lesions	H&E WSIs of primary and secondary liver lesions	Tile-level accuracy 89% and case-level accuracy 94%; significantly distinguished liver metastasis from benign lesions at case level.
HistoCAE, 2020 (15)	Convolutional autoencoder (HistoCAE)	Segmentation of viable HCC tumor regions	WSIs of liver cancer	Accurately segments viable tumor areas, capturing fine spatial details often missed by traditional visual assessment.
Wang *et al*., 2023 (MVI-DL) (16)	Multimodal deep-learning model (MVI-DL)	Histological detection of microvascular invasion (MVI) and preoperative prognostic assessment	Histological WSIs of HCC (within a multimodal preoperative framework)	Deep-learning model focused on identifying MVI on histological images; successfully applied to preoperative prognostic assessment of HCC patients.
Aatresh *et al*., 2021; Chanchal *et al*., 2024 (LiverNet / LiverNet2.x) (17, 18)	LiverNet and LiverNet2.x CNN architectures	Automated grading and subtype classification of HCC	H&E WSIs of liver tumors	First-generation LiverNet enabled accurate AI-assisted grading and subtype classification; second-generation LiverNet2.x achieved >97% diagnostic accuracy.
Hang *et al*., 2025 (19)	Deep CNN combined with hyperspectral imaging	Differentiation of high-grade HCC from cirrhosis	Hyperspectral histological images of liver tissue	Deep CNN captured subtle cellular modifications and effectively distinguished high-grade HCC from cirrhotic tissue.

It shows that most AI applications in liver tumor histopathology are H&E whole-slide image–based and mainly address diagnostic and phenotyping tasks, with promising performance but still heterogeneous validation across cohorts and settings. AI: Artificial Intelligence; DL: Deep Learning; CNN: Convolutional Neural Network; MFC: Multiple Fully Connected (layers); ELM: Extreme Learning Machine; CAE: Convolutional Autoencoder; HCC: Hepatocellular Carcinoma; H&E: Hematoxylin and Eosin; WSI / WSIs: Whole-Slide Image(s); TCGA: The Cancer Genome Atlas; HnAIM: Hepatocellular-Nodular Artificial Intelligence Model; FNH: Focal Nodular Hyperplasia; HCA: Hepatocellular Adenoma; MVI: Microvascular Invasion: AUC: Area Under the (ROC) Curve; OR: Odds Ratio.

Table 2. *AI models for prognosis, recurrence prediction and molecular profiling in hepatocellular carcinoma.*

STUDY (FIRST AUTHOR, YEAR)	AI MODEL / APPROACH	ENDPOINT	INPUT DATA	KEY FINDINGS (AS REPORTED IN THE REVIEW)
Saillard *et al*., 2020 (CHOWDER / SCHMOWDER) (14)	CHOWDER web-scalable DL system and SCHMOWDER DL model	Post-resection prognosis (overall survival) in HCC	H&E WSIs of resected HCC; SCHMOWDER uses pathologist-annotated aggressive tumor areas	Both DL models provided refined survival prediction; SCHMOWDER outperformed CHOWDER thanks to expert annotation of aggressive neoplastic areas, highlighting the benefit of pathologist–AI interaction.
Saito *et al*., 2021 (36)	Machine-learning model on digital pathology	Early recurrence of HCC after surgical resection	Digital histopathology images combined with clinicopathological variables	ML model predicted early recurrence of HCC; performance was promising but derived from a relatively limited dataset.
Yamashita *et al*., 2021 (HCC-SurvNet) (37)	Deep-learning model (HCC-SurvNet)	Postsurgical recurrence risk and survival in HCC	Histological WSIs of resected HCC	Concordance indices 0.724 (internal cohort) and 0.683 (external cohort), both higher than the prognostic performance of traditional TNM staging.
Shi *et al*., 2021 (38)	Weakly supervised DL model with AI-derived tumor risk score	Overall prognosis via histology-based tumor risk score	H&E WSIs of HCC with survival follow-up	AI-derived tumor risk score was an independent prognostic factor with predictive value superior to clinical staging; associated histologic features included sinusoidal capillarization, macro-nucleoli, high nucleus-to-cytoplasm ratio and inflammatory infiltrate.
Li *et al*., 2025 (39)	Denoised recurrence-label deep-learning model	Postoperative recurrence risk and benefit from adjuvant Sorafenib	Histology-derived features integrated with clinical data	Model achieved AUROC 0.818 and 0.811 for 1- and 2-year recurrence (internal), and 0.713 and 0.707 in external validation; also identified patients most likely to benefit from Sorafenib as adjuvant therapy.
Wang *et al*., 2023 (MVI-DL) (16)	Multimodal DL model (MVI-DL)	Presence of microvascular invasion and postoperative outcome	Histological images plus additional clinical/imaging variables	Novel multimodal DL model for preoperative prediction of MVI and patient outcome, supporting risk-adapted surgical planning.
He *et al*., 2025 (20)	AI-based prediction model for portal hypertension	Clinically significant portal hypertension in HCC	Combined clinical variables and imaging features	Deep-learning model accurately predicted clinically significant portal hypertension, enabling earlier and better-targeted therapeutic interventions.
Fu *et al*., 2020 (PC-CHiP) (41)	Pan-cancer computational histology (PC-CHiP)	Inference of molecular alterations from histology (including HCC, focus on TP53)	17,355 H&E slides from 10,452 patients (28 cancer types, including HCC)	Histology-derived features were quantitatively associated with multiple molecular alterations; in HCC, computational features were significantly associated with TP53 mutational status.
Liao *et al*., 2020 (29, 42)	DL-based mutation-prediction models	Prediction of key gene mutations in HCC	H&E WSIs of HCC	Linked specific histologic patterns to mutations in TP53, MUC4, ALB, CSMD3, RYR2 and OBSCN, demonstrating the feasibility of mutation prediction directly from routine histology.

