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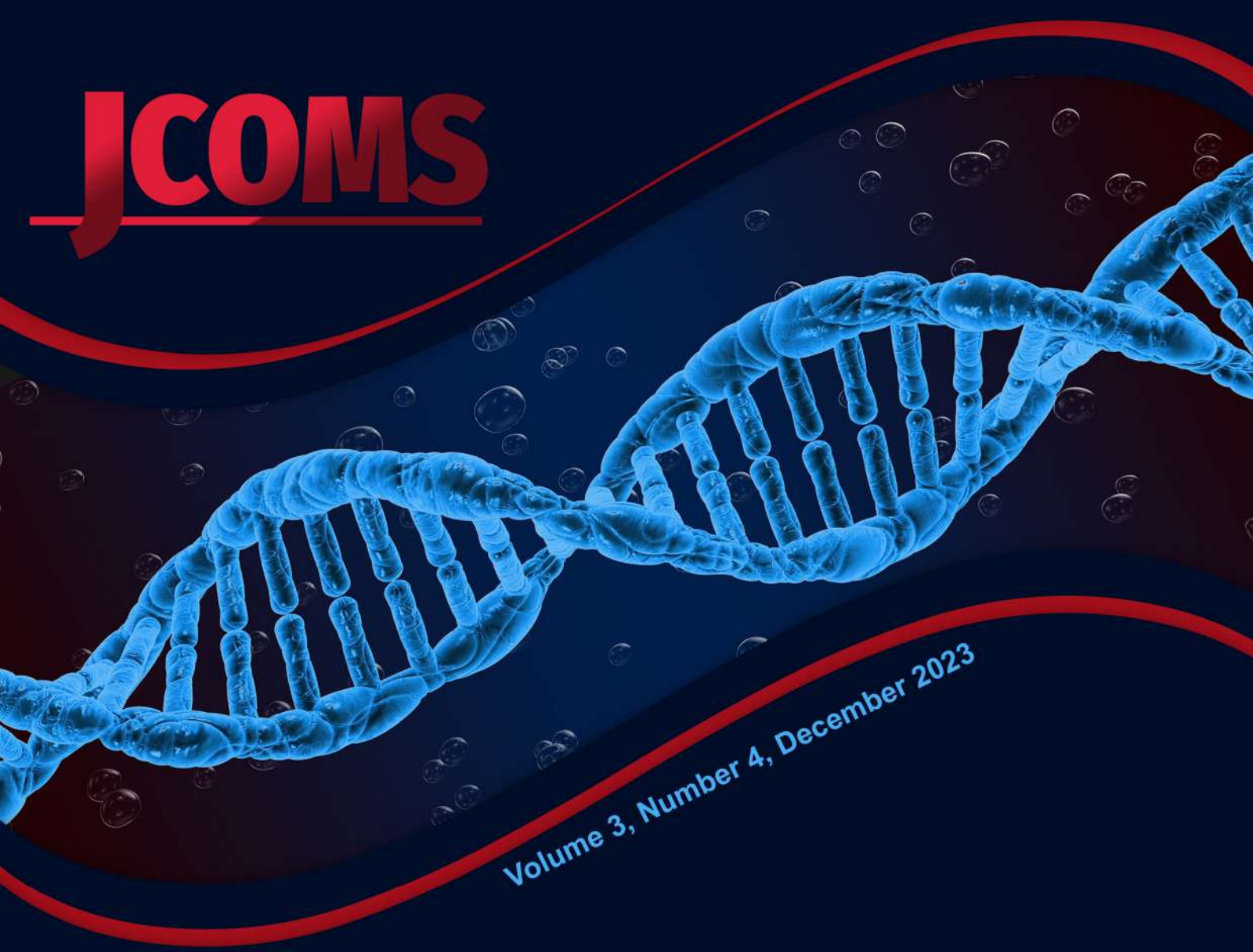

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Molecular mechanisms associated with cutaneous melanoma biology, pathogenesis, and diagnosis

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Abstract

Introduction: Melanoma is considered the most lethal skin cancer, with poor prognosis in advanced stages. The 2018 World Health Organization (WHO) Classification classified melanoma into nine different subgroups depending on the cumulative sun damage, with its respective genetic alterations, which are necessary to investigate for targeted therapies. Nevertheless, the epigenetic alterations aren't included at all in the new molecular classification. It is understanding the molecular mechanisms associated with melanoma pathogenesis and its poor prognosis.

Methods: To analyze the molecular mechanisms implicated in melanoma carcinogenesis, we reviewed the most recent papers using PubMed database and Google Scholar, the search was carried out using the following medical subject headings (MeSH) in the search engine: "melanoma epigenetic mechanisms", "miRNAs and melanoma", immunology and melanoma", "melanoma pathogenesis", in combination with boolean connectors 'AND' and 'OR'. A total of 83 articles were reviewed, published between 2000 and 2022.

Conclusion: Given the importance of genetic and epigenetic mechanisms implicated in the prognosis and progression of cancer, this paper aims to review the literature about its respective regulators, and how they have a relationship between them in several metabolic, apoptotic, physiological, and biological processes. It is essential to understand the molecular and immunological mechanisms involved in melanoma pathogenesis and how the alteration of any of them leads to the genesis of cancer, to foster the development of novel targeted therapy strategies.

Keywords: Melanoma, Molecular mechanisms, Skin neoplasm, Genetic, Epigenetic

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Introduction

Skin cancer is the most common form of cancer in the world. It is categorized into melanoma and non-melanoma skin cancers. Melanoma only accounts for about 1% of all skin cancers, but is the most aggressive with a poor prognosis, it accounts 90% of all skin cancer deaths, and it's more frequent in patients between 25 and 40 years old (1). According to the "Surveillance, Epidemiology, and End Results" program (SEER), the rate of new cases of melanoma in the U.S. of the skin was 22.8 per 100,000 men and women per year, with a death rate of 2.2 per 100,000 based on data from 2014 to 2018. In 2019, an estimated 1,294,886 people were living with melanoma of the skin. Melanoma is associated with different risk factors; they can be divided in modifiable and non-modifiable risk factors (2).

The Cancer Genome Atlas (TCGA) classified tumors according to their genomic characteristics, the most prevalent mutated genes are BRAF, NRAS, NF1-loss and triple wild type (TWT). However, there are more mutations associated with tumorigenesis of melanoma, such as CDKN2A (25-35%), TP53 (15%), ARID2 (13.32%), IDH1, PPP6C, PTEN (14%), DDX3X, RAC1 (9.2%), MAP2K1/2 (10%), RB1, ATRX (9.11%), SETD2 (9.48%), SF3B1 (33%), TERT (14%), and ERBB2/4 (3.29%) (3,4).

Modifiable risk factors

Modifiable risk factors are related by a high occurrence of oncogenesis, some external factors such as ultraviolet A (320-400 nm) (5) and B (280-315 nm) exposure (6), Intermittent sun exposure has a relative risk of 1.61, sunburn has a relative risk of 2.03 (7), citrus consumption greater than 1.6 servings of citrus fruits daily increases melanoma risk, high Body Mass Index (BMI) is inversely correlated with telomerase length with the consequence of high risk of melanoma than people with normal BMI (8), immunosuppression states, such as Kidney transplants confers a relative risk of 3.6 developing melanoma. Nowadays, gut microbiome is recognized as a potentially modifiable risk factor associated with immunotherapy response (9).

Nonmodifiable risk factors

Genetic syndromes such as Xeroderma pigmentosum, Neurofibromatosis, Charcot Marie Tooth, Familiar Atypical Multiple Mole and Melanoma Syndrome (FAMMM) and Amyotrophic Lateral Sclerosis (ALS) (8,10), familiar history (5-10% of melanomas occur in families with hereditary predisposition) (6), personal history of melanoma (32% higher risk of developing second primary malignancy) (11), race (Caucasians have a lifetime risk of 2-6%, African Americans 0.1% and Hispanics 0.58%) (11), people with blue eyes have a relative risk of 1.06 to 2.45 compared to those who have dark eyes, blonde hair 1.6-9.7 compared with dark hair (6), gender (in females are more common on the legs, in males are more common on the trunk) (8), giant congenital nevus >20 cm of diameter (the malignant transformation estimates between 4-40%) (6), atypical nevi (one has a relative risk of 1.45, two have 2.1, three have 3.03, four have 4.39 and five have 6.36 of transforming in melanoma) (10), high number of common nevi (>100 common nevi) is associated with almost 7 times higher risk of melanoma (7). Instead, people affected with vitiligo have a decreased risk of melanoma, while for melanoma patients, vitiligo is associated with better prognosis, with spontaneous regression of melanoma (12).

Genes involved in melanoma pathogenesis and prognosis

The mutations of BRAF (incidence of 45%), NRAS (15%), GNAQ, and GNA11 (80-90%) (involved in the G alpha signaling pathway) (13) are known to be responsible for the hyperactivity of mitogen-activated protein kinase (MAPK), which is involved in tumor proliferation and progression. ALK fusions are found in about 10-20% of Spitz nevi and 1% of Spitz melanomas (14). BRAF encodes a cytoplasmic serine/threonine kinase in the MAPK pathway (15). Their functions and mutations confer different molecular mechanisms associated with tumorigenesis (16). CDKN2A is a tumor suppressor gene, it encodes two transcripts (p16 and p14ARF, both needed to ubiquitination of p53), its mutation is observed in FAMMM. Another protein, CDK4 inhibits the binding of p16 leading to phosphorylation of RB (mutated in FAMMM and atypical nevi) (16), TERT encodes a reverse transcriptase that creates a template for telomer addition.

In addition, *ACD, TERF21P, TERF1, TERF2, TINF2 and POT1* are implicated in telomere maintenance and their mutations increase telomere length and fragility (16), BAP1 is a tumor suppressor gene that encodes deubiquitinating enzyme and a binding partner to BCRA1, implicated in chromatin modulation, transcriptional regulation, and DNA damage repair (16).

TP53, involved in the control of the progression of the cell cycle from G1 to S phase, its mutation is associated with high risk of melanoma (17), the loss of one copy of chromosome 3 is associated with high risk of metastasis and death (17).

The protein phosphatase 2 scaffold subunit A alpha (PPP2R1A) may mediate the survival and resistance of apoptosis of the type B malignant melanoma cell lines (18), Aurora B kinase (AURKB) is a chromosomal passenger protein regulating early mitotic stage transition from prophase to metaphase, which is overexpressed in melanoma (18). STAT3, a gene involved in cytokine signaling, regulates the expression of genes implicated in survival, cell cycle progression and angiogenesis (19). The nuclear factor-kappaB (NFkB) is a transcription factor that regulates a variety of mechanisms by its signal pathway, such as immune and inflammatory responses. Its activation is regulated by tumor necrosis factor-alpha (TNF-α), IL-1 and Toll-like receptors: TNFR, IL-1R, and TLR, however, NFkB can be activated by dysregulations of MAPK and PI3K signaling pathways, increasing the risk of proliferation and drug resistance (20). Those pigmentated subtypes showed a higher expression of microphthalmia-associated transcription factor (MITF) compared with non-pigmented melanomas. *MITF* encodes a melanocytic-lineage-specific transcription factor that regulates the differentiation, proliferation, and survival of melanocytes (16). Its mutation confers higher cell growth, increased synthesis of melanin pigment, and poor prognosis (3).

microRNAs (miRNAs) are non-coding RNAs and are important gene regulators. They are considered as a new potential therapeutic strategy and fundamental prognostic factor. miR-21-5p reduces cell proliferation and promotes apoptosis by increasing PDCD4, PTEN, and BTG2. miR-146a-5p is upregulated by BRAF and NRAS, promoting cell proliferation, cell migration and invasion (21).

Another molecular mechanism implicated in tumorigenesis of melanoma is the DNA methylation alterations. The DNA hypermethylation of PTEN, CDKN2A and RASSF1A have been reported in melanomas. Tellez C.S. et.al reported an elevated methylation status in their melanoma cell lines: ESR1 (50%), MGMT (50%), RARB2 (44%), RIL (82%), RASSF1A (69%), PAX7 (31%), PGRB (56%), PAX2 (38%), NKX2-3 (63%), OLIG2 (63%), HAND1 (63%), ECAD (88%), CDH13 (44%), and CDKN2A/p16 (6%) (22). (Table1)

Table 1. The 2018 World Health Organization (WHO) classification of cutaneous, mucosal, and uveal melanoma (23–31).

Melanomas typically associated with Cumulative Solar Damage	Melanomas not consistently associated with Cumulative Solar Damage	
Pathway I. Superficial spreading melanoma/low-CSD melanoma	Pathway IV. Spitz melanoma	Nodular melanoma
Pathway II. Lentigo maligna melanoma/high-CSD melanoma	Pathway V. Acral melanoma	
Pathway III. Desmoplastic melanoma	Pathway VI. Mucosal melanoma	
	Pathway VII. Melanomas arising in congenital nevi	
	Pathway VIII. Melanomas arising in blue nevi	
	Pathway IX. Uveal melanoma	

The 2018 World Health Organization (WHO) classification of cutaneous, mucosal, and uveal melanoma is based on its arising sun-exposure skin, the role of ultraviolet (UV) radiation, precursors, and driving and/or recurrent genomic changes (23–31). In general terms, melanoma can be divided into two groups: UV-related and UV-unrelated melanomas.

UV-related group is more frequent in white population, it arises from epithelium associated-melanocytes in cutaneous sites with cumulative sun damage (CSD), which includes pathways I-III, while UV-unrelated group is more frequent in non-white population, it arises from non-epithelium associated-melanocytes regardless of CSD and it is associated with IV-X pathways (32).

Pathway I. Superficial spreading melanoma/low-CSD melanoma

Pathway I is the route by which melanocytes acquire the genetic aberrations necessary to become melanoma, however, it is associated with lower CSD. This pathway contributes to the appearance of superficial spreading melanoma. Superficial spreading melanoma is the most common form of melanoma. This kind of melanoma is particularly localized in parts of the body with intermittent sun exposure like in vacation or weekends. In men, its most frequent localization is in the back while in women is the back of the legs or calf region. They typically express BRAF V600E mutations, TERT, and NRAS mutations in less proportion (33).

Pathway II. Lentigo maligna melanoma/high-CSD melanoma

Pathways II and III are the pathways necessary to transform melanocytes in melanoma, however, in contrast with pathway I, these two types of pathways are associated with high CSD. Through pathway II, melanocytes acquire various genetic mutations, including NF1, BRAF V600K, NRAS, KIT, CCND1, MITF and TP53 which are associated with high CSD, and leads to lentigo maligna melanoma (LMM) transformation. LM is a melanoma subtype considered a melanoma in situ; it represents about 4-15% of all melanomas. The most frequent site of this subtype is in head and neck (78.3%). They can be presented as an amelanotic/hypomelanotic macule or patch, especially in fair-skinned individuals on chronically sun-damaged skin. There's described a sex-related preferential location of LM, developing on the right side of the face in males and on the left side in females (24,25).

Pathway III. Desmoplastic melanoma

As mentioned above, pathway III is associated with an extremely high mutation burden with high CSD. Desmoplastic melanoma (DM) arises from this pathway. DM is a rare variant of cutaneous melanoma; it accounts for about 1% of all melanomas. They're commonly amelanotic or sparsely pigmented and are typically endophytic (33). The genetic alterations associated with this kind of melanoma is the inactivation of NF1 and RAS mutations, which results in the activation of MAPK pathway, however, the genetic mutations and the genesis of this type of melanoma is not yet completely known (33).

Pathway IV. Spitz melanoma

Previously to WHO classification, Spitz melanoma (SM) was classified based on the cytomorphologic features in spitzoid melanomas. Nowadays, SMs are classified based on their morphologic and genomic alterations such as HRAS, ALK, NTRK1, MAP3K8, BRAF, and CDKN2A mutations, in contrast with its counterpart Spitz Nevi (SN). SMs are rare, they represent about 1-2% of all melanocytic lesions. The mean age of diagnosis in SM is 22 years old. They can be localized in any part of the body but is more frequent in lower extremities (40-50%), trunk (20%), upper limbs (15%), and head/neck (5%). SM are elevated lesions, mostly of them are larger than 1 cm in diameter and can have pink to black coloration. The majority are asymmetrical, with coloration variety, present shiny white lines, and polymorphous vascular patterns. (26).

Pathway V. Acral melanoma

Acral melanomas arise on the non-hair bearing skin, especially in the lower extremities (78%), comprises about 2-3% of all melanomas. They have a high number of structural chromosomal changes and lower frequencies of BRAF mutations (10-23%), KIT mutations (3-29%), amplification of CCND1 and CDK4, and deletion/mutations in CDKN2A, PTEN, NF1 and hTERT (27). They have a high expression of melanoma markers, such as S-100 (95%), SOX10 (100%), Melan-A (70%), and HMB-45 (80%) observed in immunohistochemistry (IHC) (27). The most characteristic alteration on the signal pathways is the mitogen activated protein kinase (MAPK), the most frequent of these are BRAF mutations at position 600 (V600E, V600K, V600D and V600R). The MAPK

pathway contributes to many aspects of the oncogenic behavior of melanoma cells including uncontrolled proliferation by enhanced Cyclin D1 (CCND1) expression and suppression of p27, immune escape by inhibiting expression of major histocompatibility complex-I (MHC-I), and invasion by regulation of integrins and cytoskeleton proteins. In addition, TERT promoter mutations, were observed in about 5-10%, which can be associated with melanoma progression (27).

Pathway VI. Mucosal melanoma

Primary mucosal melanomas (MM) are derived from neural crest cells that migrate to several sites, they can be found in the respiratory, gastrointestinal, and genitourinary tract, it represent about 0.8-3.7% of all melanomas. They are associated with aggressive and less favorable prognoses. C-KIT is overexpressed (80%), BRAF mutations are less common (<10%) and SF3B1 mutations (12%) cause directly aberrant gene transcripts which lead to mRNA degradation or abnormal protein function in MM. There are some specific risk factors such as tobacco, and formaldehyde (associated with oral and sinonasal MM), and human immunodeficiency virus (HIV) infection (associated with rectal MM) (28,29).

Pathway VII. Melanoma arising from congenital melanocytic nevi

Congenital melanocytic nevi (CMN) are hamartomas of the neuroectoderm, they are seen in about 1-6% of all birth, and they are caused by genetic mosaicism. Large/giant CMN occur in 1/20,000-50,000 births. They can be classified by its size in small (<1.5 cm), medium (1.5-20 cm), and large (>20 cm). BRAF mutations are mostly presented in small nevi, and NRAS mutations in large/giant CMN. Melanoma risk is difficult to quantify, but there is a high risk in lesions that lie across the spine or those who has numerous satellite lesions (10-15% of risk) (30). Large/giant CMN have a risk of 2% to transformation to malignant melanoma (31).

Pathway VIII. Melanoma arising from blue nevi

As mentioned, pathway VIII is an UV-unrelated group. This type of pathway is associated with chromosomal aberrations added to a precursor lesion, blue nevi. Blue Nevis are uncommon lesions. They express GNAQ and GNA11 mutations, and infrequently in PLCB4 or CYSLTR2, EIF1AX, SF3B1 and BAP1 mutations. In addition, the gain of chromosomal arms 1q, 4p, 6p and losses of 1p and 4q have been identified (33).

Pathway IX. Uveal melanoma

The eye is an immune-privileged organ, so, intraocular environment is considered an immunosuppressive environment, where melanoma can proliferate, invade, and progress to metastasis. Uveal melanoma (UM) is a rare disease, and it has been demonstrated that it is different from its cutaneous counterpart. More than 90% involve choroid, 6% are confined to the ciliary body and 4% to the iris. They represent the most frequent intraocular primary tumor in adults (34). They are usually unilateral and associated with light-colored eyes, congenital ocular melanocytosis, melanocytoma and the BAP1-tumor predisposition syndrome. About 85% of UMs carry GNAQ and GNA11 mutations, 10% LI29 CYSTLTR2 and D630 PLCB4 mutations (35).

Pathway X. Nodular melanoma

Nodular melanomas arise from any of the pathways mentioned above, that's why they have heterogeneous epidemiologic and genomic features. They are characterized to be nodular or papular at the clinical examination, with homogeneous or heterogeneous pigment. BRAF and NRAS mutations have been demonstrated in these kinds of tumors, however, its genomic alterations are still unknown (33).

Nowadays, there is a molecular classification of melanoma, with prognostic importance, however it has not yet been added to the current WHO classification.

I. BRAF-mutant: about 60% presents CDKN2A mutation, TP53 mutation (10%), ARID2 mutated (15%), PPP6C mutated (10%), PDL1 and MITF amplification (36).

II. RAS-mutant: CDKN2A mutated (about 70%), CCDN1 amplification (10%), TP53 mutation (20%), ARID2 mutation (15%) and PPP6C mutation (15%) (36).

III. NF1-mutant: CDKN2A mutation (70%), RB1 mutation (10%), TP53 mutation (30%), and ARID2 mutation (30%) (36).

IV. Triple Wildtype: CDKN2A mutation (40%), CDK4 amplification (15%), CCDN1 amplification (10%), and MDM2 amplification (15%) (36).

Tumorigenesis in melanoma cells is regulated by multiple signaling pathways, modulated by genetic and epigenetic mechanisms, with a straight interrelation between them (3,4,13–22,36–38) (Figure 1).

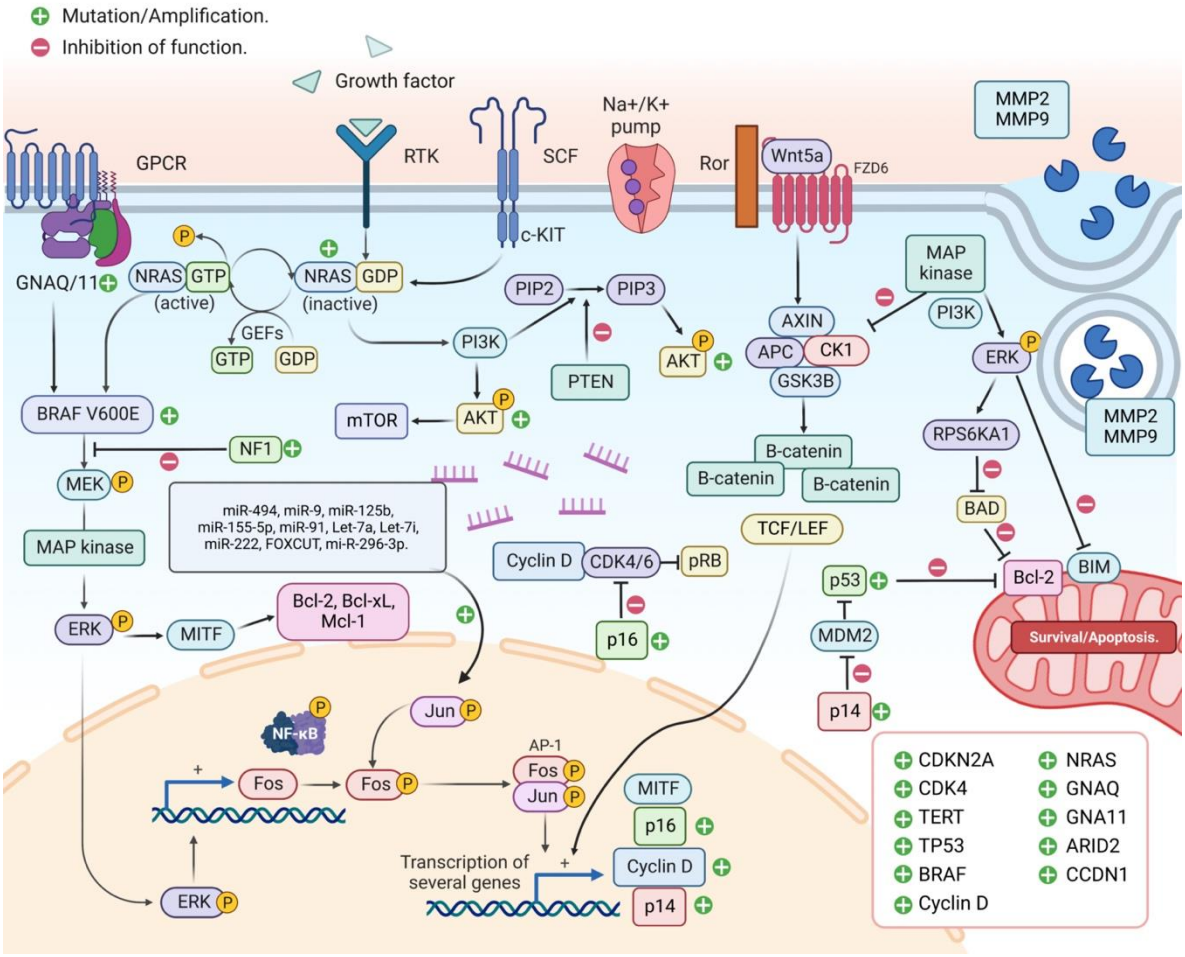

Figure 1. Genetic and epigenetic mechanisms of malignancy in melanoma.

