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REVIEW

CYCLIN-DEPENDENT KINASES: MECHANISMS OF ACTION AND THERAPEUTIC POTENTIAL

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ABSTRACT: Cyclin-dependent kinases (CDKs) are critical regulators of the cell cycle and play essential roles in DNA repair and maintenance of genome stability. Importantly, dysregulation of CDK activity is a key contributor to oncogenesis. CDKs functionally interact with various regulatory molecules, including cyclins, CDK protein inhibitors, and transcription factors such as p53 and retinoblastoma (RB) proteins. Upon mitogenic stimuli, CDK4/6 binds to cyclin D proteins, leading to hyperphosphorylation of RB proteins, activation of E2F-dependent transcriptional programs, as well as cell cycle initiation and progression. Dysregulation of CDK4/6 action is associated with cancer, and CDK4/6 inhibitors have emerged as very important anticancer therapies. This review will focus on the molecular mechanisms by which CDKs and cyclins regulate the cell cycle, their roles in maintaining genomic integrity, their interactions with RB, and the therapeutic potential of CDK 4/6 inhibitors.

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Impact statement: The transition from broad-spectrum kinase inhibitors to selective CDK4/6 inhibitors represents an important step in oncology, moving away from high-toxicity "pan-CDK" approaches toward targeted, precision medicine.

Key words: *CDKs; Cyclins; CDK inhibitors; RB Proteins; Cell cycle.*

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INTRODUCTION

Cyclin-dependent kinases (CDKs) are a family of serine/threonine kinases with crucial roles in many cellular processes, including cell cycle progression and transcriptional regulation (1). CDKs were originally identified and categorized based on their functions in cell cycle regulation and their association with activating partners, such as cyclins and CDK activating kinases (2-4). CDKs involved in cell cycle regulation include CDK1, CDK2, CDK3, CDK4, CDK5, CDK6, CDK14, CDK15, CDK16, CDK17, and CDK18. Whereas CDK7, CDK8, CDK9 (5), CDK10, CDK11, CDK12, CDK13, CDK19, and CDK20 are involved in transcriptional regulation. CDK2, CDK4 and CDK7 play a role in immune response activation, while CDK1, CDK2, CDK5 and CDK12 contribute to the DNA damage response (DDR). Accordingly, CDK activity is tightly regulated in a cell cycle phase-dependent manner by specific cyclins, changes in phosphorylation, and other regulatory molecules including CDK protein inhibitors (6). Beyond their key role in triggering cell cycle entry (**Figure 1**), CDKs orchestrate DNA synthesis and chromosome segregation (7), which are crucial processes for cell cycle progression and cell fate determination.

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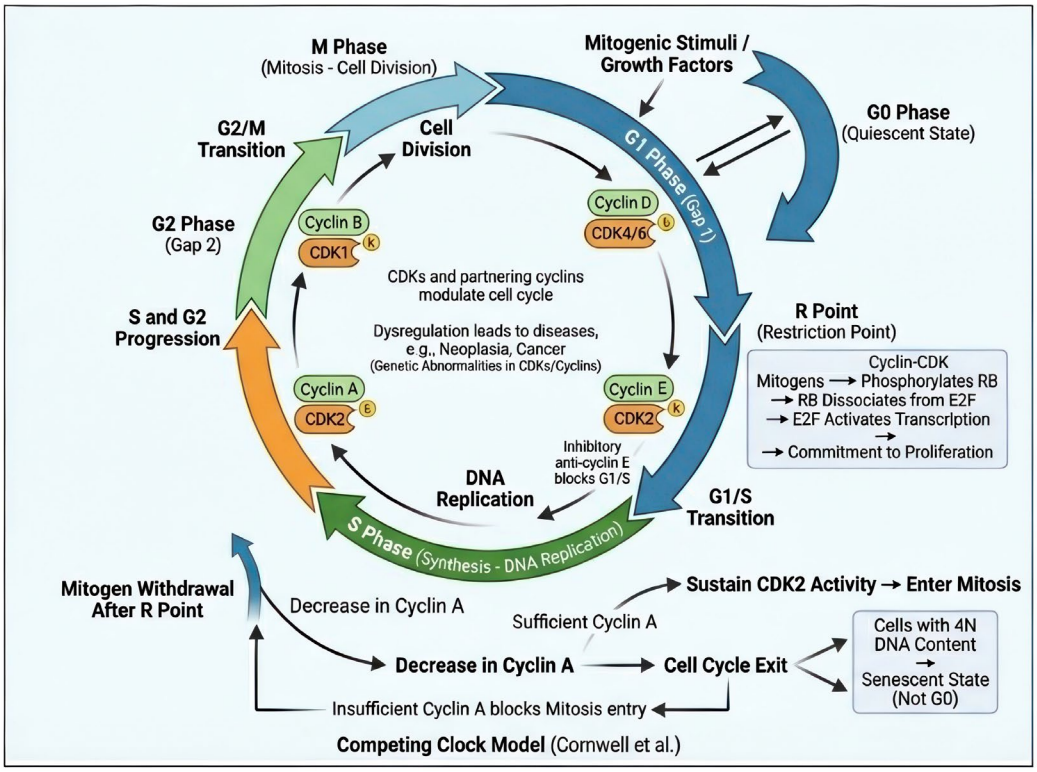

Figure 1. *Overview of cell cycle regulation and the revised "Competing Clock" model.*
This diagram illustrates the major phases of the mammalian cell cycle (G1, S, G2, M) and the quiescent state (G0). Cycle progression is driven by mitogenic stimuli and governed by specific CDK and cyclin complexes (e.g., Cyclin D-CDK4/6, Cyclin E-CDK2). The canonical Restriction Point (R Point) mechanism is detailed on the right. Cyclin-CDK-mediated phosphorylation of RB proteins releases the E2F transcription factor, leading to commitment to proliferation. The lower section presents the revised "Competing Clock Model" (Cornwell et al., 2023). Contrary to the canonical view of irreversible commitment after the R point, this model proposes that mitogen withdrawal post-R point leads to a decrease in Cyclin A levels. Insufficient Cyclin A fails to sustain necessary CDK2 activity, resulting in a cell pool exiting cycle in a senescent state with 4N DNA content, rather than entry into mitosis.

CDK4 and CDK6 (CDK4/6) are the main CDKs responsible for cell cycle initiation and proliferation, as they modulate retinoblastoma (RB) family tumor suppressor protein activity by promoting their hyperphosphorylation (8). Hyperphosphorylated RBs are inactivated, thereby releasing E2F-dependent transcriptional programs (9-11). Dysregulation of CDKs, specifically (CDK4/6), are associated with cancer initiation. Thus, CDK4/6 inhibitors are very attractive targets for cancer therapy. In this review, we will discuss molecular properties of CDKs and cyclins, including their main protein interactors, role in modulating the cell cycle, and focus on CDK4/6 inhibitors currently used in clinical practice. Although CDK4/6 inhibitors have transformed the therapeutic landscape of hormone-receptor-positive (HR^+) breast cancer, substantial unmet needs remain, including mechanisms of drug resistance, incomplete understanding of CDK4 *versus* CDK6 dependency across tumor types, lack of robust predictive biomarkers, and limited benefit in RB-deficient or Triple-Negative Breast Cancer (TNBC). Moreover, the expanding evidence that CDK inhibition modulates tumor immunogenicity underscores the need to redefine CDK targeting beyond cytostatic control and toward rational combination strategies. Thus, a comprehensive reassessment of CDK biology and therapeutic targeting is both timely and necessary.

THE CELL CYCLE

The cell cycle is crucial for controlling cell development and cell regulation (12). The cell cycle, as shown in **Figure 1**, is divided into G0, G1, S, G2, and M phases. When cells are withdrawn from the cell cycle, they are in a quiescent state, defined as the G0 phase (13). The S and M phases are parted by the G1 and G2 phases, which are defined also as "Gap Phases." Upon mitogenic stimuli, cells enter

the cell cycle in the G1 phase and then proceed through the synthesis (S) phase, where DNA replicates, followed by the G2 phase (13). In the mitosis (M) phase, cell division occurs. CDKs and partnering cyclins are essential components in modulating the cell cycle (**Figure 1**), and dysregulation of this process is associated with many diseases, including neoplasia. Genetic abnormalities such as gene amplification (*i.e.*, *CCND1, CDK4*), overexpression (*i.e.*, Cyclin E), translocation (*i.e.*, t11;14), and 3'-UTR mutations can often cause dysregulations in CDKs, cyclins, affecting cell cycle progression and possibly leading to cancer development (12, 14-16).
In mammalian cells, growth factor stimulation promotes cell exit from the G0 phase and initiation of the G1 phase (2). Importantly, CDK4/6 interaction with cyclin D is the primary contributor to starting the G1 phase. Furthermore, the activation of CDK2 and cyclin E begins at the G1/S phase transition, as confirmed by using inhibitory anti-cyclin E antibodies, which blocked the cells at the start of the G1/S phase transition (17, 18).
The restriction (R) point of the cell cycle is a crucial checkpoint in late G1 when cells commit to proliferation and DNA replication at the G1/S transition and become largely independent of continued mitogenic stimulation (18). In early G1, mitogen-dependent induction of D-type cyclins promotes the formation of cyclin-D-CDK4/6 complexes, which initiate RB phosphorylation and weaken RB-mediated repression of E2F transcription factors (2, 19). As mitogens promote the assembly of cyclin-CDK complexes, they activate cyclin-CDKs, which progressively phosphorylate RB, destabilizing inhibitory RB-E2F complexes and releasing E2F proteins to activate transcription of genes required for S-phase entry (9, 20). Consistent with this sequential model, cyclin E-CDK2 activity rises at the G1/S transition, further reinforcing RB inactivation through RB hyperphosphorylation and establishing a CDK-E2F positive feedback loop that consolidates commitment to S phase (9, 17).
Before passage through the R point, mitogen withdrawal reduces upstream cyclin D-CDK4/6 signaling and favors re-establishment of RB-dependent repression, in part through increased CDK inhibitor activity (*e.g.*, p21/p27), thereby preventing RB hyperphosphorylation and blocking E2F-dependent transcription (21, 22). By contrast, after cells pass the R point, RB remains functionally inactivated and the E2F-driven transcriptional program sustains progression through S phase even when mitogenic signals decline, while subsequent cyclin-CDK complexes coordinate orderly transitions across the remaining phases of the cell cycle (**Figure 1**) (18).
Cornwell and colleagues recently presented a revised model, the competing clock model, in which they proposed that mitogen withdrawal results in a decrease of cyclin A protein level resulting in cell cycle exit, even after passing the R point (18). Notably, cells with 4N DNA content exiting the cell cycle (about 15%) are not in G0 but in a sort of senescent state. Cells enter mitosis only when sufficient cyclin A protein is available to sustain CDK2 protein activity (18).

CDKS

CDKs are conserved across eukaryotes, ranging from unicellular organisms such as yeast to complex mammalian organisms (23). In humans, the CDK family comprises 20 members classified into two main categories: "cell cycle CDKs" and "transcriptional CDKs" (24-26). The "cell cycle CDKs" are CDK1, CDK2, CDK3, CDK4, CDK6, and CDK7, and primarily regulate cell cycle progression, whereas the "transcriptional CDKs", CDK7, CDK8, CDK9, first identified as proteins containing the "PITALRE" motif (27), CDK10, originally cloned as a "PISSLRE"-containing protein (27, 28) and CDK11, CDK12, and CDK13, modulate the transcription of specific target genes (24).
All CDK proteins share a conserved 250 amino acid catalytic domain with two-lobe structures: the amino-terminal (N) lobe and the carboxy-terminal (C) lobe (29). The N lobe presents β-sheets while the carboxy-terminal lobe presents α-helices and has an active sandwich-like site in between. The N-lobes have a glycine-rich inhibitory element, known as the G-loop, while C-lobes have an activation segment which contains the conserved PSTAIRE sequence in CDK1. The C-lobes also contain an activation domain, which extends from the Asp-Phe-Gly (DPG) motif to the Ala-Pro-Glu (APE) motif (30), while including phosphorylation-sensitive residues in their T-loops (31). For all CDKs except CDK5, phosphorylation of T-loops is required for kinase activation.
CDK4/6 are homologous CDKs that are essential for mammalian cell proliferation (19), as they drive progression through the cell cycle from the G1 phase to S phase. CDK4/6 function differs from CDK1 and CDK2, which work during later stages of the cell cycle. The activity of CDK4/6 is tightly regulated by cyclin D isoforms (D1, D2, and D3), whose expression is modulated by extracellular signals, including mitogens, cytokines, and cell-cell contact (19, 32). Under mito-

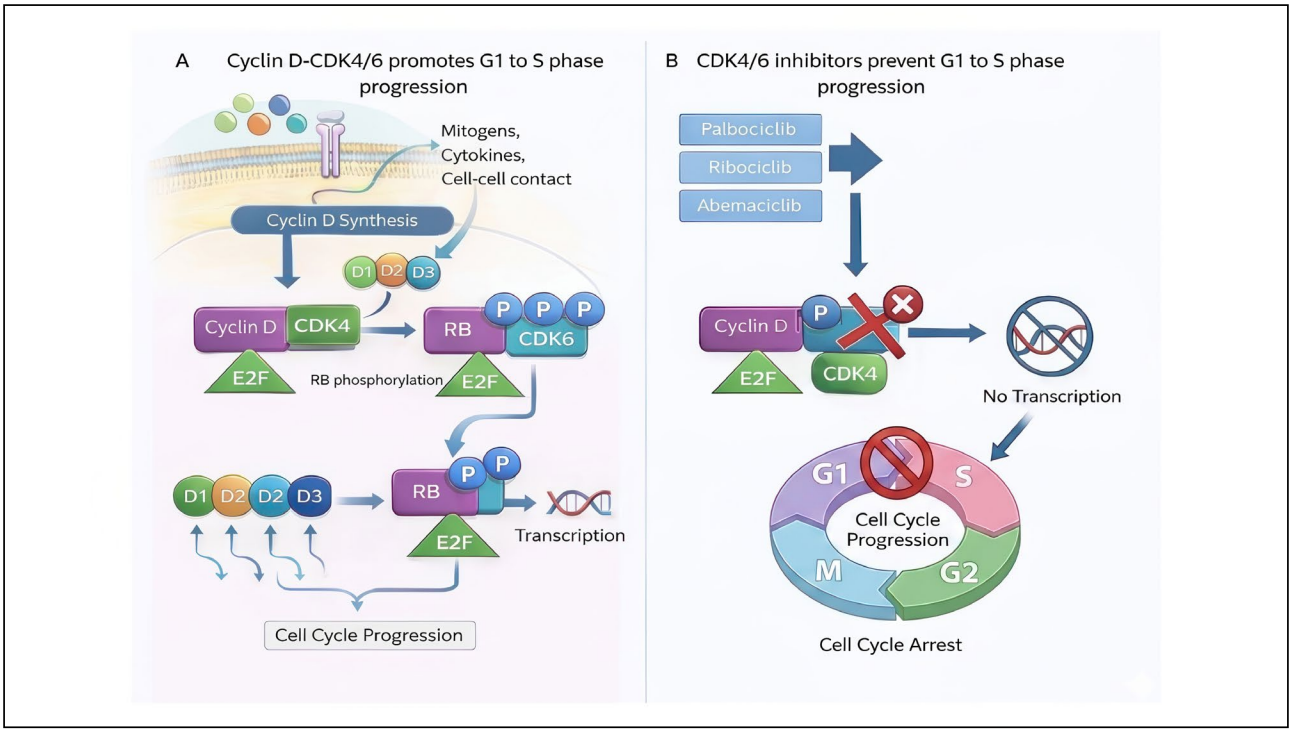

Figure 2. *Regulation of G1-S phase transition by Cyclin D-CDK4/6 and selective CDK4/6 inhibitors action.*
(A) *Mitogenic signals, including growth factors, cytokines, and cell–cell contact, induce cyclin D during early G1 phase. Cyclin D isoforms (D1, D2, and D3) associate with CDK4 and CDK6 to form active Cyclin D-CDK4/6 complexes, which phosphorylate RB proteins. RB phosphorylation disrupts its inhibitory action on E2F transcription factors, resulting in E2F activation and transcription of genes required for G1-S phase transition and cell cycle progression.* ***(B)*** *Pharmacological inhibition of CDK4/6 by selective inhibitors, including palbociclib, ribociclib, and abemaciclib, block Cyclin D-CDK4/6 kinase activity and prevents RB phosphorylation. Thus, RB remains bound to E2F, suppressing E2F-dependent transcription and leading to G1 cell cycle arrest, thereby preventing progression into S phase.*

genic stimuli, D-type cyclin levels increase, leading to the formation and activation of cyclin D-CDK4/6 complexes and subsequent phosphorylation and inactivation of RB proteins, releasing their inhibitory action on E2F transcription factors, thereby promoting transcription of genes essential for cell cycle progression and entry into S phase (**Figure 2A**). It is important to specify that CDK7 plays a dual role within the cell. In addition to being a crucial component of the general transcription factor TFIIH, it functions as a Cyclin-Activating Kinase (CAK). In this capacity, CDK7 phosphorylates the T-loop activation segment of several key kinases, including CDK1, CDK2, CDK4, and CDK6, thereby enabling their catalytic activity (33).

CYCLINS

Cyclins are key for cell cycle progression and work with their cell cycle-specific CDK partners (34). The cyclin-CDKs complexes are essential modulators of the cell cycle, each complex working at specific phases of the cell cycle. Cyclin proteins contain cyclin box domains (CBDs) or CBD-like domains which regulate cyclin-CDK binding (35). CBDs were first discovered in the cell cycle proteins of sea urchins. There are 46 human proteins which contain CBDs or CBD-like domains, where 29 of them are direct CDK activators, such as Speedy A and p35 (35). However, not all CBDs and CBD-like domains-containing cyclins have a CDK partner, like G-type cyclin.

D-type cyclins are crucial for controlling cell cycle transition to the G1 phase as well as cell cycle progression (36) through the G1/S transition phase. D-type cyclins are essential, especially during mitogenic signaling, where they are required for initiating cell cycle in response to growth factor stimulation. D-type cyclins form a complex with CDK4 or CDK6 to modulate the transition from the G1 phase to S phase. D-type cyclins are essential prognostic biomarkers, especially for neoplasia since they are predictors of cell proliferative capacity (34). Three genes encode for specific D-type cyclins, each located

on a different chromosome (36). Those genes are *CCND1:*11q13.3, *CCND2:*12p12.23, and *CCND3:* 6p21.1, which encode for a specific D-type cyclin that share approximately 57% protein sequence identity and are expressed during the G1 phase and G1/S phase of the cell cycle.

During S phase, the cyclin A-CDK2 complex promotes DNA replication and facilitates the S to G2 transition (37). As cells progress into late S/G2, cyclin A increasingly associates with CDK1, supporting early mitotic events and faithful chromosome segregation, in part by regulating the stability of kinetochore-microtubule attachments (37).

Eukaryotic species have three B-type cyclin genes (*CCNB1*, *CCNB2*, and *CCNB3*), which are expressed during the G2/M phase transition period of the cell cycle (38, 39). In yeast model organisms, the Mcm1 transcription factor interacts with Swi Five Factor (SFF), and SFF mediates cell cycle-dependent promoter activation of *CCNB1* and *CCNB2*. In addition, the *CCNB1* promoter is regulated by *cis*-acting elements, which require the activation of a G2/M promoter; therefore, cyclin B works with CDK1 for activation of the G2/M transition period, indicating a phase-specific CDKs activation, and expression of their accompanying cyclins, including regulation during the transition phase (38).

E-type cyclins are also essential proteins during the G1 and G1/S transition phase of the cell cycle (40, 41). Mammals express two E-type cyclins, cyclin E1 and cyclin E2, which activate CDK2 to promote cell cycle progression via phosphorylation of cellular proteins during the G1 and S phases. Cyclin E1 and cyclin E2 are expressed in all proliferating cell types and share similar amino acid sequences. In addition, E-type cyclins can also activate CDK1 and CDK3 proteins, and elevated cyclin E-CDK2 activities were observed in various human tumors, as they are correlated to anticancer drug resistance (41). However, in normal growth conditions, the cyclin E-CDK2 complex is most active in cells that are in the G1 phase (40).

Cyclin M (FAM58A), in association with CDK10, regulates transcription by phosphorylating ETS2, which in turn modulates the expression of targets such as c-RAF. Scientific evidence highlights the role of CDK10 in the G2/M transition and in maintaining genome stability. Clinically, the loss of function of the cyclin M/CDK10 complex has been linked to endocrine therapy resistance in breast cancer, specifically contributing to the mechanisms of tamoxifen resistance (33). Low levels of the Cyclin M/CDK10 complex are associated with a broad form of endocrine resistance in breast cancer extending beyond tamoxifen to other estrogen receptor (ER)-targeted therapies, including aromatase inhibitors and fulvestrant (42). Loss or reduced expression of CDK10 promotes activation of alternative proliferative signaling pathways, most notably the RAF-MEK-ERK MAPK cascade, through stabilization of the transcription factor ETS2 and increased expression of c-RAF. Activation of this pathway enables tumor cell proliferation in an estrogen-independent manner, thereby diminishing their reliance on ER signaling for growth and survival. As a result, therapies inhibiting estrogen production or blocking ER activity become less effective. Consequently, low CDK10 expression has been proposed as a potential biomarker of poor response to endocrine therapies (43-45).

CELL DIVISION CYCLE 25 (CDC25)

CDC25 proteins are key members of the CDK phosphatase family and play an essential role in positively regulating cell division by activating CDKs (46-48). The CDC25 family includes three isoforms: CDC25A, CDC25B, and CDC25C (49). Results from immunofluorescent analysis showed that CDC25A localizes primarily in the nucleus, suggesting that it may be also important for DNA replication. Additionally, CDC25A is regulated throughout the cell cycle, and this protein plays a central role in regulating cell cycle progression, and it is especially active at the G1/S transition phase and in the M phase (28, 48). Its constitutive activity or overexpression can disrupt normal checkpoints and lead to oncogenesis (48). CDC25B exhibits dual specificities for phosphatase enzymes, which promote cell division by activating the cyclin B-CDK1 complex (49). CDC25B phosphatases dephosphorylate phosphotyrosine (pTyr15) and phosphothreonine (pThr14) residues on CDK1, which results in mitotic entry. CDC25C is active during the S phase and M phase (50). CDC25B begins activating cyclin B-CDK1 in the G2/M transition phase, but then the activation is completed by CDC25C at the start of the M phase (50). Furthermore, CDC25A is stabilized upon cyclin B-CDK1 phosphorylation, and creates a positive feedback loop with CDC25C, which finishes the activation of cyclin B-CDK1, which then leads to mitotic entry.

Although there is little knowledge about the functions of CDC25C, it was demonstrated that it can prevent progression to the S phase (50). CDC25A and CDC25B protein levels change in a cell-cycle-dependent man-

ner, while CDC25C protein levels remain constant throughout the cell cycle. Clinically, the *CDC25* gene was one of the ten most predominant genes associated with gastric cancer (GC) when mutated (49). The CDC25 proteins are also targets of the MYC transcription factor, which regulates their function (49).

RB PROTEINS

RB protein, RB1(p105), was identified over 30 years ago as the first human tumor suppressor protein (51-55). In addition to p105, the RB family includes RB-like RBL1(p107) and RBL2(p130), which all share structural similarities and function as tumor suppressor proteins. RBs are known as pocket proteins and consist of three main structural domains: the N-terminal domain, the A/B pocket, and the C-terminal domain (56, 57). Each domain has phosphorylation sites that are important for modulating p105 activities and mediating functional interactions with transcription factors and RB-binding proteins, such as p53. Published data indicated that p105 and p53 work in concert and are critical for cell growth arrest and apoptosis upon DNA damage (2). The direct physical interaction between p53 and Rb is limited to specific cellular contexts, but p53 and RB tumor suppressor pathways are well known to intersect through multiple regulatory mechanisms. One important point of convergence involves the DREAM (DP, RB-like, E2F and MuvB) complex, which contains the RB family members p107 and p130 and mediates p53-dependent transcriptional repression of a large set of cell cycle genes. Through this complex, p53 regulates hundreds of genes involved in cell cycle progression, DNA replication, and mitosis, thereby coordinating cell cycle arrest and maintaining genomic stability (58, 59). The N-terminal domain is essential for RB functions as a tumor suppressor, stabilization of p105, and phosphorylation-dependent regulation of RB activity (56, 57). The A/B pocket domain works as a binding site for a diverse array of viral and cellular proteins and plays key roles in maintaining the regulatory functions of p105. The C-terminal domain facilitates binding to the E2F-DP complex, which is critical for inhibiting cell proliferation and controlling the cell cycle, especially in the G1/S transition period (20).

In the G0 phase, p130 is the most abundant RB family member. After cells are stimulated to enter the cell cycle, p105 and p107 expression is induced, as they are E2F target genes. When expression levels of p105 and p107 increase, the expression levels of p130 decrease accordingly. In subsequent cell cycle phases, p105 and p107 expression levels remain constant while p130 levels are relatively low under these specific growth conditions (20). Pocket proteins, including p107 and p130, function as key regulators of cell cycle control and cellular fate decisions. During differentiation, these proteins act as molecular "brakes" enabling cells to exit the cell cycle and initiate specialized developmental programs, such as muscle or bone formation (60). Consistent with this role, p107 and p130 loss can lead to premature or defective differentiation as cells could fail to properly withdraw from proliferation, as observed in cartilage development. Pocket proteins also contribute to the regulation of cellular senescence by mediating cell cycle arrest. For example, p107 is required for the initiation of senescence in certain settings when p105 is absent. In addition, pocket proteins often promote cell survival by restraining the activity of E2F transcription factors, particularly E2F1, which can induce apoptosis. By inhibiting E2F activity, p107 and p130 help prevent E2F-driven cell death. This regulatory role is highlighted by studies showing that the simultaneous loss of all three pocket proteins (p105, p107, and p130) results in broad ectopic apoptosis in the brain. Although elevated levels of pocket proteins can facilitate certain forms of cell cycle-associated cell death in certain context, their predominant role during development is to maintain cellular homeostasis by preventing premature or inappropriate apoptosis (61). These pocket proteins were first identified as a homolog of p105 and target points for transforming domains of viral oncogenic proteins (62).

p105 tightly regulates the E2F transcription factors, which are key players in regulating cell proliferation (63). Among them, E2F1-E2F3 function primarily as transcriptional activators that stimulate the expression of genes required for S-phase entry, whereas E2F4 and E2F5 act predominantly as transcriptional repressors. p105 preferentially binds and inhibits the activation of E2Fs (E2F1, E2F2, E2F3a) to regulate the G1/S transition. Conversely, the p105 protein preferentially associates with E2F1-E2F5 transcription factors, while p107 and p130 form complexes exclusively with E2F4 and E2F5 (64). This interaction is crucial for maintaining cellular quiescence (G0) and preventing unscheduled reentry into the cell cycle. The p105 family members control different aspects of E2F activity, but the specific functions of different RB family members are not

yet well defined. To characterize the specific role of these pocket proteins, Hurford *et al*. (1997) investigated how the expression of E2F-regulated genes is affected in cells deleted of each of the RB family members (65). There were no differences detected in the expression of E2F-target genes in cells deleted of either p107 or p130, but dysregulated expression of E2F target genes was evident in cells deleted of p105 and in cells that were deleted of both p107 and p130. These results proved that p105, p107, and p130 play different roles in regulating E2F. Hurford *et al*. studied these RB proteins and discovered that cells with a triple knockout of p105, p107, and p130 had an increased expression of E2F target genes (65). Additionally, the p105/E2F, p107/E2F, and p130/E2F complexes are active repressors on E2F sites, each with different roles for E2F regulation. Thus, E2F sites are critical targets for p105 family proteins, which regulate cell proliferation (65).

Early studies investigating the specific roles of the RB pocket proteins, p105, p107, and p130, demonstrated that these proteins regulate E2F-dependent transcription through distinct yet overlapping mechanisms (66-69). In one of the early genetic analyses, Hurford *et al*. showed that deletion of individual RB family members had differential effects on the expression of E2F-regulated genes. Loss of either p107 or p130 alone had minimal impact on E2F target gene expression, whereas deletion of p105, or combined deletion of p107 and p130, resulted in dysregulated expression of E2F-responsive genes (65). Moreover, cells lacking all three pocket proteins exhibited a strong increase in E2F target gene expression, demonstrating that RB family members collectively repress E2F-dependent transcription and control cell-cycle progression (70).

Subsequent work expanded this model by identifying the DREAM complex (DP, RB-like, E2F, and MuvB) as a central regulator of cell-cycle gene repression in quiescent and early G1 cells (71, 72). Seminal studies from the laboratory of James DeCaprio demonstrated that p130 and p107 associate with E2F4/5 and the MuvB core complex to form the DREAM complex, which represses a large set of cell-cycle genes during quiescence (71). Later studies from Kurt Engeland and others further established that DREAM coordinates transcriptional repression of genes required for S phase and mitosis by binding to E2F and CHR promoter elements (73-75). Upon cell-cycle entry, phosphorylation of pocket proteins by cyclin-dependent kinases disrupts the DREAM complex, allowing the MuvB complex to transition into transcriptionally activating complexes such as MMB (Myb-MuvB) and FOXM1-MuvB, thereby promoting expression of genes required for DNA replication and mitosis (71, 76-78).

Collectively, these findings refined the earlier RB-E2F model by demonstrating that RB proteins function within larger multiprotein complexes that dynamically regulate cell-cycle gene expression. In particular, p130-containing DREAM complexes play a dominant role in maintaining transcriptional repression during cellular quiescence, whereas p105 primarily regulates E2Fs during the G1/S transition. This integrated regulatory network ensures tight control of cell proliferation through coordinated repression and activation of E2F target genes.

p105 is frequently inactivated in a wide range of tumors, and *RB1* loss or functional mutations are associated with pediatric RB cancers (4, 56). Mutations or deletions in the *RB1* gene can lead to uncontrolled cell proliferation, thereby promoting cancer initiation (4).

p105 interacts with the adenovirus early region 1A (E1A) oncoprotein in adenoviruses (79). The binding between p105 and E1A prevents p105 interactions with E2Fs, thereby inducing cell proliferation and replication of adenoviral DNA (80). p105 binds to E1A via the LXCXE peptide motif, while E1A utilizes its conserved region 1 (CR1) domain to interact with E2F and inhibits the p105-E2F interactions.

CDK PROTEIN INHIBITORS

CDK inhibitors are critical regulators of cell cycle progression and play essential roles in coordinating cell cycle arrest during processes such as the DDR (2). These inhibitors are broadly classified into two major families: the INK4 (Inhibitor of CDK4) family and the CIP/KIP (CDK-interacting protein/Kinase inhibitory protein) family, which differ in both their CDK specificity and their mode of interaction with CDK complexes (81). The INK4 family, which includes $p16^{INK4A}$, $p15^{INK4B}$, $p18^{INK4C}$, and $p19^{INK4D}$, selectively inhibits CDK4 and CDK6 by binding monomeric CDK4/6, thereby preventing their association with D-type cyclins and ultimately blocking phosphorylation of the RB (82). In contrast, members of the CIP/KIP family, including $p21^{CIP1}$, $p27^{KIP1}$, and $p57^{KIP2}$, interact with and inhibit a broad spectrum of cyclin-CDK heterodimeric complexes, particularly cyclin E-CDK2 and cyclin A-CDK2 complexes, thereby restraining progression through the G1/S transition (22, 81).

Among these inhibitors, p21 was the first identified CDK inhibitor and represents a key transcriptional target of p53. By inhibiting cyclin E-CDK2 and cyclin A-CDK2 complexes, p21 prevents RB hyperphosphorylation and enforces cell cycle arrest, thereby contributing to tumor suppression (21, 83-85). Similarly, p18 functions as an inhibitor of CDK4/6, primarily during the G1 phase (2). Interestingly, there were attempts made to generate kinase inhibitors by altering cyclin-CDK complexes by resembling the functions of typical p21, p16, and p27, or via regulation of the cyclin-CDK targets (86).

A central regulatory node linking the RB and p53 tumor suppressor pathways is the *CDKN2A* locus, which encodes two distinct tumor suppressor proteins through alternative reading frames. $p16^{INK4A}$, a member of the INK4 family that regulates the RB pathway, and $p14^{ARF}$ in humans ($p19^{ARF}$ in mice), which functions in the p53 regulatory network (87, 88). $p16^{INK4A}$ induces G1 arrest by inhibiting CDK4/6 activity, thereby preventing RB phosphorylation and maintaining RB in its growth-suppressive state (19, 89). In contrast, ARF functions primarily through the p53-Murine Double Minute 2 (MDM2) pathway. Studies using mouse models demonstrated that murine p19ARF interacts with both p53 and the E3 ubiquitin ligase MDM2, highlighting the importance of ARF in regulating p53 stability. MDM2 is a major negative regulator of p53 as in fact it promotes p53 ubiquitination and proteasomal degradation (88). ARF antagonizes this process by binding to MDM2 and inhibiting its ligase activity, thereby stabilizing and activating p53. This regulatory axis forms a critical checkpoint in which ARF-mediated inhibition of MDM2 leads to p53 accumulation, resulting in transcriptional activation of p53 target genes involved in cell cycle arrest, apoptosis, and DDR (90).

Activation of p53 further reinforces cell cycle control by promoting transcription of p21, which suppresses CDK activity and reduces CDK-mediated phosphorylation of RB, thereby limiting the release of E2F transcription factors and inhibiting cell cycle progression (91). Post-translational modifications of p53, including phosphorylation at residues such as Ser15, Thr18, Ser20, Ser37 and Ser106, disrupt the p53-MDM2 interaction and contribute to p53 stabilization. In response to DNA damage, Ser15 is phosphorylated by ATM and can also be targeted by ATR (92, 93) while Ser37 is phosphorylated by ATR and DNA-PK (93, 94). Phosphorylation of Thr18 is mediated by casein kinase 1 (CK1) in Ser15-primed cascade (95). Ser20 is phosphorylated by the checkpoint kinase Chk2 (96), and Ser106 has been identified as an Aurora-A phosphorylation site that inhibits p53-MDM2 binding and prolongs p53 half-life (97). These phosphorylation events are often associated with acetylation events that further inhibit MDM2-mediated ubiquitination of p53, enhancing its tumor suppressor activity (88).