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STUDY (FIRST AUTHOR, YEAR)	AI MODEL / APPROACH	ENDPOINT	INPUT DATA	KEY FINDINGS (AS REPORTED IN THE REVIEW)
Chen *et al*., 2020 (11)	Inception V3a CNN	Prediction of recurrently mutated genes with potential therapeutic implications	H&E WSIs of liver cancer	Model trained on 10 frequently mutated genes (ARID1A, ASH1L, CSMD1, CTNNB1, EYS, FMN2, MDM4, RB1, TP53, ZFX4); external AUC 0.71–0.89, with particularly strong performance for CTNNB1, potentially useful to identify candidates for TTK inhibitors.W

It summarizes the rapid expansion of histology-based AI models for prognostic stratification (including recurrence/MVI-related endpoints) and molecular inference, highlighting clinically relevant outputs that nonetheless require robust external and prospective validation prior to routine adoption. AI: artificial intelligence; DL: deep learning; CNN: convolutional neural network; HCC: hepatocellular carcinoma; WSI: whole-slide image; MVI: microvascular invasion; AUROC: area under the receiver operating characteristic curve; TNM: tumor–node–metastasis.

molecular cancer profiles based on WSIs-H&E histological specimens (11, 40).

By applying a pan-cancer computational histology (PC-CHiP) analysis, Fu and coauthors tested the reliability of AI in associating histological cancer phenotypes with specific molecular disarrays (41). The algorithm was tuned on 17,355 H&E frozen tissue image slides, including 28 cancer histotypes from 10,452 individuals. In their study, the authors documented that "computational histopathological features" may infer quantitative associations with a spectrum of molecular disarrangements, and significantly associated *p53* mutations with HCC phenotype.

In the experience of Liao and coauthors, deep learning-based algorithms linked HCC histological specimens to specific molecular patterns involving TP53, MUC4, ALB, CSMD3, RYR2, and OBSCN (29, 42).

Chen and coauthors used whole-slide images WSIs-H&E liver tissue samples to train the Inception V3a CNN to predict HCC-associated genetic disarrangements with potential predictive impact (11). The DL model was trained and validated on ten of the most significantly mutated genes in HCC (ARID1A, ASH1L, CSMD1, CTNNB1, EYS, FMN2, MDM4, RB1, TP53, and ZFX4). Based on the WSIs-H&E histology, the model consistently revealed mutations involving CTNNB1, FMN2, TP53, and ZFX4, with external AUC values ranging from 0.71 to 0.89. In particular, the model highly predicted CTNNB1 mutations, identifying potential responders to targeted therapies with TTK inhibitors. The findings above emphasize the potential of Deep Learning Convolutional Neural Networks (DL-CNN) for expanding the diagnostic message of histological phenotyping with valuable insights into the cancer molecular profile. This approach significantly simplifies the management of cancer patients and improves the efficiency and effectiveness of the diagnostic workflow.

Liquid biopsy can be integrated with histology-based AI to provide a minimally invasive and longitudinal source of information. In HCC, AI and ML approaches are currently being investigated for liquid biopsy signals, including circulating tumor DNA (ctDNA) and cell-free DNA (cfDNA) mutations, methylation patterns, and fragmentation or end-motif features, as well as circulating tumor cells and extracellular vesicles. These methods aim to support early detection, treatment monitoring, and assessment of recurrence or minimal residual disease (MRD) risk (43-45). Blood-based models can complement WSIs-derived predictors by capturing tumor dynamics over time and by facilitating the prioritization of confirmatory molecular testing when tissue samples are limited. Nevertheless, successful clinical translation requires rigorous standardization of pre-analytical procedures and robust external or prospective validation, especially in cirrhotic patients where tumor fraction may be low and confounding signals are prevalent (43-45).

CURRENT CHALLENGES IN AI EMPLOYMENT IN HCC HISTOLOGICAL ASSESSMENT

Pathologists' propensity to embrace the new vision of AI-based pathology is key to moving from traditional human-microscope-based pathology to the

WDS-GAI dimension. GAI was not part of the training of the previous generation of pathologists; fostering this profound innovation requires both personal aptitude and technical skills from mentors and residents, the latter are more receptive to digital innovation than their predecessors. Reluctance in novelty acceptance may be grounded in the "romantic" dimension of handcrafted work, but the current commitment to efficiency demands a timely, cost-effective, and more efficient "machine-made" dimension (46). While AI performance still needs nontrivial refinements, its rapid progress will quickly achieve the required operational levels (47).

In this context, the clinical value of AI for HCC histological assessment is best viewed in pragmatic terms: as decision support within a digital pathology workflow, rather than as a stand-alone diagnostic tool. The most credible near-term uses include slide/case triage, assistance in standardizing challenging diagnostic and grading scenarios, and objective quantification of relevant morphologic features (for instance, tumor segmentation and recognition of patterns that correlate with microvascular invasion or a higher likelihood of recurrence), which may complement routine reporting and multidisciplinary discussions. A further area of interest is the use of histology-based AI outputs to prioritize reflex molecular testing, helping to allocate limited tissue more efficiently and to support selection for targeted strategies or clinical trial enrollment.

From an implementation standpoint, supervised algorithms may further accelerate clinical translation because they rely on pathologist-labeled endpoints, which define clear targets for development and enable more straightforward validation and safer workflow integration (48). In this process, pathologists remain central: they define clinically meaningful labels and reference standards, curate representative cohorts (including borderline cases), guide annotation and error analysis, and oversee deployment through ongoing quality assurance, drift monitoring, and periodic re-validation.

Accordingly, successful clinical adoption requires outputs that are interpretable and auditable, with an appropriate governance framework that preserves the pathologist's central role in the diagnostic process.

Additionally, clinical translation comes at a cost, and converting microscope-based pathology to the digital format is expensive. Whole slide scanners require investments in high-performance machines and dedicated support technicians. Prioritizing, selecting, acquiring, and implementing diagnostic algorithms involves scientific, operational, marketing, and ethical challenges.

CONCLUSIONS

This review focused on the benefits and limitations of AI employment in the pathology setting of liver cancer diagnosis (49). Extraordinary progress has been made over the past decade, with paramount advancements covering AI-based HCC histological phenotyping (potentially overcoming the human-related diagnostic variability), molecular profiling (providing clinically crucial histology-based information), prognostication (enhanced by the multimodal assessment of the cancer disease), and prediction of the treatment outcomes for targeted therapies (supporting personalized oncology). However, this is the beginning of the journey. While current experimental algorithms are more than just promising, their performance needs to be reinforced by well-structured data collections, validated by robust clinical trials, and supported by a more defined regulatory framework (5, 50).