Molecular mechanisms implicated in pathogenesis

Melanoma biology

Melanocytes are a heterogeneous group of cells, derived from the neural crest. They produce the protective skin-darkening pigment melanin in epidermis, hair, and iris, which is responsible of the protection of DNA from UV-mediated damage (39). Nowadays it has been observed that melanocytes are essential not only for UV-mediated damage protection since they have been found in the inner ear, nervous system, and heart (40).

Cutaneous melanoma is the most aggressive skin cancer; it derives from melanocytes. It accounts about 90% of melanomas including mucosal and uveal melanomas and represents about 1% of all skin cancers. The biology of the tumor is associated with the microenvironment, it has been demonstrated the hypoxic and acidity of microenvironment as an important role in melanoma biology (37). Like other solid cancers, melanomas need to increase glucose uptake, to support the high proliferation rate by upregulating glucose transporters and carbonic anhydrase, with the generation of L-lactate by the Warburg Effect, and the actively exporting protons in the extracellular microenvironment (41). The acidity generated by protons exported inhibits the proliferation of CD4+ and CD9+ Cytotoxic T Lymphocytes (CTL), Dendritic cells and Natural Killer cells (NK's), and activates proteolytic enzymes, such as matrix

metalloproteinases 2 (MMP2), cathepsin B, and cathepsin L, which are responsible for the degradation of extracellular matrix and the potential ability of invasion and metastasis. Hypoxic environment is common in advanced cancers, with local destruction, and necrosis, which activates hypoxia-inducible factors (HIF), such as HIF1, HIF2, and HIF3, promoting adaptation of cancer and stromal cells. In addition, tumor cells secret proteins, lipids, and nucleic acids by extracellular vesicles, which can dysregulate the physiological functions of extracellular matrix and cells (37). Currently, it has been described nanosized vesicles (30-120 nm), known as Exo, are involved in angiogenesis, tumor growth, and metastasis, by transporting active molecules, such as interleukins, vascular endothelial growth factor (VEGF), MMP-2, MMP-9 (42,43), and miRNAs, which are small non-coding RNAs (miR-494 (44), miR-9 (45), miR-125b (45,46), miR-155-5p (47), miR-91, Let-7a, Let-7i, miR-222 (46), FOXC promoter upstream transcript (FOXCUT) and miRNA has-miR-296-3p (38)). Mexo, an important protein needed to vehiculate molecules, can enter through the lymphatic vessels and their role is the formation of the pre-metastatic niche (PMN), by inducing members of LOX family (LOXL2 and LOXL4) and recruiting CD11b+ Ly6CmedLy6G+ myeloid cells and Cd4+ CD25 hiFOXP3+ Tregs, which secrete anti-inflammatory and pro-angiogenic factors, associated with the poor immunological response (37).

Acidosis plays an additional role by the dedifferentiation of cancer cells, to an immature phenotype, commonly known as Cancer Stem-like Cells (CSC), with the ability to self-renew and keep them in a quiescent state responsible for chemotherapy and radiotherapy resistance (37). These types of cells are immature and poor differentiated cells, so, they can be identified as the high expression of dedifferentiated surface cell markers and a low differentiated surface cell marker. The markers commonly associated with an immature melanocyte state are CD271 (known as p75) and CD133, drug resistance (ATP-binding cassette transporters, ABCs) and a high activity of ALDH1A1 and ALDH1A3 (48).

Nowadays, it is demonstrated that lipid metabolism is implicated in promoting melanoma progression. Carnitine palmitoyltransferase 2 (CPT2), phospholipase D3 (PLD3), inositol triphosphate protein kinase B (ITPKB), and inositol triphosphate receptor 3 (ITRP3), genes that encode lipid metabolism proteins, are significantly upregulated genes in melanomas compared with benign nevi, and their expression is associated with melanoma pathogenesis. However, the role of this kind of proteins in melanoma pathogenesis is still unclear (41).

Microenvironment

Melanoma is one of the most immunogenic tumors, so its microenvironment has a high concentration of infiltrating immune cells, however, most of them, are inhibitory immune populations, including T regulatory (T reg) cells, tissue-associated macrophages (TAMs) and myeloid-derived immunosuppressive cells (MDSCs) (27). Melanoma, like most of the tumors, manipulates immune defenses by intrinsic and extrinsic pathways (2). These pathways are known as “hallmarks” of cancer(49). At the same time that melanoma acquires chromosomal alterations, it also acquires different characteristics in contrast with melanocytes, which include resisting cell death, deregulating cellular metabolism, sustaining proliferative signaling (50), evading growth suppressors, avoiding immune destruction, enabling replicative immortality, tumor-promoting inflammation, activating invasion and metastasis (51), inducing or accessing vasculature, unlocking phenotypic plasticity (52), no mutational epigenetic reprogramming (53,54) and senescence (55). Immune evasion is necessary for tumor growth and progression. The microenvironment is the most important component of its immune response protection (56,57).

The most frequent inflammatory cells in the melanoma microenvironment are CD163+ histiocytes, CD3+ T lymphocytes, CD68+ histiocytes, cytotoxic CD8+ T lymphocytes, CD4+ regulatory T cells (58,59) and, CD20+ B lymphocytes. The low expression of p16 protein expression, low density of CD3+, and CD8+ cells is associated with poor prognosis by immunosuppressive statement, and with melanoma immune escape (27). CD4+ regulatory T cell is an important subtype of T cell in charge of downregulating the intensive inflammation, by secreting immunosuppressive cytokines (IL-10, IL-35, and TGF-B), inducing cytolysis by CD8+ T cells, targeting dendritic cells, and disrupting the immune

function of the cells. CD4+ regulatory T cells are increased in the tumor microenvironment (58,59), lymph nodes (60), and peripheral blood, so they are involved in melanoma progression and metastasis (2). Melanoma cells induce differentiation of myeloid cells in the bone marrow, into MDSC (61–63). MDSCs differentiate into TAMs, which are subdivided into M1 and M2 phenotypes. M1 phenotype is an anti-tumor subtype of TAM, while M2 phenotype promotes tumor progression and invasion (64,65).

The immune system has an efficient recognition of tumor cells, by presenting melanoma antigens to T cells, which can expand and become effector melanoma-specific T cells. Two immune checkpoints can upregulate or downregulate the immune stimulation: cytotoxic T lymphocyte antigen 4 (CTLA-4), a coinhibitory molecule on T cells that inhibits cells activation by ligation with CD86 and CD80; and programmed death 1 (PD-1), another immune checkpoint, that can be inhibited by programmed death 1 ligand (PD-L1 and PD-L2) expressed in tumor cells (66,67). PD-1/PD-L1 acts as a negative regulator of immune response. Healthy cells express PD-L1 in their membrane surfaces, which interacts with PD-1 receptors in T lymphocytes and prevents T lymphocyte activation. This immune protection mechanism is observed in surrounding healthy cells in an infection site (66,67); however, this physiological mechanism is used by tumor cells to evade immune response (68), which is upregulated by HIF-1, AP-1, and NF-KB transcription factors (69).

In addition to PD-1, CTLA-4 is the second most frequently known immune suppressive checkpoint regulator, its function is associated with immune suppressive activities by inhibiting T cell activation. CTLA-4 outcompetes CD28 for the ligands, CD80/CD86, in consequence, T cells become anergic (70).

Diagnosis

The diagnostic approach starts with dermoscopic evaluation, it's necessary to describe the skin lesion with the mnemotechnic ABCDE (Asymmetry (the most common criterion: 84.5%), Border, Color (the multicomponent pattern is the most characteristic and most common patient associated with melanoma), Diameter and Evolution) as seen in Figure 2. Dermoscopy is a fundamental for early diagnosis and in the preoperative estimate of the Breslow index (71), however there are some characteristics in the visual examination that it's necessary to be considered before the examination: we can recognize different dermoscopic structures with their different accuracy, such as atypical pigment network (Sensitivity: 21-100%; Specificity: 46-88.5%), angulated lines (Sensitivity: 16.7%; Specificity: 91.7%), negative network (Sensitivity: 22-34.6%; Specificity: 77.2-95%), atypical streaks (Sensitivity: 4.8-23%; Specificity:32-58%), atypical dots/globules (Sensitivity: 13-39.6%; Specificity: 74.3-92%), blue-white veil (Sensitivity: 11.4-92%; Specificity: 74-99%), atypical blotch (Sensitivity: 18-71.3%; Specificity: 30.5-92.6%), regression structures (Sensitivity: 11.4-79%; Specificity: 63-99%), peripheral tan structureless area (Sensitivity: 19-62.5%; Specificity: 92.6-96.1%), shiny white structures (Sensitivity: 70%; Specificity: 80.6%), and finally, atypical vascular structures (Sensitivity: 9.4-62.9%; Specificity: 53.8-96.1%) (72).

Most patients with cutaneous melanoma are asymptomatic, and they come to clinical care only in the presence of a suspicious injury. At the same time, patients with UM are asymptomatic (>40%), those who present symptoms may develop blurred or distorted vision, visual field loss of photopsia, or other ocular symptoms, rarely large tumors induce vitreous hemorrhage (34,35) To make easier the diagnosis of distance and nodular metastasis, extension to adjacent structures and recurrence, there are some diagnostic methods, from examination by imaging to molecular biomarkers, and their accuracy is compared to each other, like seen in Table 2.

The visual inspection of a suspicious skin lesion is the first step in melanoma diagnosis, its sensitivity is about 76% (66-85%) and specificity 75% (57-87%) (71–73) (Figure 2).

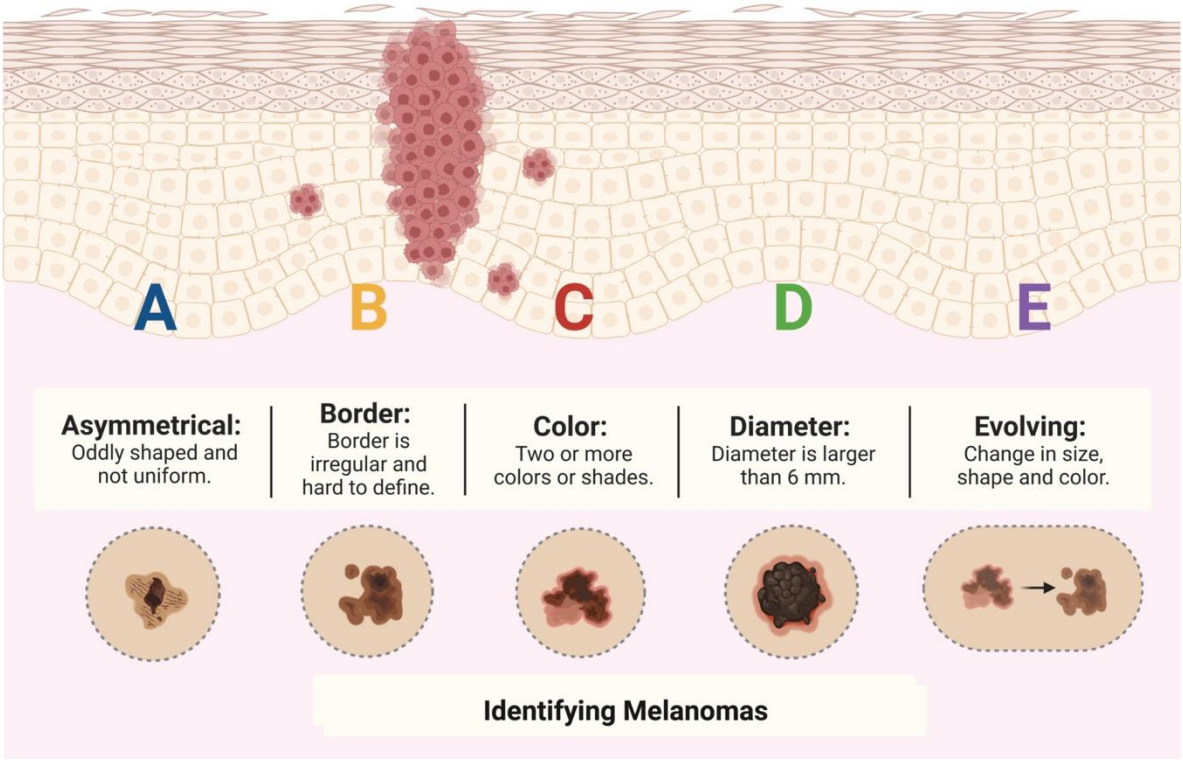

Figure 2. ABCDE for identifying melanoma.

Table 2. Accuracy of several methods used in melanoma diagnosis and staging.

Diagnostic method.	Accuracy.		Characteristics.
	Sensitivity.	Specificity.	
Visual inspection	76% (66-85%) (73)	75% (57-87%) (73).	Clinical inspection of pigmented skin lesions using the mnemonic ABCDE (73)
Dermoscopy	Without Artificial Intelligence Support (53.3-65.5%) With Artificial Intelligence Support (81.9-87.6%) (74)	Without Artificial Intelligence Support (62.3-78.9%) With Artificial Intelligence Support (74.8-83.4%) (74)	It's the examination of pigmented and non-pigmented skin lesions with the naked eye (75) With artificial intelligence support like reflectance confocal microscopy increases accuracy (74)
Histopathology	91% (84-95%) (73)	94% (86-98%) (73).	The histological examination of a pigmented skin lesion. It's considered the gold standard for melanoma diagnosis (73)
Immunohistochemistry (IHC)	Adjuvant to histopathology, it consists in the examination of melanoma antigens using anti-H4K20me and anti-H3K27me3 monoclonal antibodies, which interact with their respective antigens (76)		
Comparative Genomic Hybridization (CGH)	92-96% (77)	87-100% (77)	Adjuvant to histopathology detects genome-wide changes in DNA copy number, but it doesn't detect actual mutations. It can detect genetic anomalies in chromosomes 6p, 1q, 7p, 7q, 8q, 17q and 20q and/or losses of 9p, 9q, 10q, 10p, 6q and 11q (77)
Fluorescent In Situ Hybridization (FISH)	43-100% (77)	29-80% (77)	Adjuvant to histopathology detects cytogenetic abnormalities by direct visualization (77)
Ultrasound. (US)	Nodal metastasis 35.4% (17-59. 4%) (78)	Nodal metastasis 93.9% (86.1-97.5%) (78)	Ultrasound uses high-frequency sound waves to create images in the body, it can be used to assist in detection of lymph node metastasis (78)

Ultrasound with Fine Needle Aspiration Cytology (US FNAC)	Nodal metastasis 18% (3.58-56.5%) (78)	Nodal metastasis 99.8% (99.1-99.9%) (78)	The cytologic examination of skin lesions using a fine needle aspiration guided by ultrasound (78)
Computed Tomography (CT)	Nodal metastasis 87.2 (76.5-93.4%). Distant metastasis 73.4% (63.6-81.3%) (78)	Nodal metastasis 69.2% (34.6-90.5%). Distant metastasis 72% (64.3-78.5%) (78)	Uses ionizing radiation in the form of X-rays to take cross sectional images of the body, is used to evaluate metastasis (78)
Magnetic Resonance Imaging. (MRI)	Nodal metastasis 83.7% (68.8-92.3%). Distant metastasis 74.5% (62.1-83.9%) (78)	Nodal metastasis 77.7% (72.4-82.1%). Distant metastasis 85.8% (70.4-93.9%) (78)	Uses large magnets and non-ionizing radiation in the form of radio waves to generate images of the body, is used to evaluate metastasis (78)
Positron Emission Tomography (PET/CT).	Nodal metastasis 86.5% (80.2-91.1%). Distant metastasis 92.5% (85.3-.96.4%) Detection of bone metastasis 90.2% (78.5-95.9%) (78)	Nodal metastasis 92.5% (85-96.4%). Distant metastasis 89.7% (78.8-.95.3%) Detection of bone metastasis 88.2% (72.5-95.5%) (78)	A nuclear medicine imaging technique, it uses a radioactive component (^{18}FDG intravenous) which is taken up by cancer cells (78)

New treatment strategies

Most patients are diagnosed in early-stage disease, in which surgical excision is the treatment of choice, because it's curative in most of the cases (79). About 10% of new-diagnosed patients, presents an advanced-stage disease (unresectable or metastatic disease). They can be treated with kinase inhibitors (BRAF and MEK inhibitors) alone or in combination with immune checkpoint inhibitors (anti-CTLA4 and anti-PD1 monoclonal antibodies) (80).

BRAF is a serine/threonine protein kinase, encoded on chromosome 7q34, which activates the MAPK/ERK-signaling pathway. The most frequent BRAF mutation (90%) is located at codon 600, in which a single nucleotide mutation results in the substitution of glutamic acid for valine (V600E) (81). Melanomas with BRAF V600E mutation are associated with poor prognosis by promoting angiogenesis, immune evasion, invasion, and metastasis, whose can be used BRAF inhibitors such as dabrafenib and vemurafenib, however, the upregulation of MEK 1/2 is associated with a prominent escape from the mechanism, so it's necessary to use a combination of BRAF inhibitor and a MEK inhibitor (such as trametinib), demonstrating a survival advantage in both resectable and unresectable/metastatic disease (80).

As mentioned above, melanoma cells express PD-L1 in their membrane surfaces, and the interaction of CTLA-4 in T cells membrane surfaces results in T cell anergy. These two immune checkpoints are important for an effective immune response. Immune checkpoint inhibitors play key roles, when a tumor does not have targeted mutations, or it does not respond to BRAF/MEK inhibitors. There are two types of immune checkpoint inhibitors, PD-1 inhibitors (nivolumab and pembrolizumab) and CTLA-4 antibody inhibitors (ipilimumab). The inhibition of these two immune checkpoints helps the immune system to recognize cancer cells by suppressing melanoma's immune evasion system (82). The combination of both types is associated with a high inflammatory cell infiltration compared with ipilimumab alone (80). Nowadays, these new treatment strategies are considered the backbone of systemic therapy, while chemotherapy is considered the second line of treatment (83) since in one systematic review made by Pasquali S, et al. using Cochrane Library Database concluded that the combination of anti-CTLA4 plus anti-PD1 monoclonal antibodies was associated with better progression-free survival (HR 0.40, 95% CI 0.35 to 0.46, 2 studies, 738 participants); and the combination of BRAF plus MEK

inhibitors was associated with better overall survival (HR 0.70, 95% CI 0.59 to 0.82, 4 studies, 1784 participants) (82).

Future directions

Numerous phase I and II clinical trials are currently underway to explore innovative agents and multimodal approaches to enhance the prognosis of patients facing melanoma. Many of these trials are centered on monoclonal antibodies, which represent vital components of targeted strategies in the era of precision medicine. While monoclonal antibodies hold considerable promise, their mechanism of action often entails inhibiting critical pathways associated with melanoma pathogenesis. Consequently, these interactions can lead to adverse effects.

Discussion

Various researchers have conducted exhaustive investigations into the mechanisms discussed earlier, underscoring their significance in driving carcinogenesis in melanocytes and their correlation with various molecular subclassifications. While new treatment strategies have emerged based on these mechanisms, some still lack targeted therapies, necessitating further research into the yet uncharted direct and indirect contributors to tumorigenesis. Genetic, epigenetic alterations and tumor microenvironment have all been associated with this unfavorable prognosis due to their facilitation of uncontrolled proliferation of malignant cells. Therefore, this article seeks to consolidate valuable insights on melanoma, to contribute to the formulation of treatment strategies.

Conclusions

Melanoma is the most aggressive skin cancer, with poor prognosis and high mortality. Its pathogenesis encompasses many molecular mechanisms, incorporating genetic and epigenetic factors. These mechanisms operate within various signaling pathways, often displaying interconnectedness and interplay. They exert their influence on pro- and anti-apoptotic proteins, sculpting the microenvironment by regulating cell proliferation, invasiveness, and immune evasion. Intriguingly, these emerging mechanisms are not confined to melanoma but are also observed in other solid tumors, including breast, colorectal, urogenital, pancreatic, and lung tumors. Nowadays, these new molecular mechanisms open the possibility of investigating new alternatives for possible targeted therapies. The primary objective of this review article is to provide a comprehensive account of the molecular mechanisms involved in melanoma pathogenesis and how the alteration of any of them leads to the genesis of cancer, to foster the development of novel targeted therapy strategies.

Author contribution

This manuscript was written entirely by **JMM**.

Conflict of interest

The authors report no conflict of interest.