Importantly, alterations in endogenous cell cycle inhibitors are frequent events in human cancers. These alterations can occur through genetic deletion, transcriptional downregulation, or abnormal subcellular localization of CDK inhibitors. Notably, loss of the *CDKN2A* locus simultaneously disrupts both the RB pathway (via loss of $p16^{INK4A}$) and the p53 regulatory axis (via loss of ARF), representing a common oncogenic event that contributes to uncontrolled proliferation and tumor development.

CDK4/6 INHIBITORS

Although CDKs rapidly emerged as attractive therapeutic targets, the development of effective CDK inhibitors initially faced significant challenges (98). CDKs are highly conserved kinases across eukaryotic species, from yeast to humans, and their catalytic domains share strong homology, particularly within the ATP-binding pocket (15, 31). As a result, early generations of ATP-competitive CDK inhibitors lacked sufficient selectivity and inhibited multiple CDK family members (99). While these compounds showed promising activity in preclinical models, their clinical efficacy was limited because they targeted not only cell-cycle CDKs such as CDK4 and CDK6 but also transcriptional CDKs (*e.g.*, CDK7, CDK9) and kinases involved in DDR pathways (12). This broad inhibition often resulted in significant toxicity and limited therapeutic windows, highlighting the need for more selective strategies targeting specific CDK complexes in cancer.

In response to these limitations, alternative strategies for targeting CDKs have been explored. These include the development of highly selective ATP-competitive inhibitors specifically targeting CDK4/6, as well as non-ATP-competitive approaches designed to interfere with CDK-cyclin interactions, substrate recognition, or regulatory complex assembly (100). More recently, emerging technologies such as proteolysis-targeting chimeras (PROTACs) and molecular glues have been investigated to selectively degrade CDKs or disrupt CDK-associated regulatory networks (12). These approaches are aimed at improving spec-

ificity while minimizing off-target effects associated with earlier broad-spectrum CDK inhibitors.

Because dysregulation of CDK4/6 is frequently associated with cell cycle perturbance and cancer initiation, these kinases have always been attractive targets for therapeutic intervention but only recently they finally were successfully introduced in the clinical setting (101). CDK4/6 inhibitors work by blocking CDK4/6 activity (**Figure 2B**), thereby inhibiting RB phosphorylation, and preventing the transition from the G1 to S phase (102, 103). In clinical practice, CDK4/6 inhibitors have substantially improved the therapeutic options for $HR^+/HER2^-$ breast cancer (104). Several CDK inhibitors are in clinical trials, which can function as single agents and in combination with anticancer therapies (105). In this section, we will describe first-, second- and last generation CDK inhibitors and their main features.

Roscovitine is first-generation CDK inhibitor (106), and it is a broad-range purine inhibitor acting through direct competition at the conserved kinase ATP-binding site (34). Roscovitine inhibits CDK1, CDK2, CDK5, CDK7, and CDK9 but not CDK4 or CDK6 (107, 108). Also, this inhibitor primarily targets CDK2 (108). Roscovitine demonstrated therapeutic efficacy in diffuse pleural mesothelioma (DPM) cells when used in combination with AKT inhibitors (8, 109, 110) and enhanced the therapeutic efficacy of anti-PD1 in non-small cell lung cancer (109). This CDK inhibitor can also induce apoptosis through a p53-dependent pathway (111). An additional first-generation CDK inhibitor is flavopiridol (112), which blocks the activity of multiple CDKs, as in fact it is considered a pan-CDK inhibitor. Flavopiridol was developed after animal model studies demonstrated a tissue-specific necessity of single CDKs (113). However, flavopiridol is highly cytotoxic for patients, as in fact it is associated with gastrointestinal toxicity and extreme neutropenia, especially in metastatic breast cancer (112).

Palbociclib, ribociclib, and abemaciclib are defined as second-generation CDK inhibitors and were designed to specifically target CDK4/6 by binding to the ATP-binding pocket of CDK4/6, and block CDK4/6 activity (8, 103, 114). In contrast to the highly selective CDK4/6 inhibitors, dinaciclib is a broader-spectrum CDK inhibitor that targets CDK1, CDK2, CDK5, and CDK9, and has been evaluated in clinical trials for multiple malignancies rather than being approved for breast cancer therapy (115). Palbociclib was the first CDK4/6 inhibitor developed and was first approved by the FDA in 2015 for breast cancer therapy (4), followed by ribociclib and abemaciclib, which were approved by the FDA in 2017 (116). Accordingly, these three agents share the same core cytostatic mechanism CDK4/6 inhibition leading to blockade of RB phosphorylation and G1/S progression, whereas clinically relevant differences are primarily reflected in their safety profiles (10, 101).

The integration of palbociclib into the therapeutic regimen for $HR^+/HER2^-$ advanced breast cancer, in combination with endocrine therapy, has demonstrated a substantial increase in progression-free survival compared to endocrine monotherapy alone. Safety profile analysis highlights that grade 3-4 neutropenia represents the most significant adverse event, with a frequency of approximately 66% (117). However, the cytostatic nature and reversibility of this toxicity render febrile neutropenia a rare event, occurring in less than 2% of cases (117). In terms of relative comparison with other CDK4/6 inhibitors, palbociclib is characterized by superior gastrointestinal tolerability, a feature that clearly distinguishes it from the safety profile of abemaciclib (117).

Abemaciclib differs from other CDK4/6 inhibitors for its better selectivity for CDK4, a biochemical property that allows for continuous daily administration and results in a unique toxicity profile (118). In combination with endocrine therapy, the efficacy of abemaciclib has been extensively demonstrated in the MONARCH 3 trial, where it showed significant clinical benefit in both progression-free survival and overall survival (118). Unlike palbociclib and ribociclib, abemaciclib exhibits significantly lower rates of grade 3-4 neutropenia, at approximately 25-27% (117). However, the most characteristic adverse event is gastrointestinal toxicity; diarrhea reached grade 3 in 10-15% of cases, usually during the first treatment cycles (119). Other specific complications include an increased risk of venous thromboembolic events and an elevation in serum creatinine levels, the latter due to the inhibition of renal transporters and not indicative of actual organ damage (118).

Ribociclib, used in combination with endocrine therapy, has demonstrated robust clinical efficacy across various therapeutic settings, as confirmed by the results of the MONALEESA-2 and MONALEESA-3 clinical trials (120, 121). Similar to palbociclib, the safety profile of ribociclib is characterized by significant hematological toxicity, with grade 3-4 neutropenia being the most common adverse event, occurring in approximately 60% of patients (122). However, ribociclib is distinguished by specific non-hematological toxicities that require rigorous clinical mon-

itoring. In particular, it is associated with a risk of dose-dependent QTc interval prolongation, which occurs in about 3% of cases, necessitating periodic electrocardiographic monitoring (121). Furthermore, the drug shows a relevant incidence of hepatotoxicity, with grade 3-4 increases in transaminases (ALT/AST) reported in approximately 8-9% of patients, typically within the first six months of treatment (120). Dosage adjustments are frequently necessary to manage these specific toxicities; therefore, CDK4/6 inhibitors should be utilized in clinical practice to select the most appropriate drug, taking into account both efficacy and safety (104).

CDK4/6 inhibitors have demonstrated high efficacy as single agents and in combination therapies, particularly in HR^+ breast cancer. Multiple phase III clinical trials evaluating CDK4/6 inhibitors such as Palbociclib, Ribociclib, and Abemaciclib have consistently shown improved progression-free and overall survival when combined with endocrine therapy in $HR^+/HER2^-$ metastatic breast cancer (123). Importantly, these trials generally enroll patients whose tumors retain functional p105, as RB activity is required for CDK4/6 inhibitor-mediated cell-cycle arrest. In contrast, clinical efficacy in TNBC has been limited. TNBC frequently exhibits low p107 expression or p107 loss, which renders tumors intrinsically resistant to CDK4/6 inhibition because these drugs rely on RB-dependent suppression of E2F-driven transcription. Consequently, most clinical trials have focused on HR^+ disease rather than TNBC, and TNBC patients have largely been excluded or have shown minimal benefit. However, emerging evidence suggests that CDK4/6 inhibition may exert additional effects independently of their effect on the canonical RB-dependent pathway. For example, in RB-deficient TNBC, CDK4/6 inhibition can reduce mutant p53 protein stability through the RBM38 pathway, suggesting that mutant p53 status may serve as an additional biomarker of response and expanding the potential therapeutic scope of these agents (124). To overcome resistance and broaden their therapeutic applicability, CDK4/6 inhibitors are also actively explored in combination strategies. These include combinations with chemotherapeutic agents in breast cancer patients resistant to endocrine therapy (103), as well as with endocrine therapies, immunotherapy, and targeted therapies. Although early-phase studies have examined these strategies in TNBC, clinical responses remain modest and appear largely restricted to tumors retaining RB function.

Importantly, paclitaxel is a commonly used taxane that can be administered alone via IV infusion or in combination with additional anticancer drugs (125). Paclitaxel is used in combination with CDK4/6 inhibitors, especially with palbociclib (103). This dual targeting strategy effectively regulates the cell cycle and increases anticancer treatment efficacy. The combination therapy using paclitaxel and the CDK4/6 inhibitor palbociclib was highly effective and significantly minimized side effects associated with paclitaxel alone. A clinical study by Clark *et al*. (2019), on RB^+ advanced breast cancer patients treated with a paclitaxel-palbociclib combination therapy, showed improved patient outcomes without severe side effects (126). Patients first received intermittent doses of oral palbociclib and paclitaxel every other week. Patients who had a plateau response at more than 6 cycles in this clinical study design were able to continue their treatments with just palbociclib. Their dose increased one level higher than their previous combinatorial dose. Palbociclib and paclitaxel in RB^+ advanced breast cancer patients proved to be safe and effective without toxicity in RB^+ breast cancer.

CDK4/6 inhibitors, especially abemaciclib, can also be effectively used in combination with aromatase inhibitors to treat breast cancer (118). The aromatase inhibitors are classified into two groups: steroidal (type I) and nonsteroidal (type II). Anastrozole and letrozole are nonsteroidal aromatase inhibitors, which are highly effective in breast cancer therapy. For treatment of HER^+ and $HER2^-$ advanced breast cancer, abemaciclib was used in combination with these two aromatase inhibitors in the MONARCH-3 (randomized phase III) study, abemaciclib and anastrozole or letrozole greatly improved progression-free survival as well as an objective response rate. These treatments were safe for women with these advanced breast cancers (118).

Collectively, abemaciclib is considered highly effective for the treatment of HR^+ and HER^- breast cancer and exhibited significant efficacy when used as a second-line treatment in combination with fulvestrant, an anti-estrogen drug (8, 118). Sledge Jr *et al.* (2017) demonstrated that the abemaciclib-fulvestrant dual treatment was more effective than fulvestrant alone, as it was associated with increased cancer progression-free survival in patients with $HER^+/HER2^-$ advanced breast cancer (127). In advanced breast cancer, abemaciclib or palbociclib were effective in combination with fulvestrant (128). The PALOMA-3 phase III trial was conducted in a 2:1 randomized ratio, multicenter, double-blind, and placebo-con-

trolled to examine the safety and efficacy of a palbociclib-fulvestrant combined therapy (128). Patients treated with palbociclib-fulvestrant showed improvement in progression-free survival, while patients treated with placebo-fulvestrant did not significantly improve.

Similar to breast cancers, abemaciclib was effective in endometrial cancers (ECs) to prevent tumor progression (129). Treating ECs is very crucial as ECs are the sixth most common cancer in women, and the thirteenth most lethal globally (29, 130). ECs have dysregulated CDK4/6, but CDK4 plays a more prevalent role (129). Abemaciclib targets CDK4/6 with higher efficacy towards CDK4 inhibition, which is therefore highly beneficial for treating ECs. In addition, a combination therapy approach using the aromatase inhibitor letrozole and abemaciclib demonstrated encouraging efficacy in recurrent ER$^+$ endometrioid EC (131).

Costa *et al*. (2024) recently demonstrated that treating cisplatin-resistant DPM cell lines with abemaciclib was very effective in inhibiting growth and 3D spheroid formation of these cells. It was suggested that abemaciclib treatment may help in overcoming cisplatin-resistance in mesothelioma cells (8) (**Table 1**).

CDK4/6 inhibitor resistance is associated with abnormalities in the critical molecules of the cyclin D-CDK4/6-RB regulatory axis (114). FAT atypical cadherin 1 (FAT1) protocadherin tumor suppressor protein modulates CDK4/6 activity and may play a role in modulating resistance to CDK4/6 inhibitors (103), as in fact loss of function (LOF) mutations impact

Table 1. *Combination therapies involving CDK4/6 inhibitors.*

CDK4/6 INHIBITOR	COMBINATION PARTNER	CANCER TYPE	STUDY / TRIAL	KEY FINDINGS	REFERENCE
Palbociclib	Paclitaxel	RB$^+$ advanced breast cancer	Phase I/II clinical study	Combination improved patient outcomes and was well tolerated.	(126)
Abemaciclib	Aromatase inhibitors (anastrozole or letrozole)	HR$^+$/HER2$^-$ advanced breast cancer	MONARCH-3 (Phase III)	Significant improvement in progression-free survival and objective response rate compared to endocrine therapy alone	(118)
Abemaciclib	Fulvestrant	HR$^+$/HER2$^-$ advanced breast cancer	MONARCH-2	Combination significantly improved progression-free survival *versus* fulvestrant alone	(127)
Palbociclib	Fulvestrant	HR$^+$/HER2$^-$ advanced breast cancer	PALOMA-3 (Phase III)	Improved progression-free survival compared to placebo-fulvestrant	(128)
Palbociclib / Abemaciclib	Fulvestrant	Advanced breast cancer	Multiple clinical studies	Endocrine therapy plus CDK4/6 inhibition improved outcomes compared to endocrine therapy alone	(128)
Abemaciclib	Letrozole	ER$^+$ endometrioid endometrial cancer	Clinical evaluation	Combination showed encouraging efficacy in recurrent ER$^+$ endometrial cancer	(131)
Abemaciclib	Cisplatin-resistant tumor models	Mesothelioma	Preclinical study	Abemaciclib inhibited growth and 3D spheroid formation in cisplatin-resistant mesothelioma cells	(8)
Atirmociclib (CDK4-selective)	Endocrine therapy ± CDK2 inhibition / HER2 antibodies / immune checkpoint inhibitors	HR$^+$ breast cancer	Preclinical / translational studies	Selective CDK4 targeting improves anti-tumor efficacy and combination therapies may overcome resistance	(132)

Table 2. *Mechanisms of resistance to CDK4/6 inhibitors.*

RESISTANCE MECHANISM	MOLECULAR PATHWAY INVOLVED	BIOLOGICAL EFFECT	CLINICAL RELEVANCE	REFERENCE
FAT1 loss-of-function mutations	Hippo pathway activation	Increased CDK6 expression leading to resistance to CDK4/6 inhibitors	Observed in several cancers. Potential predictive biomarker	(103, 133)
Dysregulation of cyclin D-CDK4/6-RB axis	Cell cycle pathway	Reactivation of cell cycle progression despite CDK4/6 inhibition	Major driver of intrinsic and acquired resistance	(114)
Hippo pathway alterations (YAP, MST1, LATS1, NF2)	Hippo signaling	Increased proliferation and reduced sensitivity to CDK4/6 inhibition	Potential therapeutic targets	(103)
Wnt/β-catenin pathway activation	Wnt signaling	Promotes tumor progression and CDK4/6 inhibitor resistance	Linked to FAT1 mutations	(103)
MAPK/ERK pathway activation	MAPK signaling	Increased cell proliferation and survival	Contributes to therapeutic resistance	(103)

FAT1 and mediate CDK4/6 inhibitor resistance (132). FAT1 LOF activates the Hippo pathway, as it induces CDK6 expression, leading to resistance to CDK4/6 inhibitors (103, 132). FAT1 is often mutated in human cancers, therefore leading to tumor progression and an impact on cancer prognosis (103). Genomic analysis has demonstrated that FAT1 loss-of-function (LOF) mutations occur in approximately 5.8% of patients with metastatic breast cancer. The clinical relevance of this mechanism is underscored by a significant reduction in progression-free survival (PFS), which decreased from 10.1 months in FAT1 wild-type patients to 2.4 months in patients carrying the mutation, identifying *FAT1* loss as a robust predictive biomarker of poor response (132). FAT1 regulates the Wnt/β-catenin signaling pathway as well as the Hippo pathway and the MAPK/ERK pathways via protein-protein interactions, which affect cell proliferation, cell migration, and invasion (103). The YAP, MST1, LATS1, and NF2 proteins can also affect resistance to CDK4/6 inhibitors. While FAT1 targeting shows promise for reversing CDK4/6 inhibitor resistance, there is still a need for additional research to fully understand how FAT1-dependent regulatory mechanisms can cause resistance to CDK4/6 therapy. Despite the remarkable clinical success of CDK4/6 inhibitors in HR^+ breast cancer, both intrinsic and acquired resistance often limit long-term therapeutic benefits. Resistance mechanisms can arise through multiple molecular alterations that restore cell-cycle progression or activate compensatory proliferative pathways (114, 133). These mechanisms include loss or functional inactivation of *RB1*, amplification of *CCNE1* leading to increased CDK2 activity, upregulation of CDK6 expression, and activation of parallel signaling pathways such as PI3K/AKT/mTOR and MAPK signaling (103). Additional mechanisms involve alterations in upstream regulators of the CDK4/6-RB axis, epigenetic changes affecting cell cycle gene expression, and tumor microenvironment-mediated adaptive responses (101, 114, 134). Understanding these diverse resistance pathways is essential for designing rational combination strategies aimed at improving clinical efficacy of CDK4/6 inhibition (**Table 2**).

There are significant side effects associated with CDK4/6 inhibitors (1). Nonetheless, CDK4/6 inhibitors showed better efficacy than endocrine therapies (135). Abemaciclib is associated with the most significant adverse events, including diarrhea, anemia, neutropenia, nausea, and fatigue, especially when combined with endocrine therapies (10, 101). Abemaciclib can also trigger gastrointestinal-linked toxicities in comparison to palbociclib. Palbociclib can cause leukopenia, and fatigue, and ribociclib can lead to decreased leukocyte counts, neutropenia, elevated liver enzymes and prolonged QT levels. Ribociclib can also cause skin rash, fatigue, hypokalemia, and hyponatremia (107). Ribociclib is the CDK4/6 inhibitor with the highest risk of mortality, especially when used in combination with endocrine therapies. The use of ribociclib necessitates extreme caution, especially in patients with preexisting cardiovascular conditions. Patients put on ribociclib are therefore recommended to be closely monitored. These three inhibitors can all lead to mye-

Table 3. *Pharmacological properties of second-generation CDK4/6 inhibitors. They all have greater efficacy over CDK4 and CDK6, but can also inhibit CDK1 and CDK5.*

DRUG	PRIMARY TARGETS	ADDITIONAL TARGETS	PHARMACOKINETICS (HALF-LIFE, TMAX)
Palbociclib	Comparable activity against CDK4 and CDK6: • Cyclin D1-CDK4 • Cyclin D1/2/3-CDK6	• CDK1-Cyclin B • CDK5-p25	Half-life: ~26-27 h Tmax: 4-6 h
Ribociclib	Greater selectivity for CDK4 over CDK6: • Cyclin D1-CDK4 • Cyclin D1/2/3-CDK6	• CDK1-Cyclin B • CDK5 – -p25	Half-life: ~32-42 h Tmax: 1-5 h
Abemaciclib	Preferential inhibition of CDK4 over CDK6: • Cyclin D1-CDK4 • Cyclin D1/2/3-CDK6	• CDK1-Cyclin B • CDK5-p25	Half-life: ~17-38 h Tmax: 4-6 h
Atirmociclib (PF-07220060)	Highly selective for CDK4: • Cyclin D1-CDK4	• Cyclin E-CDK2	Terminal half-life: ~26.5 h Tmax: Not yet determined

losuppression and neutropenia (101). Collectively, these observations support the notion that palbociclib, ribociclib, and abemaciclib share a common on-target cytostatic mechanism, while drug-specific toxicity patterns likely reflect differences in target engagement and tissue dependence on CDK4 *versus* CDK6 (136).

Significantly, very recent work by Palmer *et al*. (2025) demonstrated that HR$^+$ breast cancer cells are significantly more dependent on CDK4 activity than CDK6, which is instead critical for hematopoiesis (136). Based on this observation, the researchers developed a next-generation selective CDK4 inhibitor, atirmociclib (PF-07220060), which showed enhanced anti-tumor efficacy and reduced toxicity compared to dual CDK4/6 inhibitors. Notably, the combination of endocrine therapy with either CDK2 inhibition, HER2 antibodies, or immune checkpoint inhibitors further increased atirmociclib efficacy, suggesting that dual therapy might counteract acquired resistance to CDK4 selective targeting (136). These results are very important as, in fact, they strongly support the expansion of more specific cell cycle-targeted therapy. A summary of current CDK4/6 inhibitor is listed in **Table 3**.

ANTI-CDK-RELATED CANCER THERAPIES

Dihydroartemisinin (DHA) is an immunotherapeutic anticancer drug that blocks cell proliferation and prevents tumor metastasis and angiogenesis in patients with colorectal cancer (CRC) (137). DHA also promotes immunogenic cell death (ICD) in hepatocellular carcinoma (HCC) (138). DHA therapies can even remodel the TME, boost cancer immunotherapy and enhance the efficacy of chimeric antigen receptor-T (CAR-T) cell-mediated tumor suppression and anti-programmed death-1 (anti-PD-1) antibody (**Figure 3**).

DHA leads to CDK inhibition (CDK1/2/4/5/6), as DHA upregulates intracellular reactive oxygen species (ROS), and an increase in ICD. ROS are critical factors associated with ICD since its immunogenicity weakens the N-Acetyl-L-cysteine antioxidant. The accumulation of ROS increases the amount of damage-associated molecular patterns (DAMPs) during the pre-apoptosis phase, thereby leading to the increase of ICD.

As DHA can inhibit CDKs, it can increase intracellular ROS and subsequently trigger apoptosis, cell cycle arrest, and autophagy (138). DHA can inhibit CDK4/6, but it mainly inhibits CDK4 (139). For example, DHA effectively inhibits cyclin D1-CDK4-RB signaling pathway in GC. DHA prevents GC cell proliferation and promotes cell cycle arrest. Fan *et al.*, discovered from flow cytometry analysis that DHA delivery increased the number of GC cells in G0/G1 transition phase, thereby lowering the number of cells in the S phase (139).

As demonstrated in HCC mouse model studies by Zhou *et al*. (2024), DHA treatment of HCC significantly increases the number of tumor-infiltrating CD8$^+$ T cells, which express CD25 and CD69 (138). Clinically, patients successfully treated with DHA

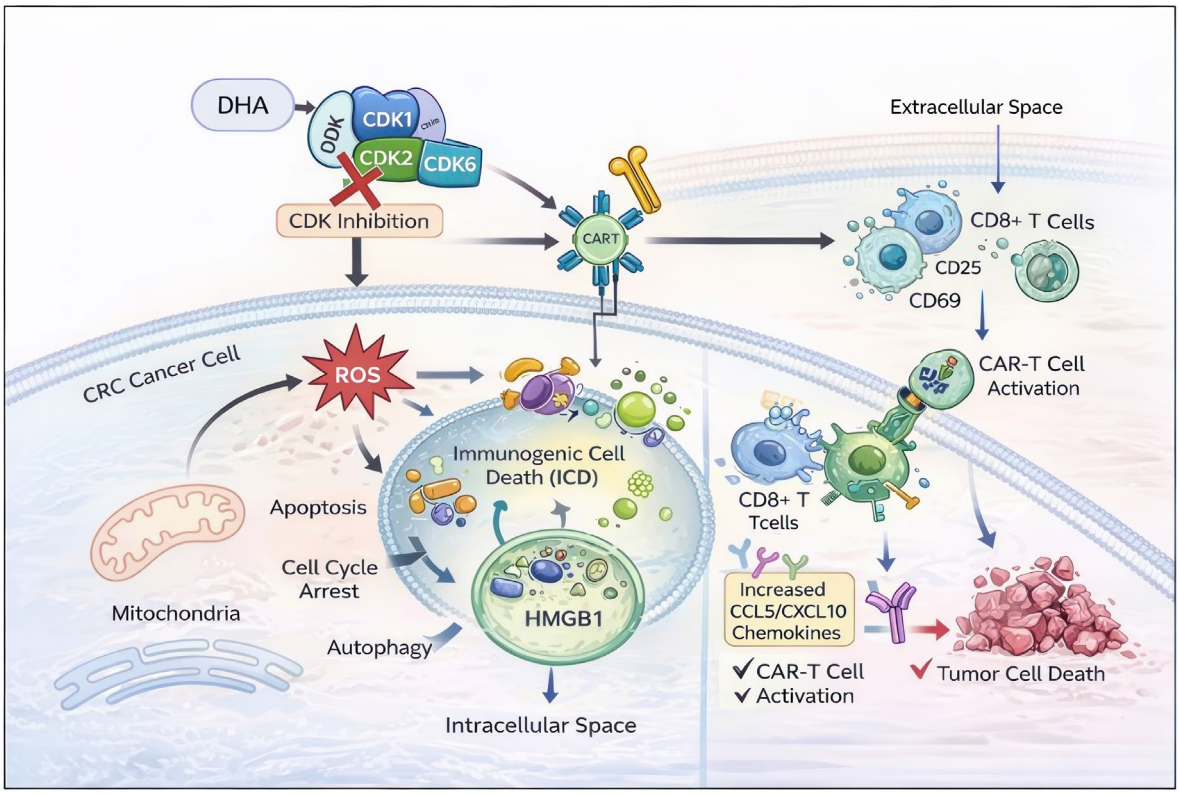

Figure 3. *DHA-mediated CDK inhibition promotes ROS-dependent immunogenic cell death and enhances antitumor immune responses. DHA inhibits multiple CDKs (CDK1, CDK2, CDK4, CDK5, and CDK6), with predominant inhibition of CDK4/6, leading to suppression of cyclin-dependent cell cycle progression in CRC cells. CDK inhibition by DHA results in increased intracellular ROS, which trigger apoptosis, cell cycle arrest, and autophagy. Elevated ROS levels promote ICD, characterized by the release of DAMPs, including HMGB1, during the pre-apoptotic phase. ICD subsequently enhances antitumor immunity by increasing tumor immunogenicity and stimulating immune cell infiltration. DHA treatment is associated with increased expression of chemokines CCL5 and CXCL10, facilitating recruitment and activation of CD8+ T cells expressing activation markers CD25 and CD69. In parallel, DHA remodels the TME and potentiates CAR-T–mediated antitumor responses, ultimately leading to enhanced tumor cell death.*

had increased mRNA levels of chemokines CCL5 and CXCL10. These clinical results indicate how DHA can inhibit dysregulated CDKs. The clinical prosperous results reaffirm the potential of ROS to suppress CDK-caused tumors. Also, CCL5 and CXCL10 decrease the amount of tumor-infiltrating $CD8^+$ T cells in colon cancer and melanoma (138).

In addition, results from experiments conducted in Huh7 cells (immortalized human hepatoma cells) treated with DHA downregulated CDK1/2/4/5/6 expression (138). The same results were obtained in Hepa1-6 (murine hepatoma – spontaneous liver tumor), H22 (mouse hepatocellular carcinoma), and HCCLM3 (human hepatocellular carcinoma) cell lines and were then further validated by qPCR and Western blotting analysis in HCC tumors (138). Notably, while conventional cytotoxic therapies act by damaging DNA and activating cell-cycle checkpoints, leading to efficient inhibition of CDKs, by contrast, DHA-associated CDK suppression works predominantly downstream of ROS induction and ICD/TME remodeling (140).

An additional important aspect currently under investigation is the optimal timing and sequential treatment of CDK4/6 inhibitor-based therapies relative to chemotherapy, radiotherapy, or other neo-adjuvant or adjuvant treatments. Because CDK4/6 inhibitors induce a cytostatic G1 cell-cycle arrest, their concurrent administration with cytotoxic agents targeting actively proliferating cells may reduce therapeutic efficacy (14, 141, 142). Consequently, several studies have explored sequential treatment strategies in which CDK4/6 inhibition is temporarily paused during chemotherapy or radiation exposure (143, 144). Conversely, in other contexts, CDK4/6 inhibitors may enhance antitumor immunity or promote tumor cell senescence, thereby potentiating the efficacy of immunotherapies or endocrine treatments (145, 146). Determining the optimal therapeutic scheduling of CDK4/6 inhibitors, therefore,

represents an important area of ongoing clinical investigation.

DISCUSSION AND CONCLUSION

CDKs are essential kinases for cell cycle progression, cell proliferation, differentiation, and DDR and act in concert with cyclins and RB proteins. CDK4/6 activity at the G1/S checkpoint is required for cell cycle progression, and dysregulation of CDK4/6 disrupts the cell cycle and promotes cancer initiation. CDK4/6 inhibitors have demonstrated significant efficacy in cancer therapies, as a monotherapy or in a combinational approach. Additionally, atirmociclib, a recently developed CDK4-specific inhibitor has shown great promises and enhanced safety as compared to classic CDK4/6 dual inhibitors. Nevertheless, some limitations should be acknowledged, including the limited availability of long-term clinical data and the rapidly expanding landscape of CDK-targeted therapies, which may lead to the emergence of additional therapeutic strategies beyond those discussed here. In conclusion, CDK inhibitors are emerging as important tools for cancer therapy. Future studies are aimed at better integrating CDK4/6 inhibitors with new immunotherapeutic therapies and/or personalized treatment modalities to further improve safety and clinical response.

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Conflicts of interest

The authors declare no competing interests.

Availability of data and materials

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Authors' contributions

SBA: writing – original draft, writing – review & editing. VS, GM: writing – review & editing, visualization. SS: writing – review & editing. AM: supervision, writing – review & editing. AG: funding acquisition, writing – review & editing.