The safe implementation of GAI in HCC diagnostic (phenotyping and molecular) pathology requires further demonstrating its clinical advantages over "traditional" human approaches. Nevertheless, the already achieved evidence reveals a strong foundation and well-established potential for a near-bright future.

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Authors' contributions

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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

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PERSPECTIVE

TARGETING PROGESTERONE SIGNALING FOR BREAST CANCER PREVENTION

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ABSTRACT: Effective strategies for primary prevention of breast cancer in premenopausal women remain limited, largely due to the modest tolerability and uptake of existing endocrine interventions. In our recent study, we demonstrate that short-term antagonism of progesterone receptor (PR) signaling targets multiple biological determinants of breast cancer risk, including epithelial progenitor activity, extracellular matrix remodeling and tissue mechanics. In this Perspective, we reflect on the conceptual and translational implications of these findings. We discuss how progesterone-driven endocrine-mechanical feedback loops shape increased risk tissue states and how their pharmacological disruption enables coordinated attenuation of epithelial and stromal susceptibility. We further consider the integration of multi-omics profiling and imaging biomarkers as a framework for prevention trials. PR antagonism may represent a promising foundation for biomarker-guided risk-reduction approaches in younger women and highlight broader opportunities for targeting tissue architecture in cancer prevention.

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Impact statement: Progesterone receptor antagonism targets drivers of breast cancer risk across epithelial and stromal tissue compartments, establishing a new approach to prevention in younger women.

Key words: *Breast cancer prevention; progesterone receptor antagonists; mammographic density; extracellular matrix remodeling.*

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INTRODUCTION

Breast cancer remains the leading cause of cancer-related mortality among women worldwide and continues to represent a major public health challenge, particularly for younger individuals at increased genetic or familial risk (1). Although substantial advances have been made in early detection and treatment, effective strategies for primary prevention in premenopausal women remain limited. Most established preventive interventions have focused on modulation of estrogen signaling, approaches that are often associated with poor tolerability, long treatment durations and limited uptake (2). In this context, our recent work proposes an alternative framework for breast cancer prevention based on targeted inhibition of progesterone receptor (PR) signaling (3).

PROGESTERONE AND BREAST CANCER RISK

Progesterone has long been recognized as a central regulator of mammary gland development, coordinating cyclical epithelial expansion and differentiation across the menstrual cycle. Experimental studies in both murine and human systems have demonstrated that progesterone drives proliferation of stem and progenitor cell populations, resulting in increased ductal branching and tissue complexity.

These effects are mediated predominantly through paracrine signaling from PR-positive luminal mature cells to PR-negative luminal progenitors, a population increasingly implicated as a likely cell of origin for aggressive breast cancer subtypes. Epidemiological data further support this biological model, linking exposure to exogenous progestins, through hormonal contraception or hormone replacement therapy, to increased breast cancer incidence. The relative contribution of each progesterone receptor isoform (PR-A and PR-B) to these effects remains incompletely understood. In addition, crosstalk with other steroid hormone receptors, including estrogen, androgen and glucocorticoid receptors, may further influence epithelial and stromal responses to progesterone signaling.

THE BC-APPS1 CLINICAL STUDY

Guided by the progesterone mechanistic insights, we designed the Breast Cancer-Anti-Progestin Prevention Study 1 (BC-APPS1) to interrogate the impact of PR antagonism on normal breast tissue biology *in vivo*. By integrating paired tissue sampling with multi-omics profiling, functional assays and advanced imaging, our study moves beyond conventional pharmacological trials and establishes a comprehensive experimental paradigm for early-phase cancer prevention research. This approach demonstrates how molecular and cellular profiling can be embedded within clinical studies to generate mechanistic understanding alongside translational relevance. As with many early window-of-opportunity studies, the trial involved a limited number of participants and relatively short exposure to treatment. Consequently, the biological effects observed should be interpreted as early mechanistic signals rather than evidence of long-term clinical benefit, and the generalizability of these findings across populations with diverse ethnic and genetic backgrounds remains to be determined. Short-term window studies in other tissues could similarly deconvolute causal pathways before large-scale clinical trials are undertaken.

EFFECTS OF PR ANTAGONISM ON BREAST TISSUE

A central contribution of the study is the demonstration that short-term treatment with the PR antagonist ulipristal acetate, selected because of its well-characterized pharmacological profile and its prior clinical use, induces consistent suppression of epithelial proliferation and luminal progenitor activity. Selective targeting of luminal progenitors in the premenopausal breast is of particular relevance given their susceptibility to oncogenic transformation and accumulation of genomic instability.