References

1. Dubbini N, Puddu A, Salimbeni G, Malloggi S, Gandini D, Massei P, et al. Melanoma prevention: Comparison of different screening methods for the selection of a high risk population. Int J Environ Res Public Health. 2021 Feb 2;18(4):1–10.
2. Eddy K, Chen S. Overcoming immune evasion in melanoma. Vol. 21, International Journal of Molecular Sciences. MDPI AG; 2020. p. 1–48.
3. Oba J, Woodman SE. The genetic and epigenetic basis of distinct melanoma types. Vol. 48, Journal of Dermatology. Blackwell Publishing Ltd; 2021. p. 925–39.
4. Amalinei C, Grigoraș A, Lozneanu L, Căruntu ID, Giușcă SE, Balan RA. The Interplay between Tumour Microenvironment Components in Malignant Melanoma. Vol. 58, Medicina (Lithuania). MDPI; 2022.
5. Emri G, Paragh G, Tósaki Á, Janka E, Kollár S, Hegedűs C, et al. Ultraviolet radiation-mediated development of cutaneous melanoma: An update. J Photochem Photobiol B. 2018 Aug 1;185:169–75.
6. Dzwierzynski WW. Melanoma Risk Factors and Prevention. Vol. 48, Clinics in Plastic Surgery. W.B. Saunders; 2021. p. 543–50.
7. Raimondi S, Suppa M, Gandini S. Melanoma epidemiology and sun exposure. Acta Derm Venereol. 2020;100(100-year theme Skin malignancies):250–8.
8. Bataille V. It's not all sunshine: Non-sun-related melanoma risk-factors. Vol. 100, Acta

Dermato-Venereologica. Medical Journals/Acta D-V; 2020. p. 259–65.
9. Warner AB, McQuade JL. Modifiable Host Factors in Melanoma: Emerging Evidence for Obesity, Diet, Exercise, and the Microbiome. Vol. 21, Current Oncology Reports. Current Medicine Group LLC 1; 2019.
10. O'Neill CH, Scoggins CR. Melanoma. Vol. 120, Journal of Surgical Oncology. John Wiley and Sons Inc.; 2019. p. 873–81.
11. Carr S, Smith C, Wernberg J. Epidemiology and Risk Factors of Melanoma. Vol. 100, Surgical Clinics of North America. W.B. Saunders; 2020. p. 1–12.
12. Motofei IG. Malignant Melanoma: Autoimmunity and Supracellular Messaging as New Therapeutic Approaches. Vol. 20, Current Treatment Options in Oncology. Springer New York LLC; 2019.
13. van Poppelen NM, de Bruyn DP, Bicer T, Verdijk R, Naus N, Mensink H, et al. Genetics of ocular melanoma: Insights into genetics, inheritance and testing. Vol. 22, International Journal of Molecular Sciences. MDPI AG; 2021. p. 1–19.
14. Pawlowska E, Szczepanska J, Szatkowska M, Blasiak J. An interplay between senescence, apoptosis and autophagy in glioblastoma multiforme—role in pathogenesis and therapeutic perspective. Vol. 19, International Journal of Molecular Sciences. MDPI AG; 2018.
15. Quan VL, Panah E, Zhang B, Shi K, Mohan LS, Gerami P. The role of gene fusions in melanocytic neoplasms. Vol. 46, Journal of Cutaneous Pathology. Blackwell Publishing Ltd; 2019. p. 878–87.
16. Toussi A, Mans N, Welborn J, Kiuru M. Germline mutations predisposing to melanoma. Vol. 47, Journal of Cutaneous Pathology. Blackwell Publishing Ltd; 2020. p. 606–16.
17. Fang S, Lu J, Zhou X, Wang Y, Ross MI, Gershenwald JE, et al. Functional annotation of melanoma risk loci identifies novel susceptibility genes. Carcinogenesis. 2020 Jun 17;41(4):452–7.
18. Chen J, Sun W, Mo N, Chen X, Yang L, Tu S, et al. Identification of key genes involved in the pathogenesis of cutaneous melanoma using bioinformatics analysis. Journal of International Medical Research. 2020 Jan 1;48(1).
19. Kulesza DW, Ramji K, Maleszewska M, Mieczkowski J, Dabrowski M, Chouaib S, et al. Search for novel STAT3-dependent genes reveals SERPINA3 as a new STAT3 target that regulates invasion of human melanoma cells. Laboratory Investigation. 2019 Nov 1;99(11):1607–21.
20. Teixido C, Castillo P, Martinez-Vila C, Arance A, Alos L. Molecular markers and targets in melanoma. Vol. 10, Cells. MDPI; 2021.
21. Dika E, Riefolo M, Porcellini E, Broseghini E, Ribero S, Senetta R, et al. Defining the Prognostic Role of MicroRNAs in Cutaneous Melanoma. Journal of Investigative Dermatology. 2020 Nov 1;140(11):2260–7.
22. Santourlidis S, Schulz WA, Araúzo-Bravo MJ, Gerovska D, Ott P, Bendhack ML, et al. Epigenetics in the Diagnosis and Therapy of Malignant Melanoma. Vol. 23, International Journal of Molecular Sciences. MDPI; 2022.
23. Elder DE, Bastian BC, Cree IA, Massi D, Scolyer RA. The 2018 World Health Organization classification of cutaneous, mucosal, and uveal melanoma detailed analysis of 9 distinct subtypes defined by their evolutionary pathway. Vol. 144, Archives of Pathology and Laboratory Medicine. College of American Pathologists; 2020. p. 500–22.
24. Deluca E V., Deluca E V., Perino F, Distefani A, Coco V, Fossati B, et al. Lentigo maligna: Diagnosis and treatment. Vol. 155, Giornale Italiano di Dermatologia e Venereologia. Edizioni Minerva Medica; 2020. p. 179–89.
25. Iznardo H, Garcia-Melendo C, Yélamos O. Lentigo maligna: Clinical presentation and appropriate management. Vol. 13, Clinical, Cosmetic and Investigational Dermatology. Dove Medical Press Ltd; 2020. p. 837–55.
26. Cheng TW, Ahern MC, Giubellino A. The Spectrum of Spitz Melanocytic Lesions: From Morphologic Diagnosis to Molecular Classification. Front Oncol. 2022 Jun 7;12.
27. Chen YA, Teer JK, Eroglu Z, Wu JY, Koomen JM, Karreth FA, et al. Translational pathology, genomics and the development of systemic therapies for acral melanoma. Vol. 61, Seminars in Cancer Biology. Academic Press; 2020. p. 149–57.
28. Yde SS, Sjoegren P, Heje M, Stolle LB. Mucosal Melanoma: a Literature Review. Vol. 20, Current Oncology Reports. Current Medicine Group LLC 1; 2018.

29. Ma Y, Xia R, Ma X, Judson-Torres RL, Zeng H. Mucosal Melanoma: Pathological Evolution, Pathway Dependency and Targeted Therapy. Vol. 11, Frontiers in Oncology. Frontiers Media S.A.; 2021.
30. Farabi B, Akay BN, Goldust M, Wollina U, Atak MF, Rao B. Congenital melanocytic naevi: An up-to-date overview. Vol. 62, Australasian Journal of Dermatology. Blackwell Publishing; 2021. p. e178–91.
31. Moustafa D, Blundell AR, Hawryluk EB. Congenital melanocytic nevi. Vol. 32, Current opinion in pediatrics. NLM (Medline); 2020. p. 491–7.
32. Ferrara G, Argenziano G. The WHO 2018 Classification of Cutaneous Melanocytic Neoplasms: Suggestions From Routine Practice. Vol. 11, Frontiers in Oncology. Frontiers Media S.A.; 2021.
33. Elder DE, Bastian BC, Cree IA, Massi D, Scolyer RA. The 2018 World Health Organization classification of cutaneous, mucosal, and uveal melanoma detailed analysis of 9 distinct subtypes defined by their evolutionary pathway. Vol. 144, Archives of Pathology and Laboratory Medicine. College of American Pathologists; 2020. p. 500–22.
34. Branisteanu D, Bogdanici C, Branisteanu D, Maranduca M, Zemba M, Balta F, et al. Uveal melanoma diagnosis and current treatment options (Review). Exp Ther Med. 2021 Oct 11;22(6).
35. Jager MJ, Shields CL, Cebulla CM, Abdel-Rahman MH, Grossniklaus HE, Stern MH, et al. Uveal melanoma. Nat Rev Dis Primers. 2020 Dec 1;6(1).
36. Tímár J, Ladányi A. Molecular Pathology of Skin Melanoma: Epidemiology, Differential Diagnostics, Prognosis and Therapy Prediction. Vol. 23, International Journal of Molecular Sciences. MDPI; 2022.
37. Boussadia Z, Gambardella AR, Mattei F, Parolini I. Acidic and hypoxic microenvironment in melanoma: Impact of tumour exosomes on disease progression. Vol. 10, Cells. MDPI; 2021.
38. Napoli S, Scuderi C, Gattuso G, Bella V Di, Candido S, Basile MS, et al. Functional Roles of Matrix Metalloproteinases and Their Inhibitors in Melanoma. Vol. 9, Cells. NLM (Medline); 2020.
39. Ostrowski SM, Fisher DE. Biology of Melanoma. Vol. 35, Hematology/Oncology Clinics of North America. W.B. Saunders; 2021. p. 29–56.
40. Brito FC, Kos L. Timeline and distribution of melanocyte precursors in the mouse heart. Pigment Cell Melanoma Res. 2008 Aug;21(4):464–70.
41. Fischer GM, Vashisht Gopal YN, McQuade JL, Peng W, DeBerardinis RJ, Davies MA. Metabolic strategies of melanoma cells: Mechanisms, interactions with the tumor microenvironment, and therapeutic implications. Vol. 31, Pigment Cell and Melanoma Research. Blackwell Publishing Ltd; 2018. p. 11–30.
42. Ekström EJ, Bergenfelz C, von Bülow V, Serifler F, Carlemalm E, Jönsson G, et al. WNT5A induces release of exosomes containing pro-angiogenic and immunosuppressive factors from malignant melanoma cells. Mol Cancer. 2014 Apr 26;13(1).
43. Hood JL. Melanoma exosome induction of endothelial cell GM-CSF in pre-metastatic lymph nodes may result in different M1 and M2 macrophage mediated angiogenic processes. Med Hypotheses. 2016 Sep 1;94:118–22.
44. Li J, Chen J, Wang S, Li P, Zheng C, Zhou X, et al. Blockage of transferred exosome-shuttled miR-494 inhibits melanoma growth and metastasis. J Cell Physiol. 2019 Sep 1;234(9):15763–74.
45. Alegre E, Sanmamed MF, Rodriguez C, Carranza O, Martín-Algarra S, González Á. Study of circulating MicroRNA-125b levels in serum exosomes in advanced melanoma. Arch Pathol Lab Med. 2014;138(6):828–32.
46. Xiao D, Barry S, Kmetz D, Egger M, Pan J, Rai SN, et al. Melanoma cell-derived exosomes promote epithelial-mesenchymal transition in primary melanocytes through paracrine/autocrine signaling in the tumor microenvironment. Cancer Lett. 2016 Jul 1;376(2):318–27.
47. Zhou X, Yan T, Huang C, Xu Z, Wang L, Jiang E, et al. Melanoma cell-secreted exosomal miR-155-5p induce proangiogenic switch of cancer-associated fibroblasts via SOCS1/JAK2/STAT3 signaling pathway. Journal of Experimental and Clinical Cancer Research. 2018 Oct 3;37(1).
48. Marzagalli M, Raimondi M, Fontana F, Montagnani Marelli M, Moretti RM, Limonta P. Cellular and molecular biology of cancer stem cells in melanoma: Possible therapeutic implications. Vol. 59, Seminars in Cancer Biology. Academic Press; 2019. p. 221–35.
49. Hanahan D. Hallmarks of Cancer: New Dimensions. Cancer Discov [Internet]. 2022 Jan 1 [cited 2023 Oct 23];12(1):31–46. Available from: https://dx.doi.org/10.1158/2159-8290.CD-21-1059

50. Cheng N, Chytil A, Shyr Y, Joly A, Moses HL. Transforming growth factor-beta signaling-deficient fibroblasts enhance hepatocyte growth factor signaling in mammary carcinoma cells to promote scattering and invasion. Mol Cancer Res. 2008 Oct 1;6(10):1521–33.
51. Tan SH, Barker N. Stemming Colorectal Cancer Growth and Metastasis: HOXA5 Forces Cancer Stem Cells to Differentiate. Cancer Cell. 2015 Dec 14;28(6):683–5.
52. Perekatt AO, Shah PP, Cheung S, Jariwala N, Wu A, Gandhi V, et al. SMAD4 Suppresses WNT-Driven Dedifferentiation and Oncogenesis in the Differentiated Gut Epithelium. Cancer Res. 2018 Sep 1;78(17):4878–90.
53. Yu X, Qiu W, Yang L, Zhang Y, He M, Li L, et al. Defining multistep cell fate decision pathways during pancreatic development at single-cell resolution. EMBO J. 2019 Apr 15;38(8).
54. Darwiche N. Epigenetic mechanisms and the hallmarks of cancer: an intimate affair. Am J Cancer Res. 2020;10(7):1954.
55. Wang B, Kohli J, Demaria M. Senescent Cells in Cancer Therapy: Friends or Foes? Trends Cancer. 2020 Oct 1;6(10):838–57.
56. Tao J, Li Y, Liu YQ, Wang L, Yang J, Dong J, et al. Restoration of the expression of transports associated with antigen processing in human malignant melanoma increases tumor-specific immunity. Journal of Investigative Dermatology. 2008;128(8):1991–6.
57. Gajewski TF, Schreiber H, Fu YX. Innate and adaptive immune cells in the tumor microenvironment. Vol. 14, Nature Immunology. 2013. p. 1014–22.
58. Fujii H, Josse J, Tanioka M, Miyachi Y, Husson F, Ono M. Regulatory T Cells in Melanoma Revisited by a Computational Clustering of FOXP3+ T Cell Subpopulations. The Journal of Immunology. 2016 Mar 15;196(6):2885–92.
59. Magnuson AM, Kiner E, Ergun A, Park JS, Asinovski N, Ortiz-Lopez A, et al. Identification and validation of a tumor-infiltrating Treg transcriptional signature conserved across species and tumor types. Proc Natl Acad Sci U S A. 2018 Nov 6;115(45):E10672–81.
60. Viguier M, Lemaître F, Verola O, Cho MS, Gorochov G, Dubertret L, et al. Foxp3 Expressing CD4+CD25high Regulatory T Cells Are Overrepresented in Human Metastatic Melanoma Lymph Nodes and Inhibit the Function of Infiltrating T Cells. The Journal of Immunology. 2004 Jul 15;173(2):1444–53.
61. Umansky V, Sevko A, Gebhardt C, Utikal J. Myeloid-derived suppressor cells in malignant melanoma. Vol. 12, JDDG - Journal of the German Society of Dermatology. Wiley-VCH Verlag; 2014. p. 1021–7.
62. Gabrilovich DI. Myeloid-derived suppressor cells. Cancer Immunol Res. 2017 Jan 1;5(1):3–8.
63. Condamine T, Ramachandran I, Youn JI, Gabrilovich DI. Regulation of tumor metastasis by myeloid-derived suppressor cells. Annu Rev Med. 2015 Jan 14;66:97–110.
64. Fujimura T, Kambayashi Y, Fujisawa Y, Hidaka T, Aiba S. Tumor-associated macrophages: Therapeutic targets for skin cancer. Vol. 8, Frontiers in Oncology. Frontiers Media S.A.; 2018.
65. Jayasingam SD, Citartan M, Thang TH, Mat Zin AA, Ang KC, Ch'ng ES. Evaluating the Polarization of Tumor-Associated Macrophages Into M1 and M2 Phenotypes in Human Cancer Tissue: Technicalities and Challenges in Routine Clinical Practice. Vol. 9, Frontiers in Oncology. Frontiers Media S.A.; 2020.
66. Nishimura H, Honjo T. PD-1: an inhibitory immunoreceptor involved in peripheral tolerance. Trends Immunol. 2001 May;22(5):265–8.
67. Freeman GJ, Long AJ, Iwai Y, Bourque K, Chernova T, Nishimura H, et al. Engagement of the PD-1 Immunoinhibitory Receptor by a Novel B7 Family Member Leads to Negative Regulation of Lymphocyte Activation [Internet]. Vol. 192, J. Exp. Med. 2000. Available from: http://www.jem.org/cgi/content/full/192/7/1027
68. Zerdes I, Matikas A, Bergh J, Rassidakis GZ, Foukakis T. Genetic, transcriptional and post-translational regulation of the programmed death protein ligand 1 in cancer: biology and clinical correlations. Vol. 37, Oncogene. Nature Publishing Group; 2018. p. 4639–61.
69. Furue M, Ito T, Wada N, Wada M, Kadono T, Uchi H. Melanoma and Immune Checkpoint Inhibitors. Vol. 20, Current Oncology Reports. Current Medicine Group LLC 1; 2018.
70. Sansom DM. CD28, CTLA-4 and their ligands: Who does what and to whom? Vol. 101, Immunology. 2000. p. 169–77.

71. Trindade FM, de Freitas MLP, Bittencourt FV. Dermoscopic evaluation of superficial spreading melanoma. An Bras Dermatol. 2021 Mar 1;96(2):139–47.
72. Marghoob NG, Liopyris K, Jaimes N. Dermoscopy: A review of the structures that facilitate melanoma detection. Vol. 119, Journal of the American Osteopathic Association. American Osteopathic Association; 2019. p. 380–90.
73. Dinnes J, Deeks JJ, Grainge MJ, Chuchu N, Ferrante di Ruffano L, Matin RN, et al. Visual inspection for diagnosing cutaneous melanoma in adults. Vol. 2018, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2018.
74. Maron RC, Utikal JS, Hekler A, Hauschild A, Sattler E, Sondermann W, et al. Artificial intelligence and its effect on dermatologists' accuracy in dermoscopic melanoma image classification: Web-based survey study. J Med Internet Res. 2020 Sep 1;22(9).
75. Dinnes J, Deeks JJ, Chuchu N, Ferrante di Ruffano L, Matin RN, Thomson DR, et al. Dermoscopy, with and without visual inspection, for diagnosing melanoma in adults. Vol. 2018, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2018.
76. Davis LE, Shalin SC, Tackett AJ. Utility of histone H3K27me3 and H4K20me as diagnostic indicators of melanoma. Melanoma Res. 2020;159–65.
77. High WA. Detection of Genetic Aberrations in the Assessment and Prognosis of Melanoma. Vol. 35, Dermatologic Clinics. W.B. Saunders; 2017. p. 525–36.
78. Dinnes J, Di Ruffano LF, Takwoingi Y, Cheung ST, Nathan P, Matin RN, et al. Ultrasound, CT, MRI, or PET-CT for staging and re-staging of adults with cutaneous melanoma. Vol. 2019, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2019.
79. Testori AAE, Blankenstein SA, van Akkooi ACJ. Primary Melanoma: from History to Actual Debates. Vol. 21, Current Oncology Reports. Springer; 2019.
80. Aderhold K, Wilson M, Berger AC, Levi S, Bennett J. Precision Medicine in the Treatment of Melanoma. Vol. 29, Surgical Oncology Clinics of North America. W.B. Saunders; 2020. p. 1–13.
81. Wellbrock C, Karasarides M, Marais R. The RAF proteins take centre stage. Vol. 5, Nature Reviews Molecular Cell Biology. 2004. p. 875–85.
82. Pasquali S, Hadjinicolaou A V., Chiarion Sileni V, Rossi CR, Mocellin S. Systemic treatments for metastatic cutaneous melanoma. Vol. 2018, Cochrane Database of Systematic Reviews. John Wiley and Sons Ltd; 2018.
83. Leonardi GC, Falzone L, Salemi R, Zanghì A, Spandidos DA, Mccubrey JA, et al. Cutaneous melanoma: From pathogenesis to therapy (Review). Vol. 52, International Journal of Oncology. Spandidos Publications; 2018. p. 1071–80.

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Nonalcoholic fatty liver scoring panels shortcut for fibro scanning results or not

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Abstract

Introduction: Liver steatosis has a wide range of conditions from simple steatosis to non-alcoholic steatohepatitis, fibrosis, and eventually cirrhosis. Several panels and scoring systems have been introduced to differentiate steatosis with or without advanced fibrosis and also the degree of fibrosis. This study aimed to evaluate eleven different scoring panels in patients with steatosis and compare their results with Fibro Scan.

Methods: The study was performed on 122 NAFLD patients who were confirmed by ultrasound. The patients were referred to the gastroenterologist in Razi hospital in the north of Iran from September 2017 to April 2018. All patients underwent Fibro Scan. Multiple scoring systems were calculated using the laboratory values. These results were compared with the results of Fibro Scan. AUC for each panel was calculated.

Results: In This study, 62 (50.8%) were men. The mean age of the patients was 47.1±11.7 years. There were significant differences between patients with or without advanced fibrosis in three panels of APRI, NIPPON, and FIB4 (p=0.03, p=0.01, p=0.005, respectively). AUROC for APRI, NIPPON, and FIB4 were, 0.695 (CI=0.58-0.8, p=0.001), 0.642 (CI: 0.5-0.74, p=0.015) and 0.684 (CI: 0.5-0.7, p=0.002), respectively. None of the other panels had enough sensitivity for the diagnosis of advanced fibrosis.

Conclusion: Given the cost-effectiveness of panels, their ease of calculation, and noninvasiveness, FIB4, NIPPON and APRI can be used as useful tools for following, and also for predicting progression to advanced fibrosis.

Keywords: Nonalcoholic Fatty Liver Disease, Scoring Panels, Predicting

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Introduction

Non-alcoholic fatty liver (NAFLD) is formed with the pathological accumulation of fat in the liver (1) which is defined as the accumulation of fat in more than 5% of hepatocytes (2). Over the past 3 decades, fatty liver has become one of the most important chronic liver diseases in the world (3, 4). The highest prevalence of this disease belongs to western countries (5, 6). The prevalence of NAFLD in Asia is variable between 12-24 %. The prevalence of NAFLD is 2.9- 7.1% in Iran (7). The incidence of fatty liver is about 20 out of every 10,000 people per year. This disease has a wide range of conditions from simple steatosis to non-alcoholic steatohepatitis, fibrosis, and eventually cirrhosis and hepatocellular carcinoma (9).

Liver biopsy is the gold standard method for evaluating inflammation and severity and ranking fibrosis in NAFLD and non-alcoholic steatohepatitis (10). The biopsy is an invasive and also a difficult procedure that is associated with pain, the risk of complications, measurement errors, high cost, and the patient's unwillingness (11); therefore, the biopsy is not realistic for all NFLD patients and it is impractical (12, 13).

Alternative methods, and various tools for NAFLD are magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), ultrasound (absence of steatosis only), the enhanced liver fibrosis (ELF) score, transient elastography and NAFLD fibrosis score (13). These methods have some limitations, thus non-invasive, and reliable tests for this highly prevalent disease is important(14). Several panels and scoring systems from a combination of laboratory and clinical variables have been introduced to differentiate NAFLD with and without advanced fibrosis and to determine the degree of liver fibrosis. Most of them, to a large extent, have acceptable accuracy in distinguishing NAFLD with and without advanced fibrosis (10, 15, 16).

Our study aimed to evaluate 11 different scoring panels such as FIB4 [Age, AST, ALT, Platelets], APRI [AST platelet ratio index], AAR [Age, ALT/AST ratio], NFS [NAFLD fibrosis score], AP [Age, Platelets], BAAT [BMI, Age, ALT, TG] Score, BARD [BMI, AST/ALT ratio, DM) score, PLALA [platelet, albumin, AST/ALT ratio] score, N [Nippon]Score, FI [Platelets, Albumin], Forns index [platelet count, GGT, Age, total cholesterol] in patients with NAFLD and compare their results with Fibro Scan.

Methods

Patient

The sample size of this cross-sectional study was set as 122 patients. All patients with age 13-69 years were referred to the gastroenterologist in Razi hospital in the north of Iran from September 2017 to April 2018. The protocol of this study was approved by a local ethical committee of Guilan University of Medical Sciences (No. IR.GUMS.1396.114) and was based on the Declaration of Helsinki. Informed consent was obtained from all patients and all securities were applied to their data.