Publication ethics

Plagiarism

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

Data falsification and fabrication

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

REFERENCES

1. Abate AA, Pentimalli F, Esposito L, Giordano A. ATP-noncompetitive CDK inhibitors for cancer therapy: an overview. Expert Opin Investig Drugs. 2013;22(7):895-906. doi: 10.1517/13543784.2013.798641.
2. MacLachlan TK, Sang N, Giordano A. Cyclins, cyclin-dependent kinases and cdk inhibitors: implications in cell cycle control and cancer. Crit Rev Eukaryot Gene Expr. 1995;5(2):127-56. doi: 10.1615/critreveukargeneexpr.v5.i2.20.
3. Tanriverdi O, Yildiz A. The Role of Speedy/RINGO Protein in Breast Cancer as a Future Biomarker. Cancer Diagn Progn. 2024;4(3):209-213. doi: 10.21873/cdp.10310.
4. Huang Y, Liu W, Zhao C, Shi X, Zhao Q, Jia J, Wang A. Targeting cyclin-dependent kinases: From pocket specificity to drug selectivity. Eur J Med Chem. 2024;275:116547. doi: 10.1016/j.ejmech.2024.116547.
5. Alfano L, Iannuzzi CA, Barone D, Forte IM, Ragosta MC, Cuomo M, et al. CDK9-55 guides the anaphase-promoting complex/cyclosome (APC/C) in choosing the DNA repair pathway choice. Oncogene. 2024;43(17):1263-1273. doi: 10.1038/s41388-024-02982-w.
6. Bagella L, Sun A, Tonini T, Abbadessa G, Cottone G, Paggi MG, et al. A small molecule based on the pRb2/p130 spacer domain leads to inhibition of cdk2 activity, cell cycle arrest and tumor growth reduction in vivo. Oncogene. 2007;26(13):1829-39. doi: 10.1038/sj.onc.1209987.
7. Zhang M, Zhang L, Hei R, Li X, Cai H, Wu X, et al. CDK inhibitors in cancer therapy, an overview of recent development. Am J Cancer Res. 2021;11(5):1913-1935.
8. Costa A, Forte IM, Pentimalli F, Iannuzzi CA, Alfano L, Capone F, et al. Pharmacological inhibition of

CDK4/6 impairs diffuse pleural mesothelioma 3D spheroid growth and reduces viability of cisplatin-resistant cells. Front Oncol. 2024;14:1418951. doi: 10.3389/fonc.2024.1418951.
9. Brown VD, Phillips RA, Gallie BL. Cumulative effect of phosphorylation of pRB on regulation of E2F activity. Mol Cell Biol. 1999;19(5):3246-56. doi: 10.1128/MCB.19.5.3246.
10. Dubey R, Makhija R, Sharma A, Sahu A, Asati V. Unveiling the promise of pyrimidine-modified CDK inhibitors in cancer treatment. Bioorg Chem. 2024;149:107508. doi: 10.1016/j.bioorg.2024.107508.
11. Huang MF, Wang YX, Chou YT, Lee DF. Therapeutic Strategies for RB1-Deficient Cancers: Intersecting Gene Regulation and Targeted Therapy. Cancers (Basel). 2024;16(8):1558. doi: 10.3390/cancers16081558.
12. Marei HE, Bedair K, Hasan A, Al-Mansoori L, Gaiba A, Morrione A, et al. Targeting CDKs in cancer therapy: advances in PROTACs and molecular glues. NPJ Precis Oncol. 2025;9(1):204. doi: 10.1038/s41698-025-00931-8.
13. Mademtzoglou D, Relaix F. From cyclins to CDKIs: Cell cycle regulation of skeletal muscle stem cell quiescence and activation. Exp Cell Res. 2022;420(1):113275. doi: 10.1016/j.yexcr.2022.113275.
14. Goel S, DeCristo MJ, McAllister SS, Zhao JJ. CDK4/6 Inhibition in Cancer: Beyond Cell Cycle Arrest. Trends Cell Biol. 2018;28(11):911-925. doi: 10.1016/j.tcb.2018.07.002.
15. Łukasik P, Załuski M, Gutowska I. Cyclin-Dependent Kinases (CDK) and Their Role in Diseases Development-Review. Int J Mol Sci. 2021;22(6):2935. doi: 10.3390/ijms22062935.
16. Hydbring P, Malumbres M, Sicinski P. Non-canonical functions of cell cycle cyclins and cyclin-dependent kinases. Nat Rev Mol Cell Biol. 2016;17(5):280-92. doi: 10.1038/nrm.2016.27.
17. Ohtsubo M, Roberts JM. Cyclin-dependent regulation of G1 in mammalian fibroblasts. Science. 1993;259(5103):1908-12. doi: 10.1126/science.8384376.
18. Cornwell JA, Crncec A, Afifi MM, Tang K, Amin R, Cappell SD. Loss of CDK4/6 activity in S/G2 phase leads to cell cycle reversal. Nature. 2023;619(7969):363-370. doi: 10.1038/s41586-023-06274-3.
19. Sherr CJ, Beach D, Shapiro GI. Targeting CDK4 and CDK6: From Discovery to Therapy. Cancer Discov. 2016;6(4):353-67. doi: 10.1158/2159-8290.CD-15-0894.
20. Henley SA, Dick FA. The retinoblastoma family of proteins and their regulatory functions in the mammalian cell division cycle. Cell Div. 2012;7(1):10. doi: 10.1186/1747-1028-7-10.
21. Harper JW, Adami GR, Wei N, Keyomarsi K, Elledge SJ. The p21 Cdk-interacting protein Cip1 is a potent inhibitor of G1 cyclin-dependent kinases. Cell. 1993;75(4):805-16. doi: 10.1016/0092-8674(93)90499-g.
22. Orend G, Hunter T, Ruoslahti E. Cytoplasmic displacement of cyclin E-cdk2 inhibitors p21Cip1 and p27Kip1 in anchorage-independent cells. Oncogene. 1998;16(20):2575-83. doi: 10.1038/sj.onc.1201791.
23. Suryadinata R, Sadowski M, Sarcevic B. Control of cell cycle progression by phosphorylation of cyclin-dependent kinase (CDK) substrates. Biosci Rep. 2010 Mar 17;30(4):243-55. doi: 10.1042/BSR20090171.
24. Roy R, Gampa SC, Garimella SV. Role of specific CDKs in regulating DNA damage repair responses and replication stress. Curr Opin Pharmacol. 2024;79:102485. doi: 10.1016/j.coph.2024.102485.
25. Song G, Liu J, Tang X, Zhong J, Zeng Y, Zhang X, et al. Cell cycle checkpoint revolution: targeted therapies in the fight against malignant tumors. Front Pharmacol. 2024;15:1459057. doi: 10.3389/fphar.2024.1459057.
26. Khan MGM, Wang Y. Cell Cycle-Related Clinical Applications. Methods Mol Biol. 2022;2579:35-46. doi: 10.1007/978-1-0716-2736-5_3.
27. Graña X, Claudio PP, De Luca A, Sang N, Giordano A. PISSLRE, a human novel CDC2-related protein kinase. Oncogene. 1994;9(7):2097-103.
28. Brambilla R, Draetta G. Molecular cloning of PISSLRE, a novel putative member of the cdk family of protein serine/threonine kinases. Oncogene. 1994;9(10):3037-41.
29. Mangone L, Marinelli F, Bisceglia I, Braghiroli MB, Mastrofilippo V, Fortunato M, et al. Incidence And Clinical Outcome Of Endometrial Cancer: A Population-Based Cancer-Registry Study. ARO. 2023;3(4):147-154. doi: 10.48286/aro.2023.77.
30. Modi V, Dunbrack RL Jr. Defining a new nomenclature for the structures of active and inactive kinases. Proc Natl Acad Sci U S A. 2019;116(14):6818-6827. doi: 10.1073/pnas.1814279116.
31. Malumbres M. Cyclin-dependent kinases. Genome Biol. 2014;15(6):122. doi: 10.1186/gb4184.
32. Lapenna S, Giordano A. Cell cycle kinases as therapeutic targets for cancer. Nat Rev Drug Discov. 2009;8(7):547-66. doi: 10.1038/nrd2907.

33. Guen VJ, Gamble C, Lees JA, Colas P. The awakening of the CDK10/Cyclin M protein kinase. Oncotarget. 2017;8(30):50174-50186. doi: 10.18632/oncotarget.15024.
34. Darzynkiewicz Z, Gong J, Juan G, Ardelt B, Traganos F. Cytometry of cyclin proteins. Cytometry. 1996;25(1):1-13. doi: 10.1002/(SICI)1097-0320(19960901)25:1<1::AID-CYTO1>3.0.CO;2-N.
35. Tatum NJ, Endicott JA. Chatterboxes: the structural and functional diversity of cyclins. Semin Cell Dev Biol. 2020;107:4-20. doi: 10.1016/j.semcdb.2020.04.021.
36. Saleban M, Harris EL, Poulter JA. D-Type Cyclins in Development and Disease. Genes (Basel). 2023;14(7):1445. doi: 10.3390/genes14071445.
37. Dumitru AMG, Compton DA. Identifying Cyclin A/Cdk1 Substrates in Mitosis in Human Cells. Methods Mol Biol. 2022;2415:175-182. doi: 10.1007/978-1-0716-1904-9_13.
38. Ito M. Factors controlling cyclin B expression. Plant Mol Biol. 2000;43(5-6):677-90. doi: 10.1023/a:1006336005587.
39. Yam CH, Fung TK, Poon RY. Cyclin A in cell cycle control and cancer. Cell Mol Life Sci. 2002;59(8):1317-26. doi: 10.1007/s00018-002-8510-y.
40. Koff A, Giordano A, Desai D, Yamashita K, Harper JW, Elledge S, et al. Formation and activation of a cyclin E-cdk2 complex during the G1 phase of the human cell cycle. Science. 1992;257(5077):1689-94. doi: 10.1126/science.1388288.
41. Chu C, Geng Y, Zhou Y, Sicinski P. Cyclin E in normal physiology and disease states. Trends Cell Biol. 2021;31(9):732-746. doi: 10.1016/j.tcb.2021.05.001.
42. Bazzi ZA, Tai IT. CDK10 in Gastrointestinal Cancers: Dual Roles as a Tumor Suppressor and Oncogene. Front Oncol. 2021;11:655479. doi: 10.3389/fonc.2021.655479.
43. Swanton C, Downward J. Unraveling the complexity of endocrine resistance in breast cancer by functional genomics. Cancer Cell. 2008;13(2):83-5. doi: 10.1016/j.ccr.2008.01.021.
44. Iorns E, Turner NC, Elliott R, Syed N, Garrone O, Gasco M, et al. Identification of CDK10 as an important determinant of resistance to endocrine therapy for breast cancer. Cancer Cell. 2008;13(2):91-104. doi: 10.1016/j.ccr.2008.01.001.
45. Guen VJ, Gamble C, Flajolet M, Unger S, Thollet A, Ferandin Y, et al. CDK10/cyclin M is a protein kinase that controls ETS2 degradation and is deficient in STAR syndrome. Proc Natl Acad Sci U S A. 2013;110(48):19525-30. doi: 10.1073/pnas.1306814110.
46. Zhou X, Lu D, Yi W, Xu D. Downregulation of CDC25C in NPCs Disturbed Cortical Neurogenesis. Int J Mol Sci. 2023;24(2):1505. doi: 10.3390/ijms24021505.
47. Dakilah I, Harb A, Abu-Gharbieh E, El-Huneidi W, Taneera J, Hamoudi R, et al. Potential of CDC25 phosphatases in cancer research and treatment: key to precision medicine. Front Pharmacol. 2024;15:1324001. doi: 10.3389/fphar.2024.1324001.
48. Hoffmann I, Draetta G, Karsenti E. Activation of the phosphatase activity of human cdc25A by a cdk2-cyclin E dependent phosphorylation at the G1/S transition. EMBO J. 1994;13(18):4302-10. doi: 10.1002/j.1460-2075.1994.tb06750.x.
49. Bona AB, Calcagno DQ, Ribeiro HF, Muniz JAPC, Pinto GR, Rocha CAM, Lacreta Junior ACC, de Assumpção PP, Herranz JAR, Burbano RR. Menadione reduces CDC25B expression and promotes tumor shrinkage in gastric cancer. Ther Adv Gastroenterol. 2020;13:1756284819895435. doi: 10.1177/1756284819895435.
50. Boutros R, Dozier C, Ducommun B. The when and wheres of CDC25 phosphatases. Curr Opin Cell Biol. 2006;18(2):185-91. doi: 10.1016/j.ceb.2006.02.003.
51. Flores M, Goodrich DW. Retinoblastoma Protein Paralogs and Tumor Suppression. Front Genet. 2022;13:818719. doi: 10.3389/fgene.2022.818719.
52. Friend SH, Bernards R, Rogelj S, Weinberg RA, Rapaport JM, Albert DM, et al. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature. 1986;323(6089):643-6. doi: 10.1038/323643a0.
53. Fung YK, Murphree AL, T'Ang A, Qian J, Hinrichs SH, Benedict WF. Structural evidence for the authenticity of the human retinoblastoma gene. Science. 1987;236(4809):1657-61. doi: 10.1126/science.2885916.
54. Lee WH, Bookstein R, Hong F, Young LJ, Shew JY, Lee EY. Human retinoblastoma susceptibility gene: cloning, identification, and sequence. Science. 1987;235(4794):1394-9. doi: 10.1126/science.3823889.
55. Ma M, Zhu Y, Xiao C, Li R, Cao X, Kang R, et al. Novel insights into RB1 in prostate cancer lineage plasticity and drug resistance. Tumori. 2024;110(4):252-263. doi: 10.1177/03008916231225576.
56. Ventura E, Iannuzzi CA, Pentimalli F, Giordano A, Morrione A. RBL1/p107 Expression Levels Are

Modulated by Multiple Signaling Pathways. Cancers (Basel). 2021;13(19):5025. doi: 10.3390/cancers13195025.
57. Cao L, Peng B, Yao L, Zhang X, Sun K, Yang X, et al. The ancient function of RB-E2F pathway: insights from its evolutionary history. Biol Direct. 2010;5:55. doi: 10.1186/1745-6150-5-55.
58. Engeland K. Cell cycle arrest through indirect transcriptional repression by p53: I have a DREAM. Cell Death Differ. 2018;25(1):114-132. doi: 10.1038/cdd.2017.172.
59. Pentimalli F. Updates from the TP53 universe. Cell Death Differ. 2018;25(1):10-12. doi: 10.1038/cdd.2017.190.
60. Indovina P, Marcelli E, Casini N, Rizzo V, Giordano A. Emerging roles of RB family: new defense mechanisms against tumor progression. J Cell Physiol. 2013;228(3):525-35. doi: 10.1002/jcp.24170.
61. Indovina P, Pentimalli F, Casini N, Vocca I, Giordano A. RB1 dual role in proliferation and apoptosis: cell fate control and implications for cancer therapy. Oncotarget. 2015;6(20):17873-90. doi: 10.18632/oncotarget.4286.
62. Classon M, Dyson N. p107 and p130: versatile proteins with interesting pockets. Exp Cell Res. 2001;264(1):135-47. doi: 10.1006/excr.2000.5135.
63. Gaubatz S, Lindeman GJ, Ishida S, Jakoi L, Nevins JR, Livingston DM, et al. E2F4 and E2F5 play an essential role in pocket protein-mediated G1 control. Mol Cell. 2000;6(3):729-35. doi: 10.1016/s1097-2765(00)00071-x.
64. Liban TJ, Thwaites MJ, Dick FA, Rubin SM. Structural Conservation and E2F Binding Specificity within the Retinoblastoma Pocket Protein Family. J Mol Biol. 2016;428(20):3960-3971. doi: 10.1016/j.jmb.2016.08.017.
65. Hurford RK Jr, Cobrinik D, Lee MH, Dyson N. pRB and p107/p130 are required for the regulated expression of different sets of E2F responsive genes. Genes Dev. 1997;11(11):1447-63. doi: 10.1101/gad.11.11.1447.
66. Classon M, Salama S, Gorka C, Mulloy R, Braun P, Harlow E. Combinatorial roles for pRB, p107, and p130 in E2F-mediated cell cycle control. Proc Natl Acad Sci U S A. 2000;97(20):10820-5. doi: 10.1073/pnas.190343497.
67. Dick FA, Sailhamer E, Dyson NJ. Mutagenesis of the pRB pocket reveals that cell cycle arrest functions are separable from binding to viral oncoproteins. Mol Cell Biol. 2000;20(10):3715-27. doi: 10.1128/MCB.20.10.3715-3727.2000.
68. Mäkelä JA, Toppari J. Retinoblastoma-E2F Transcription Factor Interplay Is Essential for Testicular Development and Male Fertility. Front Endocrinol (Lausanne). 2022;13:903684. doi: 10.3389/fendo.2022.903684.
69. Swaidan R, Daher A, Bejjani AT, El Atie YE, Chehab Y, Bou Hamdan R, et al. Rb substantially compensates for the double loss of p130 and p107 in adult but not embryonic neural stem cell lineages. Cell Death Dis. 2025;16(1):511. doi: 10.1038/s41419-025-07815-6.
70. Stengel KR, Thangavel C, Solomon DA, Angus SP, Zheng Y, Knudsen ES. Retinoblastoma/p107/p130 pocket proteins: protein dynamics and interactions with target gene promoters. J Biol Chem. 2009;284(29):19265-71. doi: 10.1074/jbc.M808740200.
71. Sadasivam S, DeCaprio JA. The DREAM complex: master coordinator of cell cycle-dependent gene expression. Nat Rev Cancer. 2013;13(8):585-95. doi: 10.1038/nrc3556.
72. Bujarrabal-Dueso A, Sendtner G, Meyer DH, Chatzinikolaou G, Stratigi K, Garinis GA, et al. The DREAM complex functions as conserved master regulator of somatic DNA-repair capacities. Nat Struct Mol Biol. 2023;30(4):475-488. doi: 10.1038/s41594-023-00942-8.
73. Uxa S, Bernhart SH, Mages CFS, Fischer M, Kohler R, Hoffmann S, et al. DREAM and RB cooperate to induce gene repression and cell-cycle arrest in response to p53 activation. Nucleic Acids Res. 2019;47(17):9087-9103. doi: 10.1093/nar/gkz635.
74. Engeland K. Cell cycle regulation: p53-p21-RB signaling. Cell Death Differ. 2022;29(5):946-960. doi: 10.1038/s41418-022-00988-z.
75. Quaas M, Kohler R, Nöltner L, Schmidbauer LF, Uxa S, Müller GA, et al. BRCA1 and BRCA2 gene expression: p53- and cell cycle-dependent repression requires RB and DREAM. Cell Death Differ. 2026;33(1):51-63. doi: 10.1038/s41418-025-01566-9.
76. Mages CF, Wintsche A, Bernhart SH, Müller GA. The DREAM complex through its subunit Lin37 cooperates with Rb to initiate quiescence. Elife. 2017;6:e26876. doi: 10.7554/eLife.26876.
77. Pattschull G, Walz S, Gründl M, Schwab M, Rühl E, Baluapuri A, et al. The Myb-MuvB Complex Is Required for YAP-Dependent Transcription of Mitotic Genes. Cell Rep. 2019;27(12):3533-3546.e7. doi: 10.1016/j.celrep.2019.05.071.
78. Koliopoulos MG, Alfieri C. Cell cycle regulation by complex nanomachines. FEBS J. 2022;289(17):5100-5120. doi: 10.1111/febs.16082.

79. Whyte P. The retinoblastoma protein and its relatives. Semin Cancer Biol. 1995;6(2):83-90. doi: 10.1006/scbi.1995.0011.
80. Talluri S, Dick FA. Regulation of transcription and chromatin structure by pRB: here, there and everywhere. Cell Cycle. 2012;11(17):3189-98. doi: 10.4161/cc.21263.
81. Csergeová L, Krbušek D, Janoštiak R. CIP/KIP and INK4 families as hostages of oncogenic signaling. Cell Div. 2024;19(1):11. doi: 10.1186/s13008-024-00115-z.
82. Roussel MF. The INK4 family of cell cycle inhibitors in cancer. Oncogene. 1999;18(38):5311-7. doi: 10.1038/sj.onc.1202998.
83. Fischer M, Quaas M, Steiner L, Engeland K. The p53-p21-DREAM-CDE/CHR pathway regulates G2/M cell cycle genes. Nucleic Acids Res. 2016;44(1):164-74. doi: 10.1093/nar/gkv927.
84. Kreis NN, Louwen F, Yuan J. The Multifaceted p21 (Cip1/Waf1/CDKN1A) in Cell Differentiation, Migration and Cancer Therapy. Cancers (Basel). 2019;11(9):1220. doi: 10.3390/cancers11091220.
85. Shamloo B, Usluer S. p21 in Cancer Research. Cancers (Basel). 2019;11(8):1178. doi: 10.3390/cancers11081178.
86. Cirillo D, Pentimalli F, Giordano A. Peptides or small molecules? Different approaches to develop more effective CDK inhibitors. Curr Med Chem. 2011;18(19):2854-66. doi: 10.2174/092986711796150496.
87. Kamijo T, Weber JD, Zambetti G, Zindy F, Roussel MF, Sherr CJ. Functional and physical interactions of the ARF tumor suppressor with p53 and Mdm2. Proc Natl Acad Sci U S A. 1998;95(14):8292-7. doi: 10.1073/pnas.95.14.8292.
88. Kung CP, Weber JD. It's Getting Complicated-A Fresh Look at p53-MDM2-ARF Triangle in Tumorigenesis and Cancer Therapy. Front Cell Dev Biol. 2022;10:818744. doi: 10.3389/fcell.2022.818744.
89. Ohtani N, Yamakoshi K, Takahashi A, Hara E. The p16INK4a-RB pathway: molecular link between cellular senescence and tumor suppression. J Med Invest. 2004;51(3-4):146-53. doi: 10.2152/jmi.51.146.
90. Nag S, Qin J, Srivenugopal KS, Wang M, Zhang R. The MDM2-p53 pathway revisited. J Biomed Res. 2013;27(4):254-71. doi: 10.7555/JBR.27.20130030.
91. Kuganesan N, Dlamini S, Tillekeratne LMV, Taylor WR. Tumor suppressor p53 promotes ferroptosis in oxidative stress conditions independent of modulation of ferroptosis by p21, CDKs, RB, and E2F. J Biol Chem. 2021;297(6):101365. doi: 10.1016/j.jbc.2021.101365.
92. Canman CE, Lim DS, Cimprich KA, Taya Y, Tamai K, Sakaguchi K, et al. Activation of the ATM kinase by ionizing radiation and phosphorylation of p53. Science. 1998;281(5383):1677-9. doi: 10.1126/science.281.5383.1677.
93. Tibbetts RS, Brumbaugh KM, Williams JM, Sarkaria JN, Cliby WA, Shieh SY, et al. A role for ATR in the DNA damage-induced phosphorylation of p53. Genes Dev. 1999;13(2):152-7. doi: 10.1101/gad.13.2.152.
94. Lees-Miller SP, Sakaguchi K, Ullrich SJ, Appella E, Anderson CW. Human DNA-activated protein kinase phosphorylates serines 15 and 37 in the amino-terminal transactivation domain of human p53. Mol Cell Biol. 1992;12(11):5041-9. doi: 10.1128/mcb.12.11.5041-5049.1992.
95. Sakaguchi K, Saito S, Higashimoto Y, Roy S, Anderson CW, Appella E. Damage-mediated phosphorylation of human p53 threonine 18 through a cascade mediated by a casein 1-like kinase. Effect on Mdm2 binding. J Biol Chem. 2000;275(13):9278-83. doi: 10.1074/jbc.275.13.9278.
96. Hirao A, Kong YY, Matsuoka S, Wakeham A, Ruland J, Yoshida H, et al. DNA damage-induced activation of p53 by the checkpoint kinase Chk2. Science. 2000;287(5459):1824-7. doi: 10.1126/science.287.5459.1824.
97. Hsueh KW, Fu SL, Chang CB, Chang YL, Lin CH. A novel Aurora-A-mediated phosphorylation of p53 inhibits its interaction with MDM2. Biochim Biophys Acta. 2013;1834(2):508-15. doi: 10.1016/j.bbapap.2012.11.005.
98. Asghar U, Witkiewicz AK, Turner NC, Knudsen ES. The history and future of targeting cyclin-dependent kinases in cancer therapy. Nat Rev Drug Discov. 2015;14(2):130-46. doi: 10.1038/nrd4504.
99. Rowland RJ, Noble MEM, Endicott JA. Diversity in CDK structural mechanisms of regulation and drug discovery opportunities. Trends Biochem Sci. 2026;51(3):234-248. doi: 10.1016/j.tibs.2025.12.007.
100. Hope I, Endicott JA, Watt JE. Emerging approaches to CDK inhibitor development, a structural perspective. RSC Chem Biol. 2022;4(2):146-164. doi: 10.1039/d2cb00201a.
101. Glaviano A, Wander SA, Baird RD, Yap KC, Lam HY, Toi M, et al. Mechanisms of sensitivity and resistance to CDK4/CDK6 inhibitors in hormone receptor-positive breast cancer treatment. Drug Resist Updat. 2024;76:101103. doi: 10.1016/j.drup.2024.101103.
102. Bolzacchini E, Pomero F, Fazio M, Civitelli C, Fabro G, Pellegrino D, et al. Risk of venous and

arterial thromboembolic events in women with advanced breast cancer treated with CDK 4/6 inhibitors: A systematic review and meta-analysis. Thromb Res. 2021;208:190-197. doi: 10.1016/j.thromres.2021.11.009.
103. Xue Y, Zhai J. Strategy of combining CDK4/6 inhibitors with other therapies and mechanisms of resistance. Int J Clin Exp Pathol. 2024;17(7):189-207. doi: 10.62347/HGNI4903.
104. Yang P, Zhang H, Wu M, Zhao F, Wang M, Zhao J, et al. Dose reduction and discontinuation due to the combination of CDK4/6 inhibitors and endocrine drugs: a systematic review and meta-analysis. Eur J Clin Pharmacol. 2024;80(12):1893-1902. doi: 10.1007/s00228-024-03757-8.
105. Esposito L, Indovina P, Magnotti F, Conti D, Giordano A. Anticancer therapeutic strategies based on CDK inhibitors. Curr Pharm Des. 2013;19(30):5327-32. doi: 10.2174/1381612811319990377.
106. Schang LM. Effects of pharmacological cyclin-dependent kinase inhibitors on viral transcription and replication. Biochim Biophys Acta. 2004;1697(1-2):197-209. doi: 10.1016/j.bbapap.2003.11.024.
107. Cicenas J, Kalyan K, Sorokinas A, Stankunas E, Levy J, Meskinyte I, et al. Roscovitine in cancer and other diseases. Ann Transl Med. 2015;3(10):135. doi: 10.3978/j.issn.2305-5839.2015.03.61.
108. Federico M, Symonds CE, Bagella L, Rizzolio F, Fanale D, Russo A, et al. R-Roscovitine (Seliciclib) prevents DNA damage-induced cyclin A1 upregulation and hinders non-homologous end-joining (NHEJ) DNA repair. Mol Cancer. 2010;9:208. doi: 10.1186/1476-4598-9-208.
109. Diaz-Montero CM, Holvey-Bates EG, Rayman PA, Parker Y, Lindner DJ, Stark GR, et al. The CDK inhibitor Roscovitine enhances the therapeutic efficacy of anti-PD-1 in non-small cell lung cancer. Front Oncol. 2026;15:1745967. doi: 10.3389/fonc.2025.1745967.
110. Bonelli MA, Digiacomo G, Fumarola C, Alfieri R, Quaini F, Falco A, et al. Combined Inhibition of CDK4/6 and PI3K/AKT/mTOR Pathways Induces a Synergistic Anti-Tumor Effect in Malignant Pleural Mesothelioma Cells. Neoplasia. 2017;19(8):637-648. doi: 10.1016/j.neo.2017.05.003.
111. Benot-Dominguez R, Cimini A, Barone D, Giordano A, Pentimalli F. The Emerging Role of Cyclin-Dependent Kinase Inhibitors in Treating Diet-Induced Obesity: New Opportunities for Breast and Ovarian Cancers? Cancers (Basel). 2022;14(11):2709. doi: 10.3390/cancers14112709.
112. Finn RS, Aleshin A, Slamon DJ. Targeting the cyclin-dependent kinases (CDK) 4/6 in estrogen receptor-positive breast cancers. Breast Cancer Res. 2016;18(1):17. doi: 10.1186/s13058-015-0661-5.
113. Rizzolio F, Tuccinardi T, Caligiuri I, Lucchetti C, Giordano A. CDK inhibitors: from the bench to clinical trials. Curr Drug Targets. 2010;11(3):279-90. doi: 10.2174/138945010790711978.
114. Huang J, Zheng L, Sun Z, Li J. CDK4/6 inhibitor resistance mechanisms and treatment strategies (Review). Int J Mol Med. 2022;50(4):128. doi: 10.3892/ijmm.2022.5184.
115. Rajput S, Khera N, Guo Z, Hoog J, Li S, Ma CX. Inhibition of cyclin dependent kinase 9 by dinaciclib suppresses cyclin B1 expression and tumor growth in triple negative breast cancer. Oncotarget. 2016;7(35):56864-56875. doi: 10.18632/oncotarget.10870.
116. Byers KF. Ribociclib and Abemaciclib: CDK4/6 Inhibitors for the Treatment of Hormone Receptor-Positive Metastatic Breast Cancer. J Adv Pract Oncol. 2021;12(1):100-107. doi: 10.6004/jadpro.2021.12.1.8.
117. Finn RS, Martin M, Rugo HS, Jones S, Im SA, Gelmon K, et al. Palbociclib and Letrozole in Advanced Breast Cancer. N Engl J Med. 2016;375(20):1925-1936. doi: 10.1056/NEJMoa1607303.
118. Goetz MP, Toi M, Campone M, Sohn J, Paluch-Shimon S, Huober J, et al. MONARCH 3: Abemaciclib As Initial Therapy for Advanced Breast Cancer. J Clin Oncol. 2017;35(32):3638-3646. doi: 10.1200/JCO.2017.75.6155.
119. Sledge GW Jr, Toi M, Neven P, Sohn J, Inoue K, Pivot X, et al. The Effect of Abemaciclib Plus Fulvestrant on Overall Survival in Hormone Receptor-Positive, ERBB2-Negative Breast Cancer That Progressed on Endocrine Therapy-MONARCH 2: A Randomized Clinical Trial. JAMA Oncol. 2020;6(1):116-124. doi: 10.1001/jamaoncol.2019.4782.
120. Slamon DJ, Neven P, Chia S, Fasching PA, De Laurentiis M, Im SA, et al. Phase III Randomized Study of Ribociclib and Fulvestrant in Hormone Receptor-Positive, Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer: MONALEESA-3. J Clin Oncol. 2018;36(24):2465-2472. doi: 10.1200/JCO.2018.78.9909.

121. Hortobagyi GN, Stemmer SM, Burris HA, Yap YS, Sonke GS, Paluch-Shimon S, et al. Ribociclib as First-Line Therapy for HR-Positive, Advanced Breast Cancer. N Engl J Med. 2016;375(18):1738-1748. doi: 10.1056/NEJMoa1609709.
122. Spring LM, Wander SA, Andre F, Moy B, Turner NC, Bardia A. Cyclin-dependent kinase 4 and 6 inhibitors for hormone receptor-positive breast cancer: past, present, and future. Lancet. 2020;395(10226):817-827. doi: 10.1016/S0140-6736(20)30165-3.
123. Buonaiuto R, Fordellone M, Caltavituro A, Cataldo ML, Criscitiello C, Dieci MV, et al. Efficacy and safety of systemic therapies following progression on CDK4/6 inhibitors in patients with $HR^+/HER2^-$ metastatic breast cancer: a systematic review and network meta-analysis. EClinicalMedicine. 2025;89:103535. doi: 10.1016/j.eclinm.2025.103535.
124. Zhang J, Wen K, Nakajima KI, Shi Y, Chen X. CDK4/6 Inhibitors Suppress RB-Null Triple-Negative Breast Cancer by Inhibiting Mutant P53 Expression via RBM38 RNA-Binding Protein. Cancers (Basel). 2025;17(20):3339. doi: 10.3390/cancers17203339.
125. Burk S, Morrione A. A brief overview of several recent advancements of targeted-therapies and antibody-conjugate drugs for advanced triple-negative breast cancer. ARO. 2023;3(2):86-98. doi: 10.48286/aro.2023.65.
126. Clark AS, McAndrew NP, Troxel A, Feldman M, Lal P, Rosen M, et al. Combination Paclitaxel and Palbociclib: Results of a Phase I Trial in Advanced Breast Cancer. Clin Cancer Res. 2019;25(7):2072-2079. doi: 10.1158/1078-0432.CCR-18-0790.
127. Sledge GW Jr, Toi M, Neven P, Sohn J, Inoue K, Pivot X, et al. MONARCH 2: Abemaciclib in Combination With Fulvestrant in Women With $HR^+/HER2^-$ Advanced Breast Cancer Who Had Progressed While Receiving Endocrine Therapy. J Clin Oncol. 2017;35(25):2875-2884. doi: 10.1200/JCO.2017.73.7585.
128. Sammons S, Kornblum NS, Blackwell KL. Fulvestrant-Based Combination Therapy for Second-Line Treatment of Hormone Receptor-Positive Advanced Breast Cancer. Target Oncol. 2019;14(1):1-12. doi: 10.1007/s11523-018-0587-9.
129. Awada A, Ahmad S. Therapeutic Prospects of Abemaciclib for Patients with Endometrial Cancer. Curr Oncol. 2024;31(9):5374-5383. doi: 10.3390/curroncol31090397.
130. Martinelli C, Vidali A, Di Chiara F, Mazzarotti G, El Messaoudi S, Alfano L, Ercoli A, Giordano A. Endometriosis: A Cancer-Mimicking Disease And The Need For A Translational Perspective. ARO. 2025;5(2):75-94. doi: 10.48286/aro.2025.107.
131. Konstantinopoulos PA, Cai M, Lee EK, Krasner C, Campos SM, Liu JF, et al. Serial Circulating Tumor DNA Sequencing to Monitor Response and Define Acquired Resistance to Letrozole/Abemaciclib in Endometrial Cancer. JCO Precis Oncol. 2025;9:e2400882. doi: 10.1200/PO-24-00882.
132. Li Z, Razavi P, Li Q, Toy W, Liu B, Ping C, et al. Loss of the FAT1 Tumor Suppressor Promotes Resistance to CDK4/6 Inhibitors via the Hippo Pathway. Cancer Cell. 2018;34(6):893-905.e8. doi: 10.1016/j.ccell.2018.11.006.
133. von Manstein V, Yang CM, Richter D, Delis N, Vafaizadeh V, Groner B. Resistance of Cancer Cells to Targeted Therapies Through the Activation of Compensating Signaling Loops. Curr Signal Transduct Ther. 2013;8(3):193-202. doi: 10.2174/15743624096661402062219 31.
134. Sawant M, Wilson A, Sridaran D, Mahajan K, O'Conor CJ, Hagemann IS, et al. Epigenetic reprogramming of cell cycle genes by ACK1 promotes breast cancer resistance to CDK4/6 inhibitor. Oncogene. 2023;42(29):2263-2277. doi: 10.1038/s41388-023-02747-x.
135. Petroni G, Formenti SC, Chen-Kiang S, Galluzzi L. Immunomodulation by anticancer cell cycle inhibitors. Nat Rev Immunol. 2020;20(11):669-679. doi: 10.1038/s41577-020-0300-y.
136. Palmer CL, Boras B, Pascual B, Li N, Li D, Garza S, et al. CDK4 selective inhibition improves preclinical anti-tumor efficacy and safety. Cancer Cell. 2025;43(3):464-481.e14. doi: 10.1016/j.ccell.2025.02.006.
137. Dai X, Zhang X, Chen W, Chen Y, Zhang Q, Mo S, et al. Dihydroartemisinin: A Potential Natural Anticancer Drug. Int J Biol Sci. 2021;17(2):603-622. doi: 10.7150/ijbs.50364.
138. Zhou Z, Lei J, Fang J, Chen P, Zhou J, Wang H, et al. Dihydroartemisinin remodels tumor micro-environment and improves cancer immunotherapy through inhibiting cyclin-dependent kinases. Int Immunopharmacol. 2024;139:112637. doi: 10.1016/j.intimp.2024.112637.
139. Fan HN, Zhu MY, Peng SQ, Zhu JS, Zhang J, Qu GQ. Dihydroartemisinin inhibits the growth and invasion of gastric cancer cells by regulating cyclin D1-CDK4-Rb signaling. Pathol

Res Pract. 2020;216(2):152795. doi: 10.1016/j.prp.2019.152795.
140. Benada J, Macurek L. Targeting the Checkpoint to Kill Cancer Cells. Biomolecules. 2015;5(3):1912-37. doi: 10.3390/biom5031912.
141. Jin D, Tran N, Thomas N, Tran DD. Combining CDK4/6 inhibitors ribociclib and palbociclib with cytotoxic agents does not enhance cytotoxicity. PLoS One. 2019;14(10):e0223555. doi: 10.1371/journal.pone.0223555.
142. Liu Y, Park S, Li Y. Breaking Cancer's Momentum: CDK4/6 Inhibitors and the Promise of Combination Therapy. Cancers (Basel). 2025;17(12):1941. doi: 10.3390/cancers17121941.
143. Brion T, Quéro L. Radiotherapy and CDK inhibitors: Opportunities and risks. Cancer Radiother. 2022;26(6-7):968-972. doi: 10.1016/j.canrad.2022.06.017.
144. Roberts PJ, Kumarasamy V, Witkiewicz AK, Knudsen ES. Chemotherapy and CDK4/6 Inhibitors: Unexpected Bedfellows. Mol Cancer Ther. 2020;19(8):1575-1588. doi: 10.1158/1535-7163.MCT-18-1161.
145. Scordamaglia D, Talia M, Zicarelli A, Mondino AA, De Rosis S, Di Dio M, et al. Alternate actions of CDK4/6 inhibitors beyond cell cycle blockade: unexplored roles in therapy resistance. Cancer Metastasis Rev. 2025;44(4):90. doi: 10.1007/s10555-025-10307-w.
146. Si Y, Li H, Shi Y. The Dual Effects of CDK4/6 Inhibitors on Tumor Immunity. Cancers (Basel). 2025;17(24):3997. doi: 10.3390/cancers17243997.