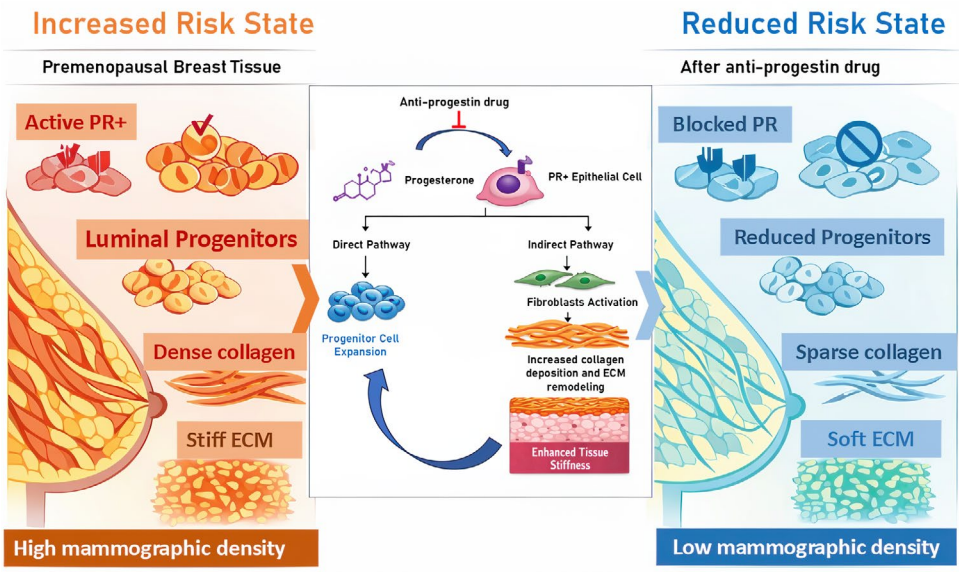

Figure 1. *Progesterone receptor antagonism disrupts endocrine–mechanical feedback in the premenopausal breast, leading to reduced luminal progenitor cell activity, decreased collagen organization and lower tissue stiffness. The schematic breast panels illustrate the tissue-level effects associated with anti-progestin therapy, including reductions in mammographic density, extracellular matrix remodeling and collagen deposition, consistent with a less stiff and mechanically active tissue microenvironment.*

Beyond its effects on epithelial compartments, the study reveals a profound influence of PR antagonism on the stromal microenvironment, thereby broadening the functional scope of PR biology (**Figure 1**). Integrated transcriptomic and proteomic analyses identify coordinated downregulation of extracellular matrix (ECM) components, most notably collagens I, IV and VI. These molecular changes are accompanied by reduced collagen fiber alignment and decreased tissue stiffness, as demonstrated by histological analysis and biomechanical measurements. Together, these findings establish progesterone signaling as a regulator not only of cellular behavior but also of tissue architecture and mechanical properties. This raises the possibility that PR activity functions upstream of mechano-transduction pathways, positioning endocrine signaling as a modulator of physical forces that shape oncogenic susceptibility. However, further mechanistic studies will be required to dissect how endocrine signaling interfaces with mechanotransduction pathways.

Of note, collagen VI emerged as an important element of the luminal progenitor niche. Imaging mass cytometry revealed a spatial association between collagen VI-rich regions and SOX9-positive luminal progenitor cells, providing evidence that biochemical and biomechanical cues converge within defined microenvironments to sustain high-risk cellular states. Complementary *in vitro* experiments further demonstrated that stiffness-induced progenitor activation can be antagonized by anti-progestins, reinforcing the proposed mechanistic model. Future work should determine whether collagen VI deposition precedes progenitor expansion or is instead a downstream consequence of progenitor activation. Collectively, these findings support a conceptual shift in how breast cancer risk is understood. Rather than being driven solely by cumulative genetic damage or isolated proliferative signals, risk emerges as a property of dynamic tissue ecosystems shaped by hormonal, stromal and mechanical interactions. Progesterone signaling appears to operate within a positive feedback loop, in which hormone-responsive epithelial cells stimulate fibroblast-mediated matrix deposition, increasing tissue stiffness and amplifying progenitor responsiveness. PR antagonism disrupts this circuit, leading to coordinated attenuation of both epithelial and stromal risk determinants. This system-level perspective warrants quantitative modelling of endocrine-mechanical feedback loops to define thresholds beyond which tissue states become self-sustaining or, conversely, reversible.

IMAGING CORRELATES AND RISK BIOMARKERS

The integration of molecular, histological and imaging data further enhances the translational relevance of the study. The observed reduction in fibroglandular volume on magnetic resonance imaging provides a clinically accessible correlation of underlying biological remodeling. Mammographic density is among the strongest established risk factors for breast cancer, yet remains largely untargeted in preventive strategies. By demonstrating that PR antagonism can modulate this parameter, the study establishes a direct link between molecular intervention and population-level risk markers. However, these findings are based on surrogate tissue and imaging markers and therefore do not directly demonstrate a reduction in breast cancer incidence. A key next step will be to assess whether density reduction persists after treatment cessation and whether imaging could serve as a surrogate endpoint in prevention trials.

Particularly noteworthy is the stronger effect of anti-progestin therapy on breast tissue in women with high mammographic density, suggesting that baseline density may serve as a predictive biomarker of response to anti-progestin therapy, although this requires validation in larger studies. Such stratification could enable more precise prevention strategies, targeting intervention to individuals most likely to derive meaningful risk reduction. As density reporting becomes increasingly routine, imaging could guide individualized risk-reduction strategies, potentially reducing overtreatment while ensuring that high-risk individuals receive targeted intervention. This approach aligns with broader trends in precision medicine and underscores the importance of integrating biological and imaging-based profiling into preventive frameworks.

FUTURE DIRECTIONS FOR PR ANTAGONISTS

If validated in larger and longer-term studies, PR antagonism could represent a new class of pharmacological prevention for premenopausal women at elevated risk. Nevertheless, such strategies would likely require repeated or prolonged treatment, making careful evaluation of safety in hormone-sensitive tissues essential. The societal implications of this possibility are substantial. Preventive options for younger women are currently limited, with surgical risk reduction and

Table 1. *Future directions for progesterone receptor antagonism in breast cancer prevention.*

DOMAIN	KEY QUESTIONS	FUTURE RESEARCH DIRECTIONS
Long-term efficacy	Are short-term biological changes durable?	Longitudinal prevention trials with extended follow-up
Safety	What are the systemic effects of prolonged PR antagonism?	Evaluation of endocrine, metabolic and reproductive safety
Mechanisms	How do endocrine-mechanical feedback loops operate at the molecular level?	Integrated spatial multi-omics and mechanobiology studies
Downstream progesterone targets	Which PR-regulated pathways could be therapeutically targeted beyond PR antagonism?	Investigation of downstream effectors such as RANKL (denosumab), CXCL13, Wnt4, and extracellular matrix regulators including collagens
Biomarkers	Can imaging markers predict response?	Validation of mammographic density and fibroglandular volume as predictive biomarkers
Population diversity	Do responses vary across genetic or ethnic backgrounds?	Inclusion of diverse cohorts and stratified analyses
Prevention strategies	How should PR antagonists be administered?	Intermittent or cyclic dosing regimens aligned with hormonal cycles

long-term endocrine therapies posing significant physical and psychological burdens. The repositioning of PR antagonists for cancer prevention therefore warrants ethical and regulatory consideration, particularly with respect to long-term safety, fertility, acceptability and equitable access (**Table 1**). In this context, intermittent or cyclic dosing strategies aligned with endogenous hormonal rhythms merit exploration as potentially more physiological prevention regimens.