Inclusion criteria were patients with NAFLD confirmed by ultrasound. People with viral hepatitis (hepatitis B and C), autoimmune hepatitis, drug-induced liver disease, consumption of hepatotoxicity drugs including glucocorticoid, methotrexate, amiodarone, isoniazid, and tamoxifen during 6 months, consumption of vitamin E or glitazon, primary biliary cirrhosis, sclerosing cholangitis, genetic, metabolic, and cholestatic liver diseases, hemochromatosis, Wilson's disease, alpha-1-antitrypsin deficiency related to liver disease, recent or past alcohol consumption of >21 standard drinks per week for men and >14 standard drinks per week for women, past and present alcohol side effects, evidence of HCC or liver cancers, and history of bariatric surgery were excluded.

Then, the patients underwent Fibro Scan (FibroScan; Echosens, Paris, France) to determine the degree of fibrosis (F0-F4) and steatosis (S1-S3) in the liver. All patients underwent Fibro Scan by one expert person.

Clinical and biochemical measurements

Clinical and biochemical parameters were assessed for each participant. Underlying comorbidities including diabetes, hypertension, dyslipidemia, hypothyroidism, and polycystic ovary syndrome (PCOS) were also recorded. The history of pharmacotherapy for diabetes, hypertension, hypothyroidism, dyslipidemia, and other drugs was also reviewed .

Laboratory tests including white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb), hematocrit (Hct), platelet (Plt), aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, direct bilirubin, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), triglycerides (TGs), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total cholesterol, albumin (Alb), ferritin, total iron-binding capacity (TIBC), gamma-glutamyl transpeptidase (GGT), ceruloplasmin, transferrin saturation, fasting blood glucose (FBS), and alpha-fetoprotein (AFP) were checked.

Then the scores of multiple scoring systems including AAR, APRI, FIB4, NFS, AP index, FI, Forms Index, BARD, BAAT, N Score, PLALA Score were calculated using the laboratory values, and the diagnostic value of the clinical indicators and the scoring systems was compared with the results of Fibro Scan. To determine the diagnostic value of each panel, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic accuracy were calculated.

Statistical analysis

Information on patients was classified, and the demographic data were analyzed in two groups with or without advanced fibrosis in SPSS 22. The qualitative parameters were analyzed through the Chi-Square test and the quantitative parameters through t-test in both groups. The results of Fibro Scan were divided into two groups without fibrosis (F0)/with mild fibrosis equivalent (F0F1) and advanced fibrosis (F2, F3, F3F4, and F4).

The results of the 11 panels were analyzed using a t-test in both groups. In addition, considering the cutoff point, the results of each panel were divided into two groups of no advanced fibrosis (no fibrosis or slight fibrosis) and advanced fibrosis. These results were compared with the results of Fibro Scan (no advanced fibrosis (F0 and F0F1) and advanced fibrosis (F2, F3, F3F4, F4), and then sensitivity, specificity, PPV, NPV, and accuracy of each panel were calculated. The area under the receiver operating characteristic (AUROC) curve and the confidence interval were also calculated for each panel. P-values less than 0.05 were considered significant. Finally, sensitivity, specificity, PPV, NPV, and accuracy of all panels were compared and the ROC curves of all panels were plotted on a single chart to compare the AUROCs. The formula and cutoff point for each panel are as follows in Table 1.

Table 1. The formula and cutoff point for each panel were as follows.

	Panel	Formula	cutoff point
1	FIB4 panel	(Age(year) × AST(IU/L))/(PLT(10^9/L) × √ALT(IU/L))	1.45 and 3.25 (21)
2	APRI panel	([AST/ULN]/PLT(10^9/L)) × 100	0.88 (17)
3	AAR panel	AST(IU/L)/ALT(IU/L)	0.8 (22)
4	NAFLD fibrosis score(NFS) panel	-1.675 + (0.037×Age(year)) + (0.094×BMI(Kg/M^2)) + (1.13×diabetes/IFG (yes=1, no=0)) + 0.99×(AST/ALT) - (0.013 × PLT (×10^9/L)) - (0.66 × ALB(g/dl))	-1.455 and 0.676 (23)
5	AP Panel	PLT(10^9/L) Age (years)	6 (24)

		>225-0 point 200-224-1 point 175-199-2 point 150-174-3 point 125-149-4 point <125-5 point	<30-0 point 30-39-1 point 40-49-2 point 50-59-3 point 60-69-4 point ≥70-5 point
		Score is the sum of two (0-10)	
6	BAAT Score panel	Sum of the items: BMI(Kg/M^2) ≥28, 1 point Age ≥50 years, 1 point ALT ≥ twice upper limit normal (80 U/L), 1 point TG ≥150 mg/dL, 1 point	2 (25)
7	BARD Score panel	Sum of the items: Diagnosis of Diabetes, 1 point BMI(Kg/M^2) ≥28, 1 point AST/ALT ≥0.8, 2 point	2 (26)
8	PLALA panel	Sum of the items: PLT <15.3(10^4/μL), 1 point Alb <4 (g/dl) 1 point AST/ALT> 0.9, 1 point	2 (3)
9	Nippon(N Score) Panel	Sum of the items: Female sex, 1 point Age >60, 1 point Type 2 Diabetes, 1 point Hypertension, 1 point	2 (27)
10	FI Panel	8.28 - (PLT(10^9/L) × 0.01) - (Alb(g/dl) × 1.08)	2.1 (28)
11	Forns index panel	7.811 - 3.131× In(PLT(10^9/L)) + 0.781× In(GGT(IU/L)) + 3.467× In(Age) - 0.014 × Cholesterol(mg/dl)	4.2 and 6.9 (29)

AST: aspartate aminotransferase, PLT: platelet count, ALT: alanine aminotransferase, ULN: upper limit of normal, BMI: body mass index, IFG: impaired fasting glucose, ALB: albumin, TG: triglyceride, GGT: gamma glutamyl transpeptidase

Results

Out of 122 samples, 62 (50.8%) were men. The mean age of the patients was 47.1±11.7 years. The mean BMI and waist circumferences were 31.3±4.9 kg/m2 and 105.3±11.4 cm, respectively. The demographic and disease characteristics of NAFLD patients with and without advanced fibrosis are compared in Table 2. The only significant difference between the two groups with and without advanced fibrosis was the presence of diabetes in these groups (p=0.001).

Table 2. Comparison of some different characteristics in NAFLD patients with and without advanced fibrosis.

Variable	Total	No fibrosis or slight fibrosis (F0, F0F1) (n=88)	Advanced fibrosis (F2, F3, F3F4, F4) (n=34)	*p*-value
Age (years) [Mean ± SD]	47.1±11.7	46±11	50±10	NS*
Gender: Male/Female [N (%)]	62 (50.8) / 60 (49.1)	45/43	17/17	NS**
BMI (kg/m^2) [Mean ± SD]	31.3±4.9	31.2±4.6	31.5±5.5	NS*
Waist circumference (cm) [Mean ± SD]	105.3±11.4	104.5±11	107.4±12	NS*
Diabetes [N (%)]	29 (23.8)	12	17	0.001**
Hypertension [N (%)]	17 (13.9)	12	5	NS**
Dyslipidemia [N (%)]	67 (54.9)	44	23	NS**
Hypothyroidism [N (%)]	6 (4.8)	2	4	NS**
Polycystic Ovary Syndrome (PCO) [N (%)]	1 (0.8)	1	0	NS**

* Analyzed with *t*-test

** Analyzed with Chi-square test

The mean fibrosis among the patients was 6.4±2.5 kPa, with the highest and lowest fibrosis of 16.1 kPa and 2.6 kPa, respectively. Regarding the fibrosis grade, 50 (41%) were F0, 38 (31.1%) were F0F1, 15 (12.3%) were F2, 13 (10.7%) were F3, 5 (4.1%) were F3F4 and 1 (0.8%) was F4.

The mean steatosis among the patients was 308.8±36.3 dB/m2, with the highest and lowest steatosis of 400 dB/m2, and 241 dB/m2, respectively. Regarding the steatosis grade, 13 patients (10.7%) were S1, 26 patients (21.3%) were S2, and 83 patients (68%) were S3. The mean percentage of steatosis was 66.3±20.5%, with the highest and lowest rate of 100% and 13%, respectively (Table 3).

Table 3. The status of fibrosis and steatosis in the participants based on the Fibro Scan results.

Fibrosis Grade (Number (%)	F0	50 (41)
	F0F1	38 (31.1)
	F2	15 (12.3)
	F3	13 (10.7)
	F3F4	5 (4.1)
	F4	1 (0.8)
Fibrosis (kPa) Mean ± SD		6.4±2.5
Steatosis	S1	13 (10.7)
	S2	26 (21.3)
	S3	83 (68)
Steatosis (in terms of CAP) Mean ± SD		308.8±36.3
Steatosis percent Mean ± SD		66.3±20.5

According to Table 4, there were significant differences between the two groups of patients with and without advanced fibrosis in three panels of APRI, NIPPON, and FIB4 (p=0.03, p=0.01, p=0.005, respectively).

Table 4. Comparison of different types of NAFLD severity scoring panels based on the fibrosis severity in FibroScan.

Panel	No fibrosis or slight fibrosis (Mean ± SD)	Advanced fibrosis (Mean ± SD)	T	*p*-value
APRI	0.3±0.2	0.4±0.2	-2.2	0.03
BAAT	1.8±0.8	1.9±0.9	-0.3	NS
AP	2.8±1.5	3.4±1.7	-1.8	NS
BARD	1.7±1.1	2.2±1.3	-1.9	NS
PLALA	0.5±0.4	0.5±0.5	0.02	NS
NIPPON	0.9±0.8	1.3±1	-2.5	0.01
AAR	0.7±0.2	0.8±0.3	-1	NS
NAFLD fibrosis score	-1.9±1.1	-1.8±1.7	-0.3	NS
FIB4	0.8±0.3	1.1±0.5	-2.9	0.005
FORNS	4.5±0.7	4.2±1.3	0.8	NS
FI	1.2±0.6	0.8±0.7	1.6	NS

According to Table 5, sensitivity, specificity, PPV, NPV, and accuracy were 2.9, 95, 20, 69.7, and 67.5% for the APRI panel, 35.3, 78.4, 38.7, 75.8, and 66.3 for the NIPPON panel, 21.2, 92.5, 35, 74, and 71.6 for the FIB4 panel at cutoff point of 1.45, and 0, 100, 0, 70.7, and 70.7 for the FIB4 panel at cutoff point of 3.25.

Table 5. Comparison of the ability of each test to detect advanced fibrosis in patients with NAFLD.

Panel	Cutoff point	AUC	Confidence interval	Sensitivity*	Specificity*	PPV*	NPV*	Accuracy
APRI	0.88	0.695	0.58-0.8	2.9	95	20	69.7	67.5
FIB4	1.45	0.684	0.57-0.8	21.2	92.5	35	74	71.6
	3.25			0	100	0	70.7	70.7
NIPPON	2	0.642	0.5-0.74	35.3	78.4	38.7	75.8	66.3
BARD	2	0.607	0.48-0.7	66.7	53.8	37.9	79.2	57.6
AP	6	0.586	0.47-0.7	9.1	95.3	42.8	73.2	71.4
NAFLD fibrosis score	-1.455	0.569	0.39-0.7	0	100	0	65.3	65.3
	0.676			11.1	94.1	50	66.6	65.3
BAAT	2	0.529	0.4-0.6	72.7	32.4	33.3	71.8	45.2
AAR	0.8	0.521	0.4-0.63	48.5	59.3	32.6	73.8	56.1
PLALA	2	0.500	0.3-0.66	0	97.1	0	65.3	64.1
FORNS	4.2	0.402	0.19-0.6	46.2	27.3	27.2	46.1	34.2
	6.9			0	100	0	62.8	62.8
FI	2.1	0.383	0.2-0.54	5.3	94.4	25	64	61.1

*** Values are in percent**

In addition, AUC for each panel is shown in figure 1.

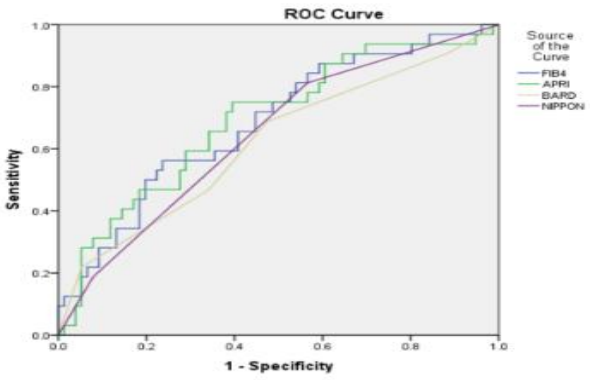

Figure 1. Comparison of the area under the ROC curve in panels with AUC >0.6.

Also, a diagnostic algorithm for clinical use of these panels is presented in Figure 2.

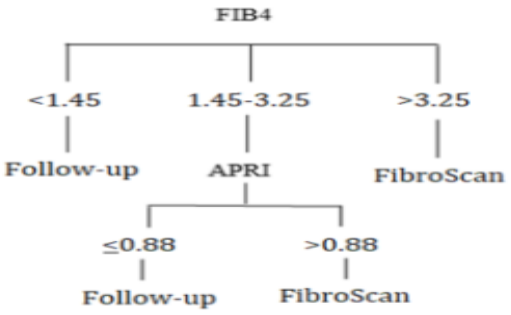

Figure 2. Proposed diagnostic algorithm for patients with NAFLD.

Discussion

This study aimed to compare the scoring panels of NAFLD with Fibro Scan. NAFLD is a common liver disease that may progress to steatohepatitis and cirrhosis. The liver biopsy is a gold standard but, invasive diagnostic procedure that is not without flaws. Therefore, there has been increasing interest in identifying non-invasive, surrogate diagnostic methods such as scoring panels and Fibro Scan.

Scoring panels can play an important role in the diagnosis of NAFLD along with Fibro Scan. There was no significant age difference between the two groups of patients with and without advanced fibrosis. However, in the study of Kessuko et al, Cichoz-Lach et al, Ratziu et al, McPherson et al, and Mohamed et al, the two groups had a significant age difference (17-21). In our study, there was no significant difference in BMI between patients with advanced fibrosis and patients without it, which is similar to the results of Kessuko et al and McPherson et al and in contrast to the results of Ratziu et al, A. Mohamed et al, and Cichoz-Lach et al (17-20).

APRI Panel

Sensitivity, specificity, PPV, NPV, and accuracy of the APRI panel were 2.9%, 95%, 20%, 69.7%, and 67.5%, respectively, indicating that the panel has a low sensitivity for the diagnosis of fibrosis, but high specificity of this panel with relatively good NPV indicates its high strength in ruling out advanced fibrosis. This panel was able to distinguish the two groups of the patient with and without advanced fibrosis (p=0.03). In addition, by calculating the area under the ROC curve, it was found that this panel had a relatively good diagnostic value (AUROC=0.695, CI=0.58-0.8, p=0.001). The cutoff point suggested by the ROC curve was 0.26 at which sensitivity and specificity were 73% and 62%, respectively.

According to the study of Atay et al, sensitivity, specificity, PPV, and NPV of the APRI panel at cutoff point of 0.61 were 35%, 95.7%, 85.7%, and 66.7%, respectively. Atay et al, stated that this panel is useful for ruling out rather than diagnosing advanced fibrosis (22). In a study by Shin et al on patients with chronic liver disease, sensitivity, specificity, PPV, and NPV of this panel were 93%, 48%, 75%, and 80% at the cutoff point of 0.5, and sensitivity, specificity, and PPV were 58%, 88% and 89% at the cutoff point of 1.5, respectively (23). In a study by Kruger et al, sensitivity, specificity, PPV, and NPV were 75%, 86%, 54%, 93%, respectively, at the cutoff point of 0.95 (24). While, in the study of Sumida et al, sensitivity, specificity, PPV, and NPV were 67%, 81%, 31%, and 95%, respectively (25). A cohort study showed that sensitivity and specificity of APRI score was 30 % and 92.8 % respectively (26).

Similar to the study of Mohamed, et al (p=0.001), the present study found a significant difference between two groups of patients with and without advanced fibrosis. In the study of Mohamed et al, sensitivity, specificity, PPV, NPV, accuracy, and AUROC were 21.1%, 93%, 50%, 77.9%, 75%, and 0.907, respectively, at the cutoff point of 1 (95%CI: 0.839-0.974). It was also stated that if the liver biopsy was considered only for individuals with a panel score of 1, 89.4% of unnecessary biopsies would be avoided (20). According to Macpherson's et al study, sensitivity, specificity, PPV, and NPV were 27%, 89%, 37%, and 84%, respectively, and AUROC was 0.67 at the cutoff point of 1 (95%CI: 0.54-0.8). Given that the NPV of this panel is suitable for ruling out advanced fibrosis. According to this study, the weak PPV of the panel indicates that it cannot replace liver biopsy (19). These were reported in the French cohort study as 66%, 90%, 72%, and 87%, respectively (27). The results of the Peres-Gutieierrez et al, were similar to those of McPherson et al, study (19, 28). According to Ding's study, AUROC was 0.795 and sensitivity, specificity, PPV, NPV, and accuracy were 80%, 73%, 33%, 96%,

and 65%, respectively (29). According to the study of Rath et al, sensitivity, specificity, PPV, NPV, and AUROC were 29.1%, 97.22%, 87.5%, 83.3%, and 0.36, respectively (10).

Similar to the results of Atay et al, and Rath et al, regarding the APRI panel, sensitivity was low and specificity was high in this study; sensitivity was much lower in our study than those studies (10, 22). On the other hand, there was a significant difference between the two groups of patients with and without advanced fibrosis; therefore, the low sensitivity of this panel may be attributed to the improper cutoff point. This cutoff point cannot properly diagnose patients with advanced fibrosis, but it can rule it out well. Therefore, using the ROC curve, 0.26 was selected as the cutoff point for the APRI panel in our study population. Assuming a new cutoff point for this panel, sensitivity and specificity were obtained 73% and 62%, respectively. As AST level in the group with advanced fibrosis was significantly higher than the other group (p=0.03), the significant difference between the two groups in the APRI panel is justifiable. But in general, given the low sensitivity and high specificity of the APRI panel, it is more useful to rule out than to diagnose advanced fibrosis.

NIPPON panel

Sensitivity, specificity, PPV, NPV, and accuracy of this panel were 35.3%, 78.4%, 38.7%, 75.8%, and 66.3%, respectively. This panel was able to make a significant difference between the two groups of patients with and without advanced fibrosis (p=0.01) (Table 4).

In addition, the area under the ROC curve showed that this panel had a good diagnostic value (AUROC=0.642, CI: 0.5-0.74, p=0.015). A limited number of studies have been performed on this panel. In a study by Sumida et al, this panel differentiated the groups of patients with and without advanced fibrosis (p<0.0001). The AUROC of this panel was 0.715 and sensitivity, specificity, PPV, and NPV were 80%, 58%, 19%, 96%, respectively. It was also stated that this panel can prevent 53% of unnecessary biopsies (25). Considering that diabetes was a parameter involved in this panel and also diabetes was differentiated in the two groups of patients with and without advanced fibrosis in this study (p=0), we could justify the ability of this panel to differentiate between these two groups.

FIB4 panel

Sensitivity, specificity, PPV, NPV and accuracy of this panel were 21.2%, 92.5%, 35%, 74%, and 71.6%, at the cutoff point of 1.45 and 0, 100%, 0, 70.7%and 70.7% at the cut point of 3.25, respectively.

This panel was able to significantly differentiate the two groups of patients with and without advanced fibrosis (p=0.005). In addition, the area under the ROC curve indicated that this panel has a good predictive value (AUROC=0.684, CI: 0.5-0.7, p=0.002). According to the ROC curve, the panel sensitivity and specificity will be 75% and 53% at the cutoff point of 0.82.

In the study of Atay et al, sensitivity, specificity, PPV, and NPV were 65%, 69.6%, 61.1%, and 72.7% at the cutoff point of 1.08, respectively. They stated that this panel has moderate sensitivity and specificity (22).

In a study by Shah et al, sensitivity, specificity, PPV, and NPV were 74%, 71%, 43%, and 90% at the cutoff point of 1.3 and 33%, 98%, 80%, and 83% at the cut point of 2.67, respectively (30). Sensitivity, specificity, PPV, and NPV in the study of Sumida et al, were 90%, 64%, 24%, and 98% at the cutoff point of 1.45, respectively. In addition, based on the ROC curve, sensitivity, specificity, PPV, and NPV were 48%, 95%, 53%, and 94% at the cutoff point of 3.25 in this study (25).

In the study of Mohamed et al, sensitivity, specificity, PPV, and NPV were 84.2%, 86.9%, 66.6%, and 94.2% at the cutoff point of 1.3 and 63.2%, 93%, 75%, and 88.3% at the cutoff point of 2.6, respectively. The accuracy and AUROC of this panel were 89.7 and 0.936 (95%CI: 0.884-0.898). The FIB4 panel was able to differentiate the two groups of patients with and without advanced fibrosis (p<0.001). It was also stated that this panel can prevent 68% of unnecessary biopsies at levels less than 1.3, and that it is suitable for both ruling out and diagnosis of advanced liver fibrosis [16]. In the study of McPherson et al, the FIB4 panel was able to differentiate the groups of patients with and without advanced fibrosis (p<0.001). In Cheah et al study FIB4 was introduced as available parameters to

identify fibrosis (6). AUROC for this panel was 0.86 (95%CI: 0.78- 0.94) and sensitivity, specificity, PPV, and NPV were 85%, 65%, 36%, and 95% at the cutoff point of 1.3 and 26%, 98%, 75%, and 85% at the cutoff point of 3.25, respectively. It was also stated that this panel can prevent 62% of unnecessary biopsies at levels less than 1.3, that this panel can rule out advanced fibrosis and its use can reduce unnecessary biopsy for people with mild fibrosis (19).

The panel's ability to differentiate the two groups of patients with and without advanced fibrosis and its good AUROC indicates the acceptable diagnostic power of this panel in the study population. Despite the panel's low sensitivity, its high specificity indicates that it can rule out rather than detecting advanced fibrosis.

In general, none of the panels had enough sensitivity for the diagnosis of advanced fibrosis. Given their relatively good specificity, these panels are generally better to rule out rather than to diagnose advanced fibrosis by comparison of the panels' diagnostic power (Table 5), the APRI and FIB4 panels are introduced as panels with high diagnostic power .

Conclusion

We concluded that the FIB4 panel is calculated first for the patient with NAFLD. For values less than 1.45, it is recommended to follow-up patients with other tests and examinations; for values greater than 3.25, it is recommended to perform more detailed investigations through Fibro Scan; and for values between 1.45 and 3.25, it is recommended to measure the APRI panel; in this regard, cases with APRI values of <0.88 and >0.88 are recommended to follow-up and perform Fibro Scan, respectively. Given the cost-effectiveness of these panels, their ease of calculation, and noninvasiveness, they can be used as useful tools for following up the patients and also for predicting progression to advanced fibrosis. It is recommended to develop a new and more accurate index for clinical use, based on the criteria of the three panels of FIB4, APRI, and NIPPON, and perform further studies on these panels. As a limitation of this study, the results of Fibro Scan were considered as the standard method, while the biopsy was the gold standard in other studies; this has somewhat diminished the accuracy of this study.