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REVIEW

GENETIC AND EPIGENETIC CONTRIBUTORS TO COVID-19 OUTCOMES: A COMPREHENSIVE REVIEW

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ABSTRACT: SARS-CoV-2 infection results in a broad spectrum of COVID-19 disease, from mild or no symptoms to hospitalization and death. The degree of the adaptive immune response to SARS-CoV-2 and some pre-existing diseases has been linked to the severity of COVID-19 disease, and a recent genome-wide association study (GWAS) of the risk of critical illness found a strong genetic component. Many assume that the diversity of HLA increases the likelihood that a species can survive pandemics. The requirement for a species to have a diversified immune system to survive a pandemic is believed to be the cause of the HLA system's widespread polymorphism. Two genomic regions are associated with severe COVID-19: one region on chromosome 3, which contains six genes, and one region on chromosome 9 that determines ABO blood groups. Numerous ACE2 and TMPRSS2 polymorphisms that affect the expression of COVID-19-related receptors have been linked to risk factors and disease susceptibility. Differential cytokine production in COVID-19 patients may be linked to genetic variations in the regulatory regions of cytokine genes. The plasma miRNA expression profile at an early stage of COVID-19 is profoundly disrupted by SARS-CoV-2 infection, which makes miRNAs extremely useful as early indicators of severity and mortality. An inflammatory outburst and lymphopenia are associated with severe COVID-19, which may worsen the prognosis for cancer. Through mechanisms including cytokine storm, tissue hypoxia, poor T-cell responses, autophagy, neutrophil activation, and oxidative stress, SARS-CoV-2 infection may increase cancer susceptibility and speed cancer progression.

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Impact statement: This review clarifies how genetic and epigenetic factors shaper COVID-19 susceptibility, severity, and outcomes, offering a framework to improve risk stratification, personalized care, and oncology-oriented research in future clinical studies.

Key words: *COVID-19; HLA; ACE2; ABO blood group; microRNAs; cancer.*

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INTRODUCTION

SARS-CoV-2 is a member of the same coronavirus family (Betacoronavirus) as the SARS and MERS viruses, which were responsible for two of the most devastating epidemics in recent years (1). SARS-CoV-2 infects alveolar epithelial cells through receptor-mediated endocytosis. The SARS-CoV-2 spike protein (S) binds to the ACE2 receptor, which is expressed in several organs, including the lung, heart, kid-

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ney, and intestine. For the detection of viruses, the innate immune system utilizes a variety of pattern recognition receptors (PRRs). Plasmacytoid dendritic cells detect the incoming viral genomic RNA in the endosome through Toll-like receptor 7 (TLR7). Other cell types express endosomal TLR3 (variety of cells) and TLR8 (myeloid cells) that can also recognize endocytosed double-stranded RNA (dsRNA) or single-stranded RNA (ssRNA), respectively (2). Alveolar and interstitial macrophages contribute to the immune response against SARS-CoV-2 and can adopt either pro-inflammatory (M1) or anti-inflammatory (M2) phenotypes depending on the local microenvironment (3). Macrophage Activation Syndrome may further explain the high serum levels of CRP, which are normally lacking in viral infections (4). Certain proteases, such as Transmembrane Serine Protease 2 (TMPRSS2) and Cathepsin L (CTSL), cleave to S domains to mediate membrane fusion and virus infectivity after the receptor binds, allowing the virus to enter the host cell cytosol through acid-dependent proteolytic cleavage of the S protein (3). Pro-inflammatory cytokines, Interleukin (IL)-1b and IL-18, are released by macrophages, epithelial cells, and endothelial cells when their inflammasomes are activated. These cytokines cause neutrophilia and leukopenia, which add to the pathogenic inflammation that causes the severity of COVID-19 symptoms (5). Higher blood plasma levels of IL-2, IL-7, IL-10, granulocyte colony-stimulating factor (G-CSF), IP-10, MCP-1, macrophage inflammatory protein 1α (MIP1α), and tumor necrosis factor (TNF) were seen in patients with severe COVID-19 who needed intensive care in hospitals (6). The infected cells display viral peptides inside major histocompatibility complex (MHC) class I antigens during the course of the viral infection cycle. Viral peptides shown in class I will activate CD8+ T lymphocytes, which can lyse tissue cells infected with viruses. Professional antigen presenting cells, such as dendritic cells, macrophages, and B-lymphocytes, use MHC class II molecules to deliver viral peptides to CD4+ T cells in the early stages of infection (7). Adaptive immune responses are particularly important because SARS-CoV-2 suppresses antigen presentation by downregulating MHC class I and II molecules, which in turn suppresses T cell mediated immune responses (8). Like antibodies, SARS-CoV-2 T cells are detected within a week from the onset of symptoms (9). CD4+ and CD8+ T cells elicited by SARS-CoV-2 infection are directed against a range of antigens, including structural and non-structural proteins, and are significantly associated with milder disease (2). Chronic viral infections can result if CD8+ or CD4+ T cells have difficulty identifying the HLA class I or II antigens on the cell surface or lower expression levels of the HLA molecules (10). Environmental, demographic, and geographic factors have also been proposed as contributors to differences in COVID-19 outcomes across populations (11). Despite major advances in understanding COVID-19 pathogenesis, substantial variability remains in disease susceptibility, severity, and mortality among individuals and populations. While environmental, demographic, and clinical factors contribute to this heterogeneity, increasing evidence suggests that host genetic and epigenetic determinants also play a critical role in shaping immune responses and clinical outcomes. The following sections review the current evidence regarding the genetic and epigenetic factors associated with COVID-19 susceptibility, severity, and prognosis.

COVID-19 AND THE HLA SYSTEM

HLA biology and antigen presentation

Many assume that the diversity of HLA increases the likelihood that a species can survive pandemics. The extensive polymorphism of the HLA system is thought to result from the need for a species to be immunologically diverse to survive a pandemic. For example, HLA-B27 confers some protection against HIV and hepatitis C (12). The expression of HLA genes is known to be influenced by age, sex, and obesity, all identified as key factors in the severity of COVID-19 (13). It is generally known that several chronic illnesses and viral infections, including HIV, HBV, H1N1, and HCV, are linked to specific HLA alleles (14). After being vaccinated, people who exhibit HLA class I and/or class II molecules with low affinity for SARS-CoV-2 peptides are likely to be more susceptible to serious infections and experience weak or non-sterilizing immunity (15). The MHC, located on the short arm of chromosome 6, is the most complex genetic system in the human genome and includes the human leukocyte antigen (HLA) genes. The primary function of the HLA transmembrane proteins expressed by the classical (A, B, C, DR, DQ, and DP) HLA genes is to present tiny pathogen-derived peptides to T cells as antigens, which sets off an immune response (13) (**Figure 1A** (16)).

The peptide-binding site is formed by the α1 and α2 domains of HLA class I (A, B, and C) molecules. Cyto-

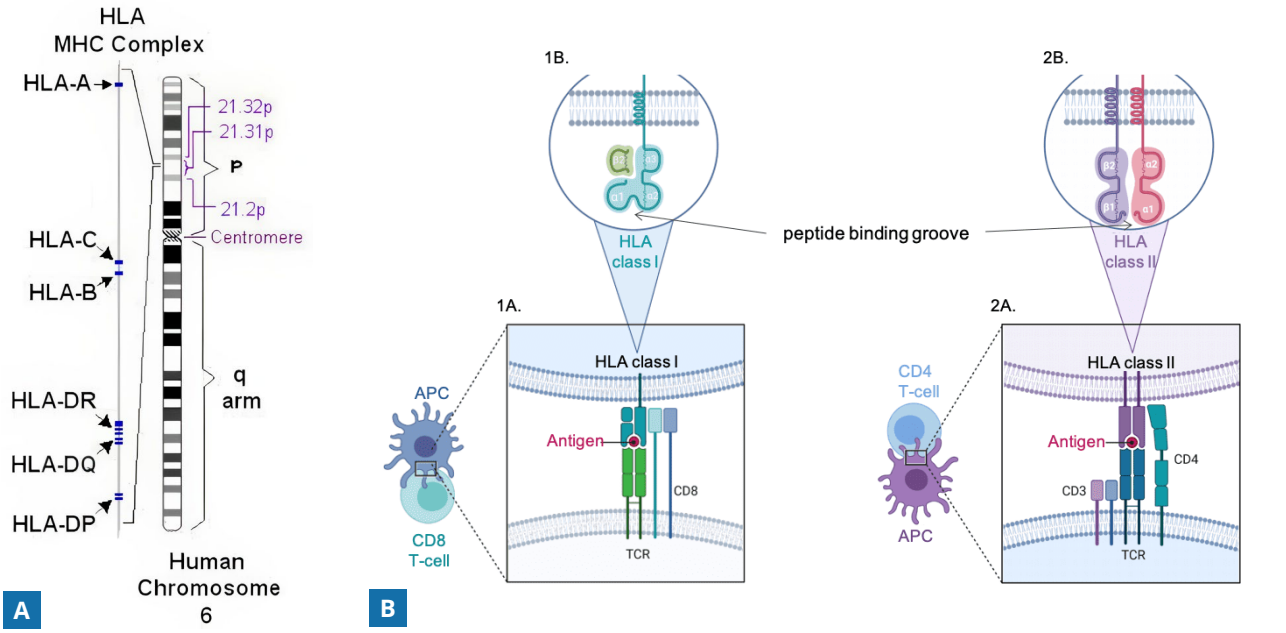

Figure 1. *HLA locus organization and main MHC functions.*

toxic CD8+ lymphocytes are presented with short peptides of 8-10 amino acids that are generated from viral proteins produced by an infected cell. Helper CD4+ cells are presented with 13-25 amino acid peptides from endocytosed antigens by the α1 and β1 domains of HLA class II (DR, DQ, DP) molecules (17). There may be four sets of digits in each HLA allele name, separated by colons. An HLA prefix is followed by a particular HLA locus, such as HLA DQ-A1. A star (HLA-DQA1*) separates the HLA locus from the first two digits. The allele group (HLA-DQA1-01) is designated by the first two digits. A specific HLA allele is indicated by the third and fourth digits (HLA-DQA1*01 02). The mutations in the allele are described by the second four digits (10). Cytotoxic CD8+ T lymphocytes identify immunogenic peptide-MHC class I complexes that are shown on nucleated cells. Antigen-presenting cells, such as dendritic cells (DCs), macrophages, or B cells, can activate CD4+ T lymphocytes by presenting immunogenic peptide–MHC class II complexes. This results in the coordination and control of effector cells (18) (**Figure 1B** (19)).

Overall, HLA class I and class II molecules play a central role in shaping antiviral immunity by regulating antigen presentation and T-cell activation, thereby potentially influencing susceptibility to and severity of SARS-CoV-2 infection.

HLA-Cw15:02, DR*03,01, and HLA-B*46:01, B*07:03, DRB1*12:02 were linked to resistance and severity of SARS-CoV, respectively, according to Wang *et al*. (20). Numerous studies have investigated whether specific HLA alleles influence susceptibility to SARS-CoV-2 infection or the clinical severity of COVID-19. However, findings have often varied across populations, reflecting differences in ethnic background, study design, and sample size. Langton *et al*., (13) using next generation sequencing (NGS), analyzed and compared the class I and class II classical HLA genes of 49 patients admitted to the hospital with severe respiratory disease following COVID-19 infection with those obtained from a group of 69 asymptomatic hospital workers who had evidence of COVID-19 exposure based on blood antibody testing. They discovered that the severe patient's allele frequency of HLA-DRB1*04:01 differed significantly from that of the staff group that did not exhibit any symptoms. There was a significantly lower frequency of the haplotype DQA1*01:01-DQB1*05:01-DRB1*01:01 in the asymptomatic group compared to the background population. The population of North-Western Europe has higher frequency of these alleles. The various statistical analyses presented in the current article indicate that while DRB1*04:01 may be protective, other DRB1*04 alleles, such as DRB1*04:02 and DRB1*04:05, may be associated with an increase in disease severity. In the current study, the alleles most strongly related to COVID-19 severity (DRB1*01:01 and DRB1*04:01) were the only alleles to show significant, positive correlations to latitude and inverse correlations to longitude (13).

These findings suggest that some HLA alleles that are more prevalent in certain European populations may be associated with a reduced risk of severe COVID-19. However, such associations should be interpreted cautiously because HLA effects are strongly influenced by population structure, environmental factors, and viral evolution (13).

Weiner *et al.* (21) examined the relationship between COVID-19 severity and HLAs in 435 participants who registered between March 2020 and August 2020 and came from Germany (n = 135), Spain (n = 133), Switzerland (n = 20), and the United States (n = 147). They tested their results by meta-analyzing data from prior genome-wide association studies (GWAS). They described a potential association of HLA-C*04:01 with a severe clinical course of COVID-19. Carriers of HLA-C*04:01 had twice the risk of intubation when infected with SARS-CoV-2. These findings are biologically plausible, as HLA-C*04:01 has fewer predicted binding sites for relevant SARS-CoV-2 peptides compared to other HLA alleles. Also, their findings suggest that HLA class I alleles have a relevant role in immune defense against SARS-CoV-2. An ecological study strongly suggests a permissive role of HLA-C*01 and B*44 towards SARS-CoV-2 infection across Italy (22). Wang *et al*. (23) reported the first host genetic study in the Chinese population by deeply sequencing and analyzing 332 COVID-19 patients categorized by varying levels of severity from the Shenzhen Third People's Hospital. They found that the patients' worst outcomes are considerably predisposed by the HLA-A*11:01, B*51:01, and C*14:02 alleles.

Collectively, these findings suggest that several HLA class I and class II alleles may contribute to increased susceptibility or disease severity. However, many associations have been reported in specific populations and require validation in larger multiethnic cohorts.

Protective and Risk HLA Variants

Several HLA alleles have been proposed as protective or risk factors for COVID-19, largely depending on their ability to efficiently present SARS-CoV-2-derived peptides and promote effective T-cell responses.

Nguyen *et al*. (24) performed a comprehensive *in silico* analysis of viral peptide MHC class I binding affinity across 145 HLA-A, -B, and -C genotypes for all SARS-CoV-2 peptides. A variety of HLA alleles were used to successfully sample and represent the SARS-CoV-2 proteome. They discovered that the allele with the fewest predicted binding peptides for SARS-CoV-2 was HLA B*46:01, indicating that people with this allele would be more susceptible to COVID-19. Conversely, they found that HLA-B*15:03 showed the greatest capacity to present highly conserved SARS-CoV-2 peptides that are shared among common human coronaviruses, suggesting that it could enable cross-protective T-cell-based immunity. Poulton *et al*. (25) analyzed data from 80 patients who tested positive for SARS-CoV-2 RNA who had previously been HLA typed to support transplantation. They reported a significant HLA association with HLA DQB1*06 and infection. In Italy, only the HLA-C*01 and HLA-B*44 alleles, which are present with a higher frequency in the northern regions of Italy, remained positively associated with COVID-19 (10). Correale *et al*. (22) suggested that healthy individuals carrying HLA-B*44 and/or C*01, and to a lesser extent, HLA-A*25, HLA-B*08 alleles may be more susceptible to SARS-CoV-2 infection. Correale *et al*. (26) revealed later that the direct correlation of HLA-C*01, and HLA-B*44 gene expression and COVID-19 risk was completely lost just after the first pandemic wave in Italy. On the contrary, the expression of the HLA-B*49 allele in specific populations emerged as inversely correlated to the risk of COVID-19 and could be considered as a protective factor.

The frequency of the two most prevalent HLA haplotypes in the Italian population varies significantly between the northern, central, and southern regions, according to a study conducted in Italy using a geographic epidemiological analysis, with HLA-A*01:01 g-B*08:01 g-C*07:01 g DRB1*03:01 g (the most frequent haplotype nationwide) showing a decreasing frequency gradient, and HLA-A* 02:01 g-B*18:01 g-C*07:01 g-DRB1*11:04 g (the second most frequent haplotype) an increasing frequency gradient from North to South (10). A study conducted with 82 Chinese patients found that the HLA-C07:29 and HLA-B15:27 alleles were more frequently detected in the COVID-19 group than in the control population (27). Novelli *et al*. (28) analyzed the HLA allele frequency distribution in a group of 99 Italian patients affected by a severe or extremely severe form of COVID-19. After the application of Bonferroni's correction for multiple tests, a significant association was found for HLA-DRB1*15:01, -DQB1*06:02, and -B*27:07, after comparing the results to a reference group of 1017 Italian individuals, previously typed in their laboratory. Yung *et al*. (29) investigated the HLA-B genotypes in 190 unrelated Chinese patients with confirmed COVID-19, identified a significant positive association between the B22 serotype and SARS-CoV-2

infection. According to the study by Barquera *et al*. (30), the HLA class II alleles DRB1*01:01, DRB1*10:01, DRB1*11:02, and DRB1*13:01 present more SARS-CoV-2 peptides, while the HLA-DRB1*03:02, DRB1*03:03, and DQA1*01:02/DQB1*06 were found to be the worst presenters of SARS-CoV-2-derived peptides. HLA-A*:01:01-g-B*08:01 g C*07:01g-DRB1*03:01g and HLA-A*02.01g-B*18.01g-C*07.01g-DRB1*11.04g, the two most prevalent HLA haplotypes in the Italian population, had a regional distribution that overlapped that of COVID-19 and demonstrated a significant positive (suggestive of susceptibility) and negative (suggestive of protection) correlation with both COVID-19 incidence and mortality, according to Pisanti *et al*. (31). Littera *et al*. (32) showed that the extended haplotype HLA-*02:05, B*58:01, C*07:01, DRB1*03:01 has a protective effect against SARS-CoV-2 infection in the Sardinian population. Genetic factors that resulted in having a negative influence on the disease course were presence of the HLA-DRB1*08:01 allele and G6PDH deficiency, but not the beta thalassemic trait. Shkurnikov *et al*. (33) identified HLA-A, HLA-B, and HLA-C genotypes of n = 111 deceased patients with COVID-19 (Moscow, Russia) and n = 428 volunteers with NGS. Three HLA-A alleles were highly overrepresented in these groups: HLA A*02:01 and HLAA* 03:01 were tightly associated with low risk while HLAA* 01:01 contributed to the high-risk group. Yu *et al*. (34) verified that HLAB*15:27 and HLA-DRB1*04:06 were linked to COVID-19 susceptibility in China by comparing against many subpopulation groupings as a control. Weak binding affinities toward viral proteins were expected for both alleles. Wang *et al*. (35) used NGS to genotype 82 COVID-19 patients for the HLA -A, -B, -C, -DRB1, -DRB3/4/5, -DQA1, -DQB1, -DPA1, and -DPB1 loci. They demonstrated that, when the revised P-value was taken into account, only HLA-C*07:29 and B*15:27 were significant. According to Romero Lopez *et al*. (36), there is a positive association with HLA A*03:02 and a negative correlation with the cumulative incidence per million people for both A*31:01 and HLA-A*02:03 frequencies. The authors concluded that, with this result, HLA A*02:03 and A*31:01 were associated with better immunity against the infection and that HLA A*03:02 can be considered as a risk factor. HLA-A*02:02, HLA-B*15:03, HLA-C*12:03, HLAA*02:03, and HLA-A*31:01 were identified as protective alleles in a number of studies, while HLA-A*25:01, HLA-B*46:01, HLA-C*01:02, HLA-A*24:02, HLADPA1*02:02, HLA DPB1*05:01, HLA-DQB1*03:01, and HLA-DRB4*01:01 were identified as risk alleles (37).

Furthermore, population-specific studies continued to identify additional alleles associated with either susceptibility or protection. Farahani *et al*. (38) reported significant associations between severe COVID-19 and HLA-B38, HLA-A68, HLA-A24, and HLA-DRB101 in an Iranian cohort. Augusto *et al*. (46) found that HLA-B15:01 was significantly associated with asymptomatic SARS-CoV-2 infection, while Letovsky *et al*. (39) identified several alleles associated with either increased (HLA-A68:01, HLA-C01:02, HLA-DQB103:02, HLA-DRB108:02 and HLA-DRB114:06) or decreased (HLA-DRB108:03, HLA-C05:01 and HLA-B*38:01) likelihood of SARS-CoV-2 infection. The list of potentially relevant HLA variants continues to expand as additional studies are performed.

HLA and immune dysregulation

Beyond genetic susceptibility, HLA-related mechanisms may also contribute to immune dysregulation and hyperinflammatory responses during COVID-19. The CDC issued a notice on May 14, 2020, alerting medical professionals to the multisystem inflammatory syndrome (MIS-C) linked to COVID-19. MIS-C presents clinical features resembling Kawasaki disease (KD). The HLA region, particularly HLA-B and HLA-C variants, has been proposed among the genetic factors potentially involved in susceptibility to KD-like manifestations.

Other studies explored the relationship between HLA polymorphisms and markers of disease severity. Elevated serum ferritin levels, which are associated with hyperinflammation and poor clinical outcomes in COVID-19, were reported more frequently in patients carrying specific HLA variants such as HLA-C*03.

Spinetti *et al*. (40) compared ICU patients with severe COVID-19 to noncritically ill hospitalized COVID-19 patients and showed reduced mHLA-DR expression on circulating CD14+ monocytes at ICU admission, indicating a dysfunctional immune response and impaired antigen-presenting capacity during severe disease.

The immune cell, cytokine, and HLA-G (including receptor) levels of a COVID-19 patient during his hospital stay were documented in a case study by Zhang *et al*.(41). In general, HLA-G levels rose following viral clearance and decreased during the active replication phase, suggesting a possible relationship with cytokine-mediated immune regulation.

A recent study documented evidence of SARS-CoV-2 antigens circulating in the blood up to 14 months after infection and reported an association between

Table 1. *Summary of selected studies investigating the association between HLA polymorphisms and COVID-19 susceptibility, severity, immune response, and clinical outcomes.*

CATEGORY	REPRESENTATIVE HLA ALLELE(S)	REPORTED ASSOCIATION	KEY REFERENCE(S)
Protective alleles	HLA-B*15:03	Enhanced presentation of conserved SARS-CoV-2 peptides; potential cross-reactive immunity	Nguyen *et al*. (24)
Protective alleles	HLA-B*15:01	Associated with asymptomatic SARS-CoV-2 infection	Augusto *et al*. (46)
Protective alleles	HLA-DRB1*04:01	Increased frequency among asymptomatic individuals	Langton *et al*. (13)
Risk alleles	HLA-C*04:01	Severe disease and increased risk of intubation	Weiner *et al*. (21)
Risk alleles	HLA-B*46:01	Low predicted peptide-binding capacity; increased susceptibility	Nguyen *et al*. (24)
Risk alleles	HLA-A*11:01, HLA-B*51:01, HLA-C*14:02	Associated with worse clinical outcomes	Wang *et al*. (23)
Risk alleles	HLA-B*15:27, HLA-DRB1*04:06	Associated with susceptibility in Chinese populations	Yu *et al*. (34)
Population-specific associations	HLA-B*38, HLA-A*68, HLA-A*24, HLA-DRB1*01	Associated with severe COVID-19 in Iranian patients	Farahani *et al*. (38)
Antigen presentation efficiency	DRB1*01:01, DRB1*10:01, DRB1*11:02, DRB1*13:01	Efficient presentation of SARS-CoV-2 peptides	Barquera *et al*. (30)
Antigen presentation efficiency	DRB1*03:02, DRB1*03:03, DQA1*01:02/DQB1*06	Poor presentation of SARS-CoV-2 peptides	Barquera *et al*. (30)
Immune dysregulation	HLA-C*03; reduced mHLA-DR expression	Hyperinflammation and impaired antigen presentation	Spinetti *et al*. (40)
Vaccine-related outcomes	HLA-A*03:01	Increased reactogenicity following Pfizer-BioNTech vaccination	Bolze *et al*. (43)

antigen positivity and post-acute sequelae of COVID-19 (PASC) involving several symptom domains (42) (**Table 1**).

COVID-19, CYTOKINES, ABO, ACE2 AND TMPRSS2 GENES

Genetic susceptibility loci identified by GWAS

SARS-CoV-2 infection results in a broad spectrum of COVID-19 disease, from mild or no symptoms to hospitalization and death. A recent genome-wide association study (GWAS) of the risk of critical illness revealed a significant genetic component (44). Genetic loci from COVID-19 GWAS in peer-reviewed publications to date represent a mixture of risk variants for SARS-CoV-2 infection (blue upward arrows) and severe COVID-19 with complications (red downward arrows). More loci are anticipated to appear as sample sizes increase. N/A indicates that the MHC on chromosome 6 was omitted from the reporting in this article due to high heterogeneity of putative associations from the individual studies in the meta-analysis (**Figure 2**) (45).

The genes identified through physical association with accessible COVID-19 variants have known roles in viral replication, interferon response, and inflammation (46). Host genetic predisposition to COVID-19 is now increasingly recognized, and whole-genome and candidate gene association studies regarding COVID-19 susceptibility have been performed. Several common and rare variants in genes related to inflammation or immune responses have been identified (47). A previous study identified two genomic regions that are associated with severe COVID-19: one region on chromosome 3, which contains six genes, and one region on chromosome 9 that determines ABO blood groups. The sole region on chromosome 3 that is strongly linked to severe COVID-19 at the genome-wide level, according to a dataset recently made public by the COVID-19 Host Genetics Initiative (48). The genes ACE2, IL6, TMPRSS2, and TNF are often highlighted. FURIN, CXCL10, OAS1, OAS2,

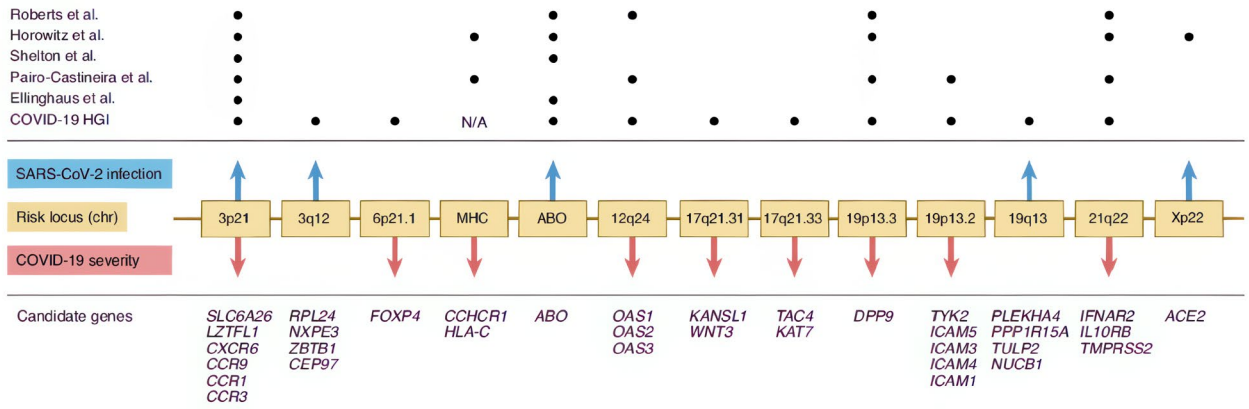

Figure 2. *Genetic loci from COVID-19 GWAS in peer-reviewed publications to date.*

OAS3, and ISG15 are emerging genes for COVID-19 (49). Using the Infinium Methylation EPIC array13, Corley *et al*. (50) examined genome-wide DNAm profiles in peripheral blood mononuclear cells from nine patients with severe COVID-19. Droplet digital PCR revealed detectable plasma SARS-CoV-2 RNA in patients with severe COVID-19. The idea that SARS-CoV-2 suppresses host IFN responses was supported by their finding of notable hypermethylation in the regulatory areas of genes implicated in the type I IFN response linked to severe COVID-19, including first-line antiviral defense genes like IFITM1 and ISG20. The SARS-CoV-2 viral host receptor ACE2 gene was also linked to abnormal levels of DNA linked to severe COVID-19, which supports research that suggests ACE2 is up-regulated during SARS-CoV-2 infection. On the other hand, they found that the cytokine genes linked to severe COVID-19 and the regulatory regions of genes related to immunological inflammation, such as the antiviral MX1 genes and the NLRP3 inflammasome, had substantial hypomethylation. Wei *et al*. (51) extracted biological concepts from the titles and abstracts of the gathered research publications using PubTator, a deep learning-based entity extraction tool created by the National Library of Medicine (NLM). They linked COVID-19 disease to the top ten genes, which include ACE2, TMPRSS2, IL6, CRP, TNF, CD4, ACE, CD8A, IFNG, and FURIN. Immune cells from patients with severe *versus* moderate COVID-19 disease showed differential expression of several of these genes (PAXBP1, IFNAR2, OAS1, OAS3, TNFAIP8L1, GART). The scientists found that the gene locus in TMEM189 (PEDS1, HGNC:16735)-UBE2V1 (HGNC:12494), which is implicated in the IL-1 signaling pathway, was associated with the severity of COVID-19. An investigation found inborn errors of Toll-like receptor 3 (TLR3, HGNC:11849) – and interferon regulatory factor 7 (IRF7, HGNC:6122) – dependent type I IFN immunity related to life-threatening COVID-19 pneumonia (52).

Compared to COVID-19 patients with powerful interferon responses, many SARS-CoV-2-infected people have blunted and/or delayed interferon responses and suffer from more severe illness (46). SARS-CoV-2 dsRNA genomes are sensed by the RIG-I/MDA5 and RNAseL pathways (53). One of the earliest known IFN-stimulated gene (ISG) antiviral pathways was the OAS/RNaseL pathway. Three catalytically active OAS genes (OAS1-3) and one inactive gene (OASL) have been identified in humans (54). The chr12q24.13 locus encoding OAS1-OAS3 antiviral proteins has been associated with COVID-19 susceptibility and severity (55). Banday *et al*. (55) analyzed patients of European (n = 2,249) and African (n = 835) ancestries with hospitalized *versus* non-hospitalized COVID-19; the risk of hospitalized disease was associated with a common OAS1 haplotype. They concluded that decreased OAS1 expression due to a common haplotype contributes to COVID-19 severity.

Cytokine genes and inflammatory responses

Genetic variation in cytokine-related genes has been proposed as an important determinant of the inflammatory response to SARS-CoV-2 infection and may contribute to disease severity.

Differential cytokine production in COVID-19 patients may be linked to genetic variations in the regulatory regions of cytokine genes. Cytokines and chemokines SNPs are associated with the severity of COVID-19 (53).

Single-nucleotide polymorphisms (SNPs) of interferons, TNF, IL1, IL4, IL6, IL7, IL10, and IL17 are among the gene variants that predispose patients to the severe form of COVID-19, also known as SARS-COV-2 (53). The COVID-19 virus causes cytokine storms, which are excessive inflammatory reactions linked to the release of proinflammatory cytokines like interleukin-6 (IL-6), IL-1β, IL-10, IL-18, IL-4, IL-33, interferon (IFN)-γ, and tumor necrosis factor alpha (TNFα) (54). In severe COVID-19 patients, an increase in IL-6 levels has been observed and is related to the disease's poor prognosis. Several gene variants in IL6 (HGNC:6018) with differential cytokine expression and with different disorders have been reported (5). The cis-regulatory landscapes of human immune cell types with established roles in disease severity were linked to putatively functional COVID-19 risk variants by Pahl *et al*. (44), who used high-resolution chromatin conformation capture to map these disease associated elements to their effector genes to obtain insight into how human genetic variation attenuates or exacerbates disease after SARS-CoV-2 infection. 16 genes related to inflammation, the interferon response, and viral replication were implicated by this functional genomic approach.

Various cytokines and VEGF have higher serum levels in COVID-19 patients compared to healthy subjects, suggesting that cytokines and their receptors play a role in disease development.

Collectively, these findings support a central role for cytokine-related genetic variation in modulating the inflammatory response and clinical severity of COVID-19.

ABO blood group and COVID-19 susceptibility

Several genome-wide association studies have identified the ABO blood group locus as a potential genetic factor influencing susceptibility to SARS-CoV-2 infection and COVID-19 severity. Ellinghaus *et al*. (56) conducted a GWAS involving 1980 patients with COVID-19 and severe disease (defined as respiratory failure) at seven hospitals in the Italian and Spanish epicenters of the SARS-CoV-2 pandemic in Europe. They found cross-replicating relationships between rs657152 at locus 9q34.2 and rs11385942 at locus 3p21.31. The association signal included the genes SLC6A20, LZTFL1, CCR9, FYCO1, CXCR6, and XCR1 at locus 3p21.31.