DISCUSSION AND CONCLUSIONS

There are several additional considerations that merit discussion. One concerns the concept of mechanical memory. ECM organization and tissue stiffness can persist long after hormonal cues change, influencing cellular behavior over extended timescales. This raises the possibility that short-term PR antagonism may induce durable protective states by reprogramming the mechanical niche. Future studies should explore whether such interventions confer long-lasting resistance to oncogenic transformation. Experimental models incorporating washout periods could directly test the durability of these mechanically imprinted states. A second implication lies in the identification of endocrine-mechanical feedback loops as drivers of cancer risk. Interrupting these self-reinforcing circuits pharmacologically represents a novel preventive paradigm, targeting not only molecular drivers but also the tissue architecture that sustain them. This concept may have relevance beyond breast cancer, particularly in fibrosis-associated malignancies or tissues where hormonal signaling and mechanical stress intersect. Finally, the potential psychosocial impact of biological risk reduction warrants consideration. Demonstrable reductions in mammographic density and tissue-based risk markers may alleviate cancer-related anxiety in high-risk individuals. Integrating biological prevention with measures of psychological well-being could represent an important dimension of future prevention trials. Such longitudinal cohorts, combining patient-reported outcomes with molecular endpoints, may also enable the development of predictive models of individual risk trajectories, thereby supporting adaptive use of PR antagonists in response to evolving tissue states rather than fixed schedules. Future prevention studies could integrate patient-reported outcomes alongside molecular and imaging markers to better understand how biological risk modulation influences patient perception and decision-making. Moreover, if PR antagonists were to be implemented as preventive interventions, issues of cost, access and regulatory approval would also need to be addressed to ensure equitable availability across different healthcare systems, including low- and middle-income countries.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflicts of interest

The authors declare no competing interests.

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Authors' contributions

AC, BMS: conceptualization, writing - original draft, writing - review & editing, final approval.

Publications ethics

Plagiarism

The article provides a perspective of a study, with accurate citations.

Data falsification and fabrication

The writing and contents of the article are entirely developed by the authors. The authors used an artificial intelligence tool to assist in the creation of **Figure 1**.

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CASE REPORT

SARCOMATOID CARCINOMA OF THE PROSTATE – RARE ENTITY WITH RARE PRESENTATION: A CASE REPORT

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ABSTRACT: Sarcomatoid carcinoma of the prostate is a rare entity constituting less than 0.1% of primary malignant tumors of the prostate. This malignancy is characterized by the presence of both glandular and sarcomatoid components, posing unique challenges in its diagnosis and management. We describe the case of a 66-year-old man who presented with a two-month history of left-sided chest pain and diffuse lower backache. Imaging revealed a mass lesion involving the prostate with widespread bone metastasis, and the serum prostate-specific antigen (PSA) level was 11.6 ng/ml. Subsequent immunohistochemical analysis confirmed sarcomatoid carcinoma of the prostate. The diagnosis and management of sarcomatoid carcinoma of the prostate are challenging due to its rarity, unusual presentation, and biphasic histologic nature. This aggressive malignancy carries a dismal prognosis and should be considered in the differential diagnosis of patients presenting with bone metastases and disproportionately low PSA levels.

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Impact statement: This case highlights the importance of considering sarcomatoid carcinoma of the prostate in atypical presentations of metastatic prostate malignancy with disproportionately low PSA levels.

Key words: *Immunohistochemistry; prostatic neoplasms; prostate-specific antigen; prognosis; sarcomatoid.*

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INTRODUCTION

Sarcomatoid carcinoma of the prostate is an exceptionally rare malignancy, accounting for fewer than 0.1% of all prostate malignancies (1). This tumor consists of mixture of both carcinomatous and sarcomatoid components, highlighting the need for meticulous histopathological evaluation. The most recent edition of World Health Organization classification of tumors, sarcomatoid carcinoma of the prostate has been categorized as a subtype of acinar adenocarcinoma (2). To date, only a few hundred cases of sarcomatoid carcinoma of the prostate have been described in the literature worldwide. We present a case of sarcomatoid carcinoma of the prostate in a 66-year-old man, illustrating the diverse clinical presentation and challenges in diagnosis, and management.

CASE PRESENTATION

We present the case of a 66-year-old man who presented with complaints of left-sided chest pain, and diffuse pain in the lower back that has been radiating to the right thigh, associated with loss of appetite for the previous 2-3 months. His physical exam-

ination revealed mild tenderness in the chest wall on the left side, otherwise was unremarkable. He presented to an outside hospital where CT chest revealed a lesion involving the left 7th rib, and an MRI of the lumbo-sacral spine which suggested marrow infiltrative changes or metastatic disease involving D11, L2, and L3 vertebrae. A soft tissue lesion involving the right foraminal region abutting the right-sided exiting nerve roots was observed at the L2 vertebral level. A pelvic and abdominal ultrasound revealed grade II prostatomegaly and early liver parenchymal disease. These results highlighted the possibility of multiple myeloma or primary originating in the prostate and metastasizing to the bone. With a negative myeloma profile, the prostate-specific antigen (PSA) levels were slightly elevated (11.6 ng/ml). A subsequent fluorodeoxyglucose positron emission tomography (FDG-PET) scan for staging showed FDG avid heterogeneously enhancing large mass lesion involving both lobes of the prostate gland, primarily involving the right half of the prostate and associated with peri-prostatic infiltration. The FDG-avid prostatic mass lesion had loss of fat planes with bilateral seminal vesicles, and the posterior wall of the urinary bladder. Multiple sclerotic-lytic lesions were observed in the bodies of multiple vertebrae, the right scapula, the left clavicle, and the sacrum, as well as the shafts of multiple ribs on both sides, the largest of which involved the shaft of the seventh rib on the left side (SUV = 7.48 gm/ml) (**Figure 1**).