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Author contributions

Conception and design: **FMGh**, **ASh**; analysis and interpretation of the data: **FJ**, **SD**, **SY**, **SFA**;formal analysis: **FJ**, **ASh**; drafting of the article: **FJ**, **SD**, **SY**, **SFA**; critical revision of the article for important intellectual content: **FJ**, **ASh**; **KA** project administration: **FMGh**, **FJ**, **SFA**; final approval of the article: **ASh**, **FJ**, **SFA**. All authors approved its final version and agreed to be accountable for all aspects of the study.

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Ethical approval and consent to participate

This study was registered in the Research Department of Guilan University of Medical Sciences with the ethics code of IR.GUMS.1396.114. This manuscript has not been published in whole or in part. All authors have read the manuscript and have agreed that the work is ready for submission and accept responsibility for its contents. Before participation, all participants received oral and written study information and signed a written consent form.

Competing of interest

None to declare.

References

1. Lotfi K, Nouri M, Askari G. The Effect of Resveratrol Supplementation on Improving Non-Alcoholic Fatty Liver: A Review on Randomized Clinical Trials. Clinical Excellence. 2020;9(4):11-22.

2. EASL-EASD-EASO Clinical Practice Guidelines for the Management of Non-Alcoholic Fatty Liver Disease. Obesity facts. 2016;9(2):65-90.

3. Younossi Z, Tacke F, Arrese M, Chander Sharma B, Mostafa I, Bugianesi E, et al. Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology (Baltimore, Md). 2019;69(6):2672-82.

4. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nature reviews Gastroenterology & hepatology. 2018;15(1):11-20.

5. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology (Baltimore, Md). 2016;64(1):73-84.

6. Cheah MC, McCullough AJ, Goh GB-B. Current modalities of fibrosis assessment in non-alcoholic fatty liver disease. Journal of clinical and translational hepatology. 2017;5(3):261.

7. BAGHERI LK, GHAFFARPASAND F, MAHMOODI M, LOTFI M, ZAMIRI N, HEYDARI ST, et al. Non alcoholic fatty liver disease in southern Iran: A population based study. 2013.

8. Barikani A, Pashaeypoor S. Lifestyle in non-alcoholic fatty liver: A review. Iranian Journal of Nursing Research. 2019;13(6):39-47.

9. Nikroo H, Mohammadian M, Nematy M, Sima HR, Attarzadeh Hosseini SR. The effect of diet and exercise on improvement of quality of life in patients with nonalcoholic steatohepatitis. Journal of Kerman University of Medical Sciences. 2014;21(1):61-72.

10. Rath MM, Panigrahi MK, Pattnaik K, Bhuyan P, Kar SK, Misra B, et al. Histological evaluation of non-alcoholic fatty liver disease and its correlation with different noninvasive scoring systems with special reference to fibrosis: a single center experience. Journal of clinical and experimental hepatology. 2016;6(4):291-6.

11. Sumida Y, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. World journal of gastroenterology: WJG. 2014;20(2):475.

12. Patel K, Sebastiani G. Limitations of non-invasive tests for assessment of liver fibrosis. JHEP Reports. 2020;2(2):100067.

13. UK NGC. Non-alcoholic fatty liver disease: assessment and management. 2016.

14. Wai C-T, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology (Baltimore, Md). 2003;38(2):518-26.

15. Angulo P, Bugianesi E, Bjornsson ES, Charatcharoenwitthaya P, Mills PR, Barrera F, et al. Simple noninvasive systems predict long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology. 2013;145(4):782-9. e4.

16. Alkhouri N, Mansoor S, Giammaria P, Liccardo D, Lopez R, Nobili V. The development of the pediatric NAFLD fibrosis score (PNFS) to predict the presence of advanced fibrosis in children with nonalcoholic fatty liver disease. PloS one. 2014;9(8):e104558.

17. Cichoż-Lach H, Celiński K, Prozorow-Król B, Swatek J, Słomka M, Lach T. The BARD score and the NAFLD fibrosis score in the assessment of advanced liver fibrosis in nonalcoholic fatty liver disease. Medical science monitor: international medical journal of experimental and clinical research. 2012;18(12):CR735.

18. Kessoku T, Ogawa Y, Yoneda M, Imajo K, Sumida Y, Eguchi Y, et al. Simple scoring system for predicting cirrhosis in nonalcoholic fatty liver disease. World Journal of Gastroenterology: WJG. 2014;20(29):10108.

19. McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut. 2010;59(9):1265-9.

20. Mohamed RA, Nabih MI, ElShobaky MB, Khattab HM. The value of noninvasive scoring systems for the diagnosis of advanced fibrosis in Egyptian patients with nonalcoholic fatty liver disease. The

Egyptian Journal of Internal Medicine. 2014;26(4):162-9.

21. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology. 2005;128(7):1898-906.

22. Atay K, Canbakan B, Alan O, Koroglu E, HATEMİ Aİ, Kepil N, et al. Evaluation of non-invasive diagnostic methods as indicators of fibrosis in patients with nonalcoholic fatty liver disease. 2017.

23. Shin WG, Park SH, Jun S-Y, Jung JO, Moon JH, Kim JP, et al. Simple tests to predict hepatic fibrosis in nonalcoholic chronic liver diseases. Gut and Liver. 2007;1(2):145.

24. Kruger FC, Daniels CR, Kidd M, Swart G, Brundyn K, Van Rensburg C, et al. APRI: a simple bedside marker for advanced fibrosis that can avoid liver biopsy in patients with NAFLD/NASH. South African Medical Journal. 2011;101(7):477-80.

25. Sumida Y, Yoneda M, Hyogo H, Yamaguchi K, Ono M, Fujii H, et al. Japan Study Group of Nonalcoholic Fatty Liver Disease (JSG-NAFLD) A simple clinical scoring system using ferritin, fasting insulin, and type IV collagen 7S for predicting steatohepatitis in nonalcoholic fatty liver disease. Journal of gastroenterology. 2011;46:257-68.

26. Imai N, Imai Y, Kido Y. Psychosocial factors that aggravate the symptoms of sick house syndrome in Japan. Nursing & health sciences. 2008;10(2):101-9.

27. Calès P, Lainé F, Boursier J, Deugnier Y, Moal V, Oberti F, et al. Comparison of blood tests for liver fibrosis specific or not to NAFLD. Journal of hepatology. 2009;50(1):165-73.

28. Pérez-Gutiérrez OZ, Hernández-Rocha C, Candia-Balboa RA, Arrese MA, Benítez C, Brizuela-Alcántara DC, et al. Validation study of systems for noninvasive diagnosis of fibrosis in nonalcoholic fatty liver disease in Latin population. Annals of hepatology. 2013;12(3):416-24.

29. Ding D, Li H, Liu P, Chen L, Kang J, Zhang Y, et al. FibroScan, aspartate aminotransferase and alanine aminotransferase ratio (AAR), aspartate aminotransferase to platelet ratio index (APRI), fibrosis index based on the 4 factor (FIB-4), and their combinations in the assessment of liver fibrosis in patients with hepatitis B. International journal of clinical and experimental medicine. 2015;8(11):20876.

30. Shah AG, Lydecker A, Murray K, Tetri BN, Contos MJ, Sanyal AJ, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clinical gastroenterology and hepatology. 2009;7(10):1104-12.

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Case report Free Access

Squamous cell carcinoma, a rare variant of primary breast carcinoma: a case report

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Abstract

Introduction: Breast cancer is the most common malignancy occurring worldwide in females but primary squamous cell carcinoma represents a very rare variant of breast carcinoma, accounting for less than 0.1%. Mostly it is grayish-white in colour with an ill-defined cut surface and has cystic areas of foci of necrosis macroscopically. Squamous elements in these neoplasms can range from well to poorly differentiated. The majority was moderately differentiated and showed cystic degeneration correlating with the macroscopic appearance.

Case presentation: A 45-year-old female presented to us with a painless progressive lump involving all quadrants of left breast that at presentation had involved the whole breast and was associated with foul-smelling discharge. The patient had toxic features and was taken up for toilet mastectomy. The wound was left open for a delayed closure. The histopathological report suggested triple negative squamous cell carcinoma involving the breast.

Discussion: Squamous cell carcinoma is commonly seen in the skin and lung, it rarely originates in breast tissue. There are reports that it may develop within a previous benign lesion such as an epidermal cyst or chronic inflammatory lesions. It may also mimic benign breast disease resulting in inappropriate or delayed management. Clinically and radiologically it is indistinguishable from adenocarcinoma, the most common presentation being cystic lesion. Because of limited data and few case reports worldwide, management strategies have been controversial. Total mastectomy with axillary clearance is usually done. As it is locally advanced, conservative surgery is not feasible most of the time. Radiotherapy has been used in locally advanced cases, though not much useful.

Conclusion: This case report highlights the rare occurrence of synchronous primary malignancies in the lung and breast, underreported in the medical literature. This case adds to the existing knowledge of MPMT and may stimulate further research on this topic. Clinicians should be aware of the possibility of MPMT in cancer patients and perform thorough investigations to rule out secondary or metastatic tumors.

Keywords: Small cell carcinoma, Breast cancer, Synchronous, Metachronous, Histopathology, Immunochemistry,

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Introduction

Breast cancer is the most common malignancy occurring worldwide in females but primary squamous cell carcinoma represents a very rare variant of breast carcinoma, accounting for less than 0.1% (1). It is a highly aggressive tumor with a greater tendency to metastasize as compared to adenocarcinoma breast, thus having a poor prognosis. The lesions are usually larger, hormone receptor-negative with lesser nodal spread. Apart from adenocarcinoma breast, it needs to be differentiated from primary squamous cell carcinoma of skin overlying breast and metastatic squamous cell carcinoma from some distant site. As clinical and radiological findings are not specific, the biopsy is a must to diagnose this variant. For diagnosis of squamous cell carcinoma, more than 90% of cells should be squamous (2).Murcia and colleague defined pure squamous cell carcinoma as:

1) No other neoplastic component such as ductal or mesenchymal element is present in tumour.

2) Tumor origin must be independent from the overlying skin and nipple.

3) Absence of an associated primary squamous cell carcinoma in a second site (3).

Pathogenesis

Gross Findings

Mostly it is grayish white with an ill-defined cut surface and has cystic areas of foci of necrosis macroscopically. A wide range of sizes was reported, often larger than other special types (4).

Microscopic Findings

Squamous elements in these neoplasms can range from well to poorly differentiated. The majority was moderately differentiated and showed cystic degeneration (resembling cutaneous inclusion cyst) correlating with the macroscopic appearance. A small (<25%) spindle cell component may be present. Spindled components may range from low to high grade. In some cases associated DCIS confirms the primary nature of the lesion (4,5).

Immunohistochemistry

Estrogen receptor (ER) assays have been variable and no reliable conclusion can be drawn and mostly regarded as ER negative. Focally tumor express cytokeratins; shows immunostaining for S100 and smooth muscle actin (4).

We report a case of this rare variant of breast carcinoma along with the management done.

Case presentation

45 year old female presented to us with a painless progressive lump involving all quadrants of the left breast that at presentation had involved the whole breast and was associated with foul-smelling discharge (Figure 1).

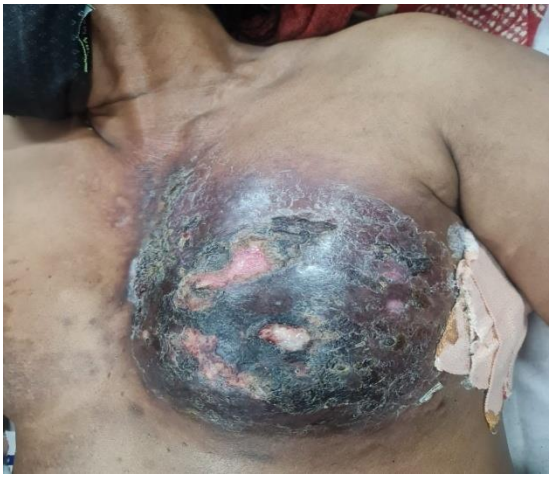

Figure 1. Gross image of breast mass.

The patient had toxic features and was taken up for toilet mastectomy. The wound was left open for a delayed closure. The histopathological report suggested triple negative squamous cell carcinoma involving the breast. (Figure 2).

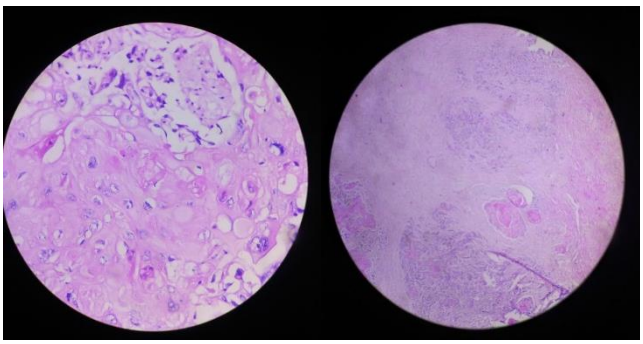

Figure 2. HP image of SCC.

Discussion

Squamous cell carcinoma is commonly seen in the skin and lungs, it rarely originates in breast tissue. Although its origin is unclear, multiple hypotheses have been proposed. Murialdo R et al(6). state that it originates from the epithelium of the mammary ducts or squamous metaplasia of adenocarcinoma. There are reports that it may develop within a previous benign lesion such as an epidermal cyst or chronic inflammatory lesions (7). It may also mimic benign breast disease resulting in inappropriate or delayed management (7).

Clinically and radiologically it is indistinguishable from adenocarcinoma, the most common presentation being a cystic lesion. The typical presentation is a hard breast lump, which may have inflammatory signs in an elderly woman. Although it is larger, the tendency for nodal spread is lesser than adenocarcinoma as stated by Vekariya M et al (1). and Carbone S et al (8). 70% of squamous cell carcinoma of the breast don't have axillary lymphadenopathy at presentation but lymph node dissection could always be performed for staging due to unpredictable lymph node dissemination. Distant metastasis is comparatively higher. Hormone receptor (ER/PR) and HER2/*neu*- are usually negative with overexpression of EGFR.

Because of limited data and few case reports worldwide, management strategies have been controversial. Total mastectomy with axillary clearance is usually done. As it is locally advanced, conservative surgery is not feasible most of the time. Radiotherapy has been used in locally advanced cases, though not very useful. They are reported to be resistant to standard chemotherapy used for adenocarcinoma, as well as hormonal therapy. Several chemotherapeutic agents have been tried to date but efficacy and response have not been estimated yet. Hennessy et al (9) reported no benefit in using anthracycline/taxane-based neoadjuvant chemotherapy. In contrast, few have also reported a good response with neoadjuvant therapy using cisplatin and 5-fluoro-uracil (10). A high incidence of recurrence had been reported in those who received adjuvant chemotherapy (11). Due to the high rates of locoregional recurrence in this disease, early adjuvant radiation therapy is thought to be prudent despite reports of frequent recurrence in irradiated fields. Adjuvant chemotherapy is used regularly given the aggressive nature, but the risk of distant metastasis remains high in SCC (12,13). Historically, anthracycline-containing regimens have been the standard; however, the use of carboplatin and taxanes has biological plausibility and have been employed.

Conclusion

Very rare incidence along with nonspecific presentation poses a major challenge in the diagnosis of primary SCC. Subsequent challenges being variable responses or resistance to standard chemotherapy regimens as well as hormonal agents. EGFR positivity had been a scope for targeting specific chemotherapeutic agents.

Author contribution

PS supervised and corresponding author, **KR**, **HK**, and **MS** contributed to some parts of the study and **RK** contributed as an anesthetist.

Conflict of interest

The authors declare no conflict of interest.

References

1. Vekariya M, Gupta V, Pednekar A, Nagur B, Mahna A et al. Primary squamous cell carcinoma of the breast: A rare case report. Int J Sci Stud 2014;2(8):226-228.
2. Kamra H, Gadgil P, Chaware S, Kanade U. Acantholytic variant of squamous cell carcinoma of breast: a rare case report. Ecancermedicalscience. 2011;5:214.
3. Macia M, Ces JA, Becerra E, Novo A. Pure squamous carcinoma of the breast. Report of a case diagnosed by aspiration cytology. Acta Cytol. 1989 Mar-Apr;33(2):201-4.
4. O'Malley FP, Pinder SE, Mulligan AN. In: Breast pathology: a volume in the series: foundation in diagnostic pathogy. 2nd ed. Elsevier: United states 2011.p.216.
5. Gobbi H, Simpson JF, Borowsky A, Jensen RA, Page DL. Metaplastic breast tumors with a dominant fibromatosis-like phenotype have a high risk of local recurrence. Cancer. 1999 May 15;85(10):2170-82.

6. Murialdo R, Boy D, Musizzano Y, Tixi L, Murelli F et al. Squamous cell carcinoma of the breast: a case report. Cases J. 2009 Jul 10;2:7336.
7. Flikweert, E.R., Hofstee, M. & Liem, M.S. Squamous cell carcinoma of the breast: a case report. World J Surg Onc 6, 135 (2008).
8. Carbone S, Lobo Alvarez R, Lamacchia A, Almenar Gil A, Martin Hernandez R et al. Primary squamous cell carcinoma of the breast: A rare case report. Rep Pract Oncol Radiother. 2012 Aug 9;17(6):363-6.
9. Hennessy BT, Krishnamurthy S, Giordano S, Buchholz TA, Kau SW et al. Squamous cell carcinoma of the breast. J Clin Oncol. 2005 Nov 1;23(31):7827-35.
10. Seddik Y, Brahmi SA, Afqir S. Primary squamous cell carcinoma of the breast: a case report and review of literature. Pan Afr Med J. 2015 Feb 18;20:152.
11 . Pandey A, Joshi K, Moussouris H, Joseph G. Case Reports on Metaplastic Squamous Cell Carcinoma of the Breast and Treatment Dilemma. Case Rep Oncol Med. 2019 Sep 18;2019:4307281.
12. Hennessy BT, Krishnamurthy S, Giordano S, Buchholz TA, Kau SW, Duan Z. Squamous cell carcinoma of the breast. *J Clin Oncol*. 2005 Nov 1;23(31):7827-7835.
13. Behranwala KA, Nasiri N, Abdullah N, et al. Squamous cell carcinoma of the breast: Clinico-pathologic implications and outcome. *Eur J Surg Oncol*. 29:386,2003-389

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Harnessing viral power: immunotherapy's synergy with targeted oncolytic viruses

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Abstract

Cancer treatment has witnessed a profound transformation in recent decades, with combination therapy emerging as a beacon of hope for patients. This review delves into the groundbreaking synergy between immunotherapy and targeted oncolytic viruses, offering a glimpse into the future of cancer conquering. Traditional methods like surgery, radiation, and chemotherapy have limitations, especially in advanced or metastatic cancers. Immunotherapy, inspired by the body's innate defenses, leverages the immune system to selectively identify and eradicate cancer cells. Immune checkpoint inhibitors, such as pembrolizumab and nivolumab, have showcased remarkable success in clinical trials, unlocking the potential of the immune system against once-intractable cancers. In tandem, oncolytic viruses exhibit precision targeting, minimizing harm to healthy tissues. Notably, herpes simplex virus type 1 (HSV-1) has proven effective against various malignancies. The fusion of immunotherapy and oncolytic viruses represents a paradigm shift in cancer treatment, harnessing the strengths of each modality. This review explores mechanisms, recent developments, clinical triumphs, and the challenges of combination therapy. The dynamic synergy of these two approaches promises to revolutionize cancer treatment, transforming it from an insurmountable foe into a manageable condition.

Keywords: Immunotherapy, Oncolytic viruses, Combination therapy, Immune checkpoint inhibitors, Cancer treatment

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Introduction

Cancer, the relentless scourge of our time, continues to cast its long shadow over the lives of millions worldwide. The global burden of this insidious disease is staggering, with an estimated 19.3 million new cancer cases and 10 million cancer-related deaths reported in 2020 alone (1). These harrowing statistics underscore the pressing need for modern, innovative, and effective cancer treatment methods that can provide a glimmer of hope amidst the daunting challenges posed by this complex ailment. Cancer, a heterogeneous group of diseases characterized by the uncontrolled growth and spread of abnormal cells, defies easy categorization (2). It infiltrates virtually every organ system, from the blood to the bone, and carries with it a diverse array of subtypes and mutations that further complicate diagnosis and treatment. In the face of this formidable adversary, the oncology community has relentlessly pursued novel strategies to combat cancer's relentless advance.

Traditionally, cancer treatment has relied on a triad of approaches: surgery, radiation therapy, and chemotherapy (3). While these modalities have been instrumental in extending the lives of countless cancer patients, they come with their own set of limitations. Surgery is often restricted to early-stage tumors, while radiation therapy can cause collateral damage to healthy tissues. Chemotherapy, although a mainstay of cancer treatment, often elicits severe side effects, leading to a diminished quality of life for patients. The epidemiological landscape of cancer further complicates the quest for effective treatments. Age, genetics, lifestyle factors, and environmental exposures all play pivotal roles in determining an individual's susceptibility to cancer (2). Moreover, the rise of cancer incidence in low- and middle-income countries adds a layer of complexity, as disparities in access to healthcare and treatment options persist (4). In the midst of these formidable challenges, a ray of hope has emerged on the horizon in the form of immunotherapy and oncolytic virotherapy (5). These groundbreaking approaches have heralded a paradigm shift in the field of oncology, offering a glimmer of optimism in the relentless battle against cancer.

I. Cancer immunotherapy

In the realm of cancer treatment, immunotherapy has emerged as a revolutionary approach, transforming the oncology landscape and providing renewed hope to patients with various malignancies. Notable recent developments in immunotherapy have propelled the field forward, paving the way for enhanced therapeutic strategies and improved patient outcomes (6).

I.a. Monoclonal Antibodies (mAbs)

Monoclonal antibodies (mAbs) have revolutionized cancer treatment through their precise targeting mechanisms. These immunoglobulins possess two Fab terminals for direct target binding and an Fc terminal for interactions with immune cell receptors, modulating their modes of action (MOA) (7). Notably, Fc-mediated effector functions encompass complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis (ADCP) (8). CDC involves Fc interaction with complement component C1q, initiating immune responses. ADCC and ADCP operate via direct Fc-FcyR interactions, engaging NK cells and macrophages, respectively, in tumor cell elimination.

mAbs can also bind and block soluble antigens and disease-related mediators. FDA-approved mAbs, such as rituximab and trastuzumab, have transformed the treatment landscape. Antibody drug conjugates (ADCs) exhibit direct cytotoxicity by delivering payloads to target cells. While hematological tumors are more accessible to mAbs due to their microenvironment, ADCs are increasingly promising in treating solid tumors. Fc-engineering enhances mAbs' antitumor and immune activation activities. For example, Tafasitamab, targeting CD-19, underwent Fc-related modifications, resulting in impressive clinical outcomes (9).

Despite mAbs' advantages, cytokine storms can induce severe side effects in some patients. Reducing immunogenicity through Fe-engineering may enhance safety. While mAbs are administered via injection, nanobodies, lacking an Fe terminal, offer higher tissue permeability and lower production costs. Combinations with chemotherapy and targeted therapies are common, emphasizing mAbs' enduring importance (10).