Shelton *et al*. (57) identified several non-genetic conditions as risk factors for hospitalization, and the genetic variants LZTFL1 rs13078854 and ABO rs9411378 were associated with COVID-19 outcome severity and diagnosis, respectively. Adjacent genes in the 3p21.31 locus, such as SLC6A20, CCR9, FYCO1, CXCR6, and XCR1, may be responsible for the association. The ABO blood group locus and the association signal at locus 9q34.2 were in co-occurrence. Pereira *et al*. (58) identified a 3p21.31 gene cluster as a genetic susceptibility locus in patients with COVID-19 with respiratory failure and confirmed a potential involvement of the ABO blood-group system. While blood type A is commonly referred to as a risk factor, blood type O is primarily linked to decreased rates of SARS-CoV-2 infection. Blood type A is most closely linked to the severity and mortality of COVID-19, although findings on the likelihood of severe consequences are more mixed. In contrast, most studies characterize blood type O as protective against disease progression. Blood antigens have been proposed as contributing to intracellular absorption, signal transduction, or adhesion, and blood groups may act as receptors and/or co-receptors for bacteria, viruses, and parasites (59).

Overall, available evidence suggests a role for the ABO blood group system in susceptibility to SARS-CoV-2 infection and clinical outcomes, although the biological mechanisms underlying these associations remain incompletely understood.

ACE2 and TMPRSS2 variants

Most African populations could be protected to some degree because they lack some genetic susceptibility risk factors or have low level expression of allelic variants, such as ACE2 and TMPRSS2, that are thought to be involved in increased infection risk or disease severity (60). Numerous ACE2 and TMPRSS2 aberrations that impact the expression of COVID-19-related receptors have been linked to risk factors and disease susceptibility. Besides its role in SARS-CoV-2 infection, ACE2 acts as a negative regulator of the renin-angiotensin system and a facilitator of amino acid transport (5). The ACE2 system is a critical protective pathway against heart failure with reduced and preserved ejection fraction, including myocardial infarction and hypertension, lung disease, and diabetes mellitus (61). Unfortunately, the function of ACE2 is lost following the binding of SARS-CoV-2. According to a recent study, South Asian and East Asian groups have genetic markers of the highest ACE2 expression, whereas Africans have the lowest levels of ACE2 expression (62). Africans showed a genetic tendency for the lowest levels of TMPRSS2 (HGNC:11876) expression, while East Asians showed the greatest levels (5). While two mutations (p. Leu-

351Val and p. Pro389His) were expected to interfere with SARS-CoV-2 spike protein binding, three common missense alterations in ACE2 (p. Asn720Asp, p. Lys26Arg, and p. Gly211Arg) have been predicted to interfere with protein structure and stabilization (60). These studies provide information on the genetic overlap between immunological factors and COVID-19, indicating possible avenues for further investigation and clinical testing (63).

COVID-19 AND MICRORNAS

Biological role of miRNAs in SARS-CoV-2 infection

COVID-19 and MicroRNAs Noncoding RNAs (ncRNAs) do not encode a protein but rather modulate chromatin regulation and gene expression. These comprise piwi-interacting RNAs (piRNAs), small nuclear RNAs (snRNAs), small nucleolar RNAs (snoRNAs), microRNAs (miRNAs), small interfering RNAs (siRNAs), ribosomal RNAs (rRNAs), transfer RNAs (tRNAs), and long noncoding RNAs (lncRNAs) (64). Human miRNAs can interact with other single-stranded RNAs, including viral genomes, in addition to post-transcriptionally regulating mRNAs (65). SARS-CoV-2 infection deeply disturbs the plasma miRNA expression profile from an early stage of COVID-19, making miRNAs highly valuable as early predictors of severity and mortality (66). miRNAs are proposed as promising biomarkers, new targets, and tools in therapeutic approaches, but also as prognostic factors in SARS-CoV-2 infection (**Figure 3**) (62).

Given their ability to regulate both viral and host gene expression, miRNAs have emerged as important modulators of SARS-CoV-2 infection and disease progression.

Several studies have investigated the role of miRNAs in regulating key host factors involved in SARS-CoV-2 entry and antiviral immune responses, including ACE2, TMPRSS2, and interferon-related pathways. In their respective 3'-UTRs, Pierce *et al*. (67) found 43 miRNAs targeting ACE2, 107 for TMPRSS2, 20 for IFN-α, 29 for IFN-β, and 47 for IFN-γ using five miRNA computational methods (miRWalk, MicroT4, miRMap, RNAhybrid, and TargetScan). There were 7 predicted lung-enriched miRNAs (the top 5 miRNAs were miR-141-3p, miR-4270, miR-331-3p, miR-200a 3p, and miR-218-5p) that targeted the ACE2 mRNA 30-UTR and 25 predicted lung-enriched miRNAs (the top 5 miRNAs were miR-4763-3p, let-7d-5p, miR-

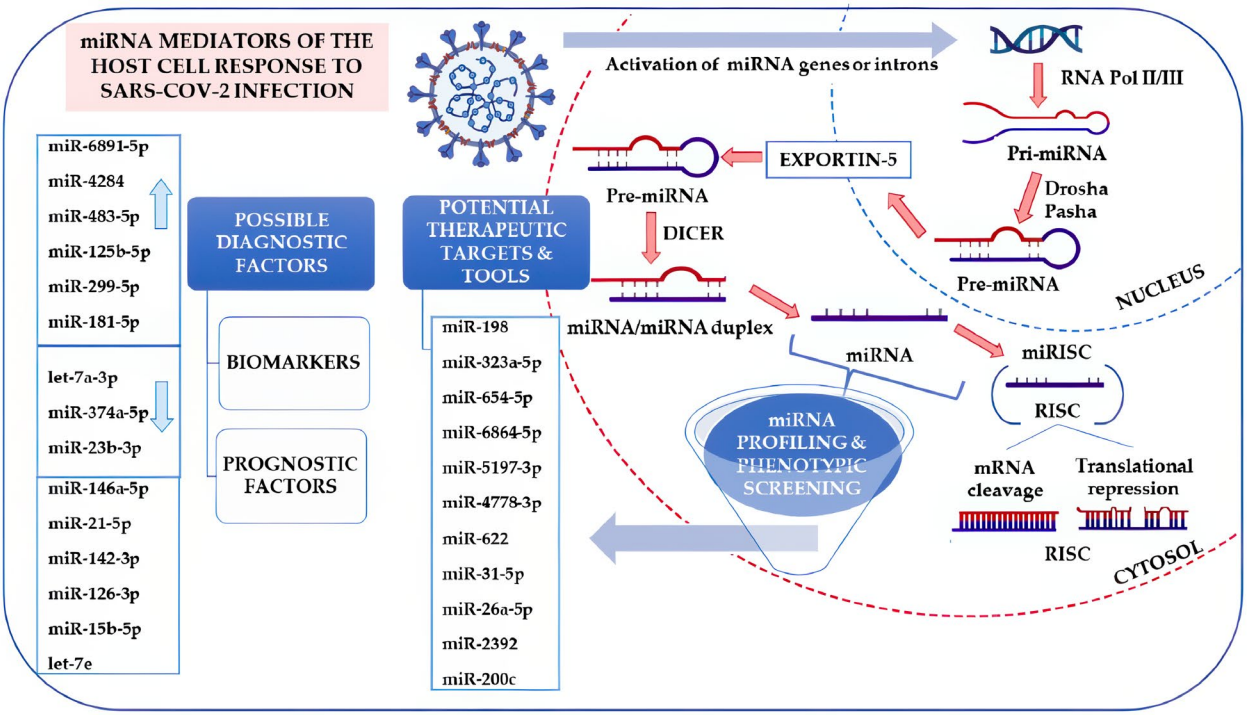

Figure 3. *Proposed roles of microRNAs in SARS-CoV-2 infection and COVID-19 pathogenesis. Host and viral miRNAs may regulate key pathways involved in viral entry (ACE2 and TMPRSS2), interferon signaling, cytokine production, immune-cell activation, and inflammatory responses. Altered miRNA expression profiles have also been associated with disease severity, prognosis, and potential therapeutic targets, highlighting their relevance as biomarkers and modulators of host-virus interactions. miRNAs Regulating Viral Entry and Host Responses.*

4530, let-7e-5p, and miR-181a 5p) that targeted the TMPRSS2 mRNA 30-UTR. The 30-UTRs of IFN-α and IFN-β mRNAs contained two (miR-203a-3p and miR-361-5p) and one (miR-145-5p) predicted binding sites, respectively, for lung-enriched miRNAs, whereas IFN-γ harbored nine (miR-128-3p, miR-143-3p, miR-181b-5p, miR-181d-5p, miR-24-3p, miR-26a-5p, miR-26b-5p, miR-340-5p, and miR-664b 3p). Interestingly, in human lung tissue, the only miRNAs that share both target mRNAs (TMPRSS2 and IFN-γ) are miR-181b-5p, miR-181d-5p, and miR-664b-3p. Using single-cell RNA-sequencing-based data, two miRNAs, hsa-miR-302c-5p and hsa-miR-16-5p, were identified to be potential virus-targeting miRNAs across multiple cell types from bronchoalveolar lavage fluid samples. The results showed that these miRNA/target pairs are involved in the ACE2 receptor network, regulating pro-inflammatory cytokines and immune cell maturation and differentiation (65).

miRNAs and immune dysregulation

Beyond viral entry mechanisms, miRNAs may contribute to immune dysregulation and inflammatory responses that characterize severe COVID-19. Increases in plasma cytokine storms, including TNF-α, IL-1β, IL-6, miR-146a, miR-146b, and IL-8, are associated with miR-125b, miR-138, miR-199a, and miR-21 in acute respiratory distress syndrome and COPD (68). Recent studies include the role of ID02510.3p miRNA, ID00448.3pmiRNA, miRNA 3154, miRNA 7114-5p, miRNA 5197-3p, ID02750.3p miRNA and ID01851.5p-miRNA, miR-5197-3p, miR-17-5p and miR-20b-5p in control COVID-19 pathogenesis by binding to the genome of SARS-CoV-2 (69). These findings suggest that miRNA-mediated regulation may influence both cytokine production and host antiviral responses during SARS-CoV-2 infection.

miRNAs as diagnostic and prognostic biomarkers

Nersisyan *et al*. (70) introduced six miRNAs, including miR-21-3p, miR-195-5p, miR-16-5p, miR-3065-5p, miR-424 5p, and miR-421, that potentially regulated all human coronaviruses through direct binding to the viral genome. The high predictive accuracy reported in these studies highlights the potential utility of miRNA signatures for disease detection and risk stratification. The miR-21-3p binds to the human coronavirus genome the best. Supervised machine learning analysis revealed that a three-miRNA signature (miR-423-5p, miR-23a-3p, and miR-195-5p) independently classified COVID-19 cases with an accuracy of 99.9% (71). The three miRNA signature identified SARS-CoV-2 infection in a ferret COVID-19 model with 99.7% accuracy and differentiated SARS-CoV-2 infection from influenza A (H1N1) infection and healthy controls with 95% accuracy (71). The inflammatory miR-31-5p was the most significantly increased of the 55 miRNAs that were changed in COVID-19 patients in the early stages of the illness (71). In addition to their diagnostic value, miRNAs may represent novel therapeutic targets because of their ability to modulate both viral replication and host immune pathways. Pawlica *et al*. (72) discovered a viral miRNA-like small RNA, named CoV2-miR-O7a (for SARS-CoV-2 miRNA-like ORF7a-derived small RNA). Its abundance ranges from low to moderate compared to host miRNAs, and it is associated with Argonaute proteins, core components of the RNA interference pathway. They discovered potential targets for CoV2-miR 7a, such as the interferon signaling-related Basic Leucine Zipper ATF-Like Transcription Factor 2 (BATF2). According to Li *et al*. (73), when comparing human COVID-19 patients to healthy controls, 35 miRNAs were elevated and 38 miRNAs were downregulated. The following is a list of the top 10 genes: hsa-miR-16-2-3P, hsa-miR-5695, hsa-miR-10399-3P, hsa-miR-6501-5P, hsa miR-361-3P, hsa-miR-361-3p, hsa-miR-4659a-3p, hsa-miR-142-5p, hsa-miR-4685-3p, hsa-miR 454-5p, and hsa-miR-30c-5p. Hsa-miR-183-5p, Hsa-miR-627-5p, Hsa-miR-941, Hsa-miR-21-5p, Hsa-miR-20a-5p, Hsa-miR-146b-5p, Hsa-miR-454-3p, Hsa-miR-18a-5p, Hsa-miR-340-5p, and Hsa-miR-17-5p were the ten genes that had the biggest decrease. Notably, miR-627-5p was the most downregulated miRNA, changing by 2.3 times in comparison to the controls, whereas miR 16-2-3p was the most upregulated miRNA, changing by 1.6 times. Four miRNAs (miR-127-3p, miR-21-5p, miR-285p, and miR-34a-5p) were found by Salem *et al*. (74) to regulate the TGF-beta signaling system, interleukin-4 and 13 signaling, IL-17 signaling pathway, and B cell receptors (BCRs) signaling pathway. They have been suggested as possible biomarkers for a variety of COVID-19 disease characteristics, including susceptibility, severity, course of complications, prognosis, and potential treatments (75). Therefore, a profile of the circulating miRNA at various phases of COVID-19 disease may offer valuable clinical information and point the way for future treatments (76).

Therapeutic potential and future perspectives

In addition to their diagnostic value, miRNAs may represent novel therapeutic targets because of

Table 2. *Representative microRNAs associated with SARS-CoV-2 infection, their major targets, level of evidence, and potential clinical relevance.*

miRNA	MAIN TARGET(S)	EVIDENCE TYPE	PROPOSED CLINICAL RELEVANCE
miR-141-3p	ACE2	Computational	Potential regulation of viral entry
miR-181b-5p	TMPRSS2, IFN-γ	Computational	Modulation of antiviral responses
miR-181d-5p	TMPRSS2, IFN-γ	Computational	Immune regulation
hsa-miR-302c-5p	ACE2 network	Computational/Transcriptomic	Potential antiviral activity
hsa-miR-16-5p	ACE2 network	Computational/Transcriptomic	Regulation of immune-cell maturation
miR-21-3p	Coronavirus genomes	Computational	Potential broad antiviral activity
miR-423-5p, miR-23a-3p, miR-195-5p	Biomarker signature	Experimental + Machine Learning	Diagnostic/prognostic biomarker
CoV-2-miR-O7a	BATF2 pathway	Experimental	Viral immune-evasion mechanism

their ability to modulate both viral replication and host immune pathways. Pawlica *et al*. (72) discovered a viral miRNA-like small RNA, named CoV2-miR-O7a (for SARS-CoV-2 miRNA-like ORF7a-derived small RNA), which associates with Argonaute proteins and may regulate interferon-related pathways through targets such as BATF2. Furthermore, several host miRNAs have been implicated in regulating inflammatory responses, cytokine production, and viral-host interactions, suggesting potential applications in both therapeutic intervention and disease monitoring.

Overall, current evidence supports a multifaceted role of miRNAs in COVID-19, ranging from the regulation of viral entry and immune responses to their potential use as biomarkers and therapeutic targets. However, many findings remain based on computational predictions or small cohorts and require validation in larger and more diverse populations. A summary of representative miRNAs, their principal targets, level of evidence, and potential clinical relevance is presented in **Table 2**.

COVID-19 AND CANCER

Impact of the COVID-19 pandemic on cancer care

Regular health service delivery was seriously hampered during the COVID-19 pandemic. The potential of COVID-19 infection for patients necessitated careful assessment, and resources were reallocated to COVID-19 services. There was a halt to cancer screening programs, fewer medical professionals were available, and some patients had trouble getting the best care in a timely way (77).

These disruptions raised concerns regarding delayed diagnosis, treatment interruptions, and potentially worse outcomes among patients with cancer.

Shared biological mechanisms between COVID-19 and cancer

Beyond healthcare-related consequences, several biological pathways appear to be shared between COVID-19 and cancer progression.

An inflammatory outburst and lymphopenia are associated with severe COVID-19, which may worsen the prognosis for cancer (78). Zong *et al*. (79) described the four main signaling pathways at the junction of COVID-19 and cancer: cytokine, type I interferon (IFN-I), androgen receptor (AR), and immunological checkpoint signaling. They also emphasized the clinical and molecular parallels between COVID-19 and cancer. Depletion of B cells, natural killer cells, and CD8+ and CD4+ T cells is linked to COVID-19 progression. COVID-19 susceptibility is increased by the expression of receptors such as transmembrane protease serine 2 (TMPRSS2) and angiotensin-converting enzyme 2 (ACE2) (80). Through mechanisms including cytokine storm, tissue hypoxia, poor T-cell responses, autophagy, neutrophil activation, and oxidative stress, SARS-CoV-2 infection may increase cancer susceptibility and speed cancer progression (81).

SARS-CoV-2 infection and cancer-related molecular pathways

According to Serwaa *et al*. (82), SARS-CoV-2 infection affects different cancer cellular phenotypes as well as the expression of molecular cancer markers and proinflammatory cytokines. They demonstrated how SARS-CoV-2 infection affects some crucial cellular processes related to prostate and colorectal cancer cell proliferation, death, and migration. The primary receptor of the SARS-CoV-2 virus, angiotensin-converting enzyme 2 (ACE2), is extensively expressed on the cell surface of pancreatic cells, including exocrine glands and pancreatic islets, making these cells a prime target for the virus (1). Moreover, CREB1, PTEN, SMAD3, and CASP3 have been reported to be differentially expressed in pancreatic adenocarcinoma according to TCGA database analyses. Although current evidence does not support a direct causal relationship between SARS-CoV-2 infection and pancreatic carcinogenesis, these observations suggest potential overlap between molecular pathways involved in cancer biology and those affected during SARS-CoV-2 infection (83).

Vaccination, immunotherapy, and clinical outcomes

Grippin *et al*. (84) demonstrated that mRNA vaccines targeting SARS-CoV-2 also make tumors more susceptible to immune checkpoint inhibitors (ICIs). For many cancer patients, ICIs prolong survival. In several large retrospective populations, receiving SARS-CoV-2 mRNA vaccinations within 100 days of starting ICI is linked to considerably enhanced median and three-year overall survival.

These findings highlight the potential interaction between vaccination-induced immune activation and the efficacy of cancer immunotherapies, warranting further investigation in prospective studies.

SARS-CoV-2 infection and cancer-related molecular pathways

Recent evidence has also highlighted the potential involvement of the cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) pathway at the intersection between COVID-19 and cancer biology. Activation of cGAS-STING signaling during the early stages of SARS-CoV-2 infection may promote antiviral immunity through induction of type I interferon responses. Conversely, persistent activation of this pathway may contribute to excessive inflammation and tissue damage. Interestingly, cGAS-STING signaling has also emerged as a promising therapeutic target in oncology because of its ability to enhance antitumor immune responses and improve the efficacy of immunotherapies (85). These observations suggest that molecular pathways involved in host antiviral defense may also influence cancer progression and therapeutic responsiveness, highlighting potential areas for future translational research.

CONCLUSIONS

Every disease has a genetic component, to varying degrees. Variations in our DNA and differences in how that DNA functions, alongside the environment, contribute to disease processes (86). By identifying the causal processes that explain why some people are more seriously affected by the disease after contracting the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, human genetics can help understand the biology and epidemiology of coronavirus disease 2019 (COVID-19) (78).

Although advanced age, male sex, and several comorbidities are recognized risk factors for severe COVID-19, these variables alone do not fully explain the marked heterogeneity observed in clinical outcomes. A growing body of evidence indicates that host genetic factors contribute to individual susceptibility, disease severity, immune responses, and clinical prognosis (45).

The studies reviewed here highlight the potential role of multiple genetic determinants, including HLA polymorphisms, cytokine-related genes, ABO blood groups, ACE2 and TMPRSS2 variants, and microRNA-mediated regulatory pathways. These genetic factors may influence viral entry, antigen presentation, inflammatory responses, immune regulation, and vaccine-related outcomes. In addition, emerging evidence suggests complex interactions between COVID-19 and cancer-related biological pathways.

Despite considerable progress, many reported genetic associations remain population-specific and have not been consistently replicated across independent cohorts. Differences in ethnicity, study design, sample size, viral variants, and environmental factors may partly explain these discrepancies. Therefore, larger multiethnic studies, functional investigations, and integrative genomic approaches are needed to clarify the biological significance of these findings and their potential clinical applications. A better understanding of host genetic variability may contribute to improved risk stratification, personalized preventive strategies, and the development of targeted therapeutic approaches for current and future viral outbreaks.

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Conflicts of interest

The authors declare no competing interests.

Availability of data and materials

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

Authors' contributions

AAN, MB, FC: conceptualization. AAN, MQ, SS, MB, FC: investigation, data curation, writing – original draft. AAN, FC: writing – review & editing.

Publications ethics

Plagiarism

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

Data falsification and fabrication

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

REFERENCES

1. Namagerdi AA, Ciani F, d'Angelo D, Napolitano F, Avallone L. COVID-19, Environment, Clinicopathologic Features, Laboratory Findings and Diagnosis, Treatment, Vaccines, Animals, and Cancer. Ann Res Oncol. 2022;02(1):34. doi: 10.48286/aro.2022.40.
2. Merad M, Blish CA, Sallusto F, Iwasaki A. The immunology and immunopathology of COVID-19. Science 2022;375(6585):1122-1127. doi: 10.1126/science.abm8108.
3. Abassi Z, Knaney Y, Karram T, Heyman SN. The Lung Macrophage in SARS-CoV-2 Infection: A Friend or a Foe? Front Immunol. 2020;11:1312. doi: 10.3389/fimmu.2020.01312.
4. Paces J, Strizova Z, Smrz D, Cerny J. COVID-19 and the immune system. Physiol Res. 2020;69(3):379-388. doi: 10.33549/physiolres.934492.
5. Fricke-Galindo I, Falfán-Valencia R. Genetics Insight for COVID-19 Susceptibility and Severity: A Review. Front Immunol. 2021;12:622176. doi: 10.3389/fimmu.2021.622176.
6. Tay MZ, Poh CM, Rénia L, Macary PA, Ng LFP. The trinity of COVID-19: immunity, inflammation, and intervention. Nat Rev Immunol. 2020;20:363-74. doi: 10.1038/s41577-020-0311-8.
7. Rijkers GT, Weterings N, Obregon-henao A, Lepolder M, Dutt TS, Overveld FJ Van, et al. Antigen Presentation of mRNA-Based and Virus-Vectored SARS-CoV-2 Vaccines. Vaccines (Basel). 2021;9(8):848. doi: 10.3390/vaccines9080848.
8. Gu W, Gan H, Ma Y, Xu L, Cheng ZJ, Li B, et al. The molecular mechanism of SARS-CoV-2 evading host antiviral innate immunity. Virol J. 2022;19(1):49. doi: 10.1186/s12985-022-01783-5.
9. Bertoletti A, Tan AT, Le Bert N. The T-cell response to SARS-CoV-2: kinetic and quantitative aspects and the case for their protective role. Oxf Open Immunol. 2021;2(1):iqab006. doi: 10.1093/oxfimm/iqab006.
10. Migliorini F, Torsiello E, Spiezia F, Oliva F, Tingart M, Maffulli N. Association between HLA genotypes and COVID-19 susceptibility, severity and progression: a comprehensive review of the literature. Eur J Med Res. 2021;26(1):84. doi: 10.1186/s40001-021-00563-1.
11. De Larochelambert Q, Marc A, Antero J, Le Bourg E, Toussaint JF. Covid-19 Mortality: A Matter of Vulnerability Among Nations Facing Limited Margins of Adaptation. Front Public Health. 2020;8:604339. doi: 10.3389/fpubh.2020.604339.
12. Neumann-Haefelin C. HLA-B27-mediated protection in HIV and hepatitis C virus infection and pathogenesis in spondyloarthritis. Curr Opin Rheumatol. 2013;25(4):426-433. doi: 10.1097/BOR.0b013e328362018f.
13. Langton DJ, Bourke SC, Lie BA, Reiff G, Natu S, Darlay R, et al. The influence of HLA genotype on the severity of COVID-19 infection. HLA. 2021;98(1):14-22. doi: 10.1111/tan.14284.
14. Mobini Kesheh M, Shavandi S, Hosseini P, Kakavand-Ghalehnoei R, Keyvani H. Bioinformatic HLA Studies in the Context of SARS-CoV-2 Pandemic and Review on Association of HLA Alleles with Preexisting Medical Conditions. Biomed Res Int. 2021;2021:6693909. doi: 10.1155/2021/6693909.
15. Jordan SC. Innate and adaptive immune responses to SARS-CoV-2 in humans: relevance to acquired immunity and vaccine responses. Clin Exp Immunol. 2021;204(3):310-320. doi: 10.1111/cei.13582.

16. Conversation T, Nguyen BA, Nellore A, Thompson R. Your genes could determine whether coronavirus puts you in the hospital – and we're starting to unravel which ones matter. 2020. p. 1-6.
17. Sanchez-Mazas A. A review of HLA allele and SNP associations with highly prevalent infectious diseases in human populations. Swiss Med Wkly. 2020;150:w20214. doi: 10.4414/smw.2020.20214.
18. Wieczorek M, Abualrous ET, Sticht J, Álvaro-Benito M, Stolzenberg S, Noé F, et al. Major Histocompatibility Complex (MHC) Class I and MHC Class II Proteins: Conformational Plasticity in Antigen Presentation. Front Immunol. 2017;8:292. doi: 10.3389/fimmu.2017.00292.
19. Douillard V, Nasir J, Pearson N, Rosenfeld J, Limou S. Exploring the puzzle of HLA immunogenetics in COVID-19 patients. 2021. Available from: https://www.covid19hg.org/blog/2021-12-15-exploring-the-puzzle-of-hla-immunogenetics-in-covid-19-patients/.
20. Wang SF, Chen KH, Chen M, Li WY, Chen YJ, Tsao CH, et al. Human-leukocyte antigen class I Cw 1502 and class II DR 0301 genotypes are associated with resistance to severe acute respiratory syndrome (SARS) infection. Viral Immunol. 2011;24(5):421-6. doi: 10.1089/vim.2011.0024.
21. Weiner J, Suwalski P, Holtgrewe M, Rakitko A, Thibeault C, Müller M, et al. Increased risk of severe clinical course of COVID-19 in carriers of HLA-C*04:01. EClinicalMedicine. 2021;40:101099. doi: 10.1016/j.eclinm.2021.101099.
22. Correale P, Mutti L, Pentimalli F, Baglio G, Saladino RE, Sileri P, et al. HLA-B*44 and c*01 Prevalence Correlates with Covid19 Spreading across Italy. Int J Mol Sci. 2020;21(15):5205. doi: 10.3390/ijms21155205.
23. Wang F, Huang S, Gao R, Zhou Y, Lai C, Li Z, et al. Initial whole-genome sequencing and analysis of the host genetic contribution to COVID-19 severity and susceptibility. Cell Discov. 2020;6(1):83. doi: 10.1038/s41421-020-00231-4.
24. Nguyen A, David JK, Maden SK, Wood MA, Weeder BR, Nellore A, et al. Human leukocyte antigen susceptibility map for SARS-CoV-2. J Virol. 2020;94(13):e00510-20. doi: 10.1128/JVI.00510-2.
25. Poulton K, Wright P, Hughes P, Savic S, Welberry Smith M, Guiver M, et al. A role for human leucocyte antigens in the susceptibility to SARS-Cov-2 infection observed in transplant patients. Int J Immunogenet. 2020;47(4):324-328. doi: 10.1111/iji.12505.
26. Correale P, Baglio G, Parrella R, Saladino RE, Cuomo N, Scarano F, et al. A rapid ecologic analysis, confirmed by a case–control study, identifies class I HLA alleles correlated to the risk of COVID-19. J Transl Med. 2025;23(1):303. doi: 10.1186/s12967-025-06285-w.
27. Gutiérrez-Bautista JF, Rodriguez-Nicolas A, Rosales-Castillo A, López-Ruz MÁ, Martín-Casares AM, Fernández-Rubiales A, et al. Study of HLA-A, -B, -C, -DRB1 and -DQB1 polymorphisms in COVID-19 patients. J Microbiol Immunol Infect. 2022;55(3):421-427. doi: 10.1016/j.jmii.2021.08.009.
28. Novelli A, Andreani M, Biancolella M, Liberatoscioli L, Passarelli C, Luigi V, et al. HLA allele frequencies and susceptibility to COVID-19 in a group of 99 Italian patients. HLA. 2020;96(5):610-614. doi: 10.1111/tan.14047.
29. Yung Y, Lau K, Chan CCH, Xia JT, Wong RSM, Wu AKL, et al. Association of HLA-B22 serotype with SARS-CoV-2 susceptibility in Hong Kong Chinese patients. HLA. 2021;97(2):127-132. doi: 10.1111/tan.14135.
30. Barquera R, Collen E, Di D, Buhler S, Sanchez-mazas A. Binding affinities of 438 HLA proteins to complete proteomes of seven pandemic viruses and distributions of strongest and weakest. HLA. 2020;96(3):277-298. doi: 10.1111/tan.13956.
31. Pisanti S, Deelen J, Gallina AM, Caputo M, Citro M, Abate M, et al. Correlation of the two most frequent HLA haplotypes in the Italian population to the differential regional incidence of Covid-19. J Transl Med. 2020;18(1):352. doi: 10.1186/s12967-020-02515-5.
32. Littera R, Campagna M, Deidda S, Angioni G, Cipri S, Melis M, et al. Human Leukocyte Antigen Complex and Other Immunogenetic and Clinical Factors Influence Susceptibility or Protection to SARS-CoV-2 Infection and Severity of the Disease Course. The Sardinian Experience. Front Immunol. 2020;11:605688. doi: 10.3389/fimmu.2020.605688.
33. Shkurnikov M, Nersisyan S, Jankevic T, Galatenko A, Gordeev I, Vechorko V, et al. Association of HLA Class I Genotypes With Severity of Coronavirus Disease-19. Front Immunol. 2021;12:641900. doi: 10.3389/fimmu.2021.641900.
34. Yu X, Ho K, Shen Z, Fu X, Huang H, Wu D, et al. The Association of Human Leukocyte Antigen and COVID-19 in Southern China. Open Forum Infect Dis. 2021;8(9):ofab410. doi: 10.1093/ofid/ofab410.

35. Wang W, Zhang W, Zhang J, He J, Zhu F. Distribution of HLA allele frequencies in 82 Chinese individuals with coronavirus disease-2019 (COVID-19). HLA. 2020;96(2):194-196. doi: 10.1111/tan.13941.
36. Romero-López JP, Carnalla-Cortés M, Pacheco-Olvera DL, Ocampo-Godínez JM, Oliva-Ramírez J, Moreno-Manjón J, et al. A bioinformatic prediction of antigen presentation from SARS-CoV-2 spike protein revealed a theoretical correlation of HLA-DRB1*01 with COVID-19 fatality in Mexican population: An ecological approach. J Med Virol. 2021;93(4):2029-2038. doi: 10.1002/jmv.26561.
37. Yoonha PJ, Rakosi-Schmidt R, Liu J. The Role of MHC System in COVID-19 Susceptibility: A Qualitative Review of Current Literature. North Am J Med Sci. 13(1). Retrieved from https://www.najms.com/index.php/najms/article/view/541.
38. Farahani RH, Esmaeilzadeh E, Asl AN, Heidari MF, Hazrati E. Frequency of HLA Alleles in a Group of Severe COVID-19 Iranian Patients. Iran J Public Health. 2021;50(9):1882-1886. doi: 10.18502/ijph.v50i9.7061.
39. Letovsky SI, Cao X, Hollenbach JA, Mack SJ, Maiers M. Association between HLA genetics and SARS-CoV-2 infection in a large real-world cohort. Genes Immun. 2025;26(3):213-221. doi: 10.1038/s41435-025-00328-4.
40. Spinetti T, Hirzel C, Fux M, Walti LN, Schober P, Stueber F, et al. Reduced Monocytic Human Leukocyte Antigen-DR Expression Indicates Immunosuppression in Critically Ill COVID-19 Patients. Anesth Analg. 2020;131(4):993-999. doi: 10.1213/ANE.0000000000000504.
41. Zhang S, Gan J, Chen BG, Zheng D, Zhang JG, Lin RH, et al. Dynamics of peripheral immune cells and their HLA-G and receptor expressions in a patient suffering from critical COVID-19 pneumonia to convalescence. Clin Transl Immunology. 2020;9(5):e1128. doi: 10.1002/cti2.1128.
42. Georgopoulos AP, James LM, Peterson PK. Human Leukocyte Antigen (HLA) at the Root of Persistent Antigens and Long COVID. J Immunol Sci. 2025;9(1):1-3. doi: 10.29245/2578-3009/2025/1.1257.
43. Bolze A, Neveux I, Schiabor Barrett KM, White S, Isaksson M, Dabe S, Lee W, Grzymski JJ, Washington NL, Cirulli ET. HLA-A*03:01 is associated with increased risk of fever, chills, and stronger side effects from Pfizer-BioNTech COVID-19 vaccination. HGG Adv. 2022;3(2):100084. doi: 10.1016/j.xhgg.2021.100084.
44. Pahl MC, Coz C Le, Su C, Sharma P, Thomas RM, Pippin JA, et al. Implicating effector genes at COVID-19 GWAS loci using promoter-focused Capture-C in disease-relevant immune cell types. Genome Biol. 2022;23(1):125. doi: 10.1186/s13059-022-02691-1.
45. Karlsen TH. Understanding COVID-19 through genome-wide association studies. Nat Genet. 2022;54(4):368-369. doi: 10.1038/s41588-021-00985-x.
46. Augusto DG, Murdolo LD, Chatzileontiadou DSM, Sabatino JJ, Yusufali T, Peyser ND, et al. A common allele of HLA is associated with asymptomatic SARS-CoV-2 infection. Nature. 2023;620(7972):128-136. doi: 10.1038/s41586-023-06331-x.
47. Velavan TP, Pallerla SR, Rüter J, Augustin Y, Kremsner PG, Krishna S, et al. Host genetic factors determining COVID-19 susceptibility and severity. EBioMedicine. 2021;72:103629. doi: 10.1016/j.ebiom.2021.103629.
48. Zeberg H, Pääbo S. The major genetic risk factor for severe COVID-19 is inherited from Neanderthals. Nature. 2020;587(7835):610-612. doi: 10.1038/s41586-020-2818-3.
49. Wu M, Zhang Y, Grosser M, Tipper S, Venter D, Lin H, et al. Profiling COVID-19 Genetic Research: A Data-Driven Study Utilizing Intelligent Bibliometrics. Front Res Metr Anal. 2021;6:683212. doi: 10.3389/frma.2021.683212.
50. Corley MJ, Patterson BK, Peluso MJ, Yeung ST, Sacha JB, Pang APS, et al. Genome-wide DNA methylation profiling of peripheral blood reveals an epigenetic signature associated with severe COVID-19. J Leukoc Biol. 2021;110(1):21-26. doi: 10.1002/JLB.5HI0720-466R.
51. Wei CH, Kao HY, Lu Z. PubTator: a web-based text mining tool for assisting biocuration. Nucleic Acids Res. 2013;41(Web Server issue):W518-22. doi: 10.1093/nar/gkt441.
52. Zhang Q, Bastard P, Liu Z, Le Pen J, et al. Inborn errors of type I IFN immunity in patients with life-threatening COVID-19. Science. 2020;370(6515):eabd4570. doi: 10.1126/science.abd4570.
53. Kazem M, Yaser V, Ghaidaa M, Lateef R, Awsi A, Hosseinipour A, et al. Individual genetic variability, mainly of Proinflammatory cytokines, cytokine receptors, and toll-like receptors, dictates the pathophysiology of COVID-19 disease. J Med Virol. 2022;94(9):4088-4096. doi: 10.1002/jmv.27849.