A biopsy from the rib lesion of our patient revealed deposits from the sarcomatoid lesion, possibly of prostatic origin. The most likely differential diagnosis was sarcoma originating from the prostate and double malignancy-carcinoma prostate and sarcoma arising from the bone. Histopathological examination of the rib lesion revealed plump, spindle-shaped cells with features of atypia in the form of nuclear

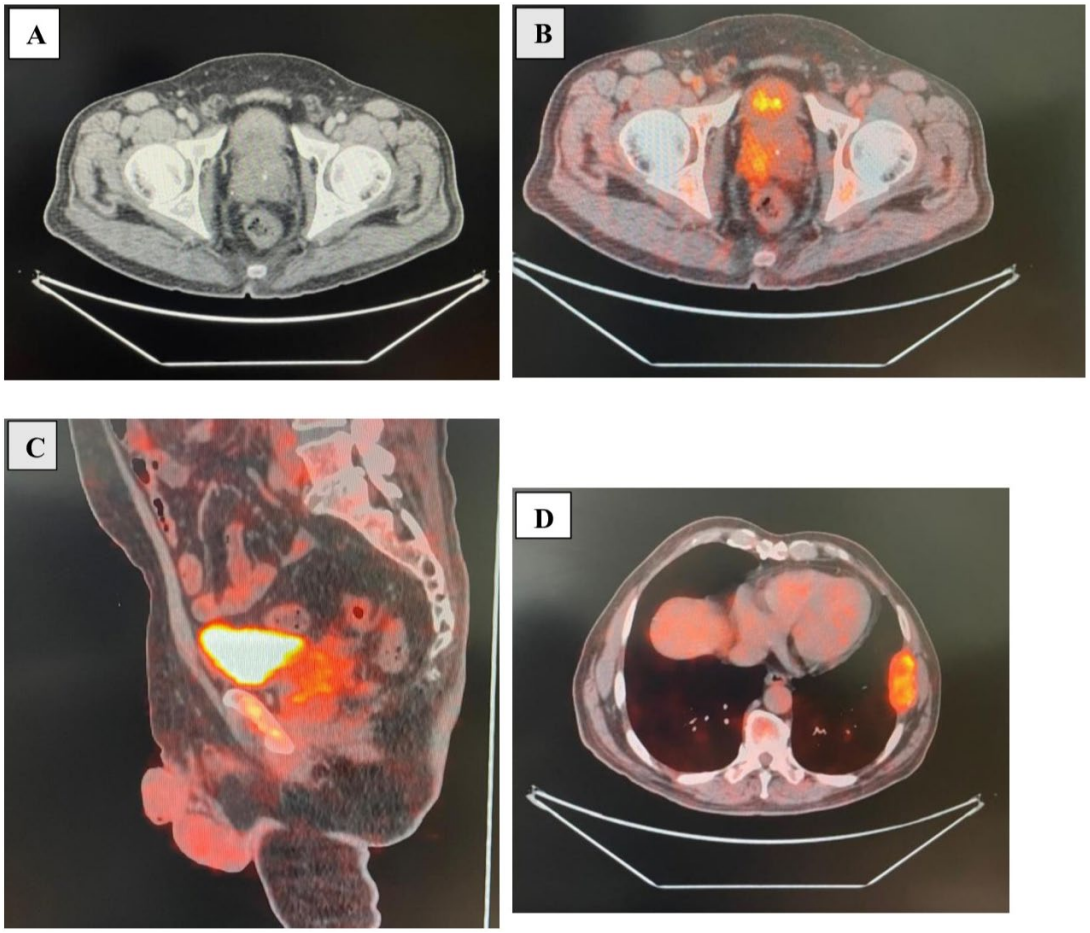

Figure 1. ***(A-D)*** *FDG avid heterogeneously enhancing large mass lesion involving both lobes of the prostate gland, predominantly involved the right half of the prostate associated with peri-prostatic infiltration with loss of fat planes with bilateral seminal vesicles and the posterior wall of the urinary bladder. FDG-avid multiple sclerotic-lytic lesions were seen involving the shafts of multiple ribs on both sides, the largest involving the shaft of the 7th rib on the left side (SUV = 7.48 gm/ml).*

pleomorphism and hyperchromatic cells arranged in fascicles. Additionally, focal areas of necrosis and haemorrhage were also noted. The diagnosis of spindle cell neoplasm with a potential for sarcomatoid carcinoma was confirmed by immunohistochemistry staining, which showed some areas to be positive for calponin, desmin, and smooth muscle actin (SMA) consistent with sarcomatous appearance. S-100, H-caldesmon, myogenin, CD117, EMA, and CD34 did not show any staining. Ki-67 proliferative activity was 18-20% (**Figure 2**).

A multiparametric MRI of the prostate revealed a heterogeneous mass with diffusion restriction and early enhancement measuring 3.2 × 3.3 × 5.8 cm that