I.b. Bispecific Monoclonal Antibodies (bsAbs)

Bispecific mAbs (bsAbs) offer enhanced antitumor effects by simultaneously binding multiple targets. They provide better stability, specificity, and fewer side effects. Blinatumomab, targeting CD19 and CD3, has achieved high response rates in clinical trials (11). Several bsAbs targeting diverse antigens are in development, including MEDl5752, which targets PD-1 and CTLA-4. Manufacturing challenges and optimal dosing strategies remain for bsAbs, especially in solid tumors. However, clinical studies are ongoing, with promising results. As more bsAbs enter the market, their potential in cancer therapy is expected to grow (12).

I.c. Immune Checkpoint Monoclonal Antibodies

Immune checkpoint mAbs target regulatory molecules like CTLA-4 and PD-1 on T cells, unleashing the immune system's antitumor potential. These therapies have revolutionized cancer treatment. CTLA-4 inhibition with ipilimumab has improved melanoma survival. PD-1/PD-L1 mAbs like pembrolizumab have shown remarkable results across various cancers, especially when combined with chemotherapy or targeted therapy (13). Fe-engineering strategies enhance the MOA of immune checkpoint mAbs. Other immune checkpoints like LAG-3, TIM-3, and TIGIT are emerging targets, with positive clinical outcomes. Combining checkpoint inhibitors further augments efficacy (14). While immune checkpoint therapy has less toxicity than chemotherapy, Immune-related adverse events (IrAEs) can occur. These are generally reversible and manageable with glucocorticoids. lrAEs are less common and less severe than chemotherapy-induced side effects (Table 1).

Table 1. Key aspects of monoclonal antibody-based immunotherapy in cancer treatment.

Aspect	Monoclonal Antibodies (mAbs)	Bispecific Monoclonal Antibodies (bsAbs)	Immune Checkpoint Monoclonal Antibodies
Overview	Precision targeting through Fab terminals	Simultaneous binding to multiple targets	Unleashing the immune system's potential
	Fc terminal modulates modes of action	Enhanced stability and specificity	Targeting regulatory molecules on T cells
	Fc-mediated effector functions (CDC, ADCC, ADCP)	Promising clinical results	Significant improvement in cancer treatment
	Challenges in manufacturing and dosing	Ongoing research on novel immune checkpoints	
Examples	Rituximab (CD20), Trastuzumab (HER-2), Bevacizumab (VEGFA)	Blinatumomab (CD19/CD3)	Ipilimumab (CTLA-4), Pembrolizumab (PD-1/PD-L1)
	Antibody-Drug Conjugates (ADCs)	MEDI5752 (PD-1/CTLA-4)	Avelumab (PD-L1)
	Amivantamab (EGFR/METR)	Emerging targets (LAG-3, TIM-3, TIGIT)	
	Challenges in manufacturing and dosing	Fc-engineering strategies	
	Promising clinical results	Combination therapy	
	Management of immune-related adverse events		
Future Prospects	Fc-engineering for safer and more effective mAbs	Overcoming manufacturing challenges	Expansion of targets and combination therapies
	Nanobodies with higher tissue permeability	Optimizing dosing strategies for solid tumors	Continued refinement of Fc-engineering
	Combinations with chemotherapy and targeted therapies	Exploring optimal routes of administration	Personalized treatment approaches
	Expanding clinical applications	Patient selection based on genetic screening	

(Fc: Stands for "fragment crystallizable," referring to the tail portion of an antibody that interacts with other immune cells or molecules. CDC: Complement-Dependent Cytotoxicity, a mechanism involving the complement system to target cells. ADCC: Antibody-Dependent Cell-Mediated Cytotoxicity, a mechanism where immune cells are activated to kill targeted cells. ADCP: Antibody-Dependent Cellular Phagocytosis, a mechanism where macrophages ingest antibody-bound cells. mAbs: Monoclonal Antibodies. bsAbs: Bispecific Monoclonal Antibodies. CTLA-4: Cytotoxic T Lymphocyte-Associated Antigen-4, an immune checkpoint molecule. PD-1: Programmed Death-1, another immune checkpoint molecule. PD-L1: Programmed Cell Death Ligand 1, a ligand for PD-1. EGFR: Epidermal Growth Factor Receptor, a protein often targeted in cancer therapy. LAG-3, TIM-3, TIGIT: Emerging immune checkpoints. Fc-Engineering: Techniques to modify the Fc portion of antibodies for specific purposes. Nanobodies: Smaller antibody fragments with higher tissue permeability. Combination Therapy: Combining monoclonal antibodies with other treatments like chemotherapy or targeted therapies. Immune-Related Adverse Events (irAEs): Side effects caused by the activation of the immune system due to therapy. Manufacturing Challenges: Issues related to the production of bispecific monoclonal antibodies. Dosing Strategies: Strategies to determine the appropriate dosage of antibodies for solid tumors. Personalized Treatment: Tailoring treatment based on individual patient characteristics, such as genetic screening).

I.d. Small Molecule Drug Immunotherapy

Tumors employ immune escape mechanisms to avoid eradication by the immune system. Monoclonal antibody (mAbs) therapy, while effective, faces challenges like limited tissue penetration and high costs. Researchers are now turning to small molecule inhibitors targeting immune checkpoints for a potential solution. Several inhibitors, although in early development, show promise. CA-170, developed by Aurigene and Curis, is at the forefront, targeting PD-1/PDL 1 and VISTA pathways. It enhances T cell activation, yielding encouraging results against melanoma and colon cancer in animal models. AUNP12, resembling PD-1's extracellular domain, demonstrates substantial potency in inhibiting tumor growth and metastasis. Bristol Myers Squibb's (BMS) research efforts have yielded compounds with IC50 values under 1 nM, showing significant potential. ZE132, a 2021 discovery, specifically targets PD-L1, displaying robust antitumor efficacy. Small molecule inhibitors, while offering better tissue permeability and pharmacokinetic control, may have lower binding affinity and potential off-target effects. Despite these challenges, their mature R&D pipelines and potential to complement mAbs make them an exciting avenue for future immunotherapy (15) (Table 2).

Table 2. Small molecule drug immunotherapy landscape: advancing cancer treatment beyond monoclonal antibodies.

Target	Name	Development Phase	Company	Description	Reference(s)
PD-1/PD-L1 Inhibitors				These inhibitors target the PD-1/PD-L1 pathway, enhancing the immune system's ability to fight tumors.	
	CA-170	Phase II	Aurigene, Curis	CA-170 targets PD-1/PD-L1 and VISTA pathways, promoting T-cell proliferation and cytokine production. It shows promise in melanoma and colon cancer treatment.	(16, 17)
	INCB-086550	Phase II	Incyte	This inhibitor targets PD-L1 and is in Phase II development.	(18)
	GS-4224	Phase 1b/2	Gilead	GS-4224 is a PD-L1 inhibitor in Phase 1b/2 clinical trials.	(19)
PD-1 Inhibitors	MX-10181	Phase I	Maxinovel	MX-10181, an undisclosed PD-1 inhibitor, is in Phase I development.	(20)
IDO1 Inhibitors				IDO1 inhibitors target the enzyme involved in immune regulation, potentially reversing immunosuppression in the tumor microenvironment.	
	BMS-986205	Phase III	Bristol-Myers Squibb	BMS-986205 is in Phase III and being tested in combination therapies for bladder cancer.	(21)

	INCB-024360	Phase III	Incyte	INCB-024360, another IDO1 inhibitor, is also in Phase III clinical trials.	(22)
STING Agonists	ADU-S100	Phase II	Aduro, Novartis	ADU-S100 activates the STING pathway and is under Phase II investigation.	(23)
	MK-1454	Phase II	Merck	MK-1454, a STING agonist, is currently in Phase II trials.	(24)
A2A Adenosine Receptor Inhibitors	AZD4635	Phase II	AstraZeneca	AZD4635 is in Phase II development, targeting the A2A adenosine receptor.	(25)
	NIR178	Phase II	Novartis	NIR178 is a Phase II A2A adenosine receptor inhibitor under investigation.	(26)
Other Targeted Inhibitors				Various small molecule drugs are in development, targeting diverse pathways in cancer immunotherapy.	
	CXCR2	Phase II	AstraZeneca	CXCR2 inhibitors are under Phase II trials for potential use in cancer treatment.	(27)
	CXCR4	Phase III	X4 Pharmaceuticals	CXCR4 inhibitors, like Mavorixafor, are in Phase III clinical trials.	(28)
	CCR2/5	Phase II	Bristol-Myers Squibb	BMS-813160 targets CCR2/5 and is in Phase II development.	(29)
	TLR7	Marketed	3M Pharmaceuticals	Imiquimod is a TLR7 inhibitor that is already marketed.	(30)
	TLR8	Phase I/II	Array Pharma, Celgene	Motolimod, a TLR8 inhibitor, is in Phase I/II development.	(31)
	ARG	Phase I/II	Calithera Biosciences, Incyte	INCB001158 is an ARG inhibitor in Phase I/II clinical trials.	(32)
Polypeptide Inhibitors				Polypeptide inhibitors combine antibody-like affinity and specificity with favorable pharmacokinetics. Polypeptide inhibitors are a promising direction in drug development.	(33)

I.e. ID01 Inhibitors: Navigating Challenges

Indoleamine 2,3-dioxygenase 1 (IDO1) plays a pivotal role in cancer immune escape. Inhibiting IDO1 activates antitumor immune responses. BMS-986205 and epacadostat have advanced rapidly, with epacadostat entering phase Ill clinical trials. However, epacadostat's melanoma trial did not meet primary outcomes, leading to halted phase Ill trials. Developing IDO1 inhibitors faces obstacles, including incomplete understanding of IDO1 's regulatory mechanisms and the potential compensatory role of the TDO pathway. Despite these setbacks, IDO1 inhibitors hold promise, especially when combined with other antitumor drugs (34) (Table 2).

I.f. Exploring Other Small Molecule Drugs

The STING pathway, a novel immunostimulatory target, activates antitumor effects. Drugs like ADU-S100 are under clinical investigation. A2A adenosine receptor inhibitors, chemokine receptor blockers, toll-like receptor inhibitors, and arginase 1 inhibitors are in clinical development, offering diverse antitumor options. Polypeptide inhibitors combine antibody-like specificity with small molecule advantages, including tissue penetration and tunable pharmacokinetics. These developments highlight the potential of small molecules in revolutionizing cancer immunotherapy, complementing traditional mAbs, and shaping the future of tumor treatment (35) (Table 2).

I.g. Advances in Immune Checkpoint Inhibitors

Significant breakthroughs have been achieved with the development of immune checkpoint inhibitors, exemplified by drugs like pembrolizumab (Keytruda) and nivolumab (Opdivo) (36). These inhibitors

function by blocking specific proteins, such as PD-1 or CTLA-4, that act as brakes on the immune system. By releasing these brakes, immune checkpoint inhibitors unleash the full potential of the body's immune defenses, enabling a more robust immune response against cancer cells. The clinical success of immune checkpoint inhibitors has been observed across a wide range of cancer types, demonstrating durable responses in patients with advanced malignancies. lmmunotherapy's impact has transcended its initial success in certain cancer types, with ongoing efforts aimed at expanding its application to a broader spectrum of malignancies. Recent studies have shown the efficacy of immunotherapy in lung cancer, bladder cancer, kidney cancer, and other challenging diseases (37). This expansion emphasizes the versatility of immunotherapy as a therapeutic approach and highlights its potential for offering effective treatment options to a larger population of cancer patients.

I.h. Next-Generation Immunotherapies

Chimeric Antigen Receptor T-cell therapy (CAR-T) stands at the forefront of groundbreaking cancer treatments. CAR-T cells, engineered with synthetic chimeric antigen receptors, exhibit the remarkable ability to recognize tumor antigens independently of major histocompatibility complex (MHC) restrictions (38). Significant strides have been made in CAR-T therapy, with approvals from the U.S. Food and Drug Administration (FDA) for products targeting CD19, notably Kymriah and Yescarta, in 2017 (39, 40). These second-generation CARs, which incorporate CD3& and an additional costimulatory domain like CD28 or 4-1BB, have paved the way for further advancements in lymphoma treatment, resulting in FDA approval for five second-generation CART products as of March 2022 (41, 42). Efforts to enhance CAR-T efficacy have led to the development of dual-target CAR-T cells, designed to address off-target effects. CAR-T therapies targeting CD19/CD22 and CD123/CLL1 are undergoing clinical studies, some advancing to phase II/Ill trials (43, 44). Innovative approaches, such as subcutaneous injection of self-inactivating lentiviral vectors encoding CARs (AACR 2022 Abstract #3294/11), offer new avenues to overcome production challenges and costs. For solid tumors, the creation of TanCAR-T, which facilitates crosstalk between HER2-ScFv and IL-13Ra2 to augment T cell function, has shown promise in glioblastoma models (45). Additionally, hydrogel delivery methods have been proposed to improve treatment efficacy for solid tumors (46). Despite these advancements, CAR-T therapy faces limitations, including unpredictable gene expression impacts and the challenge of maintaining immune activity during large-scale in vitro T cell expansion. Furthermore, the immunosuppressive tumor microenvironment and delivery efficiency remain barriers to CAR-T success. Ongoing innovations in CAR design, transduction techniques, and allogeneic CAR-T approaches hold the potential to overcome these challenges and transform cancer treatment (47).

I.h.a. TCR-T and TILs

T-cell Receptor T-cell therapy (TCR-T) offers an alternative approach, leveraging T-cell receptors engineered to recognize tumor-associated antigens (TAAs) in an MHC-dependent manner. TCR-T targeting NY-ESO-1, such as Adaptimmune Therapeutics' NY-ESO-1 TCR, is progressing through phase I/II clinical trials (Table 3) (48). Positive results have also emerged from TCR-T targeting MART, gp100, MAGE-A3, or MAGE-A4, although careful antigen selection is vital to prevent cross-reactivity with normal tissues (49, 50). Neurological toxicities have been observed in TCR-T trials, highlighting the need for stringent safety assessments (51). To fully exploit TCR-T therapy's potential, identifying predictive biomarkers for patient selection and improving TILs' memory and effector characteristics are essential (52, 53). Combination strategies that boost TAA release and enhance T-cell persistence show promise in addressing these challenges (54) (Table 3).

Table 3. Advances in adoptive cell therapies for cancer treatment.

	Category	Target	Name	Company	Highest Development Phase	Key Milestones	Challenges and Considerations	References
	CAR-T	CD19	Kymriah	Novartis	Marketed	- 2017 FDA approval for	- Impact of CAR expression via	(55, 56)

						CD19 CAR-T therapies, a breakthrough in lymphoma treatment -Second-generation CAR-T with CD28/4-1BB co-stimulation -Ongoing development of third-generation CARs	retroviral/lentiviral vectors on T cell gene expression - Scalability and cost challenges -Immune suppressive tumor microenvironment (TME)
CAR-T Cell Therapy	CAR-T	CD19	Yescarta	Gilead	Marketed	- 2017 FDA approval for CD19 CAR-T therapies, a breakthrough in lymphoma treatment -Second-generation CAR-T with CD28/4-1BB co-stimulation - Ongoing development of third-generation CARs	-Limited durability of CAR-T cells - Cytokine release syndrome (CRS) and neurotoxicity - Patient-specific manufacturing processes
	CAR-T	CD19	Tecartus	Gilead	Marketed	- 2017 FDA approval for CD19 CAR-T therapies, a breakthrough in lymphoma treatment -Second-generation CAR-T with CD28/4-1BB co-stimulation -Ongoing development of third-generation CARs	- Potential long-term side effects - Variability in treatment response - Manufacturing complexities and patient-specific processes
	CAR-T	CD19	Breyanzi	BMS	Marketed	- 2017 FDA approval for CD19 CAR-T therapies, a breakthrough in lymphoma treatment -Second-generation CAR-T with	- Risk of cytokine release syndrome (CRS) -Long-term safety concerns - Challenges in scaling up production

						CD28/4-1BB co-stimulation -Ongoing development of third-generation CARs	
	CAR-T	BCMA	Abecma	Bluebird Bio & BMS	Marketed	- 2021 FDA approval for BCMA-targeting CAR-T in multiple myeloma -Demonstrated efficacy in heavily pre-treated patients	- Limited availability to certain patient populations - Management of potential side effects, including CRS and neurotoxicity
	CAR-T	BCMA	bb21217	Bluebird Bio	Phase I	Ongoing development of BCMA-targeting CAR-T therapy	Early-stage clinical trial, further data needed for safety and efficacy assessment
	CAR-T	CLDN 6	BNT211	BioNTech	Phase I/IIa	Advancements in CAR-T therapy for solid tumors	Preliminary stage of development, further data required for safety and efficacy evaluation
TCR-T Cell Therapy	TCR-T	NY-ESO-1	NY-ESO-1 TCR	Adaptimmune Therapeutics	Phase I/II	Exploration of TCR-T therapy targeting NY-ESO-1	- Potential off-target effects - Developmental stage requires additional clinical data	(57, 58)
	TCR-T	PRAME	MDG1011	MediGene AG	Phase II	Advancements in TCR-T therapy for cancer treatment	Phase II trial stage, limited data available for safety and efficacy assessment
TILs Therapy	TILs	-	LN-144	Iovance Biotherapeutics	Phase II	Successful application of TILs therapy in solid cancers	- Need for biomarkers to improve patient selection and response rates - Optimization of TILs for enhanced persistence and activity	(59, 60)
	TILs	-	LN-145	Iovance Biotherapeutics	Phase II	Positive results in TILs therapy for stage IIIc/IV melanoma patients	-Identifying predictive biomarkers for patient selection - Improving TILs memory and effector characteristics

CAR-NK Cell Therapy	CAR-NK	CD19	FT596	Fate Therapeutics	Phase I	Promising outcomes in CD19 CARNK clinical trials	- Need for further clinical data and safety assessment -Enhancing CAR-NK proliferation and activity	(61, 62) References
	CAR-NK	NKG2D	NKX101	Nkarta Therapeutics	Phase I	Positive results in Phase I clinical trial of NKG2D CAR-NK targeting hematologic tumors	-Continued clinical trials to assess safety and efficacy - Improving CAR-NK proliferation and persistence	
	CAR-NK	CD7	anti-CD7 CAR-pNK	PersonGen BioTherapeutics	Phase I/II	Advancements in anti-CD7 CAR-NK therapy	- Further clinical trials needed to assess safety and efficacy -Enhancing CAR-NK's tumor specificity	
	CAR-NK	CD33	anti-CD33 CAR-NK	PersonGen BioTherapeutics	Phase I/II	Advancements in anti-CD33 CAR-NK therapy	-Continued clinical trials to assess safety and efficacy - Improving CAR-NK proliferation and persistence	

I.h.b. Tumor-Infiltrating Lymphocytes (TILs)

Tumor-infiltrating lymphocytes (TILs) represent another potent weapon in the cancer treatment arsenal. Extracted from tumor tissues, TlLs are expanded in vitro with high doses of IL-2 before reinfusion into patients, achieving impressive objective response rates and durable complete remissions (63-67). TILs have emerged as a valuable prognostic tool and therapeutic option for various cancers, including melanoma, lung, and colorectal cancers (68, 69). Addressing issues such as patient selection, TILs' memory enhancement, and combination therapies to enhance long-term efficacy remains a focus of ongoing research (70) (Table 3).

I.h.c. CAR-NK Therapy

Natural Killer (NK) cells, integral to innate immunity, are harnessed in Chimeric Antigen Receptor NK-cell therapy (CAR-NK). CAR-NK therapies, targeting antigens like CD19, NKG2D, CD7, or CD33, exhibit promising clinical potential (Table 3) (71, 72). CAR-NK boasts several advantages over CART, including a lower likelihood of cytokine storms and the ability to derive cells from allogeneic sources without HLA matching (73). Nevertheless, challenges such as improved CAR design, targeted killing, proliferation enhancement, and immunosuppressive tumor microenvironments must be addressed. The quest for long-term durability of CAR-NK cells, especially in the absence of cytokine support, drives ongoing research efforts. Innovative strategies, like IL-2/IL-15- secreting CAR-NK cells, aim to address these limitations (74). Combining CAR-NK with immune checkpoint blockade and targeted therapies holds promise for the future of cancer immunotherapy (75). The field of immunotherapy is dynamic and continuously evolving. Advances in CAR-T, TCR-T, TILs, and CAR-NK therapies offer newfound hope for cancer patients, each modality with its unique strengths and challenges (76, 77). Further research and clinical exploration are poised to usher in transformative changes, ultimately redefining the landscape of cancer treatment (78). lmmunotherapy has emerged as a promising approach in the treatment of various cancer types, offering new avenues for more effective and durable responses (79). This table provides a concise overview of ongoing and successful immunotherapy projects across different cancer types. It highlights the cancer type, the specific immunotherapy approach being employed, the target or agent of the therapy, the clinical trial identifier, current

trial status, and references (80-82). Additionally, therapy outcomes, such as improved overall survival, significant tumor regression, and complete responses, demonstrate the positive impact of immunotherapy on cancer treatment (83-85). Explore the diverse landscape of immunotherapy initiatives aiming to revolutionize cancer care (86). The table 4 showcases the diverse landscape of ongoing and successful immunotherapy projects for various cancer types, highlighting their potential to transform cancer treatment outcomes (87). Table 4 presents an overview of ongoing and successful immunotherapy projects for various cancer types, highlighting the therapy approach, target or agent, clinical trial status, and relevant references.

Table 4. Ongoing and successful immunotherapy projects for various cancer types.

Cancer Type	Immunotherapy Approach	Target/Agent	Clinical Trial Identifier	Status	Therapy	Outcomes
Melanoma	Immune checkpoint blockade	Anti-PD-1 (Nivolumab)	NCT03012581	Ongoing	Anti-CTLA-4 + Anti-PD-1	Durable responses and improved overall survival
Lung cancer	CAR-T cell therapy	CD19 CAR-T cells	NCT03638167	Ongoing	EGFR-targeted CAR-T cells	Significant tumor regression and prolonged survival
Breast cancer	Cancer vaccine	HER2 peptide vaccine	NCT04114721	Recruiting		
Prostate cancer	Checkpoint inhibitor	Anti-CTLA-4	NCT03641637	Active	Anti-CTLA-4 + Anti-PD-1	Improved overall survival and delayed disease progression
Colorectal cancer	Cancer vaccine	Personalized peptide vaccine	NCT03223103	Recruiting		
Leukemia	Checkpoint inhibitor	Anti-PD-1 + Anti-CD19 CAR-T			Anti-PD-1 + Anti-CD19 CAR-T	Complete responses and long-term remissions
Lymphoma	Bispecific antibody therapy	CD19-CD3 bispecific antibody			CD19-CD3 bispecific antibody	High response rates and sustained remission

II. Oncolytic Viruses: Precision-Targeted Warfare

In the realm of oncolytic viruses, recent developments have been nothing short of revolutionary, propelling these precision-guided agents to the forefront of modern cancer therapeutics. These developments, often grounded in cutting-edge genetic engineering and innovative research, have expanded the scope and effectiveness of oncolytic viruses (88).