54. Choi UY, Kang JS, Hwang YS, Kim YJ. Oligoadenylate synthase-like (OASL) proteins: dual functions and associations with diseases. Exp Mol Med. 2015;47(3):e144. doi: 10.1038/emm.2014.110.
55. Banday AR, Stanifer ML, Florez-vargas O, Onabajo OO, Papenberg BW, Zahoor MA, et al. Genetic regulation of OAS1 nonsense-mediated decay underlies association with COVID-19 hospitalization in patients of European and African ancestries. Nat Genet. 2022;54(8):1103-1116. doi: 10.1038/s41588-022-01113-z.
56. Ellinghaus, Degenhardt, Valenti, Franke, Karlsen, et al. Genomewide Association Study of Severe Covid-19 with Respiratory Failure. N Engl J Med. 2020;383(16):1522-1534. doi: 10.1056/NEJMoa2020283.
57. Shelton JF, Shastri AJ, Ye C, Weldon CH, Filshtein-Sonmez T, Coker D, et al. Trans-ancestry analysis reveals genetic and nongenetic associations with COVID-19 susceptibility and severity. Nat Genet. 2021;53(6):801-808. doi: 10.1038/s41588-021-00854-7.
58. Pereira E, Felipe S, de Freitas R, Araújo V, Soares P, Ribeiro J, et al. ABO blood group and link to COVID-19: A comprehensive review of the reported associations and their possible underlying mechanisms. Microb Pathog. 2022;169:105658. doi: 10.1016/j.micpath.2022.105658.
59. Cooling L. Blood Groups in Infection and Host Susceptibility. Clin Microbiol Rev. 2015;28(3):801-70. doi: 10.1128/CMR.00109-14.
60. Mariam SH. The Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) Pandemic: Are Africa's Prevalence and Mortality Rates Relatively Low? Adv Virol. 2022;2022:3387784. doi: 10.1155/2022/3387784.
61. Gheblawi M, Wang K, Viveiros A, Nguyen Q, Zhong JC, Turner AJ, et al. Angiotensin-Converting Enzyme 2: SARS-CoV-2 Receptor and Regulator of the Renin-Angiotensin System: Celebrating the 20th Anniversary of the Discovery of ACE2. Circ Res. 2020;126(10):1456-1474. doi: 10.1161/CIRCRESAHA.120.317015.
62. Xiao F, Han M, Zhu X, Tang Y, Huang E, Zou H, et al. The immune dysregulations in COVID-19: Implications for the management of rheumatic diseases. Mod Rheumatol. 2021;31(5):927-32. doi: 10.1080/14397595.2020.1868673.
63. Meng Z, Zhang C, Liu S, Li W, Wang Y, Zhang Q, et al. Exploring genetic loci linked to COVID-19 severity and immune response through multi-trait GWAS analyses. Front Genet. 2025;16:1502839. doi: 10.3389/fgene.2025.1502839.
64. Amirkhani Namagerdi A, d'Angelo D, Ciani F, Iannuzzi CA, Napolitano F, Avallone L, et al. Triple-Negative Breast Cancer Comparison With Canine Mammary Tumors From Light Microscopy to Molecular Pathology. Front Oncol. 2020;10:563779. doi: 10.3389/fonc.2020.563779.
65. Li C, Wang R, Wu A, Yuan T, Song K, Bai Y, et al. SARS-COV-2 as a potential microRNA sponge in COVID-19 patients. BMC Med Genomics. 2022;15(Suppl 2):94. doi: 10.1186/s12920-022-01243-7.
66. Fernández-pato A, Virseda-berdices A, Resino S, Ryan P, Martínez-gonzález O, Pérez-garcía F, et al. Plasma miRNA profile at COVID-19 onset predicts severity status and mortality. Emerg Microbes Infect. 2022;11(1):676-688. doi: 10.1080/22221751.2022.2038021.
67. Pierce JB, Simion V, Icli B, Pérez-Cremades D, Cheng HS, Feinberg MW. Computational Analysis of Targeting SARS-CoV-2, Viral Entry Proteins ACE2 and TMPRSS2, and Interferon Genes by Host MicroRNAs. Genes (Basel). 2020;11(11):1354. doi: 10.3390/genes11111354.
68. Fani M, Zandi M, Ebrahimi S, Soltani S, Abbasi S. The role of miRNAs in COVID-19 disease. Future Virol. 2021:10.2217/fvl-2020-0389. doi: 10.2217/fvl-2020-0389.
69. Srivastava SP, Srivastava R, Chand S, Goodwin JE. Coronavirus Disease (COVID)-19 and Diabetic Kidney Disease. Pharmaceuticals (Basel). 2021;14(8):751. doi: 10.3390/ph14080751.
70. Nersisyan S, Engibaryan N, Gorbonos A, Kirdey K, Makhonin A, Tonevitsky A. Potential role of cellular miRNAs in coronavirus-host interplay. PeerJ. 2020;8:e9994. doi: 10.7717/peerj.9994.
71. Farr RJ, Rootes CL, Rowntree LC, Nguyen THO, Hensen L, Kedzierski L, et al. Altered microRNA expression in COVID-19 patients enables identification of SARS-CoV-2 infection. PLoS Pathog. 2021;17(7):e1009759. doi: 10.1371/journal.ppat.1009759.
72. Pawlica P, Yario TA, White S, Wang J, Moss WN, Hui P, et al. SARS-CoV-2 expresses a microRNA-like small RNA able to selectively repress host genes. Proc Natl Acad Sci U S A. 2021;118(52):e2116668118. doi: 10.1073/pnas.2116668118.
73. Li C, Hu X, Li L, Li JH. Differential microRNA expression in the peripheral blood from human patients with COVID-19. J Clin Lab Anal. 2020;34(10):e23590. doi: 10.1002/jcla.23590.

74. Salem S, Lotfy R, Eltaweel N, Elbadry M. Association of plasma microRNAs with COVID-19 severity and outcome. J Genet Eng Biotechnol. 2024;22(4):100433. doi: 10.1016/j.jgeb.2024.100433.
75. Jankovic M, Nikolic D, Novakovic I, Petrovic B, Lackovic M, Santric-Milicevic M. miRNAs as a Potential Biomarker in the COVID-19 Infection and Complications Course, Severity, and Outcome. Diagnostics (Basel). 2023;13(6):1091. doi: 10.3390/diagnostics13061091.
76. Belete MA, Anley DT, Tsega SS, Moges N, Anteneh RM, Zemene MA, et al. The potential of circulating microRNAs as novel diagnostic biomarkers of COVID-19: a systematic review and meta-analysis. BMC Infect Dis. 2024;24(1):1011. doi: 10.1186/s12879-024-09915-8.
77. Stannard R, Lambert PC, Lyratzopoulos G, Andersson TML, Khan S, Rutherford MJ. The long-lasting impacts of the COVID-19 pandemic on population-based cancer survival: what are the implications for data analysis? Epidemiology. Br J Cancer. 2025;132(8):673-8. doi: 10.1038/s41416-024-02931-0.
78. Derosa L, Melenotte C, Griscelli F, Gachot B, Marabelle A, Kroemer G, et al. The immuno-oncological challenge of COVID-19. Nat Cancer. 2020;1(10):946-64. doi: 10.1038/s43018-020-00122-3.
79. Zong Z, Wei Y, Ren J, Zhang L, Zhou F. The intersection of COVID-19 and cancer: signaling pathways and treatment implications. Mol Cancer. 2021;20(1):76. doi: 10.1186/s12943-021-01363-1.
80. Chen VHE, Tay JK, Gurung R, Nair S, Tay DJW, Tan KS, et al. ACE2 and TMPRSS2 gene expression is reduced acutely in SARS-CoV-2 patients but returns to normal with recovery. Sci Rep. 2025;15(1):12828. doi: 10.1038/s41598-025-96279-x.
81. Tyagi S, Tyagi N, Singh A, Gautam A, Singh A, Jindal S, Singh RP, Chaturvedi R, Kushwaha HR. Linking COVID-19 and cancer: Underlying mechanism. Biochim Biophys Acta Mol Basis Dis. 2025;1871(1):167563. doi: 10.1016/j.bbadis.2024.167563.
82. Serwaa A, Oyawoye F, Owusu IA, Dosoo D, Manu AA, Sobo AK, et al. In vitro analysis suggests that SARS-CoV-2 infection differentially modulates cancer-like phenotypes and cytokine expression in colorectal and prostate cancer cells. Sci Rep. 2024;14(1):24625. doi: 10.1038/s41598-024-75718-1.
83. Zamorano Cuervo N, Grandvaux N. ACE2: Evidence of role as entry receptor for SARS-CoV-2 and implications in comorbidities. Elife. 2020;9:e61390. doi: 10.7554/eLife.61390.
84. Grippin AJ, Marconi C, Copling S, Li N, Braun C, Woody C, et al. SARS-CoV-2 mRNA vaccines sensitize tumours to immune checkpoint blockade. Nature. 2025;647(8089):488-497. doi: 10.1038/s41586-025-09655-y.
85. Augusto DG, Hollenbach JA. SARS-CoV-2 infection. Curr Opin Immunol. 2022;76:102178. doi: 10.1016/j.coi.2022.102178.
86. Jackson M, Marks L, May GHW, Wilson JB. The genetic basis of disease. Essays Biochem. 2018;62(5):643-723. doi: 10.1042/EBC20170053.

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RESEARCH ARTICLE

CLINICOPATHOLOGICAL FACTORS ASSOCIATED WITH EXTRATHYROIDAL EXTENSION IN PAPILLARY THYROID CARCINOMA

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ABSTRACT: Extrathyroidal extension (ETE) is a key pathological feature of papillary thyroid carcinoma (PTC) associated with aggressive tumor behavior and adverse outcomes. However, clinicopathological factors associated with ETE remain incompletely characterized, particularly in Southeast Asian populations. This study aimed to identify factors associated with pathological ETE in patients with PTC. This prospective observational study included 346 consecutive patients with histopathologically confirmed PTC who underwent thyroidectomy at a tertiary referral center in Vietnam between April and August 2025. ETE was defined based on postoperative histopathological examination, including both minimal and gross ETE. Clinicopathological variables were evaluated using univariable analysis and multivariable logistic regression. ETE was identified in 66 patients (19.1%). In univariable analysis, age ≥ 55 years, symptomatic presentation, multifocality, tumor size, lymph node metastasis and thyroiditis were associated with ETE (all $p < 0.05$). In multivariable analysis, age ≥ 55 years (OR = 2.321; 95%CI 1.153-4.674; $p = 0.018$), tumor size (per 10 mm increase) (OR = 1.052; 95%CI 1.016-1.090; $p = 0.005$) and lymph node metastasis (OR = 2.932; 95%CI 1.565-5.492; $p < 0.001$) were independently associated with increased odds of ETE. In contrast, coexisting thyroiditis was associated with lower odds of ETE (OR = 0.440; 95%CI 0.231-0.839; $p = 0.013$). In conclusion, older age, larger tumor size and lymph node metastasis were independently associated with pathological ETE in PTC, whereas coexisting thyroiditis showed a potentially inverse association that should be confirmed in larger cohorts. These findings provide additional insight into clinicopathological factors associated with tumor invasiveness in PTC and may contribute to improved risk assessment.

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Impact statement: This prospective study identifies age, tumor size and lymph node metastasis as key factors associated with extrathyroidal extension in papillary thyroid carcinoma.

Key words: *Papillary thyroid carcinoma; extrathyroidal extension; lymph node metastasis; thyroiditis; tumor size.*

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INTRODUCTION

Thyroid cancer is the most common endocrine malignancy worldwide, with a rapidly increasing incidence over the past decades. In 2022, approximately 821,214 new cases were diagnosed globally, ranking seventh among all cancers and fifth among women, with an incidence rate of 9.1 per 100,000 population (1). Despite this increasing incidence, thyroid cancer is generally associated with an excellent prognosis, data from the Surveillance, Epidemiology and End Results (SEER) program indicate a 5-year survival rate of up to 98.4% (2). Papillary thyroid carcinoma (PTC), the most common histological subtype, accounts for approximately 85-90% of thyroid cancer cases and is typically characterized by indolent behavior and favorable treatment outcomes (3). Extrathyroidal extension (ETE), defined as tumor invasion beyond the thyroid capsule into adjacent soft tissues, is an important pathological feature associated with more aggressive disease behavior (4, 5). ETE can be broadly classified into minimal extrathy-

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roidal extension (mETE), which is identified only on histopathological examination and gross extrathyroidal extension (gETE), which is evident clinically, radiologically, or intraoperatively (6).

Historically, mETE was classified as T3 disease in earlier editions of the American Joint Committee on Cancer (AJCC) staging system (7). However, its independent prognostic significance has been increasingly questioned due to substantial interobserver variability and its strong dependence on histopathological interpretation (8, 9). In contrast, gETE has been consistently associated with advanced tumor stage, higher recurrence rates and increased disease-specific mortality (10). Consequently, the eighth edition of the AJCC staging system removed mETE from tumor staging and placed greater emphasis on gETE (11).

Despite these differences in prognostic impact, the presence of ETE at any extent may still reflect tumor invasiveness and local tumor aggressiveness (12, 13). From a clinical perspective, identifying factors associated with the occurrence of ETE, regardless of its extent, may provide insights into tumor behavior, particularly in settings where preoperative distinction between mETE and gETE remains challenging (14, 15). Assessment of potential differences between ETE subtypes may also improve the understanding of tumor invasiveness.

Although ETE is widely recognized as an important prognostic factor, its reported prevalence varies considerably across studies (5%-45%), largely reflecting differences in definitions, assessment methods and study populations (16). Reported clinicopathological factors associated with ETE also remain inconsistent and data from certain populations, particularly those in developing countries, remain limited (17, 18). In Vietnam, prospective data on clinicopathological factors associated with ETE in PTC remain scarce.

Therefore, this study aimed to identify clinicopathological factors associated with postoperative pathological ETE in patients with PTC. This study is reported in accordance with the STROBE reporting checklist.

MATERIALS AND METHODS

Study population

This prospective observational study was conducted at a tertiary referral oncology center in Vietnam. Patients were consecutively enrolled between April 2025 and August 2025. A total of 346 patients who underwent thyroidectomy and had a final histopathological diagnosis of PTC were included in the study.

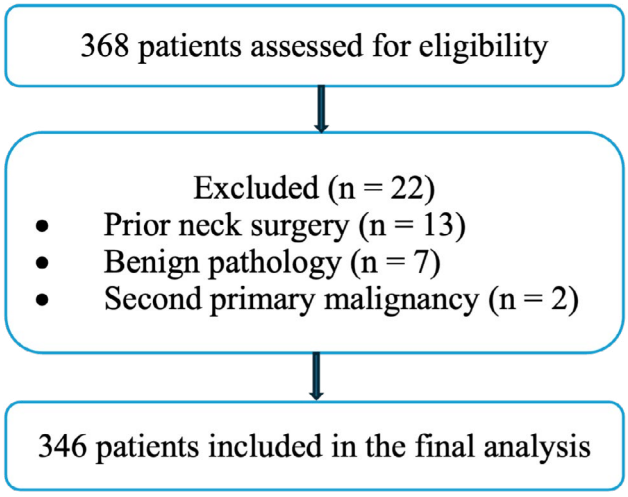

Figure 1. *Flowchart of patient selection and inclusion in the final analysis.*

The study was conducted in accordance with the principles of the Declaration of Helsinki and its subsequent amendments. Ethical approval was obtained from the Institutional Ethics Committee of Ho Chi Minh City Oncology Hospital (No. 542/BVUB-HDDD). Written informed consent was obtained from all participants prior to inclusion.

Patients were eligible for inclusion if they had preoperative cytological findings from fine-needle aspiration (FNA) suggestive of or diagnostic for PTC and subsequently underwent thyroid surgery. Patients were excluded if they had a history of previous neck surgery, prior radioactive iodine (I-131) therapy or other treatments, the presence of another primary malignancy, incomplete clinical or operative data, or a postoperative histopathological diagnosis other than PTC.

During the study period, 368 patients with preoperative FNA results of PTC or suspicious for PTC underwent thyroidectomy. Of these, 22 patients were excluded, including 2 with a second primary malignancy, 13 with a history of prior neck surgery and 7 with benign postoperative histopathological findings. Ultimately, 346 patients were included in the final analysis.

The patient selection process is illustrated in **Figure 1**.

Data collection

Patients were prospectively enrolled and clinical and histopathological data were collected using a standardized case report form according to a predefined study protocol. Tumor characteristics, lymph node status and coexisting thyroid conditions were determined based on histopathological examination of the

surgical specimens. Surgical management, including lobectomy, near-total thyroidectomy, or total thyroidectomy with or without lymph node dissection, was performed according to institutional protocols and contemporary clinical guidelines. Central and/or lateral neck dissection was performed based on clinical indications.

The following variables were analyzed:

- Demographic characteristics: age, sex and body mass index (BMI).
- Clinical characteristics: reason for consultation and Graves' disease.
- Tumor characteristics: maximum tumor diameter, multifocality and histological variant.
- Lymph node status: lymph node metastasis and extranodal extension.
- Coexisting thyroid conditions: lymphocytic thyroiditis and multinodular goiter.
- Primary outcome: presence of ETE.

The reason for consultation was initially recorded in detailed categories, including health screening and specific symptoms (neck mass, throat discomfort, dysphagia, hoarseness, dyspnea, neck pain and palpitations). For analytical purposes, these were categorized into two groups: health screening (asymptomatic) and symptomatic presentation. Patients were classified as symptomatic if they presented with at least one of the above symptoms. This categorization was performed to improve statistical power and model stability given the relatively small number of events in individual symptom subgroups.

Lymph node metastasis was defined based on postoperative histopathological examination of resected lymph nodes. Only patients who underwent lymph node dissection were considered assessable for pathological nodal status, while those without lymph node dissection were classified as having no pathological evidence of nodal metastasis.

Coexisting thyroid conditions were defined based on postoperative histopathological examination of surgical specimens. Although these conditions may be suggested preoperatively based on cytological findings, including FNA, the final diagnosis in this study was established histopathologically. Thyroiditis in this study refers to lymphocytic thyroiditis and goiter was defined as multinodular goiter (nodular hyperplasia).

ETE was determined based on postoperative histopathological examination of surgical specimens. All slides were independently reviewed by two board-certified pathologists with approximately 10 years of experience in oncologic pathology and any discrepancies were resolved by consensus.

mETE was defined according to standard histopathological criteria as microscopic tumor extension beyond the thyroid capsule that was identified exclusively on histopathological examination and was not accompanied by gross operative, radiological, or macroscopic pathological evidence of invasion. Microscopic involvement of perithyroidal soft tissue, including adipose tissue, small foci of skeletal muscle fibers, or extension around or into vascular structures or nerves, was categorized as mETE only when it was detected microscopically. Cases with gross strap muscle invasion observed intraoperatively and confirmed histopathologically were not counted as mETE; these cases were classified as gETE (pT3b), consistent with the AJCC 8th edition staging framework and contemporary thyroid pathology recommendations (11,19-21). gETE was defined as macroscopic tumor invasion beyond the thyroid capsule, including invasion into strap muscles (pT3b) or extension beyond the strap muscles into adjacent structures such as subcutaneous soft tissue, larynx, trachea, esophagus, recurrent laryngeal nerve, prevertebral fascia, or major vessels (pT4) (11, 21). gETE was initially identified intraoperatively by the operating surgeon and correlated with histopathological findings. This classification was applied consistently for prevalence estimates and subgroup comparisons.

For statistical analysis, ETE was treated as a dichotomous variable (present *vs* absent), with both mETE and gETE categorized as ETE-positive. This approach was adopted to increase statistical power and model stability given the limited number of events in each subgroup.

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Continuous variables were presented as mean ± standard deviation (SD), whereas categorical variables were expressed as frequencies and percentages. Differences between groups were assessed using the chi-square test or Fisher's exact test, as appropriate.

Univariable logistic regression analysis was performed to evaluate clinicopathological factors associated with the presence of ETE and crude odds ratios (ORs) with 95% confidence intervals (CIs) and corresponding P-values were reported. Variables with a P-value < 0.10 in the univariable analysis, together with clinically relevant variables, were entered into a multivariable logistic regression model to identify factors independently associated with ETE. Adjusted

odds ratios (ORs) with 95% confidence intervals (CIs) were calculated. For the subgroup analysis of gETE, variables were selected based on clinical relevance and consistency with the primary model.

The number of events per variable (EPV) was assessed to evaluate the risk of overfitting in the multivariable model. A total of 66 events were included with 6 variables entered into the model, resulting in an EPV of 11, which exceeds the commonly recommended threshold and supports the robustness of the model. Multicollinearity among variables was assessed using the variance inflation factor (VIF) and no significant collinearity was observed (all VIF < 2). Extranodal extension was analyzed descriptively in the univariable analysis but was not included in the multivariable model because it occurs only in the presence of lymph node metastasis and is therefore not considered an independent predictor.

Tumor size was categorized into four groups (≤ 10 mm, > 10-20 mm, > 20-40 mm and > 40 mm) based on clinically relevant cutoffs derived from established thyroid cancer staging systems, including the 10 mm threshold defining papillary thyroid microcarcinoma. Tumor size categories were used for descriptive analyses, whereas continuous tumor size was entered into logistic regression models.

Model calibration was assessed using the Hosmer-Lemeshow goodness-of-fit test and further evaluated using a calibration plot. Model discrimination was quantified using the area under the receiver operating characteristic curve (AUC). Internal validation was performed using bootstrap resampling. All statistical tests were two-sided and a P-value < 0.05 was considered statistically significant.

RESULTS

A total of 346 patients were included in the final analysis. The baseline clinicopathological characteristics of the study population are summarized in **Table 1**. The mean age was 42.6 ± 12.1 years, and most patients were aged < 55 years (82.9%). Females predominated, accounting for 86.1% of the cohort. The mean body mass index was 23.2 ± 3.3 kg/m^2, with the majority of patients having a normal BMI (64.7%).

Most tumors were incidentally detected during health screening (72.2%), whereas 27.8% of patients presented with symptoms. Graves' disease was identified in 2.6% of patients. Goiter and thyroiditis were observed in 14.7% and 14.2% of cases, respectively.

Regarding tumor characteristics, the mean tumor size was 11.3 ± 7.7 mm and 66.2% of tumors measured ≤ 10 mm. Multifocal tumors were present in 26.0% of patients. The classical variant was the predominant histological subtype (98.8%). Lymph node metastasis was identified in 28.3% of patients, extranodal extension in 3.8% and ETE in 19.1%.

Among these patients, 205 (59.2%) underwent total thyroidectomy, 3 (0.9%) underwent near-total thyroidectomy and 138 (39.9%) underwent lobectomy. Among patients who underwent total thyroidectomy, 81 (39.5%) had central neck dissection, 9 (4.4%) had lateral neck dissection and 39 (19.0%) had both central and lateral neck dissection. Among patients who underwent lobectomy, central neck dissection was performed in 9 patients (6.5%), while no lymph node dissection was performed in patients who underwent near-total thyroidectomy.

Larger tumor size was significantly associated with ETE (**Table 2**). Patients with ETE more frequently had tumors > 10 mm compared with those without ETE. In univariable analysis (**Table 3**), age ≥ 55 years, symptomatic presentation, multifocality, larger tumor size, lymph node metastasis, thyroiditis and extranodal extension were significantly associated with ETE (all p < 0.05).

In multivariable logistic regression analysis (**Table 4**), age ≥ 55 years (OR = 2.321, 95%CI 1.153-4.674; p = 0.018), tumor size (per 10 mm increase) (OR = 1.052, 95%CI 1.016-1.090; p = 0.005) and lymph node metas-

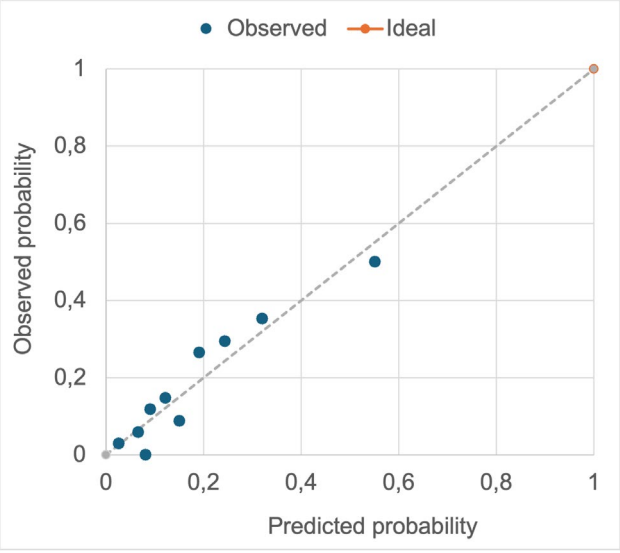

Figure 2. *Calibration plot of the multivariable logistic regression model evaluating the association between clinicopathological factors and extrathyroidal extension.*
The dashed diagonal line indicates perfect calibration.

Table 1. *Baseline clinicopathological and surgical characteristics of the study population.*

VARIABLES	VALUE (N = 346)
Age (years)	42.6 ± 12.1
<55 years	287 (82.9)
≥55 years	59 (17.1)
Sex	
Female	298 (86.1)
Male	48 (13.9)
Body mass index (kg/m²)	23.2 ± 3.3
<18.5	24 (6.9)
18.5–24.9	224 (64.7)
25.0–29.9	89 (25.7)
≥30	9 (2.6)
Reason for consultation	
Health screening	250 (72.2)
Neck mass	67 (19.4)
Throat discomfort	13 (3.7)
Dysphagia	3 (0.9)
Hoarseness	5 (1.4)
Dyspnea	2 (0.6)
Neck pain	4 (1.2)
Palpitations	2 (0.6)
Graves' disease	9 (2.6)
Maximum tumor diameter (mm)	11.3 ± 7.7
≤10	229 (66.2)
>10–20	82 (23.7)
>20–40	32 (9.2)
>40	3 (0.9)
Multifocality	
Yes	90 (26.0)
No	256 (74.0)
Histological variant	
Classic	342 (98.8)
Invasive follicular variant	4 (1.2)
Lymph node metastasis	98 (28.3)
Extranodal extension	13 (3.8)
Thyroiditis	49 (14.2)
Goiter	51 (14.7)
Extrathyroidal extension (ETE)	66 (19.1)
Gross ETE	46 (13.3)
Microscopic ETE	20 (5.8)
Surgical management	
Type of thyroidectomy	
Total thyroidectomy	205 (59.2)
Near-total thyroidectomy	3 (0.9)
Lobectomy	138 (39.9)
Lymph node dissection stratified by surgery type	
Total thyroidectomy (n = 205)	
None	76 (37.1)
Central	81 (39.5)
Lateral	9 (4.4)
Both central and lateral	39 (19.0)
Lobectomy (n = 138)	
None	129 (93.5)
Central	9 (6.5)
Near-total thyroidectomy (n = 3)	
None	3 (100.0)

Data are presented as mean ± standard deviation or number (%). ETE: extrathyroidal extension.

tasis (OR = 2.932, 95%CI 1.565-5.492; p < 0.001) were independently associated with higher odds of ETE. In contrast, thyroiditis was independently associated with lower odds of ETE (OR = 0.440, 95%CI 0.231-0.839; p = 0.013). Other variables were not significantly associated with ETE after adjustment.

The Hosmer–Lemeshow goodness-of-fit test indicated an adequate model fit (p = 0.543). The model demonstrated good discriminatory ability, with an area under the receiver operating characteristic curve (AUC) of 0.777 (95%CI 0.717-0.838).

Calibration of the model was assessed using a calibration plot, which showed good agreement between predicted and observed probabilities of ETE across deciles of risk (**Figure 2**). Most data points were closely aligned with the 45-degree reference line, indicating satisfactory calibration. Minor variability was observed in the lower-risk range, while a slight tendency toward overestimation was noted at higher predicted probabilities.

Internal validation using bootstrap resampling (1,000 iterations) demonstrated stable model performance, supporting the robustness and reliability of the model.

In a supplementary analysis, clinicopathological characteristics were further compared across ETE subgroups (no ETE, mETE and gETE) (**Supplementary Table 1**). Several key variables differed across subgroups, with a tendency toward more aggressive features in the gETE group compared with mETE

Table 2. *Comparison of clinicopathological characteristics according to extrathyroidal extension status.*

VARIABLES	ETE (N = 66)	NO ETE (N = 280)	P-VALUE
Age			0.037
<55 years	49 (74.2)	238 (85.0)	
≥55 years	17 (25.8)	42 (15.0)	
Sex			0.128
Male	13 (19.7)	35 (12.5)	
Female	53 (80.3)	245 (87.5)	
Body mass index (kg/m²)			0.336
<18.5	6 (9.1)	18 (6.4)	
18.5–24.9	44 (66.7)	180 (64.3)	
25.0–29.9	13 (19.7)	76 (27.1)	
≥30	3 (4.5)	6 (2.1)	
Reason for consultation			0.008
Health screening	39 (59.1)	211 (75.4)	
Symptomatic	27 (40.9)	69 (24.6)	
Graves' disease			1.000
Yes	1 (1.5)	8 (2.9)	
No	65 (98.5)	272 (97.1)	
Tumor size (mm)			<0.001
≤10	25 (37.9)	204 (72.9)	
>10–20	31 (47.0)	51 (18.2)	
>20–40	8 (12.1)	24 (8.6)	
>40	2 (3.0)	1 (0.4)	
Multifocality			<0.001
Yes	28 (42.4)	62 (22.1)	
No	38 (57.6)	218 (77.9)	
Histological variant			0.166
Classic	64 (97.0)	278 (99.3)	
Invasive follicular	2 (3.0)	2 (0.7)	
Lymph node metastasis			<0.001
Yes	34 (51.5)	64 (22.9)	
No	32 (48.5)	216 (77.1)	
Extranodal extension			<0.001
Yes	8 (12.1)	5 (1.8)	
No	58 (87.9)	275 (98.2)	
Thyroiditis			0.013
Yes	3 (4.5)	46 (16.4)	
No	63 (95.5)	234 (83.6)	
Goiter			0.916
Yes	10 (15.2)	41 (14.6)	
No	56 (84.8)	239 (85.4)	

Values are presented as number (%). P-values were calculated using the chi-square test or Fisher's exact test, as appropriate. ETE: extrathyroidal extension.

and no ETE. Larger tumor size and a higher frequency of lymph node **metastasis** were observed in patients with gETE.