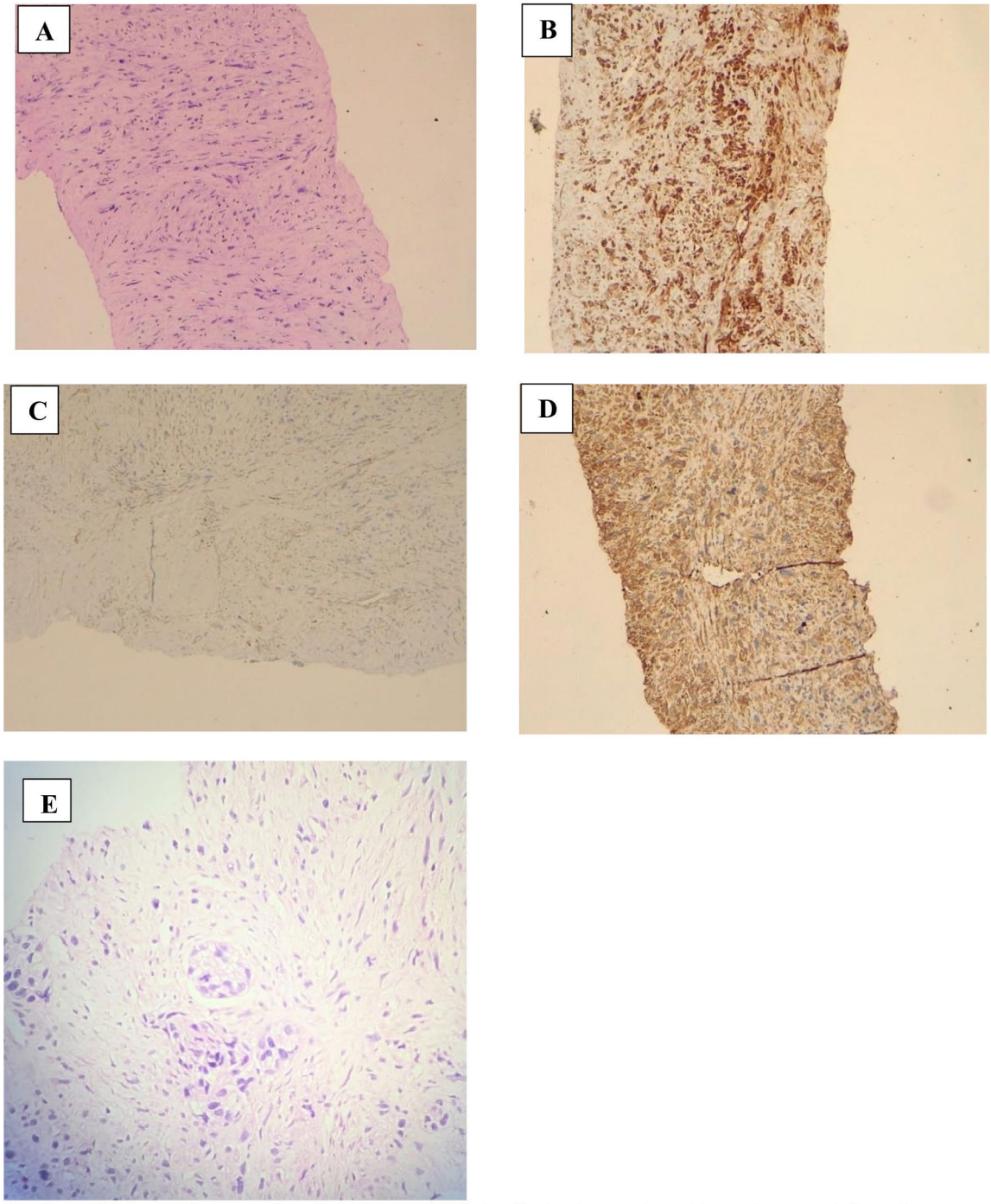

Figure 2. *Microscopic examination from the left rib lesion using **(A)** H&E staining demonstrating plump spindle-shaped cells with atypia in the form of nuclear pleomorphism and hyperchromatous in fascicles at a low power field 10x; **(B)** Immunohistochemical staining showing moderate and diffuse immunoreactivity for Calponin (IHC x400); **(C)** Strong and diffuse positive immunoreactivity for Desmin (IHC x400); **(D)** Strong and diffuse positive immunoreactivity for SMA (IHC x400); **(E)** Histopathological examination of the prostate revealed a biphasic malignant neoplasm composed of epithelial elements arranged in glandular structures with sarcomatous component characterized by plump spindle cells.*

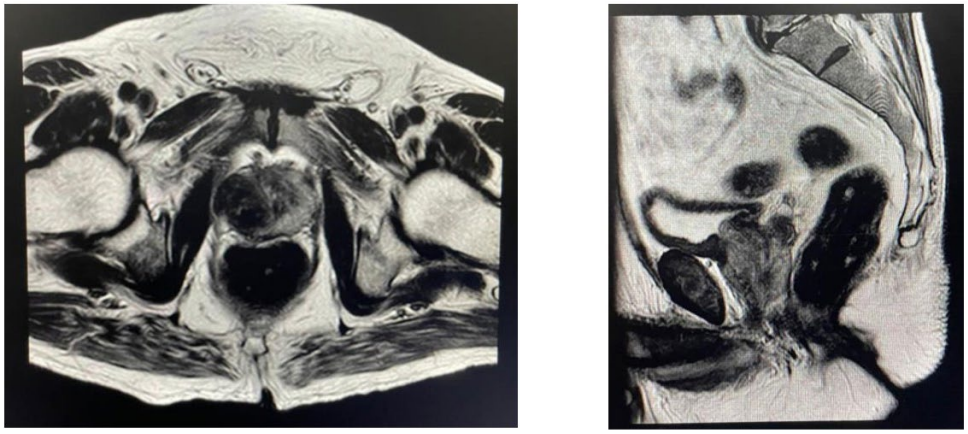

Figure 3. *Magnetic resonance imaging of the prostate revealing heterogeneous mass with diffusion restriction and early enhancement measuring 3.2 × 3.3 × 5.8 cm replacing the peripheral zone of the prostate, infiltrating the right neurovascular bundle, base, and right side of the seminal vesicle with the abutment of the right obturator internus muscle on the lateral side.*

replaced the peripheral zone of the prostate on the right side. Posteriorly, this mass infiltrated the right neurovascular bundle, base, and right side of the seminal vesicle with the abutment of the right obturator internus muscle on the lateral side (**Figure 3**). Subsequently the patient underwent prostate biopsy which revealed admixing of spindle cells and epithelial components, with the epithelial component constituting only approximately 10-15% of the prostatic tissue. No transition zone was identified between the two components. Mitotic activity was noted at 3-4 mitoses per high-power field. Areas of necrosis and perineural invasion were also present. Gleason score was 4 + 3 = 7. This favored the diagnosis of sarcomatoid carcinoma of prostate.

After a multidisciplinary team discussion, in view of advanced metastatic disease and severe left-sided chest pain, he was treated with palliative radiation therapy to the left rib lesion to a dose of 30 Gy in ten fractions using intensity-modulated radiation therapy. The patient experienced symptomatic improvement and was referred to medical oncology for systemic treatment.