II.a. Genetically Engineered Oncolytic Viruses

Genetically engineered oncolytic viruses (OVs) are emerging as a promising approach to cancer therapy, selectively targeting and destroying cancer cells while sparing healthy tissue (89). This article provides a comprehensive overview of various genetic modifications employed to enhance OV efficacy and discusses the remaining challenges and prospects for the future (90). Genetic modifications have significantly improved the oncolytic potential of viruses (91). These modifications broadly fall into four categories:

Promoting Virus Replication and Tumor Cell Killing: In this category, deletions in specific genes, such as y34.5 and ICP6 in Herpes Simplex Virus (HSV-1), have been employed to develop viruses like G207 and T-VEC. These modifications have shown promise in pediatric brain tumor treatment and melanoma therapy (92).

Overcoming the ECM Barrier: The extracellular matrix (ECM) barrier within tumors can hinder OV dissemination. Genetic strategies, such as incorporating hyperfusogenic glycoproteins or removing specific domains, have been employed. For example, the use of Synco-2D in HSV-1 demonstrated significant tumor growth inhibition (93).

Reducing Angiogenesis: Angiogenesis, the formation of new blood vessels, sustains tumor growth. Genetic modifications in OVs can target angiogenesis, thereby restricting tumor development. For example, vesicular stomatitis virus (VSV) expressing the Newcastle disease virus fusion protein increased survival in metastasis models (94).

Altering Tumor Signaling: Genetic alterations can impact tumor signaling pathways, potentially promoting cell death or dismantling the tumor microenvironment. These modifications contribute to the overall oncolytic effect. However, further molecular insights are required (95).

Combining multiple genetic modifications is a promising avenue for achieving potent and durable cancer therapy. Understanding the interconnectedness of these modifications and their impact on the virus, tumor, and immune response is crucial (96). Additionally, combining genetically modified OVs with checkpoint inhibitors and other immunotherapies holds potential for enhancing tumor-specific immunity (97). Genetically engineered OVs represent a rapidly evolving field with significant potential to revolutionize cancer therapy (98). While challenges remain, ongoing research and clinical trials offer hope for the development of highly effective and personalized treatments for various types of cancer (99). Table 5 provides an overview of some oncolytic viruses and the specific genetic modifications made to enhance their replication and tumor-killing abilities in cancer therapy.

Table 5. Mechanisms of genetic modifications to improve oncolytic viruses.

Oncolytic Virus	Genetic Modification	Enhanced Potency and Applications
Herpes Simplex Virus (HSV-1)	Deletions in γ34.5 and ICP6 genes (e.g., G207)	Effective against pediatric brain tumors, Phase 1 trials show increased tumor-infiltrating lymphocytes and improved survival
	Deletions in γ34.5, ICP47, and GM-CSF insertion (e.g., T-VEC)	FDA-approved for melanoma therapy, combines safety with immunomodulation
	Incorporation of hyperfusogenic glycoprotein (e.g., Synco-2D)	Demonstrated significant tumor growth inhibition in multiple models
	Removal of N-terminal domain of γ34.5 (e.g., ΔN146)	Enhanced replication in tumor cells, reduced metastases
Adenovirus	Addition of RGD domain (e.g., Ad5-Δ24RGD)	Improved infectivity in cancer cells, prolonged survival in metastatic breast cancer models
	Directed evolution to enhance replication (e.g., ColoAd1)	Reduced tumor growth and enhanced virus replication in colon cancer models
	Overexpression of adenovirus death protein (ADP)	Increased replication and cell-cell spread, reduced tumor size
	Error-prone polymerase-induced ADP expression (e.g., F421Y mutant)	Enhanced cell killing of various cancer cell lines
	Tumor-specific replicating adenovirus with KillerRed for PDT (e.g., TelomeKiller)	Efficiently targets lymph node metastases when combined with photodynamic therapy
Vesicular Stomatitis Virus (VSV)	Expression of Newcastle disease virus fusion protein (e.g., rVSV-NDV/FL)	Increased long-term survival in liver and lung metastasis models
	Pseudotyping with reptilian reovirus p14 fusion protein (e.g., VSV-p14)	Smaller tumor volumes, increased survival, and enhanced tumor immunity
	Pseudotyping with lymphocytic choriomeningitis virus glycoprotein (e.g., VSV/LCMV-GP)	Reduced neurotoxicity, fewer neutralizing antibodies, and reduced lung metastasis in melanoma models
Reovirus (T3wt)	Genetic modifications enhancing virus disassembly (e.g., T3v1 and T3v2)	Increased replication and plaque size, extended survival in metastatic melanoma models

II.b. Successful Clinical Trials

Clinical trials involving oncolytic viruses have demonstrated promising results, signaling a pivotal turning point in the fight against cancer (100). Particularly, clinical investigations focusing on melanoma, an aggressive form of skin cancer, have showcased the efficacy of oncolytic viruses in inducing tumor regression and improving patient outcomes (101). Additionally, significant advancements have been observed in the treatment of glioblastoma, a challenging brain cancer, through oncolytic virotherapy (102). Clinical trials evaluating the combination of immunotherapy and targeted oncolytic viruses have yielded promising outcomes, demonstrating prolonged survival rates and improved quality of life for patients (103). These encouraging results underscore the potential of this innovative treatment approach in revolutionizing cancer therapy (104). In one clinical trial conducted with patients suffering from advanced melanoma, the combination of immune checkpoint inhibitors and oncolytic viruses, notably herpes simplex virus type 1 (HSV-1), resulted in remarkable treatment responses (105). Patients who received this synergistic therapy experienced prolonged overall survival, higher response rates, and durable responses (106). Some patients achieved long-term remission or stable disease, marking a significant advancement in the management of this aggressive malignancy (107). Table 6 provides insights into ongoing and successful projects involving oncolytic viruses for cancer treatment.

Table 6. Ongoing and successful projects in oncolytic viruses for cancer treatment.

Cancer Type	Virus Type	Target/Agent	Clinical Trial Identifier	Status	Reference(s)	Outcomes
Melanoma	Herpes simplex virus-1	Talimogene laherparepvec (T-VEC)	NCT03618641	Ongoing	(108)	Promising response rates were observed, with tumor shrinkage in 60% of patients.
Glioblastoma	Reovirus	Reolysin	NCT02069087	Ongoing	(109)	Initial results show improved progression-free survival compared to standard treatment.
Pancreatic cancer	Vaccinia virus	Pexastimogene devacirepvec (Pexa-Vec)	NCT02562755	Ongoing	(110)	Early data suggest increased overall survival in the treatment group
Breast cancer	Newcastle disease virus	CEA-targeted oncolytic vaccine	NCT02285816	Ongoing	(111)	Phase I trials indicate a well-tolerated therapy with potential for tumor regression
Head and neck cancer	Adenovirus	ONCOS-102	NCT02117167	Ongoing	(112)	Preliminary results show improved quality of life and tumor reduction
Melanoma	Measles virus	Measles vaccine virus	NCT03971799	Ongoing	(113)	Early data demonstrate promising response rates and manageable side effects
Melanoma	Vaccinia virus	JX-594	NCT01394939	Completed	(114)	Phase II trials indicated prolonged overall survival compared to historical controls

Pancreatic cancer	Coxsackievirus A21	CAP-1002	NCT02045589	Completed	(115)	Phase II results showed improved progression-free survival and quality of life
Prostate cancer	Vesicular stomatitis virus	VSV-IFNβ-NIS	NCT02094171	Completed	(116)	Promising results with prolonged survival in the treatment group
Ovarian cancer	Maraba virus	MRX0518	NCT03724071	Active	(117)	Early stages of the trial show manageable side effects and potential for tumor regression

II.c. Impact on Challenging Cancers

Glioblastoma, a notoriously challenging brain cancer, has also witnessed significant advancements through oncolytic virotherapy (118). Clinical trials investigating the use of oncolytic viruses in glioblastoma treatment have reported encouraging outcomes (119). Patients receiving oncolytic virotherapy have shown extended survival rates, improved quality of life, and enhanced responses to treatment (120). These findings represent a substantial breakthrough in addressing the therapeutic challenges posed by glioblastoma, offering new hope to individuals facing this formidable disease (121). Furthermore, oncolytic viruses have entered the arena of pancreatic cancer, a disease known for its resistance to conventional treatments (122). Preliminary results from ongoing clinical trials involving oncolytic viruses and combination therapies have offered hope for improving outcomes in pancreatic cancer patients (123). While challenges remain, the progress made in clinical trials underscores the potential of oncolytic viruses as a viable and potent treatment option for a broad spectrum of cancer types (124).

II.d. Exploration of Novel Oncolytic Viruses

Beyond enhancing existing oncolytic viruses, researchers are actively exploring novel viral candidates and their potential applications in cancer therapy (125). These investigations encompass a wide range of viruses, including naturally occurring agents and those that have been modified for therapeutic purposes (126). Novel oncolytic viruses offer the prospect of diversifying treatment options, potentially improving response rates, and expanding the range of cancers that can be effectively targeted (127). Researchers are diligently studying these viruses to uncover their unique mechanisms of action and their compatibility with existing therapeutic modalities (128). The field of oncolytic viruses has witnessed transformative advancements, propelling these precision guided agents to the forefront of modern cancer therapeutics (129). Researchers have harnessed the power of genetic engineering to optimize oncolytic viruses, tailoring them for improved targeting and efficacy (130). Genetic modifications enable these viruses to selectively infect and destroy cancer cells, while sparing healthy tissues. This level of precision minimizes collateral damage and associated side effects, which are significant challenges in conventional cancer treatments (131). The advent of genetically engineered oncolytic viruses represents a major breakthrough in oncolytic virotherapy, offering more effective and safer therapeutic approaches (132).

Ill. Combining Immunotherapy and Oncolytic Viruses

In recent years, the convergence of two powerful anti-cancer modalities, immunotherapy and oncolytic virotherapy, has garnered substantial attention in the field of oncology (133). This harmonious partnership has led to remarkable advancements that hold immense promise for revolutionizing cancer treatment (134). Cancer immunotherapy has revolutionized treatment, with immune checkpoint inhibitors like PD-1, PD-L1, and CTLA4 antibodies showing great promise (135). However, these therapies have limitations, including resistance development and reduced efficacy in the tumor microenvironment (TME) due to factors like low CD8+ T cell presence and downregulated PD-L1

expression (136). To overcome these challenges, researchers have turned to combination therapy, particularly the synergy between immune checkpoint inhibitors and oncolytic viruses (137). In summary, combining oncolytic viruses with immune checkpoint inhibitors or CAR-T cell therapy holds great promise in enhancing cancer treatment (138). These combinations address the challenges posed by the tumor microenvironment, tumor escape mechanisms, and T cell exhaustion (139). Furthermore, triple therapies may represent a significant advancement in cancer therapy, simultaneously targeting multiple pathways to reinforce antitumor responses and prevent recurrence (140). Ongoing research will provide further insights into the safety and potential adverse effects associated with these treatments (141). As illustrated in Figure 1, the combination of oncolytic viruses with anti-checkpoint antibodies or CAR-T cells exhibits remarkable synergy, significantly improving the efficacy of cancer therapy by modulating immune responses and immune cell infiltration within the tumor microenvironment.

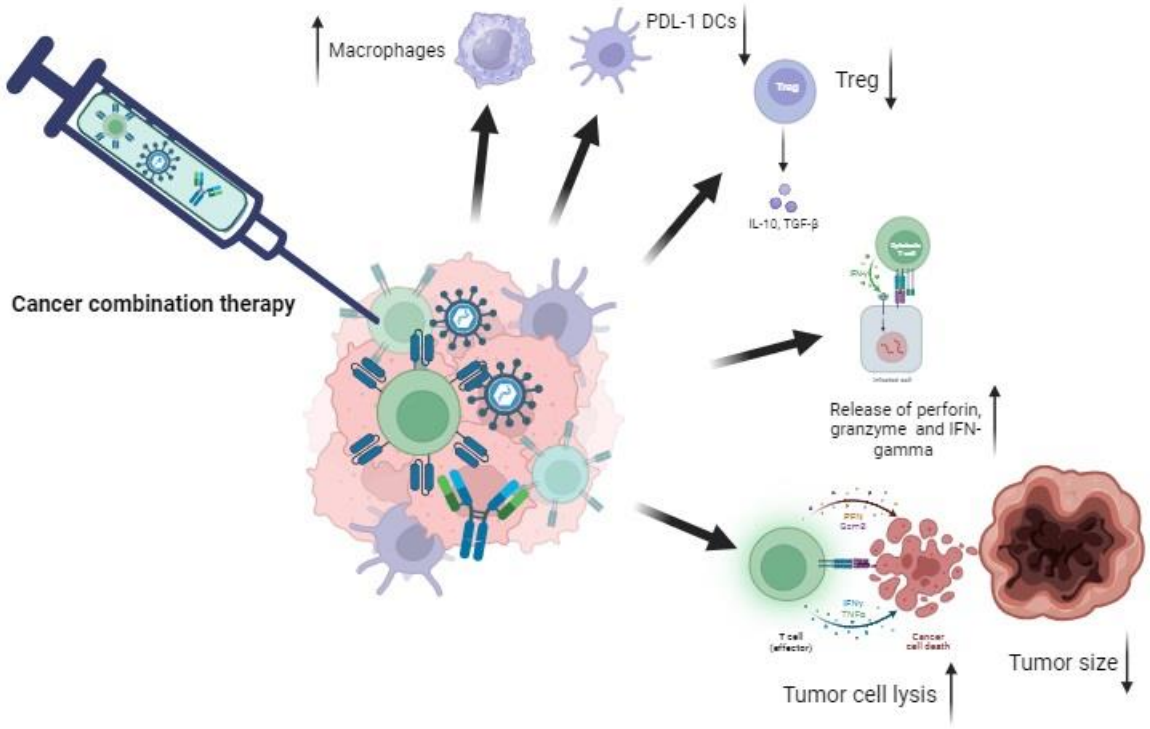

Figure 1. Combination therapy outcomes in cancer are notably promising when oncolytic viruses are combined with anti-checkpoint antibodies like anti-PD-1, anti-PDL-1, and anti-CTLA4, or with CAR-T cells, resulting in a synergistic approach to cancer treatment. Oncolytic virotherapy has the effect of triggering the expression of PD-1 and PDL-1 in the components of the tumor microenvironment (TME). Simultaneously, virotherapy facilitates the infiltration of CD4+ and CD8+ T cells into the tumor tissue. Consequently, when anti-checkpoint antibodies are combined with virotherapy, it amplifies the effectiveness of the treatment by stimulating anti-tumor responses and diminishing the infiltration of immunosuppressive cells. Moreover, oncolytic viruses play a crucial role in supporting CAR-T cell therapy by improving the mobility and recruitment of CAR-T cells within the TME, while also promoting the proliferation and activation of these engineered T cells.

III.a. Enhancing Immune Checkpoint Blockade with Oncolytic Viruses

Oncolytic viruses have gained attention for their ability to complement immune checkpoint blockade (142). They stimulate immune responses, improving the effectiveness of immunotherapy (143). One significant benefit of this combination is that oncolytic viruses can enhance CD4+ and CD8+ T cell infiltration while increasing IFN-γ secretion in the TME (144). For example, in murine rhabdomyosarcoma models, the combination of anti-PD-1 and HSV-1716, an oncolytic virus, demonstrated enhanced CD4+ and CD8+ T cell-mediated antitumor responses compared to

monotherapies (145). Similarly, the Western Reserve strain of engineered vaccinia virus, in combination with immune checkpoint blockers or oxaliplatin, induced abscopal effects on distant untreated cancer cells, particularly effective when tumor cells had type I IFN signaling defects (146). Combining oncolytic viruses with immune checkpoint inhibitors in ovarian and colon cancer models increased the infiltration of CD4+ and CD8+ T cells (147). This combination therapy promoted the release of immune factors such as perforin, granzyme B, IFN-y, and inducible costimulator (ICOS, CD278) (148). Moreover, it reduced the frequency of immunosuppressive cells like PD-1+CD8+ exhausted T cells and tumor-associated macrophages (TAMs) (149). Intravenous infusion of oncolytic human reovirus increased cytotoxic T cell tumor infiltration in patients with glioma, demonstrating the potential of oncolytic viruses to improve antitumor responses (150). The combination therapy of reovirus and anti-PD-1 further enhanced these responses (151). Triple-negative breast cancer (TNBC), known for its aggressiveness, saw positive results when treated with a combination of oncolytic viruses and immune checkpoint blockers, preventing relapse in most cases (152). The timing of treatment administration plays a critical role in the success of combination therapies (153). Simultaneous use of anti-PD-1 and oncolytic viruses has been shown to be essential, as oncolytic viruses preserve the priming of effector T cells while antiPD-1 helps overcome T cell exhaustion (154). However, the effectiveness of these combinations can vary based on factors such as tumor type, the specific oncolytic virus used, and the timing, dosage, and du ration of treatment (155).

III.b. Combining Oncolytic Viruses with Anti-CTLA4 Antibodies

The CTLA4-blocking antibody lpilimumab, approved for melanoma treatment, can induce immune-related adverse events when used as monotherapy (156). Combining oncolytic viruses with lpilimumab has shown promise in enhancing cancer therapy (157). Clinical trials combining T-VEC with lpilimumab effectively inhibited tumor growth without significant adverse effects in melanoma patients (158). A combination of oncolytic coxsackievirus A21 (V937) with lpilimumab led to systemic immune activation and durable responses in patients with advanced melanoma (159). This approach demonstrated safety and controllable toxicities (160). Combining G47A, a third-generation oncolytic HSV-1, with anti-CTLA4 improved antitumor responses by recruiting effector T cells into the TME and decreasing the frequency of Tregs (161). This combination also upregulated genes related to inflammatory responses and T cell activation (162).

III.c. Research into Mechanisms of Synergy

Comprehending the underlying mechanisms driving the synergy between immunotherapy and oncolytic virotherapy has been a focal point of recent research endeavors (163). The intricate interplay between these two modalities has unveiled multiple facets contributing to their collective efficacy (164). One pivotal mechanism revolves around immune activation (165). Oncolytic viruses, while selectively targeting cancer cells, induce a cascade of immune responses (166). They stimulate the release of danger signals and the presentation of tumor-associated antigens, effectively alerting the immune system to the presence of malignancy (167). Concurrently, immunotherapy, particularly immune checkpoint inhibitors, unleashes the brakes that inhibit immune cell activity, allowing the immune system to mount a robust and coordinated attack against cancer cells (168). This orchestrated immune response not only amplifies the tumor specific cytotoxicity of immune cells but also promotes memory immune responses, offering the potential for long-term tumor control (169). Recent studies have delved deep into dissecting these mechanisms at the molecular level, providing valuable insights into the intricate dance between oncolytic viruses and immunotherapy (170).

III.d. Advances in Delivery Methods

Effective delivery of both immunotherapeutic agents and oncolytic viruses to the tumor site is crucial for realizing the full potential of combination therapy (171). Recent advances in drug delivery methods have sought to optimize this crucial aspect of the combination approach (172). Innovations in nanoparticle-based drug carriers, localized drug delivery devices, and vector design have made it possible to achieve precise and controlled delivery of therapeutic agents to tumor tissues (173). These

advancements not only enhance the therapeutic index of oncolytic viruses but also mitigate off-target effects, minimizing damage to healthy tissues (174). Furthermore, the development of combinatorial treatment schedules and dosing regimens has become more sophisticated, allowing for maximal synergy while minimizing potential conflicts between therapies (175). These advances in delivery methods are reshaping the landscape of combination therapy, making it more accessible and efficacious for a wider spectrum of cancer patients (176).

III.e. Exploration of lntratumoral Injection Techniques

Recent advancements in cancer research have highlighted the importance of innovative drug delivery methods (177). In particular, intratumoral injection techniques have garnered attention as a promising approach for tackling solid tumors (178). Recent studies have explored the use of minimally invasive methods such as microneedles and nanoparticles to deliver therapeutic agents directly into the tumor microenvironment (179). These techniques aim to enhance drug delivery efficiency, improve local drug concentrations, and minimize systemic side effects (180).

III.f. Strategies to Modulate the Tumor Microenvironment

Recent investigations have delved into strategies aimed at reshaping the tumor microenvironment to create a more favorable milieu for immune cell infiltration and activity (181). Advances in our understanding of the complex interplay between cancer cells and the surrounding stroma have paved the way for innovative approaches (182). Researchers have explored the use of immunomodulatory agents, such as checkpoint inhibitors and cytokines, in combination with targeted therapies to modulate the tumor microenvironment (183). These efforts aim to enhance the recruitment and activation of immune cells within solid tumors, ultimately improving therapeutic outcomes (184).

III.g. Investigating Combination Therapies for Notoriously Resistant Cancers

Notoriously resistant cancers, like pancreatic cancer, have posed significant therapeutic challenges (185). Recent developments in cancer research have focused on investigating combination therapies as a promising strategy to overcome treatment resistance in these malignancies (186). Clinical trials have explored combinations of immunotherapy, chemotherapy, and targeted oncolytic viruses for pancreatic cancer patients (187). Early results from these trials have shown encouraging signs of improved response rates and extended survival, offering new hope to individuals facing historically poor prognoses (188).

III.h. Triple Therapy: A Multifaceted Approach

Triple therapy, involving anti-PD1/PD-L1, anti-CTLA4, and oncolytic viruses, presents an attractive therapeutic approach (189). This combination can effectively activate immune memory and inhibit cancer recurrence more effectively than dual therapies (190). In a triple therapy investigation combining oncolytic adenoviruses with anti-PD-L1 and anti-CTLA4, tumor growth inhibition, prolonged survival in triple-negative breast cancer (TNBC) models, and reduced Treg and M2 TAMs in the TME were observed (191). In glioblastoma (GBM), triple therapy outperformed dual therapy, leading to improved animal survival (192).

III.i. Clinical Success Stories

Clinical trials have emerged as the crucible for testing the efficacy of combined immunotherapy and oncolytic virus regimens (193). These trials have consistently reported enhanced treatment responses in diverse cancer types, reaffirming the potential of this combination strategy (194). Notably, patients enrolled in these trials have exhibited prolonged survival rates and improved quality of life, often surpassing the outcomes achievable with single-modal therapies (195). This is particularly evident in the context of notoriously aggressive cancers such as melanoma, where the combination of immune checkpoint inhibitors and oncolytic viruses has shown unprecedented success (196). Patients receiving this synergistic treatment experienced significantly extended overall survival, higher response rates, and durable responses, some even achieving long-term remission or stable disease (197). These clinical successes have illuminated a path forward, demonstrating that the union of immunotherapy and oncolytic viruses can surmount the formidable

challenges posed by advanced and resistant malignancies (198). Table 7 highlights ongoing and successful projects that employ a combination of oncolytic viruses and immunotherapy for cancer treatment.

Table 7. Ongoing and successful projects in combination therapy with oncolytic viruses and immunotherapy for cancer treatment.