In a separate multivariable analysis focusing on gETE (**Table 5**), older age (≥ 55 years), larger tumor size and lymph node metastasis remained inde-

Table 3. *Univariable logistic regression analysis of factors associated with extrathyroidal extension.*

VARIABLES	CRUDE OR	95%CI	P-VALUE
Age			
<55 years	1 (reference)		
≥55 years	1.966	1.035–3.735	0.039
Sex			
Female	1 (reference)		
Male	0.582	0.289–1.176	0.131
Body mass index (kg/m²)			
18.5–24.9	1 (reference)		
<18.5	2.045	0.492–8.501	0.325
25.0–29.9	1.500	0.284–7.934	0.633
≥30	2.923	0.649–13.174	0.163
Reason for consultation			
Health screening	1 (reference)		
Symptomatic	2.117	1.208–3.710	0.009
Graves' disease			
No	1 (reference)		
Yes	0.523	0.064–4.256	0.545
Tumor size (mm)			
≤10	1 (reference)		
>10–20	6.000	0.478–75.344	0.165
>20–40	3.290	0.286–37.811	0.339
>40	16.320	1.428–186.515	0.025
Multifocality			
No	1 (reference)		
Yes	2.591	1.474–4.553	<0.001
Histological variant			
Classic	1 (reference)		
Invasive follicular	4.344	0.601–31.420	0.146
Lymph node metastasis			
No	1 (reference)		
Yes	3.586	2.053–6.262	<0.001
Extranodal extension			
No	1 (reference)		
Yes	7.586	2.396–24.023	<0.001
Thyroiditis			
No	1 (reference)		
Yes	0.242	0.073–0.805	0.021
Goiter			
No	1 (reference)		
Yes	0.961	0.454–2.034	0.916

CI: confidence interval; OR: odds ratio. Reference categories are indicated for each variable. Tumor size was analyzed as a categorical variable. Variables with p < 0.10 in univariable analysis were considered for multivariable analysis.

pendently associated with higher odds of gETE. Specifically, each 10 mm increase in tumor size was independently associated with higher odds of gETE (OR = 1.090, 95%CI 1.048-1.134; p < 0.001), while lymph node metastasis was associated with more than a threefold increase in risk (OR = 3.486, 95%CI 1.662-

Table 4. *Multivariable logistic regression analysis of factors associated with extrathyroidal extension.*

VARIABLES	ADJUSTED OR (95%CI)	P-VALUE
Age (≥55 *vs* <55 years)	2.321 (1.153–4.674)	0.018
Reason for consultation (symptomatic *vs* health screening)	1.613 (0.863–2.983)	0.135
Multifocality (Yes *vs* No)	1.649 (0.882–3.081)	0.117
Tumor size (per 10-mm increase)	1.052 (1.016–1.090)	0.005
Lymph node metastasis (Yes *vs* No)	2.932 (1.565–5.492)	<0.001
Thyroiditis (Yes *vs* No)	0.440 (0.231–0.839)	0.013

CI: confidence interval. Tumor size was modeled as a continuous variable per 10-mm increase. Variables were selected for multivariable analysis based on clinical relevance and a univariable screening threshold of p < 0.10.

Table 5. *Multivariable logistic regression analysis of factors associated with gross extrathyroidal extension.*

VARIABLES	ADJUSTED OR (95%CI)	P-VALUE
Age (≥55 *vs* <55 years)	2.936 (1.306–6.600)	0.009
Multifocality (Yes *vs* No)	1.393 (0.665–2.920)	0.379
Tumor size (per 10-mm increase)	1.090 (1.048–1.134)	<0.001
Lymph node metastasis (Yes *vs* No)	3.486 (1.662–7.316)	<0.001
Thyroiditis (Yes *vs* No)	0.337 (0.091–1.248)	0.104

CI: confidence interval. Tumor size was modeled as a continuous variable per 10-mm increase. Variables included in the multivariable model were selected based on clinical relevance and consistency with the primary model.

7.316; p < 0.001). Age ≥ 55 years was also significantly associated with gETE (OR = 2.936, 95%CI 1.306-6.600; p = 0.009). In contrast, multifocality and thyroiditis were not significantly associated with gETE after adjustment.

DISCUSSION

ETE is a well-recognized adverse prognostic factor in PTC and is associated with an increased risk of locoregional recurrence and poorer oncologic outcomes. In this prospective observational study, older age (≥ 55 years), larger tumor size and lymph node metastasis were independently associated with ETE, whereas thyroiditis was associated with lower odds of ETE.

Although gETE is clearly associated with disease recurrence and disease-specific mortality, the clinical significance of mETE remains controversial (9, 22-24). Consistent with AJCC 8th edition concepts, mETE was not used for T upstaging in this study; it was analyzed only as a histopathological marker of local microscopic invasion (11). Macroscopic strap muscle invasion and invasion into deeper adjacent structures were classified as gETE. This distinction is important because mETE and gETE differ in prognostic relevance and in the reliability of pathological assessment.

Older age (≥ 55 years) was independently associated with ETE in our cohort, suggesting a more aggressive clinicopathological phenotype in older patients. This observation is consistent with previous studies reporting a higher likelihood of ETE or aggressive clinicopathological features in older individuals with PTC (18, 25). This association may reflect longer subclinical tumor growth, progressive local extension and the accumulation of biological alterations over time. Molecular events associated with aggressive PTC, including coexisting BRAF V600E and TERT promoter mutations, may also contribute to invasive behavior in some patients (44). Age-associated metabolic alterations, including increased reliance on aerobic glycolysis (the Warburg effect) and age-related changes in immune function have also been proposed as possible contributors to invasive cancer phenotypes (26-28). Because metabolic profiling and molecular testing were not performed, these explanations should be considered speculative. The present finding should therefore be interpreted as a clinicopathological association rather than evidence of a direct biological mechanism.

Tumor size also emerged as an independent factor associated with ETE, reinforcing its role as an important indicator of tumor aggressiveness. Larger tumors are more likely to penetrate the thyroid capsule and

invade surrounding tissues, reflecting progressive tumor growth. This observation is consistent with prior studies demonstrating a strong association between increasing tumor diameter and the risk of ETE (17, 29, 30). Notably, papillary thyroid microcarcinoma (≤ 10 mm) accounted for a substantial proportion of cases in our cohort (66.2%), reflecting the increasing detection of early-stage disease through health screening and routine imaging (31-33). Although microcarcinomas are generally considered indolent, ETE can still occur in a subset of these tumors, albeit at a lower frequency than in larger tumors (34). This pattern supports a continuum of tumor behavior, in which the risk of extrathyroidal invasion increases progressively with tumor size rather than being defined by a strict size threshold.
Lymph node metastasis was another factor independently associated with ETE, reflecting a more aggressive tumor biology characterized by enhanced invasive and metastatic potential. Previous studies have consistently reported a close association between lymph node metastasis and ETE (5, 17, 29, 35-39). From a clinical perspective, the presence of lymph node metastasis may therefore raise suspicion for concurrent ETE and should prompt careful preoperative and intraoperative evaluation. However, the detection of lymph node metastasis may be influenced by the extent of surgical dissection and the strategy of lymph node evaluation, potentially leading to underestimation in patients who do not undergo systematic lymph node dissection.
Coexisting lymphocytic thyroiditis was associated with lower odds of ETE in our study. This finding is consistent with several previous reports suggesting a potential association between autoimmune thyroiditis and less aggressive tumor behavior in PTC (40-43). Nevertheless, only 49 patients (14.2%) had thyroiditis and the confidence interval for the adjusted estimate was relatively wide, indicating uncertainty in the magnitude and stability of the association. This result should therefore be regarded as hypothesis-generating rather than evidence of a protective effect. Potential biological explanations, such as enhanced immune surveillance, have been proposed, but these mechanisms were not directly evaluated in the present study (45). Conflicting evidence has also been reported, and further studies are warranted to clarify the relationship between autoimmune thyroiditis and tumor invasiveness (18).
Although ETE was analyzed as a composite outcome in the primary analysis to reflect tumor invasion across its full spectrum, supplementary analyses focusing on gETE were performed because mETE and gETE may represent distinct biological and prognostic entities. Clinicopathological characteristics differed across ETE subgroups, with gETE showing more aggressive features, including larger tumor size and a higher prevalence of lymph node metastasis. In a separate multivariable model, these factors remained independently associated with gETE, whereas the association between thyroiditis and gETE was attenuated and no longer statistically significant. These findings are consistent with the concept that gETE represents a more advanced and clinically relevant form of tumor invasion (10, 11).
The prospective observational design, standardized histopathological review and multivariable analysis strengthen the reliability of this study. The model also showed acceptable stability, discrimination and calibration, supporting the internal consistency of the findings.
Several limitations should be acknowledged. The single-center setting and relatively short enrollment period may limit generalizability. Variations in surgical technique and intraoperative assessment were not fully controlled and may have introduced residual confounding. Assessment of lymph node metastasis was limited to patients who underwent lymph node dissection, which was generally performed in those with clinical or intraoperative suspicion of nodal involvement. Therefore, the detection of lymph node metastasis may have been influenced by the surgical strategy and extent of lymph node dissection, potentially leading to underestimation of subclinical or occult nodal disease in patients who did not undergo surgical evaluation. ETE was primarily analyzed as a composite outcome combining both minimal and gross ETE, which may have obscured differences in biological behavior and clinical significance. Although a supplementary analysis focusing on gETE was performed, the relatively limited number of mETE cases precluded a more detailed comparative analysis between mETE and gETE. Interobserver variability in the assessment of ETE cannot be entirely excluded, particularly for minimal extension, despite the use of standardized histopathological criteria. Imaging features, metabolic data and molecular markers, such as BRAF and TERT promoter mutations, were not included because the primary focus was on clinicopathological factors and molecular testing was not routinely performed during the study period. Some variables were determined based on postoperative histopathological findings; therefore, caution is needed when applying these

results to preoperative risk assessment. Long-term oncologic outcomes were not assessed. In patients with multifocal disease, only the dominant tumor was included in the analysis, which may have underestimated the contribution of smaller tumor foci to ETE. However, this approach reflects routine clinical practice and current staging principles.

Beyond discrimination, the model demonstrated adequate calibration, with only minor deviations at higher predicted probabilities. These findings support the internal consistency of the multivariable model.

CONCLUSIONS

In this study, older age (≥ 55 years), larger tumor size and lymph node metastasis were independently associated with pathological ETE in patients with PTC. Coexisting thyroiditis showed a potentially inverse association with ETE; however, given the relatively small number of thyroiditis cases and the uncertainty around the effect estimate, this finding should be interpreted cautiously and requires confirmation in larger cohorts. These results provide further insight into clinicopathological factors related to tumor invasiveness. Supplementary analyses suggested that gETE may represent a more aggressive disease phenotype, characterized by stronger associations with tumor size and lymph node metastasis, as well as a significant association with older age. Further multicenter studies with larger populations, standardized ETE classification, imaging variables and molecular data are warranted to validate these findings and to determine their potential value in preoperative risk stratification.

COMPLIANCE WITH ETHICAL STANDARDS

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None.

Conflicts of interest

The authors declare no competing of interests.

Availability of data and materials

The data underlying this article cannot be shared publicly due to patient privacy and ethical restrictions. The data can be made available from the corresponding author upon reasonable request.

Authors' contributions

KN: conceptualization. KV: project administration. ND: data curation, formal analysis, writing – original draft. All authors: writing – review & editing.

Ethical approval

Human studies and subjects

This study was performed in accordance with the principles of the Declaration of Helsinki and its subsequent amendments. Ethical approval was obtained from the Institutional Ethics Committee of Ho Chi Minh City Oncology Hospital (No. 542/BVUB-HDDD). Written informed consent was obtained from all participants.

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 work.

REFERENCES

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-263. doi: 10.3322/caac.21834.
2. National Cancer Institute. Surveillance, Epidemiology and End Results (SEER) Program Populations (1969-2020). Cancer Stat Facts: Thyroid Cancer. Available from: https://seer.cancer.gov. Accessed on January 15, 2026.
3. Harahap AS, Jung CK. Cytologic hallmarks and differential diagnosis of papillary thyroid carcinoma subtypes. J Pathol Transl Med. 2024;58(6):265-282. doi: 10.4132/jptm.2024.10.11.
4. International Collaboration on Cancer Reporting. Carcinoma of the Thyroid Histopathology Reporting Guide. Version 2.0. Sydney: ICCR; 2020.

5. Bortz MD, Kuchta K, Winchester DJ, Prinz RA, Moo-Young TA. Extrathyroidal extension predicts negative clinical outcomes in papillary thyroid cancer. Surgery. 2021;169(1):2-6. doi: 10.1016/j.surg.2020.04.003.
6. Wan F, He W, Zhang W, Zhang Y, Zhang H, Guang Y. Preoperative prediction of extrathyroidal extension: radiomics signature based on multimodal ultrasound in papillary thyroid carcinoma. BMC Med Imaging. 2023;23(1):96. doi: 10.1186/s12880-023-01049-8.
7. Greene FL, Page DL, Fleming ID, Fritz AG, Balch CM, Haller DG, et al. AJCC Cancer Staging Manual. 6th ed. New York: American Joint Committee on Cancer; 2002.
8. Parvathareddy SK, Siraj AK, Qadri Z, DeVera F, Siddiqui K, Al-Sobhi SS, et al. Microscopic extrathyroidal extension results in increased rate of tumor recurrence and is an independent predictor of patient outcome in Middle Eastern papillary thyroid carcinoma. Front Oncol. 2021;11:724432. doi: 10.3389/fonc.2021.724432.
9. Diker-Cohen T, Hirsch D, Shimon I, Bachar G, Akirov A, Duskin-Bitan H, et al. Impact of minimal extrathyroidal extension in differentiated thyroid cancer: systematic review and meta-analysis. J Clin Endocrinol Metab. 2018;103(6):2100-2106. doi: 10.1210/jc.2018-00081.
10. Moritani S. Impact of gross extrathyroidal extension into major neck structures on prognosis of papillary thyroid carcinoma according to AJCC 8th edition. Endocr J. 2020;67(9):941-948. doi: 10.1507/endocrj.EJ19-0523.
11. Tuttle RM, Haugen B, Perrier ND. Updated American Joint Committee on Cancer/Tumor-Node-Metastasis staging system for differentiated and anaplastic thyroid cancer (eighth edition): what changed and why? Thyroid. 2017;27(6):751-756. doi: 10.1089/thy.2017.0102.
12. Liu L, Oh C, Heo JH, Park HS, Lee K, Chang JW, et al. Clinical significance of extrathyroidal extension according to primary tumor size in papillary thyroid carcinoma. Eur J Surg Oncol. 2018;44(11):1754-1759. doi: 10.1016/j.ejso.2018.05.009.
13. Yuan SS, Zhang XR, Yu XQ, Hu JJ, Chen QQ, Lu F, et al. Prediction model for extrathyroidal extension in papillary thyroid carcinoma based on ultrasound radiomics. Sci Rep. 2025;15(1):19908. doi: 10.1038/s41598-025-19908-5.
14. Javid M, Mirdamadi A, Javid M, Keivanlou MH, Amini-Salehi E, Norouzi N, et al. The evolving role of MRI in the detection of extrathyroidal extension of papillary thyroid carcinoma: a systematic review and meta-analysis. BMC Cancer. 2024;24(1):1531. doi: 10.1186/s12885-024-13288-1.
15. Chung SR, Baek JH, Choi YJ, Sung TY, Song DE, Kim TY. Sonographic assessment of the extent of extrathyroidal extension in thyroid cancer. Korean J Radiol. 2020;21(10):1187-1195. doi: 10.3348/kjr.2019.0983.
16. Kwak JY, Kim EK, Youk JH, Kim MJ, Son EJ, Choi SH, et al. Extrathyroidal extension of well-differentiated papillary thyroid microcarcinoma on ultrasonography. Thyroid. 2008;18(6):609-614. doi: 10.1089/thy.2007.0345.
17. Wang H, Zhao S, Yao J, Yu X, Xu D. Factors influencing extrathyroidal extension of papillary thyroid cancer and evaluation of ultrasonography for its diagnosis: a retrospective analysis. Sci Rep. 2023;13(1):18344. doi: 10.1038/s41598-023-45642-x.
18. Kuo CY, Yang PS, Chien MN, Cheng SP. Preoperative factors associated with extrathyroidal extension in papillary thyroid cancer. Eur Thyroid J. 2020;9(5):256-262. doi: 10.1159/000506474.
19. Ghossein R. Update to the College of American Pathologists reporting on thyroid carcinomas. Head Neck Pathol. 2009;3(1):86-93. doi: 10.1007/s12105-009-0109-2.
20. Baloch ZW, Asa SL, Barletta JA, Ghossein RA, Juhlin CC, Jung CK, et al. Overview of the 2022 WHO Classification of Thyroid Neoplasms. Endocr Pathol. 2022;33(1):27-63. doi: 10.1007/s12022-022-09707-3.
21. Haddad RI, Bischoff L, Agosto Salgado S, Applewhite M, Berner V, Blomain E, et al. NCCN Clinical Practice Guidelines in Oncology: Thyroid Carcinoma. Version 1.2025. Plymouth Meeting: National Comprehensive Cancer Network; 2025. Available from: https://www.nccn.org. Accessed on January 15, 2026.
22. Marongiu A, Nuvoli S, De Vito A, Vargiu S, Spanu A, Madeddu G. Minimal extrathyroid extension (mETE) as the only risk factor in patients with papillary thyroid carcinoma: its clinical impact on recurrence and outcome during long-term follow-up. Biomedicines. 2024;12(2):350. doi: 10.3390/biomedicines12020350.
23. Amit M, Boonsripitayanon M, Goepfert RP, Tam S, Busaidy NL, Cabanillas ME, et al. Extrathyroidal extension: does strap muscle invasion alone influence recurrence and survival in patients with differentiated thyroid cancer? Ann Surg Oncol.

2018;25(11):3380-3388. doi: 10.1245/s10434-018-6563-x.
24. Tran B, Roshan D, Abraham E, Wang L, Garibotto N, Wykes J, et al. An analysis of the American Joint Committee on Cancer 8th edition T staging system for papillary thyroid carcinoma. J Clin Endocrinol Metab. 2018;103(6):2199-2206. doi: 10.1210/jc.2017-02551.
25. Kaliszewski K, Diakowska D, Nowak Ł, Wojtczak B, Rudnicki J. The age threshold of the 8th edition AJCC classification is useful for indicating patients with aggressive papillary thyroid cancer in clinical practice. BMC Cancer. 2020;20(1):1166. doi: 10.1186/s12885-020-07636-0.
26. Hsu YC, Liu CL, Yang PS, Tsai CH, Lee JJ, Cheng SP. Interaction of age at diagnosis with transcriptional profiling in papillary thyroid cancer. World J Surg. 2016;40(12):2922-2929. doi: 10.1007/s00268-016-3625-8.
27. Hay N. Reprogramming glucose metabolism in cancer: can it be exploited for cancer therapy? Nat Rev Cancer. 2016;16(10):635-649. doi: 10.1038/nrc.2016.77.
28. Wu Y, Wei J, Chen X, Qin Y, Mao R, Song J, et al. Comprehensive transcriptome profiling in elderly cancer patients reveals aging-altered immune cells and immune checkpoints. Int J Cancer. 2019;144(7):1657-1663. doi: 10.1002/ijc.31875.
29. Lee HS, Park C, Kim SW, Park T, Chun BK, Hong JC, et al. Correlation of minimal extrathyroidal extension with pathologic features of lymph node metastasis in patients with papillary thyroid carcinoma. J Surg Oncol. 2015;112(6):592-596. doi: 10.1002/jso.24051.
30. Walczyk A, Gąsior-Perczak D, Pałyga I, Kopczyński J, Kuchareczko A, Niedziela E, et al. Risk characteristics of papillary thyroid cancer 1-4 cm associated with increased tumour size. Clin Exp Med. 2025;25(1):155. doi: 10.1007/s10238-025-01596-0.
31. Park S, Oh CM, Cho H, Lee JY, Jung KW, Jun JK, et al. Association between screening and the thyroid cancer "epidemic" in South Korea: evidence from a nationwide study. BMJ. 2016;355:i5745. doi: 10.1136/bmj.i5745.
32. Xu S, Han Y. The overdiagnosis of thyroid micropapillary carcinoma: the rising incidence, inert biological behavior and countermeasures. J Oncol. 2021;2021:5544232. doi: 10.1155/2021/5544232.
33. Hau NX, Quang LV. Cervical lymph node metastasis presentation of patients with thyroid carcinoma. Vietnam Med J. 2022;515(1). doi: 10.51298/vmj.v515i1.2705. (In Vietnamese).
34. Lombardi CP, Bellantone R, De Crea C, Paladino NC, Fadda G, Salvatori M, et al. Papillary thyroid microcarcinoma: extrathyroidal extension, lymph node metastases and risk factors for recurrence in a high prevalence of goiter area. World J Surg. 2010;34(6):1214-1221. doi: 10.1007/s00268-009-0375-x.
35. Kim JW, Roh JL, Gong G, Cho KJ, Choi SH, Nam SY, et al. Extent of extrathyroidal extension as a significant predictor of nodal metastasis and extranodal extension in patients with papillary thyroid carcinoma. Ann Surg Oncol. 2017;24(2):460-468. doi: 10.1245/s10434-016-5594-4.
36. Seok J, Ryu CH, Park SY, Lee CY, Lee YK, Hwangbo Y, et al. Factors affecting central node metastasis and metastatic lymph node ratio in papillary thyroid cancer. Otolaryngol Head Neck Surg. 2021;165(4):519-527. doi: 10.1177/0194599821991465.
37. Feng JW, Qin AC, Ye J, Pan H, Jiang Y, Qu Z. Predictive factors for lateral lymph node metastasis and skip metastasis in papillary thyroid carcinoma. Endocr Pathol. 2020;31(1):67-76. doi: 10.1007/s12022-019-09599-w.
38. Mao J, Zhang Q, Zhang H, Zheng K, Wang R, Wang G. Risk factors for lymph node metastasis in papillary thyroid carcinoma: a systematic review and meta-analysis. Front Endocrinol (Lausanne). 2020;11:265. doi: 10.3389/fendo.2020.00265.
39. Wang Y, Nie F, Wang G, Liu T, Dong T, Sun Y. Value of combining clinical factors, conventional ultrasound and contrast-enhanced ultrasound features in preoperative prediction of central lymph node metastases of different sized papillary thyroid carcinomas. Cancer Manag Res. 2021;13:3403-3415. doi: 10.2147/CMAR.S299157.
40. Osborne D, Choudhary R, Vyas A, Kampa P, Abbas LF, Chigurupati HD, et al. Hashimoto's thyroiditis effects on papillary thyroid carcinoma outcomes: a systematic review. Cureus. 2022;14(8):e28054. doi: 10.7759/cureus.28054.
41. Tang Q, Pan W, Peng L. Association between Hashimoto thyroiditis and clinical outcomes of papillary thyroid carcinoma: a meta-analysis. PLoS One. 2022;17:e0269995. doi: 10.1371/journal.pone.0269995.
42. Wu K, Shi L, Wang J, Xie L. Association between papillary thyroid carcinoma and lymphocytic thyroiditis: a retrospective study. Oncol Lett. 2023;25(4):148. doi: 10.3892/ol.2023.13734.
43. Lee JH, Kim Y, Choi JW, Kim YS. The association between papillary thyroid carcinoma and his-

tologically proven Hashimoto's thyroiditis: a meta-analysis. Eur J Endocrinol. 2013;168(3):343-349. doi: 10.1530/EJE-12-0903.

44. Xing M, Liu R, Liu X, Murugan AK, Zhu G, Zeiger MA, et al. BRAF V600E and TERT promoter mutations cooperatively identify the most aggressive papillary thyroid cancer with highest recurrence. J Clin Oncol. 2014;32(25):2718-2726. doi: 10.1200/JCO.2014.55.5094.
45. Greten FR, Grivennikov SI. Inflammation and cancer: triggers, mechanisms and consequences. Immunity. 2019;51(1):27-41. doi: 10.1016/j.immuni.2019.06.025.

SUPPLEMENTARY MATERIALS

Supplementary Table 1. *Comparison of clinicopathological characteristics according to extrathyroidal extension subgroups.*

VARIABLES	NO ETE	METE	GETE	P-VALUE
Age				0.080
<55 years	238 (82.9)	16 (5.6)	33 (11.5)	
≥55 years	42 (71.2)	4 (6.8)	13 (22.0)	
Sex				0.314
Male	35 (72.9)	4 (8.3)	9 (18.8)	
Female	245 (82.2)	16 (5.4)	37 (12.4)	
Reason for consultation				0.02
Health screening	211 (84.4)	8 (3.2)	31 (12.4)	
Symptomatic	69 (71.9)	12 (2.5)	15 (15.6)	
Tumor size (mm)				<0.001
≤10	204 (89.1)	13 (5.7)	12 (5.2)	
>10–20	51 (62.2)	7 (8.5)	24 (29.3)	
>20–40	24 (75.0)	0 (0.0)	8 (25.0)	
>40	1 (33.3)	0 (0.0)	2 (66.7)	
Lymph node metastasis				<0.001
Yes	64 (65.3)	7 (7.1)	27 (27.6)	
No	216 (87.1)	13 (5.2)	19 (7.7)	
Extranodal extension				<0.001
Yes	5 (38.5)	0 (0.0)	8 (61.5)	
No	275 (82.6)	20 (6.0)	38 (11.4)	
Thyroiditis				0.035
Yes	46 (93.9)	0 (0.0)	3 (6.1)	
No	234 (78.8)	20 (6.7)	43 (14.5)	

ETE: extrathyroidal extension; mETE: minimal extrathyroidal extension; gETE: gross extrathyroidal extension. Values are presented as n (row %). P-values were calculated using the chi-square test or Fisher's exact test, as appropriate.

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RESEARCH ARTICLE

TRENDS, CLINICOPATHOLOGIC FEATURES, AND MANAGEMENT OF *IN SITU* BREAST CANCER: INSIGHTS FROM THE NORTH ITALY CANCER REGISTRY, 2000-2023

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ABSTRACT: *In situ* breast cancer represents an important subset of breast neoplasms and is frequently detected through organized screening programs. We analyzed incidence trends, clinicopathologic characteristics, and treatment patterns of *in situ* breast tumors registered in the Reggio Emilia Cancer Registry from 2000 to 2023. A total of 1,543 *in situ* breast tumors were identified, representing 13.3% of all registered breast cancers during the study period. Age-adjusted incidence rates per 100,000 were calculated using the 2013 European Standard Population, and the annual percentage change (APC) was estimated to assess temporal trends. Clinicopathologic features, including histology and hormone receptor status, were collected. Surgical management and demographic characteristics were also analyzed. Overall, *in situ* breast tumors showed a modest upward trend over the study period; however, this increase was not statistically significant (APC 0.7; 95%CI -0.6 to 2.2). A modest decline was observed in 2022–2023 following the COVID-19 pandemic, which significantly affected screening uptake in 2020–2021. Age distribution revealed that 8.5% of cases occurred in women under 45 years and 8.5% in women over 75 years. The majority (83%) were diagnosed in women aged 45-74, the primary target of screening programs, including 19.7% in women aged 45-49 years, 69% aged 50-69, and 11.3% aged 70-74. Histologically, the vast majority (93.5%) were ductal in origin. Hormone receptor analysis showed that 36.5% were estrogen receptor-positive (ER+) and 25.9% progesterone receptor-positive (PR+). Breast-conserving surgery was performed in 74.7%, while 19.1% underwent mastectomy. Sentinel lymph node biopsy (SLNB) was performed in 46.2% of cases, whereas axillary lymph node dissection was uncommon (3.3%). Women of foreign nationality represented 6.6% of the cohort. *In situ* breast tumors in our Cancer Registry demonstrated a modest, non-significant increase in incidence over time and predominantly affected women within the screening age range. Most cases were ductal and managed with breast-conserving surgery, reflecting current clinical practice. These findings highlight the sustained impact of screening programs and the importance of ongoing surveillance of early breast cancer, especially in the context of healthcare disruptions such as the COVID-19 pandemic.

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Impact statement: From 2000 to 2023, ***in situ*** breast cancers in the Reggio Emilia Cancer Registry showed a modest, non-significant increase, predominantly ductal and mainly treated with breast-conserving surgery within screening ages.

Key words: *In situ breast cancer; incidence trends; screening; breast-conserving surgery.*

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INTRODUCTION

Ductal carcinoma *in situ* (DCIS) is a non-invasive breast neoplasm characterized by the proliferation of malignant epithelial cells confined to the mammary ducts, without invasion through the basement membrane. DCIS is considered a precursor lesion of many invasive breast carcinomas, although the risk of progression is variable and not fully predictable (1).
The incidence of DCIS increases with age, with the highest rates observed after 50-60 years of age (2). Over the past decades, a marked increase in DCIS incidence has been reported in Western countries, largely attributable to the widespread implementation of population-based mammographic screening programs. The introduction of screening has led to an increase in DCIS diagnoses, reaching approximately 10-11 cases per 100,000 women-years in some populations, with the greatest increases observed among women aged 50-69 years, the primary target group for screening (3).
Some studies also suggest a modest increase in incidence may also be attributable to factors independent of screening, possibly reflecting changes in underlying risk or diagnostic practices (4). Despite the growing number of diagnosed cases, it remains unclear to what extent this trend represents a true increase in clinically relevant disease *versus* improved detection resulting from screening and advances in diagnostic techniques (2). DCIS frequently expresses estrogen (ER) and progesterone (PR) receptors, with implications for the potential use of endocrine therapy in selected cases, similarly to invasive breast carcinoma (5).
DCIS is frequently asymptomatic and is most commonly detected through mammographic screening, with microcalcifications representing the most frequent radiologic finding (6).
Primary surgical treatment options include breast-conserving surgery (lumpectomy), with or without adjuvant radiotherapy, aimed at excising the lesion while preserving the breast (7), and mastectomy, which is recommended in cases of extensive disease, multicentric involvement, or when clear surgical margins cannot be achieved (8).
The use of SLNB has increased over time and may be considered in selected DCIS cases, particularly when mastectomy is planned or when there is a high suspicion of occult invasive disease (7). Endocrine therapy (*e.g.*, tamoxifen) has been shown to reduce the risk of subsequent invasive breast cancer and recurrent DCIS in patients with ER-positive disease, as demonstrated in randomized trials and reflected in international and Italian (AIOM) clinical guidelines (9, 10).
The present study aims to describe a population-based cancer registry case series of *in situ* breast tumors over a long observation period (2000-2023).

MATERIALS AND METHODS

Reggio Emilia Cancer Registry

This study used data from the Reggio Emilia Cancer Registry (RE-CR), a population-based registry covering the Province of Reggio Emilia, Italy, and dedicated to descriptive epidemiological surveillance. Cancer

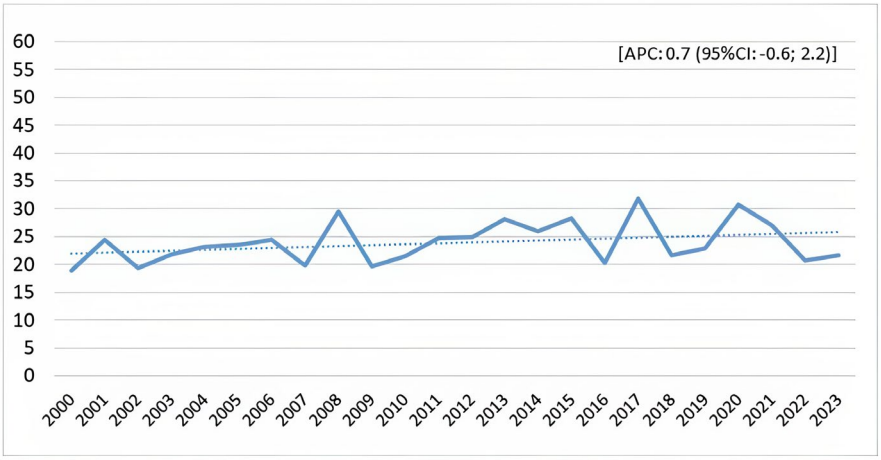

Figure 1. *Reggio Emilia Cancer Registry, 2000-2023. Number of in situ tumors per year of incidence. Agestandarded rate, per 100,000. APC: Annual Percentage Change.*

Registry operates with ethical approval from the Provincial Ethics Committee of Reggio Emilia (Protocol no. 2014/0019740; August 4, 2014). Case ascertainment is achieved through systematic linkage of multiple data sources, including pathology databases, hospital discharge records, mortality registries, laboratory datasets, diagnostic and therapeutic procedure records, and information provided by general practitioners.

The RE-CR covers approximately 532,000 residents and is recognized for the high quality and completeness of its data. Previous analyses reported histological confirmation rates exceeding 90% and a proportion of Death Certificate Only (DCO) cases of 0.2%. For breast cancer specifically, the proportion of microscopically verified cases currently reaches 99.5% (11). Reggio Emilia Cancer Registry is among the few in Italy with incidence data updated through 2023 and has contributed substantially to the epidemiological characterization of cancer burden at national and international levels.

Data analysis

The study included all DCIS of the breast diagnosed between 2000 and 2023. Tumors were classified by topography according to the International Classification of Diseases for Oncology (ICD-O) (12) and grouped by year of diagnosis. Age at diagnosis was categorized as < 45, 45-74 (the organized screening age group in our region since 2010), and ≥ 75 years. For descriptive purposes, the 45-74 age group was further subdivided into 45-49, 50-69, and 70-74 years. Information on receptor status was obtained through careful review of clinical records. Estrogen receptor (ER) and progesterone receptor (PR) were considered positive when expression exceeded 20%. Age-standardized incidence rates were calculated using the resident population of the Province of Reggio Emilia (as of 1 January of each year) as denominators. Rates were standardized by the direct method using the 2013 European Standard Population as a reference. Temporal trends were analyzed by estimating the annual percent change (APC) in age-standardized rates using Joinpoint Regression analysis. The APC represents the average annual percentage change in incidence over a specified time interval, assuming that rates change at a constant percentage of the previous year. The joinpoint model identifies points in time at which statistically significant changes in trend occur, based on predefined statistical criteria. For these analyses, the maximum number of joinpoints was set at four (13).

RESULTS

The study included 1,543 *in situ* breast tumors, representing 13.3% of all breast cancers registered between 2000 and 2023 (**Table 1**). Overall, the incidence of *in situ* tumors showed a slight upward trend over time, with age-adjusted incidence rates per 100,000 women showing a modest increase (**Figure 1**). However, this trend was not statistically significant (APC 0.7; 95%CI -0.6 to 2.2). A modest decline in the number of situ cases was observed in 2022-2023, following the COVID-19 pandemic period, which had as substantial impact on our province during 2020-2021.