DISCUSSION

Soft tissue sarcomas encompass a group of highly heterogeneous mesenchymal malignancies accounting for < 1% of all cancers. Sarcomatoid carcinoma of the prostate is a rare malignant biphasic tumor characterized by the coexistence of epithelial (carcinomatous) and mesenchymal (sarcomatous) components, with variable presence of heterologous elements (3). The exact origin of these tumors is controversial. It is hypothesized that these tumors arise from the independent development of sarcomatous and carcinomatous components within the prostate, resulting in a biphasic morphology. This tumor may arise de novo or develop through histologic transformation occurring several years after treatment for conventional prostatic adenocarcinoma (4).

The clinical presentation of sarcomatoid carcinoma of the prostate is heterogeneous, with patients presenting with lower urinary tract obstructive symptoms, including poor urinary stream, hesitancy, post-void dribbling, and a sensation of incomplete bladder emptying. In contrast, irritative symptoms such as urinary frequency, urgency, and dysuria are less frequently reported (5). Nevertheless, rare atypical presentations without urinary complaints have been described in the literature. Jayasinghe *et al*. have reported sarcomatoid carcinoma of the prostate presenting with bilateral cervical lymphadenopathy without accompanying lower urinary tract symptoms (6). Our patient demonstrated an unusual clinical presentation, with chest pain and diffuse lower back pain radiating to the right thigh, in the absence of urinary symptoms. The absence of typical symptoms of carcinoma prostate may have contributed to a delay in diagnosis in this patient.

The diagnosis of this tumor is complicated by several clinicopathologic and immunohistochemical challenges. These include disproportionately low PSA levels relative to disease burden, likely attributable to tumor dedifferentiation, as well as the predominance of a sarcomatoid component on histopathology, with heterologous elements that may be present

or absent (4). Furthermore, immunohistochemistry poses diagnostic pitfalls in this malignancy due to significant overlap with true prostatic sarcomas and other primary mesenchymal neoplasms. In the present case, immunohistochemical findings supported sarcomatoid differentiation, with tumor cells showing positivity for smooth SMA and desmin, and a high proliferative activity (Ki-67 labeling index of approximately 18–20%). However, the absence of H-caldesmon expression argued against true smooth muscle differentiation, thereby excluding leiomyosarcoma. Additionally, negative staining for myogenin, S100, CD34, and CD117 helped rule out rhabdomyosarcoma, neural or melanocytic tumors, solitary fibrous tumor, and gastrointestinal stromal tumor, respectively. These findings highlight the importance of a comprehensive immunohistochemical panel, interpreted in conjunction with histomorphology, to distinguish sarcomatoid carcinoma of the prostate from its histologic mimics. As our patient presented with metastatic disease, an initial biopsy was performed from the rib lesion, followed by immunohistochemical evaluation. Subsequently, a prostate biopsy was obtained to establish the primary site. However, the limited tissue available in the core biopsy was sufficient only to confirm a prostatic origin, and additional epithelial immunohistochemical markers that could have further strengthened diagnostic confirmation could not be performed due to tissue constraints and limited availability in our setting.

In a recent single-institutional study by Tekin *et al.* (4), eight patients were evaluated to characterize the molecular landscape of sarcomatoid carcinoma of the prostate. Of these, three patients (37.5%) had a prior history of acinar adenocarcinoma prostate treated with radiation therapy, while five patients (62.5%) demonstrated mixed histology comprising adenocarcinoma and sarcomatoid components. Notably, seven patients (87.5%) either presented with distant metastasis at diagnosis or developed metastatic disease during follow-up. Our patient presented with bone metastases, the most frequently reported site of distant spread in sarcomatoid carcinoma of the prostate. Abiodun *et al*. (7) reported distant metastases in 83.3% of cases, with bone being the predominant site, underscoring the aggressive nature of this malignancy and its tendency for early systemic dissemination. There is no established standard treatment protocol for sarcomatoid carcinoma of prostate due to its rarity (8). For resectable tumors, surgery is the primary treatment, followed by adjuvant radiation therapy with or without chemotherapy in patients with positive margins or nodal disease (3). The prognosis of sarcomatoid carcinoma prostate is poor, with a reported one-year mortality risk of approximately 20% (9).

Sarcomatoid carcinoma of prostate is exceedingly rare; consequently, data on its clinical presentation, imaging features, immunohistochemical profile, and management remain limited. This case emphasizes on timely reporting of these cases and a coordinated, multidisciplinary approach involving oncologists, pathologists, and urologists.

Limitations

This case has certain limitations. The diagnosis was primarily based on immunohistochemical evaluation of the rib lesion, and the epithelial component was not assessed. A broader immunohistochemical panel, including additional epithelial markers, may have provided further diagnostic clarification and strengthened the distinction between a primary bone sarcoma arising synchronously with prostatic adenocarcinoma and a sarcomatoid variant of prostatic adenocarcinoma with bone metastasis, which cannot be confidently excluded in this case. Therefore, the interpretation of this report should be approached with caution, acknowledging the diagnostic limitations and emphasizing the need for clinicopathologic correlation.

CONCLUSIONS

Sarcomatoid carcinoma of the prostate is a highly aggressive malignancy, for which histopathological assessment supported by immunohistochemical evaluation is central to establishing the diagnosis. This case report underscores the need for heightened clinical awareness of the sarcomatoid variant of prostate cancer in the setting of bone metastasis. Owing to its rarity, standardized treatment recommendations for this entity are lacking. Therefore, multi-institutional collaboration to report individual patient data is essential to identify effective treatment approaches and improve survival outcomes in these patients.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflicts of interest

The authors declare no competing interests.

Availability of data and material

All relevant data supporting this case report are included within the manuscript.

Authors' contributions

HSP, AR, VM: insight and valuable inputs to the manuscript. TK, DR: radiological images and data related to the case. PN, HS: writing - original draft, writing - review & editing.

Ethical approval

Ethical approval was not required for this study in accordance with institutional guidelines, as it involved the reporting of an individual case.
Written informed consent was obtained from the patient for publication of this article.

Publications ethics

Plagiarism

We hereby declare that this manuscript is an original work and 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 case report.

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