In our cohort, 190 patients with an initial diagnosis of *in situ* breast cancer subsequently developed an invasive breast tumor. This is in line with the long-

Table 1. *Distribution of in situ cancers compared with infiltrating tumors, by year of incidence. Reggio Emilia Cancer Registry, 2000-2023.*

YEAR	*IN SITU*	INFILTRATING	% *IN SITU**
2000	42	407	10.3
2001	54	415	13.0
2002	43	398	10.8
2003	49	422	11.6
2004	53	444	11.9
2005	57	439	13.0
2006	59	436	13.5
2007	47	451	10.4
2008	73	440	16.6
2009	53	437	12.1
2010	56	499	11.2
2011	66	502	13.1
2012	67	479	14.0
2013	76	497	15.3
2014	76	465	16.3
2015	82	522	15.7
2016	59	485	12.2
2017	95	522	18.2
2018	64	502	12.7
2019	67	522	12.8
2020	92	551	16.7
2021	83	579	14.3
2022	64	589	10.9
2023	66	577	11.4
Total	**1,543**	**11,580**	**13.3**

** "In situ" refers to non-invasive cancers; "Infiltrating" refers to invasive tumors. Percentages are calculated as the proportion of in situ cases relative to total cases per year.*

term natural history of DCIS documented in NSABP B-17/B-24, the 20-year SweDCIS update, EORTC 10853 at 15 years and the UK Sloane Project, which consistently identify high grade, ER-negativity, comedonecrosis and young age (< 40 years) as predictors of late invasive recurrence.

In our institution, women treated for DCIS undergo oncology-led follow-up with annual mammography for 10 years, after which they re-enter the organized regional screening program. Although our registry spans 24 years, the median follow-up of cases diagnosed after 2010 is still below 15 years, and events beyond 20 years are currently too few to support robust pattern-specific stratification (now stated as a limitation and as a prospective registry objective). Regarding age at diagnosis, 8.5% of cases were recorded in women younger than 45 years, and a similar proportion was observed among women aged 75 years or older (**Table 2**). The 45-74 age group, corresponding to the target population for organized screening programs, accounted for the majority of *in situ* tumors (83%). Within this group, 19.7% of cases occurred in women aged 45-49 years, 69% in those aged 50-69 years, and 11.3% in those aged 70-74 years.

Histologically, 93.5% of tumors were classified as DCIS. Regarding hormone receptor status, 36.5% of tumors were estrogen receptor-positive (ER+) and 25.9% were progesterone receptor-positive (PR+).

Concerning surgical treatment, 74.7% of patients underwent breast-conserving surgery (lumpectomy), while 19.1% underwent mastectomy. SLNB was performed in 46.2% of cases, while axillary lymph node dissection was carried out in 3.3%. Finally, women of foreign nationality represented 6.6% of the overall *in situ* breast cancer cohort.

Table 2. *Characteristics of in situ breast cancers (n = 1,543). Reggio Emilia Cancer Registry, 2000-2023.*

CHARACTERISTIC	N	%
Age at diagnosis		
<45	131	8.5
45–74	1,281	83.0
45–49	*252*	*19.7*
50–69	*884*	*69.0*
70–74	*145*	*11.3*
75+	131	8.5
Morphology		
Ductal	1,442	93.5
Lobular	90	5.8
Other	11	0.7
ER positive	563	36.5
PR positive	400	25.9
Surgery		
None	53	3.4
Quadrantectomy	1,153	74.7
Mastectomy	294	19.1
Tumorectomy/Lumpectomy	41	2.7
NOS	2	0.1
Sentinel lymph node biopsy		
No	830	53.8
Yes	713	46.2
Axillary lymph node dissection		
No	1,492	96.7
Yes	51	3.3
Nationality		
Italian	1,441	93.4
Foreign	102	6.6

Percentages calculated among available cases.

DISCUSSION

Our population-based registry study of 1,543 *in situ* breast tumors (13.3% of all breast cancers, diagnosed between 2000 and 2023) confirms several epidemiologic and clinical patterns consistently reported in the literature.

Incidence and age distribution

Consistent with historical evidence indicating that DCIS incidence increased markedly following the implementation of screening mammography – particularly among women aged 50 and older – our data showed a modest upward trend in age-adjusted DCIS rates over time, followed by a slight decline in 2022-2023. This recent decrease likely reflects pandemic-related disruptions in screening activities and healthcare access. Screening mammography remains one of the strongest determinants of DCIS detection, accounting for a substantial proportion of diagnoses in screened populations (14). The age distribution observed in our cohort mirrors established epidemiologic patterns: the majority of cases occurred in women aged 45-74 years, corresponding to the target population of organized screening programs. These findings further support the central role of screening practices in shaping both the incidence and age distribution of detected DCIS cases (15).

Surgical management and axillary evaluation

Breast-conserving surgery (BCS) was the predominant surgical approach, consistent with contemporary management strategies that favor conservative treatment when appropriate (16). Over time, the use of SLNB in DCIS has increased, particularly in patients undergoing mastectomy, due to the risk of occult invasive disease not detectable preoperatively (17). Large retrospective analyses have demonstrated that SLNB positivity in DCIS is rare, and the likelihood of detecting nodal micrometastases is low. These findings underscore that routine SLNB is not indicated for all DCIS patients, especially those treated with BCS. However, in selected cases – such as patients undergoing mastectomy or those presenting high-risk clinicopathologic features – SLNB may be appropriate to avoid the need for axillary staging after mastectomy. In our cohort, SLNB was performed in 46.2% of cases and axillary dissection in 3.3%, reflecting a tailored application of axillary staging in line with current evidence and guidelines. This approach supports selective rather than routine axillary evaluation in DCIS management (18).

Clinical implications

Screening and detection

The strong association between screening participation and DCIS detection reinforces the need to balance early detection with the risk of overdiagnosis (14, 15). Risk stratification tools incorporating age, imaging characteristics, and biomarkers remain essential to inform individualized screening strategies and follow-up protocols.

Axillary management

The low incidence of axillary metastasis in DCIS argues against routine SLNB in all patients, particularly those undergoing BCS. Decisions regarding axillary staging should be individualized and based on the planned surgical procedure (*e.g.*, mastectomy) and clinicopathologic features suggestive of occult invasion (17, 18).

Surgical decision-making

The high rate of breast-conservative surgery in our cohort underscores the continued emphasis on breast preservation when oncologically appropriate. However, increasing mastectomy rates reported in some settings highlight the importance of shared decision-making that integrates patient preferences with evidence regarding recurrence risk, cosmetic outcomes, and long-term prognosis (18).

Strengths and limitations

A major strength of this study is its population-based design with a long observation period and complete case capture. The analysis included all women diagnosed with *in situ* breast tumors in the Province of Reggio Emilia between 2000 and 2023, without selection criteria. This real-world setting reinforces the external validity of the findings and provides a valuable opportunity to assess the impact of screening programs, evolving clinical guidelines, and advancements in surgical and pathological practice on routine care. The observed trends are consistent with findings reported in the literature, but derive from an unselected, non-trial population, thereby reflecting the translation of evidence into everyday clinical practice. Moreover, the study provides detailed information on molecular characteristics, surgical management, and axillary staging, variables that are not always comprehensively captured in population-based registries.

However, some limitations should be acknowledged. First, this is a single-province study, which may limit generalizability beyond northern Italy, despite the use of standardized diagnostic and therapeutic protocols.

Second, long-term outcome data, such as local recurrence or progression to invasive carcinoma, were not uniformly available, limiting the ability to correlate molecular and clinical characteristics with prognosis. Finally, temporal changes in screening coverage, diagnostic techniques, and healthcare access over the study period may have influenced incidence patterns and treatment choices, potentially introducing residual confounding in trend interpretation.

CONCLUSIONS

In conclusion, this population-based study provides a comprehensive overview of *in situ* breast tumors diagnosed over 23 years period in the Province of Reggio Emilia. The findings indicate a modest increase in DCIS incidence over time, together with a predominant use of breast-conserving surgery and selective approach to sentinel lymph node evaluation. These results reflect real-world practice and are consistent with existing literature. They underscore the value of high-quality population-based cancer registry data in informing screening policies, improving risk stratification, and supporting personalized management strategies for DCIS.

COMPLIANCE WITH ETHICAL STANDARDS

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Conflicts of interest

The authors declare no competing interests.

Availability of data and materials

Data are available under reasonable request to the corresponding author.

Authors' contributions

MBB: conceptualization, investigation, writing – original draft, visualization, supervision. FM: formal analysis. IB: writing – review & editing, visualization, investigation, supervision. AC: investigation, supervision. CP, FG: supervision. MGS: investigation, visualization. LM: conceptualization, writing – original draft, investigation, supervision.

Ethical approval

Human studies and subjects

This population-based cohort study uses data from the Reggio Emilia Cancer Registry, approved by the Provincial Ethics Committee of Reggio Emilia (ref. no. 2014/0019740 of 4 August 2014). The Ethics Committee authorized, even in the absence of consent, the processing of personal data, including those suitable for revealing the state of health of patients who are deceased or untraceable for the execution of the study.

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 work.

REFERENCES

1. Tomlinson-Hansen SE, Khan M, Cassaro S. Breast Ductal Carcinoma in Situ. 2023. In: StatPearls. Treasure Island (FL): StatPearls Publishing; 2026 Jan–.
2. Iatrakis G, Zervoudis S. Epidemiology of Ductal Carcinoma In Situ. Chirurgia (Bucur). 2021;116(5 Suppl):S15-S21. doi: 10.21614/chirurgia.116.5.suppl.S15.
3. Sørum R, Hofvind S, Skaane P, Haldorsen T. Trends in incidence of ductal carcinoma in situ: the effect of a population-based screening programme. Breast. 2010;19(6):499-505. doi: 10.1016/j.breast.2010.05.014.
4. Bucchi L, Mancini S, Biggeri A, Vattiato R, Giuliani O, Ravaioli A, et al. Mammography screening and incidence of ductal carcinoma in situ of the breast in Italy: an age-period-cohort analysis. Int J Epidemiol. 2025;54(4):dyaf102. doi: 10.1093/ije/dyaf102.
5. Poulakaki N, Makris GM, Battista MJ, Böhm D, Petraki K, Bafaloukos D, et al. Hormonal receptor status, Ki-67 and HER2 expression: Prognostic value in the recurrence of ductal carcinoma in situ of the breast? Breast. 2016;25:57-61. doi: 10.1016/j.breast.2015.10.007.
6. Shehata M, Grimm L, Ballantyne N, Lourenco A, Demello LR, Kilgore MR, et al. Ductal Carcinoma in Situ: Current Concepts in Biology, Imaging, and Treatment. J Breast Imaging. 2019;1(3):166-176. doi: 10.1093/jbi/wbz039.
7. Worni M, Akushevich I, Greenup R, Sarma D, Ryser MD, Myers ER, et al. Trends in Treatment Patterns and Outcomes for Ductal Carcinoma In Situ. J Natl Cancer Inst. 2015;107(12):djv263. doi: 10.1093/jnci/djv263.
8. Czajka ML, Pfeifer C. Breast Cancer Surgery. (Updated 2023 Feb 8). In: StatPearls (Internet). Treasure Island (FL): StatPearls Publishing; 2025 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK553076/.
9. Allred DC, Anderson SJ, Paik S, Wickerham DL, Nagtegaal ID, Swain SM, et al. Adjuvant tamoxifen reduces subsequent breast cancer in women with estrogen receptor-positive ductal carcinoma in situ: a study based on NSABP protocol B-24. J Clin Oncol. 2012;30(12):1268-73. doi: 10.1200/JCO.2010.34.0141.
10. AIOM (Associazione Italiana Oncologia Medica), Carcinoma mammario in stadio precoce, Edizione 2023.

11. Mangone L, Borciani E, Michiara M, Vicentini M, Carrozzi G, Mancuso P, et al. I tumori nelle province dell'Area Vasta Emilia Nord: Piacenza, Parma, Reggio Emilia e Modena: Anni 2013-2014. Modena, Italy: Associazione Italiana Registri Tumori; 2015.
12. Fritz A, Percy C, Jack A, Shanmugaratnam K, Sobin L, Parkin DM, et al. International Classification of disease for Oncology, 3rd edition. World Health Organization 2013.
13. Surveillance Research Program, National Cancer Institute (2025). Joinpoint Regression Software, Version 5.4.0 - April 2025. Available from: https://surveillance.cancer.gov/joinpoint.
14. Kerlikowske K. Epidemiology of ductal carcinoma in situ. J Natl Cancer Inst Monogr. 2010;2010(41):139-41. doi: 10.1093/jncimonographs/lgq027.
15. Esserman LJ, Thompson IM, Reid B, Nelson P, Ransohoff DF, Welch HG, et al. Addressing overdiagnosis and overtreatment in cancer: a prescription for change. Lancet Oncol. 2014;15(6):e234-42. doi: 10.1016/S1470-2045(13)70598-9.
16. Patani N, Khaled Y, Al Reefy S, Mokbel K. Ductal carcinoma in-situ: an update for clinical practice. Surg Oncol. 2011;20(1):e23-31. doi: 10.1016/j.suronc.2010.08.007.
17. Chiu CW, Chang LC, Su CM, Shih SL, Tam KW. Precise application of sentinel lymph node biopsy in patients with ductal carcinoma in situ: A systematic review and meta-analysis of real-world data. Surg Oncol. 2022;45:101880. doi: 10.1016/j.suronc.2022.101880.
18. Papila B. The Evolution of Axillary Surgery in Breast Cancer-Towards De-escalation. Balkan Med J. 2025;42(3):183-184. doi: 10.4274/balkanmedj.galenos.2025.2025.160425.

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CASE REPORT

GIANT RETROPERITONEUM SCHWANNOMA CAUSING IPSILATERAL HYDRONEPHROSIS: A RARE CASE REPORT

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ABSTRACT: Retroperitoneal schwannomas are rare benign tumors originating from Schwann cells and are seldom diagnosed preoperatively due to their anatomical location and nonspecific clinical-radiological features. We report a case of 25-year-old woman with progressive right upper abdominal pain for eight months and a palpable right subcostal mass. Imaging showed a large, well-circumscribed, heterogeneously enhancing retroperitoneal lesion displacing right kidney with secondary hydronephrosis. With a provisional diagnosis of ganglioneuroma, laparoscopic complete excision was performed with repair of iatrogenic renal vein rents. Histopathology confirmed benign schwannoma with Antoni A and B areas. Recovery was uneventful, with no recurrence at six months follow-up period.

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Impact statement: Current case highlights the diagnostic challenge of retroperitoneal schwannoma, while illustrating the feasibility of laparoscopic complete excision for large lesions with favorable postoperative and short-term oncological outcomes.

Key words: *Antoni; hydronephrosis; retroperitoneal; schwannoma; Verocay.*

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INTRODUCTION

Schwannomas, also known as neurilemmomas, are slow-growing, benign, encapsulated tumors arising from Schwann cells of neural crest origin (1). Although they are typically benign, malignant transformation has been reported, particularly in association with neurofibromatosis type 1 (NF1) (2). These tumors most commonly occur in the head and neck region and along the flexor surfaces of the extremities. In contrast, retroperitoneal schwannomas (RPS) are exceedingly rare, accounting for approximately 4% of all retroperitoneal tumors and nearly 3% of all schwannomas (3). Owing to their deep anatomical location and nonspecific clinical presentation, diagnosis is often delayed and challenging, with definitive diagnosis usually established only after imaging and surgical excision (1). We report a rare case of RPS managed by laparoscopic excision in a 25-year-old female who presented with right flank and right hypochondrial pain.

CASE PRESENTATION

A 25-year-old female with no known comorbidities presented to our outpatient department with progressive right upper abdominal pain of 8 months' duration, which had been managed with over-the-counter analgesics. Physical examination revealed a non-tender, globular mass measuring approximately 6 × 6 cm below the right subcostal margin, which did not move with respiration. In view of the uncertain origin of the mass, an ultrasonography of

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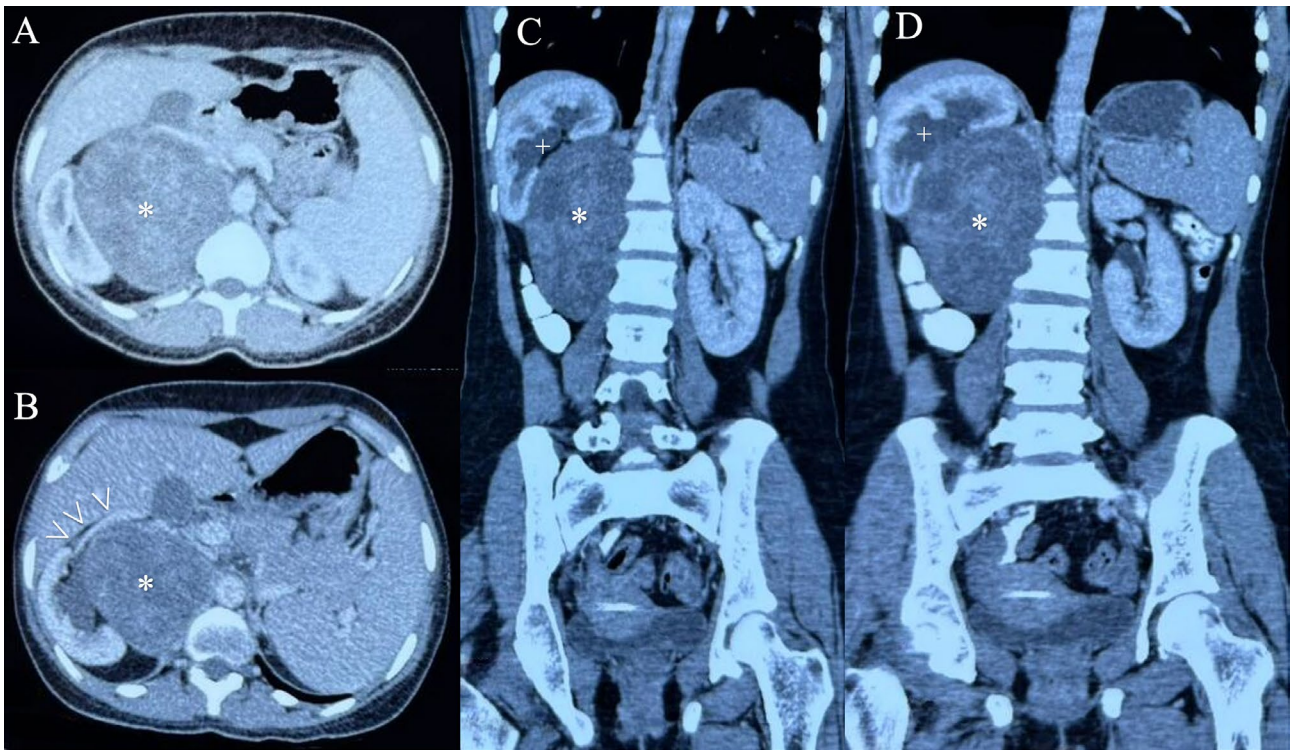

Figure 1. *Contrast-enhanced CT images depicting the retroperitoneal mass.*
(A, B) *CT Axial sections revealing a heterogeneously enhancing clearly encapsulated solid retroperitoneal mass (denoted by *) with displacing effects on the surrounding structures like IVC, Liver, Kidney and right renal vessels (denoted by arrow heads).* **(C, D)** *Coronal reconstruction section of the CT abdomen revealing a retroperitoneal large mass of size 10 × 9 cm (denoted by *) with displacing effects on the right kidney and hydronephrosis owing to proximal ureteral extrinsic compression (denoted by +).*

the whole abdomen was performed, which demonstrated a well-defined heterogeneous hypoechoic lesion in the right retroperitoneum, displacing the right kidney and associated with moderate hydronephrosis. Further evaluation with contrast-enhanced computed tomography (CECT) of the abdomen revealed a well-circumscribed, smoothly marginated, heterogeneously enhancing solid mass measuring 104 × 92 mm in the right retroperitoneum, with central non-enhancing necrotic areas. The right kidney and adrenal gland were visualized separately, confirming the retroperitoneal origin of the lesion. The right kidney was compressed and displaced superolaterally with secondary hydronephrosis. The mass was seen abutting the inferior vena cava, duodenum, and ascending colon without evidence of invasion, and no significant lymphadenopathy was noted (**Figure 1**). Given the radiological suspicion of a paraganglioma, a metabolic workup including serum metanephrines, cortisol, and dehydroepiandrosterone was performed and found to be within normal limits. With a provisional diagnosis of ganglioneuroma, the patient was planned for laparoscopic excision of the retroperitoneal mass.

Intraoperatively, a large retroperitoneal mass measuring approximately 10 × 10 cm was identified, abutting the liver and displacing the right kidney superolaterally. The ascending colon, including the hepatic flexure, and the duodenum were mobilized to expose the mass (**Figure 2A**). The right ureter and gonadal vessels were displaced inferolaterally (**Figure 2B**). The right ureter was identified, carefully mobilized, and preserved. The mass was dissected off the underlying psoas muscle, proceeding medially toward the inferior vena cava and superolaterally toward the right kidney after identification of the right renal artery and vein (**Figure 2C,D**). The right renal vessels were found to be closely adherent to the mass, and during dissection, three rents occurred in the renal vein, which were successfully repaired using prolene sutures. After complete mobilization, the specimen was extracted through a right iliac fossa incision. Post-operative course of the patient was uneventful. Drain was clamped on 3rd day and removed next day, with the patient being discharged on day 4.

On gross examination, the tumor was greyish white to yellow bosselated in appearance, measuring 10 ×

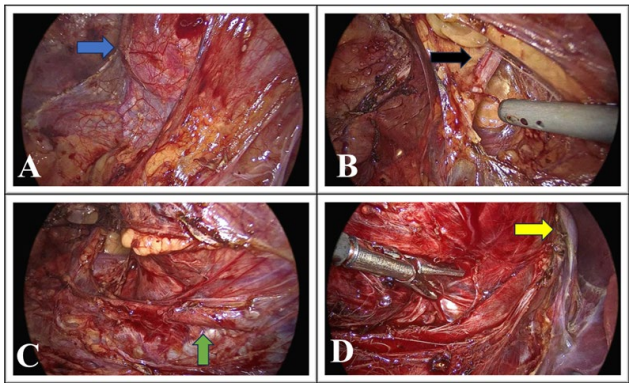

Figure 2. *Intraoperative anatomical relationships of the retroperitoneal mass.*
(A) *Inferior aspect of the retroperitoneal mass (blue arrow).* **(B)** *Right Ureter identified coursing inferolaterally to the mass (black arrow).* **(C)** *Inferior vena cava located medially in relation to the mass (green arrow).* **(D)** *Relationship of the mass with the right renal vein (yellow arrow) and right kidney.*

9 cm in size, being covered by capsule (**Figure 3A**). The cut surface was predominantly solid, lobulated and firm in consistency, and was whitish to tan-yellow with mucoid to myxoid texture. Areas of necrosis and focal hemorrhage were seen. On microscopic examination, findings were consistent with schwannoma with Antoni A and B areas. Section showed an encapsulated neoplasm composed of cytologically bland spindle cells arranged predominantly in densely cellular areas with nuclear palisading (Verocay bodies) and alternating hypocellular areas. Focal areas showed thick walled hyalinized blood vessels and microcystic change, with no mitosis/atypia seen. The pathological findings were consistent with schwannoma without any signs of malignancy (**Figure 3B-D**). At the 6-month follow-up visit, the patient was asymptomatic with no clinical or radiological evidence of recurrence.

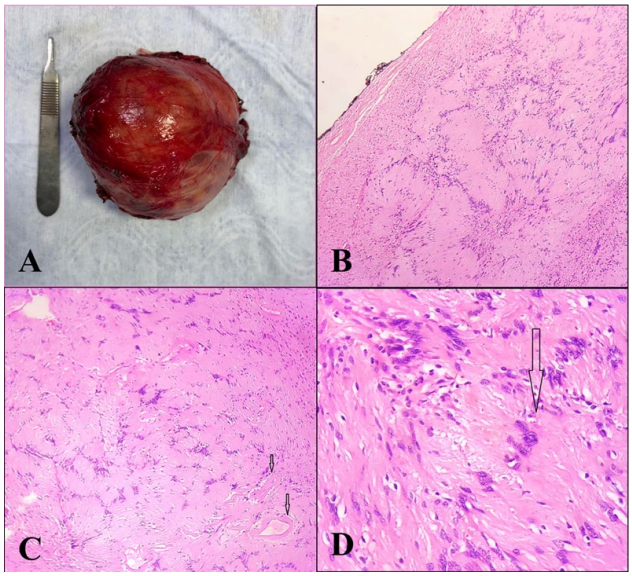

Figure 3. *Gross and histopathological features of the resected specimen.*
(A) *Gross appearance of the excised encapsulated tumor.* **(B)** *Microscopic examination revealing an encapsulated lesion composed of biphasic areas (H & E; 100X).* **(C)** *Presence of compact hypercellular Antoni A areas and myxoid hypocellular Antoni B areas along with thickened and hyalinized blood vessels (H & E; 100X).* **(D)** *High power view demonstrating Verocay bodies characterized by nuclear palisading around fibrillary process (H & E; 400X).*

DISCUSSION

Schwannomas are usually solitary benign tumors, with malignant transformation being exceptional. However, patients with NF1, particularly those harboring plexiform neurofibromas, are at increased risk of developing malignant peripheral nerve sheath tumors, with the reported lifetime risk reaching up to 60% in selected high-risk subgroups (4). Retroperitoneal involvement is rare, accounting for approximately 0.7% of benign schwannomas and 1.7% of malignant schwannomas (5, 6). The age spectrum of RPS is wide, ranging from 6 months to 70 years; however, they are most frequently reported in women between the second and fifth decades of life (7). Although extremely uncommon, schwannomas should be considered in the differential diagnosis of retroperitoneal masses in young patients, with only two cases reported in the adolescent age group to date (1).

Most RPS are detected incidentally, particularly smaller lesions identified during evaluation for unrelated conditions. When symptomatic, clinical presentation is often nonspecific, including low back pain or gastrointestinal and urinary complaints due to mass effect on adjacent organs, which contributes to diagnostic delay (8). Owing to the capacious nature of the retroperitoneum, these tumors frequently attain a large size before detection, typically ranging from 4 to 15 cm, with a reported median size of 8.7 cm (4). Preoperative imaging plays a central role in identifying the origin of retroperitoneal masses despite diagnostic challenges posed by nonspecific symptoms. Ultrasonography and computed tomography are indispensable initial modalities, allowing assessment of tumor location, density, and relationship to surrounding structures. These typically demonstrate a well-circumscribed, heterogeneous mass with peripheral enhancement, suggestive of

a benign lesion. Magnetic resonance imaging (MRI) remains the gold standard due to its superior diagnostic accuracy, characteristically showing hypo- to isointense signals on T1-weighted images and hyperintensity on T2-weighted images. MRI also facilitates better evaluation of origin, vascular involvement and possible extension into the intervertebral foramina, although such extension is rare (9).

Preoperative percutaneous biopsy remains controversial and is generally not recommended because of the risks of hemorrhage and infection, as well as the rare but serious possibility of neoplastic dissemination in the event of a malignant tumor (9). The differential diagnosis of RPS includes several mesenchymal and neuroendocrine neoplasms, such as pheochromocytoma, paraganglioma, liposarcoma, leiomyosarcoma, fibrosarcoma, and ganglioneuroma; therefore, a high index of suspicion and meticulous clinical evaluation are essential (**Table 1**).

Complete surgical excision remains the primary and most effective treatment for schwannoma. However, the necessity of extensive multiorgan resection to achieve negative margins remains debatable. Given that the majority of these tumors are benign, aggressive organ resection may be unwarranted and potentially overtreats the disease. Nevertheless, uncertainty regarding the exact nature of the tumor in the preoperative period often creates a dilemma, necessitating complete excision to exclude malignancy. To date, no clear consensus has been established. Radical excision of RPS is technically challenging because of the tumor's hypervascularity and its close adherence to major retroperitoneal vessels, which increases the risk of intraoperative bleeding (10).

A definitive diagnosis of schwannoma is established on histopathological examination of the resected specimen. Grossly, RPS are typically solitary, well-circumscribed, encapsulated, and bosselated masses. Histopathological examination reveals Schwann cells arranged in alternating compact hypercellular Antoni A areas and hypocellular myxoid Antoni B areas, with strong immunoreactivity for S-100 protein. Secondary degenerative changes such as hyalinization, calcification, cystic degeneration, and fibrosis characterize the variant known as "ancient schwannoma" (8).

The prognosis of RPS is excellent, with minimal risk of recurrence following complete resection. Recurrence rates of 5-10% have been reported after incomplete excision, underscoring the importance of complete tumor removal (1, 3). This highlights the need for a systematic postoperative follow-up protocol, although no standardized guidelines currently exist. Most centers perform imaging at 6-12 months postoperatively; however, follow-up practices vary between institutions.

Table 1. *Differential diagnosis of retroperitoneal schwannoma: comparative morphological, immunohistochemical, and molecular features of selected retroperitoneal neoplasms.*

ENTITY	MORPHOLOGICAL FEATUREZ	IMMUNOHISTOCHEMISTRY	MOLECULAR FEATURES
Schwannoma	Encapsulated; Antoni A and B areas; Verocay bodies; nuclear palisading	S100+, SOX10+; CD34−; SMA−; Desmin−	NF2 alterations occasionally present
Malignant Peripheral Nerve Sheath Tumor	Hypercellular spindle cells; pleomorphism; necrosis; high mitotic activity	Focal/weak S100, SOX10; loss of H3K27me3	NF1 mutations; CDKN2A, SUZ12, EED alterations
Solitary fibrous tumor	Patternless spindle-cell proliferation; staghorn vessels	STAT6+, CD34+, BCL2+	NAB2-STAT6 fusion
Leiomyosarcoma	Intersecting fascicles of eosinophilic spindle cells with atypia	SMA+, Desmin+, h-caldesmon+	Complex karyotype
Dedifferentiated liposarcoma	Adipocytic component with atypical stromal cells and non-lipogenic sarcoma	MDM2+, CDK4+	MDM2 amplification
Fibrosarcoma	Herringbone architecture	Vimentin+; nonspecific	Complex genetic abnormalities
Ganglioneuroma	Mature ganglion cells within Schwannian stroma	S100+, Synaptophysin+, NSE+	No characteristic alteration
Paraganglioma	Zellballen pattern; sustentacular cells	Chromogranin+, Synaptophysin+, S100 in sustentacular cells	SDHB/SDHD, RET, VHL mutations

CONCLUSIONS

RPS are uncommon tumors that present significant diagnostic challenges due to their variable and non-specific clinico-radiological features. Surgical excision remains the treatment of choice, providing both definitive histopathological diagnosis and cure. While complete resection is desirable, a conservative surgical approach should be considered whenever feasible, given the predominantly benign nature of these lesions. Early recognition and appropriate follow-up are essential to ensure optimal outcomes.

COMPLIANCE WITH ETHICAL STANDARDS

Funding

None.

Conflicts of interest

The authors declare no competing interests.

Availability of data and materials

The data that support the findings of this study are not publicly available due to institutional ethical regulations but are available from the corresponding author upon request.

Authors' contributions

BS, SY, HS: conceptualization. BS, HS, TKA: data curation. BS, HS, PP: formal analysis. BS, SY, TKA, HS: writing – original draft. SY, HS, TKA, PP: writing – review & editing. PP: resources.

Ethical approval

Human studies and subjects

Institutional Ethics Committee approval was not required for publication of this single case report. Written informed consent was obtained from patient included in the study. Consent was also obtained for publication of clinical details and images.

Publications ethics

Plagiarism

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Data falsification and fabrication

The authors declare that no data fabrication, falsification, or manipulation has been carried out in the preparation of this work.

REFERENCES

1. Zaafouri EB, Rihane S, Omry A, Ferjaoui W, Haloui N, Rebii S. Uncommon retroperitoneal mass in a young adult: A rare case report of retroperitoneal schwannoma and review of diagnostic challenges. Int J Surg Case Rep. 2025;127:110862. doi: 10.1016/j.ijscr.2025.110862.
2. Shelat VG, Li K, Naik S, Ng CY, Rao N, Rao J, et al. Abdominal schwannomas: case report with literature review. Int Surg. 2013;98(3):214-8. doi: 10.9738/INTSURG-D-13-00019.1.
3. Radojkovic M, Mihailovic D, Stojanovic M, Radojković D. Large retroperitoneal schwannoma: a rare cause of chronic back pain. J Int Med Res. 2018;46(8):3404-3410. doi: 10.1177/0300060518776474.
4. Harhar M, Ramdani A, Bouhout T, Serji B, El Harroudi T. Retroperitoneal Schwannoma: Two Rare Case Reports. Cureus. 2021;13(2):e13456. doi: 10.7759/cureus.13456.
5. Beddouche A, Fahsi O, Kallat A, El Bote H, Ziani I, El Sayegh H et al. Schwannome benin primitif retrovesical: une tumeur très rare à propos d'un cas [Primitive benign retrovesical schwannoma: an extremely rare tumor, a case report]. Pan Afr Med J. 2016;23:79. French.
6. Garcia G, Anfossi E, Prost J, Ragni E, Richaud C, Rossi D. Schwannome rétropéritonéal bénin: à propos de trois cas [Benign retroperitoneal schwannoma: report of three cases]. Prog Urol. 2002;12(3):450-3. French.
7. Hide IG, Baudouin CJ, Murray SA, Malcolm AJ. Giant ancient schwannoma of the pelvis. Skeletal Radiol. 2000;29(9):538-42. doi: 10.1007/s002560000266.
8. Çalişkan S, Gümrükçü G, Kaya C. Retroperitoneal Ancient Schwannoma: A Case Report. Rev Urol. 2015;17(3):190-3.
9. Al Skaini MS, Haroon H, Sardar A, Bazeed M, Al Zain A, Al Shahrani M, et al. Giant retroperitoneal ancient schwannoma: Is preoperative biopsy always mandatory? Int J Surg Case Rep. 2015;6C:233-6. doi: 10.1016/j.ijscr.2014.08.015.
10. Daneshmand S, Youssefzadeh D, Chamie K, Boswell W, Wu N, Stein JP, et al. Benign retroperitoneal schwannoma: a case series and review of the literature. Urology. 2003;62(6):993-7. doi: 10.1016/s0090-4295(03)00792-1.

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