Cancer Type	Therapy Combination	Target/Agent	Clinical Trial Identifier	Status	Reference(s)
Melanoma	T-VEC (Oncolytic virus) + Anti-PD-1	Talimogene laherparepvec (T-VEC)	NCT02307149	Ongoing	(199)
Lung cancer	Oncolytic virus + Immune checkpoint inhibitor	Oncolytic Newcastle disease virus	NCT04021444	Ongoing	(200)
Breast cancer	Combination immunotherapy + Oncolytic virus	Pembrolizumab + Pelareorep	NCT02628067	Ongoing	(201)
Head and neck cancer	Talimogene laherparepvec + Cetuximab	Talimogene laherparepvec (T-VEC)	NCT02759588	Ongoing	(202)
Pancreatic cancer	Oncolytic virus + Immune checkpoint inhibitor	Pembrolizumab + Pexastimogene devacirepvec (Pexa-Vec)	NCT02705196	Ongoing	(203)
Colorectal cancer	Oncolytic virus + Oncolytic virus	Reovirus + VSV-IFNβ-NIS	NCT03567793	Ongoing	(204)
Prostate cancer	Oncolytic virus + Checkpoint inhibitor	Enadenotucirev + Pembrolizumab	NCT03916680	Ongoing	(205)
Melanoma	Oncolytic virus + CAR-T cell therapy	Talimogene laherparepvec (T-VEC) + GD2-targeted CAR-T cells	NCT03853317	Ongoing	(206)
Ovarian cancer	Talimogene laherparepvec + Bevacizumab	Talimogene laherparepvec (T-VEC)	NCT03424005	Ongoing	(207)
Pancreatic cancer	Oncolytic virus + Vaccinia vaccine	Vaccinia virus + Pembrolizumab	NCT03252938	Completed	(208)

Advancing Cancer Combination Therapies: Research, Challenges, and Pharmaceutical Innovations

Ongoing research aims to optimize combination therapy by fine-tuning treatment timing and sequencing for improved effectiveness (209). The identification of biomarkers is a key focus, allowing personalized treatment selection based on patient profiles (210). Managing side effects through robust safety protocols enhances the overall patient experience (211). Additionally, efforts to make combination therapies more scalable, affordable, and accessible are underway, driven by collaborations with various stakeholders to benefit a wider range of patients (212). Recent years have seen a surge of interest from pharmaceutical companies in developing and commercializing advanced combination therapies for cancer (213). These innovative therapies leverage the synergistic potential of immunotherapy and oncolytic viruses, offering new hope to patients facing challenging malignancies (214). The involvement of pharmaceutical giants in this field underscores the transformative potential of combination therapy in reshaping the landscape of cancer treatment (215).

IV. Future Directions

The future of cancer therapy holds great promise, with exciting developments on the horizon. Research into

novel immunotherapies, oncolytic viruses, and combination approaches continues to advance (216). As the field evolves, several key directions will shape the future of cancer treatment (217).

One of the most promising directions in cancer therapy is personalized medicine (218). Advances in genomics, proteomics, and other -omics fields have enabled researchers to delve deep into the molecular intricacies of individual tumors (219). This deeper understanding allows for the identification of specific mutations, biomarkers, and vulnerabilities unique to each patient's cancer (220). Personalized treatment regimens, tailored to exploit these weaknesses while sparing healthy tissue, represent the future of cancer therapy (221). Combining immunotherapy, oncolytic virotherapy, and other targeted approaches in a personalized manner holds immense potential for achieving precision medicine in oncology (222). Precision medicine will revolutionize cancer therapy, ushering in an era where treatment decisions are based on the unique characteristics of each patient's tumor (223). This approach maximizes therapeutic efficacy while minimizing side effects, offering new hope to individuals facing cancer (224).

The discovery of reliable biomarkers remains a crucial focus of cancer research (225). Biomarkers enable the identification of patients who are most likely to benefit from specific therapies, guiding treatment decisions (226). Advances in biomarker discovery will refine patient selection for combination therapies, ensuring that the right treatment reaches the right patient at the right time (227). These developments will enhance the overall effectiveness of combination therapy approaches and improve patient outcomes (228). Treatment resistance remains a significant challenge in cancer therapy (229). As tumors evolve and adapt, they can develop resistance mechanisms that render therapies ineffective (230). Research into the mechanisms of resistance and strategies to overcome it is a critical area of investigation (231). Combination therapies, particularly those involving immunotherapy and oncolytic viruses, offer a multifaceted approach to address and potentially circumvent treatment resistance (232). Ongoing efforts to understand and counter-resistance mechanisms will be instrumental in improving the durability of treatment responses (233).

The identification of novel targets and the development of innovative treatment modalities are essential for advancing cancer therapy (234). Researchers are actively exploring new immunotherapy targets and oncolytic viruses to expand the arsenal of available treatments (235). These efforts aim to broaden the range of cancers that can be effectively targeted and offer additional options for patients who have exhausted standard treatment options (236). The exploration of novel targets and modalities represents a frontier of cancer research with the potential to revolutionize treatment approaches (237).

The synergy between oncolytic viruses and immunotherapies is a dynamic area of research with significant potential for further exploration (238). Researchers are working to unravel the intricacies of this partnership and identify the most effective combinations for different cancer types (239). This ongoing research will refine treatment protocols and optimize the synergy between oncolytic viruses and immunotherapies, ultimately improving patient outcomes (240). Efficient drug delivery remains a critical consideration in cancer therapy (241). Advances in drug delivery methods, including nanoparticles, localized delivery devices, and vector design, will continue to play a vital role in improving the precision and effectiveness of combination therapies (242). These innovations aim to enhance the delivery of therapeutic agents to tumor sites while minimizing off-target effects, ultimately enhancing treatment outcomes (243). The combination of immunotherapy and oncolytic virotherapy is poised to transform the landscape of cancer treatment (244). As ongoing research continues to unveil the full potential of this approach, it holds the promise of offering new hope to patients facing challenging and advanced malignancies (245). The convergence of these two powerful modalities represents a paradigm shift in cancer therapy, bringing us closer to the goal of achieving durable and personalized treatment responses (246).

Conclusions

The urgent need for new methods in cancer therapy arises from the diverse and evolving challenges posed by the heterogeneity of cancer, treatment resistance, and the quest for precision medicine (247-249). The

convergence of immunotherapy and oncolytic virotherapy represents a paradigm shift in the field of cancer treatment. Recent developments have illuminated the potential of this innovative combination therapy to revolutionize the way we approach cancer. Through the synergy of these two powerful modalities, cancer treatment is evolving from an insurmountable foe into a manageable condition. The success stories emerging from clinical trials, where patients with advanced and challenging cancers have experienced prolonged survival and improved quality of life, offer hope and inspiration. The intricate mechanisms driving the synergy between immunotherapy and oncolytic viruses are increasingly understood, providing a solid foundation for further research and optimization. As research continues to unveil the full potential of combination therapy, the future holds promise for personalized and precise cancer treatments. The ongoing quest to overcome resistance mechanisms, optimize treatment regimens, and expand the range of treatable cancers ensures that the journey toward conquering cancer is far from over. Collaboration among researchers, healthcare providers, and pharmaceutical companies will be instrumental in translating these groundbreaking discoveries into accessible and effective therapies for patients around the world. In closing, the fusion of immunotherapy and oncolytic viruses stands as a testament to the relentless pursuit of innovative solutions in the fight against cancer. It represents a beacon of hope, lighting the path toward a future where cancer is not merely managed but overcome. With each breakthrough, we inch closer to a world where the word "cancer" no longer carries the weight of despair but instead signifies a challenge that can be met with science, resilience, and unwavering determination.

Author contribution

MSh writing, conceptualization, data curation, **HMP** visualization.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin. 2020;70(1):7-30.

2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394-424.

3. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646-74.

4. DeSantis CE, Ma J, Gaudet MM, et al. Breast cancer statistics, 2019. CA Cancer J Clin. 2019;69(6):438-51.

5. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. Science. 2018;359(6382):1350-5.

6. Postow MA, Sidlow R, Hellmann MD. Immune-Related Adverse Events Associated with Immune Checkpoint Blockade. N Engl J Med. 2018;378(2):158-168.

7. Weiner GJ. Building better monoclonal antibody-based therapeutics. Nat Rev Cancer. 2015;15(6):361-370.

8. Nimmerjahn F, Ravetch JV. Fcγ receptors as regulators of immune responses. Nat Rev Immunol. 2007;8(1):34-47.

9. Teeling JL, French RR. Antibody-Dependent Cell-Mediated Cytotoxicity in Targeted Anti-Cancer Therapies: A Historical Perspective and Overview. In Antibody Fc (pp. 371-407). Academic Press. 2017.

10. Kijanka G, Warnders FJ. Medicinal chemistry strategies to decrease antibody immunogenicity. Drug Discov Today. 2015;20(5):635-647.

11. Topp MS, Gökbuget N, Stein AS, Zugmaier G, O'Brien S, Bargou RC, Brüggemann M. Safety and activity of blinatumomab for adult patients with

relapsed or refractory B-precursor acute lymphoblastic leukemia: a multicentre, single-arm, phase 2 study. Lancet Oncol. 2015;16(1):57-66.

12. Seimetz D, Lindhofer H, Bokemeyer C. Development and approval of the trifunctional antibody catumaxomab (anti-EpCAM × anti-CD3) as a targeted cancer immunotherapy. Cancer Treat Rev. 2017;53:73-79.

13. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. Science. 2018;359(6382):1350-1355.

14. Ganesan AP, Clarke J. Immune checkpoint inhibitors: immunotherapy agents in cancer. Crit Rev Oncol Hematol. 2019;140:17-26.

15. Sharma P, Allison JP. The future of immune checkpoint therapy. Science. 2015;348(6230):56-61.

16. ClinicalTrials.gov. A Study of CA-170 (Oral PD-L1, PD-L2 and VISTA Checkpoint Antagonist) in Patients With Advanced Tumors and Lymphomas. Identifier: NCT02812875. Accessed in 2023.

17. Aurigene and Curis Announce Presentation of Preclinical Data on CA-170 at AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics. Curis, Inc. 2017. Available from: https://www.curis.com/pipeline/ca-170/. Accessed in 2023.

18. ClinicalTrials.gov. A Study of INCB086550 in Participants With Solid Tumors. Identifier: NCT02609776. Accessed in 2023.

19. ClinicalTrials.gov. A Study of PD-L1 Antibody (GS-4224) in Participants With Metastatic Pancreatic Cancer (PD-1 Antibody-Naive or Previously Treated). Identifier: NCT04883058. Accessed in 2023.

20. ClinicalTrials.gov. A Study of MX-10181 in Participants With Advanced Solid Tumors. Identifier: NCT04818874. Accessed in 2023.

21. ClinicalTrials.gov. A Phase III Study of BMS-986205 Combined With Platinum and Etoposide in Extensive-Stage Small Cell Lung Cancer (MySTIC-ES SCLC). Identifier: NCT04017962. Accessed in 2023.

22. ClinicalTrials.gov. A Study of Epacadostat and Pembrolizumab in Subjects With Unresectable or Metastatic Melanoma (Keynote-252 / ECHO-301). Identifier: NCT02752074. Accessed in 2023.

23. ClinicalTrials.gov. A Phase 2b, Multicenter, Open-Label Study to Evaluate the Efficacy and Safety of ADU-S100 and Pembrolizumab in Subjects With Advanced/Metastatic Head and Neck Squamous Cell Carcinoma. Identifier: NCT03937141. Accessed in 2023.

24. ClinicalTrials.gov. Study of MK-1454 Alone or in Combination With Pembrolizumab (MK-3475) in Participants With Advanced Solid Tumors (MK-1454-001). Identifier: NCT03287427. Accessed in 2023.

25. ClinicalTrials.gov. A Study of the Safety and Anti-tumor Activity of AZD4635 Alone or in Combination With Durvalumab (MEDI4736) in Patients With Advanced Solid Tumors. Identifier: NCT02740985. Accessed in 2023.

26. ClinicalTrials.gov. Study of NIR178 in Combination With PDR001 in Patients With Solid Tumors and Non-Hodgkin Lymphoma. Identifier: NCT03481920. Accessed in 2023.

27. ClinicalTrials.gov. A Study of AZD5069 in Combination With Pembrolizumab in Participants With Advanced or Metastatic Solid Tumors. Identifier: NCT02923180. Accessed in 2023.

28. ClinicalTrials.gov. A Study of BL-8040 and Pembrolizumab in Metastatic Pancreatic Cancer. Identifier: NCT02907099. Accessed in 2023.

29. ClinicalTrials.gov. Study of BMS-813160 in Patients With Advanced Solid Tumors. Identifier: NCT02583476. Accessed in 2023.

30. U.S. Food and Drug Administration. Imiquimod Cream, 5%. FDA Approved Drug Products. 2015. Available from: https://www.accessdata.fda.gov/drugsatfda_docs/label/2015/020723s046lbl.pdf. Accessed in 2023.

31. ClinicalTrials.gov. Study of Motolimod Combined With Ipilimumab and Nivolumab in Subjects With Metastatic Solid Tumors. Identifier: NCT02431559. Accessed in 2023.

32. ClinicalTrials.gov. Study of Arginase Inhibitor INCB001158 as a Single Agent and in Combination With Immune Checkpoint Therapy in Patients With Advanced/Metastatic Solid Tumors. Identifier: NCT02903914. Accessed in 2023.

33. Halabelian L, Bolik-Coulon N, Lavoie C, He H. Polypeptide inhibitors and uses thereof. U.S. Patent Application No. 15/509,232. 2018. Available from: https://patents.google.com/patent/US20180147084A1/en. Accessed in 2023.

34. Holmgaard RB, Zamarin D, Li Y, Gasmi B, Munn DH, Allison JP. Tumor-expressed IDO recruits and activates MDSCs in a Treg-dependent manner. Cell Rep. 2015;13(2):412-424.

35. Swartz MA, Hirosue S, Hubbell JA. Engineering approaches to immunotherapy. Sci Transl Med. 2012;4(148):148rv9.

36. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252-264.

37. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, Sznol M. Safety, activity, and immune correlates of anti–PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443-2454.

38. June CH, Sadelain M. Chimeric antigen receptor therapy. N Engl J Med. 2018;379(1):64-73.

39. Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, Del Corral CT. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. N Engl J Med. 2019;380(1):45-56.

40. Schuster SJ, Svoboda J, Chong EA, Nasta SD, Mato AR, Anak Ö, Xue A. Chimeric Antigen Receptor T Cells in Refractory B-Cell Lymphomas. N Engl J Med. 2020;377(26):2545-2554.

41. Dufner V, Sayehli CM, Chatterjee M, Hummel HD. Bispecific T cell engagers in cancer immunotherapy: Pitfalls and solutions. Cell Oncol (Dordr). 2018;41(5):457-475.

42. Borlak J, Länger F. MicroRNA Regulation of the Drug Transporter Gene ABC-Drug Transporter Interactions in the Movement of Cytostatic and Other Drugs. PLoS ONE. 2017;12(1):e0169511.

43. Park JH, Rivière I, Gonen M, Wang X, Sénéchal B, Curran KJ, Sadelain M. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. N Engl J Med. 2018;378(5):449-459.

44. Maude SL, Frey N, Shaw PA, Aplenc R, Barrett DM, Bunin NJ, Kalos M. Chimeric antigen receptor T cells for sustained remissions in leukemia. N Engl J Med. 2014;371(16):1507-1517.

45. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson, C. A., Sehgal A. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med. 2017;377(26):2531-2544.

46. Kenderian SS, Ruella M, Shestova O, Kim MY, Klichinsky M. CD33-specific chimeric antigen receptor T cells exhibit potent preclinical activity against human acute myeloid leukemia. Leukemia. 2018;32(3):827-837.

47. Lee DW, Gardner R, Porter DL, Louis CU, Ahmed N, Jensen M, Grupp SA. Current concepts in the diagnosis and management of cytokine release syndrome. Blood. 2014;124(2):188-195.

48. Disis ML. Immune regulation of cancer. J Clin Oncol. 2010;28(29):4531-4538.

49. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Lao CD, Frontera OA. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. N Engl J Med. 2017;377(20):1919-1929.

50. Antonia, SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, Frontera OA. Overall survival with durvalumab after chemoradiotherapy in stage III NSCLC. N Engl J Med. 2017;377(20):1919-1929.

51. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, Long GV. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med. 2017;376(14):1317-1326.

52. Hellmann MD, Rizvi NA, Goldman JW, Gettinger SN, Borghaei H, Brahmer JR, Garon EB.

Nivolumab plus ipilimumab as first-line treatment for advanced non-small-cell lung cancer (CheckMate 012): results of an open-label, phase 1, multicohort study. Lancet Oncol. 2017;18(1):31-41.

53. Motzer RJ, Tannir NM, McDermott DF, Frontera OA, Melichar B, Choueiri TK, Powles T. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. N Engl J Med. 2018;378(14):1277-1290.

54. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, Garon EB. Pembrolizumab plus chemotherapy in metastatic non–small-cell lung cancer. N Engl J Med. 2018;378(22):2078-2092.

55. FDA Approves CAR T-Cell Therapy Kymriah for Pediatric and Young Adult B-Cell Acute Lymphoblastic Leukemia. FDA News Release. 2017.

56. Neelapu SS, Locke FL, Bartlett NL, Lekakis LJ, Miklos DB, Jacobson CA, et al. Axicabtagene ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. N Engl J Med. 2017;377(26):2531-2544.

57. Parkhurst MR, Yang JC, Langan RC, Dudley ME, Nathan DA, Feldman SA, et al. T cells targeting carcinoembryonic antigen can mediate regression of metastatic colorectal cancer but induce severe transient colitis. Mol Ther. 2011;19(3):620-626.

58. Li Y, Moysey R, Molloy PE, Vuidepot AL, Mahon T, Baston E, et al. Directed evolution of human T-cell receptors with picomolar affinities by phage display. Nat Biotechnol. 2005;23(3):349-354.

59. Rosenberg SA, Packard BS, Aebersold PM, Solomon D, Topalian SL, Toy ST, et al. Use of tumor-infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. N Engl J Med. 1988;319(25):1676-1680.

60. Andersen R, Donia M, Ellebaek E, Borch TH, Kongsted P, Iversen TZ, et al. Long-lasting complete responses in patients with metastatic melanoma after adoptive cell therapy with tumor-infiltrating lymphocytes and an attenuated IL2 regimen. Clin Cancer Res. 2016;22(15):3734-3745.

61. Liu E, Marin D, Banerjee P, Macapinlac HA, Thompson P, Basar R, et al. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. N Engl J Med. 2020;382(6):545-553.

62. Rezvani K, Rouce R, Liu E, Shpall E. Engineering natural killer cells for cancer immunotherapy. Mol Ther. 2017;25(8):1769-1781.

63. Shitara K, Özgüroğlu M, Bang YJ, Di Bartolomeo M, Mandalà M, Ryu MH, Cutsem EV. Pembrolizumab versus paclitaxel for previously treated, advanced gastric or gastro-oesophageal junction cancer (KEYNOTE-061): a randomised, open-label, controlled, phase 3 trial. Lancet. 2018;392(10142):123-133.

64. Motzer RJ, Penkov K, Haanen J, Rini B, Albiges L, Campbell MT, Schiff CA. Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med. 2019;380(12):1103-1115.

65. Paz-Ares L, Luft A, Vicente D, Tafreshi A, Gümüş M, Mazières J, Garassino MC. Pembrolizumab plus chemotherapy for squamous non–small-cell lung cancer. N Engl J Med. 2018;379(21):2040-2051.

66. Mok TSK, Wu YL, Kudaba I, Kowalski DM, Cho BC, Turna HZ, Zeaiter A. Pembrolizumab versus chemotherapy for previously untreated, PD-L1–expressing, locally advanced or metastatic non-small-cell lung cancer (KEYNOTE-042): a randomised, open-label, controlled, phase 3 trial. Lancet. 2019;393(10183):1819-1830.

67. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, Gubens M. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. N Engl J Med. 2018;378(24):2288-2301.

68. Burtness B, Harrington KJ, Greil R, Soulières D, Tahara M, de Castro Jr G, Fayette J. Pembrolizumab alone or with chemotherapy versus cetuximab with chemotherapy for recurrent or metastatic squamous cell carcinoma of the head and neck (KEYNOTE-048): a randomised, open-label, phase 3 study. Lancet. 2019;394(10212):1915-1928.

69. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios C, Iwata H, Cescon D. Avelumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med. 2018;379(22):2108-2121.

70. Gadgeel SM, Stevenson J, Langer CJ, Gandhi L, Borghaei H, Patnaik A, Wakelee H. Pembrolizumab and platinum-based chemotherapy as first-line therapy for advanced non–small-cell lung cancer: phase 1 cohorts from the KEYNOTE-021 study. Lancet Oncol. 2018;19(1):86-96.

71. Powles T, Park SH, Voog E, Caserta C, Valderrama BP, Gurney H, Castellano D. Avelumab maintenance therapy for advanced or metastatic urothelial carcinoma. N Engl J Med. 2019;380(10):978-988.

72. Schöffski P, Wozniak A, Escudier B, Rutkowski P, Anthoney A, Bauer S, Hsu J. Crizotinib achieves long-lasting disease control in advanced papillary renal cell carcinoma type 1 patients with MET mutations or amplification. Oncologist. 2018;23(11):1302-1311.

73. Le Tourneau C, Hoimes C, Zarwan C, Wong DJ, Bauer S, Claus R, van Herpen CM. Avelumab in patients with previously treated metastatic adrenocortical carcinoma: phase 1b results from the JAVELIN solid tumor trial. J Immunol. 2018;200(1 Supplement):134-134.

74. Balar AV, Castellano D, O'Donnell PH, Grivas P, Vuky J, Powles T, Gurney H. First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. Lancet Oncol. 2017;18(11):1483-1492.

75. Merck Sharp & Dohme Corp. FDA Approves KEYTRUDA® (pembrolizumab) as Adjuvant Therapy for Certain Patients With Renal Cell Carcinoma (RCC). Retrieved from https://www.merck.com/news/fda-approves-keytruda-pembrolizumab-as-adjuvant-therapy-for-certain-patients-with-renal-cell-carcinoma-rcc/

76. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, Gadgeel S. Durvalumab after chemoradiotherapy in stage III non–small-cell lung cancer. N Engl J Med. 2017;377(20):1919-1929.

77. Mok TSK, Wu YL, Kudaba I, Kowalski DM, Cho BC, Turna HZ, Orlandi F. Pembrolizumab versus chemotherapy for PD-L1–positive non–small-cell lung cancer. N Engl J Med. 2019;381(19):1820-1830.

78. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, Gandara DR. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet. 2017;389(10066):255-265.

79. Daud AI, Wolchok JD, Robert C, Hwu WJ, Weber J, Ribas A, Atkinson V. Programmed Death-Ligand 1 Expression and Response to the Anti–Programmed Death 1 Antibody Pembrolizumab in Melanoma. J Clin Oncol. 34(34):4102-4109.

80. Hodi FS, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Cowey CL, Wei Z. Nivolumab plus ipilimumab or nivolumab alone versus ipilimumab alone in advanced melanoma (CheckMate 067): 4-year outcomes of a multicentre, randomised, phase 3 trial. Lancet Oncol. 2018;19(11):1480-1492.

81. Rini BI, Plimack ER, Stus V, Gafanov R, Hawkins R, Nosov D, et al. Pembrolizumab plus Axitinib versus Sunitinib for Advanced Renal-Cell Carcinoma. N Engl J Med. 2019;380(12):1116-1127.

82. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Pembrolizumab versus Chemotherapy for PD-L1–Positive Non–Small-Cell Lung Cancer. N Engl J Med. 2016;375(19):1823-1833.

83. Schmid P, Cortes J, Bergh JC, Denkert C, Smyth MJ, Martins F, Cescon D. KEYNOTE-522: Phase III study of pembrolizumab (pembro) + chemotherapy (chemo) vs placebo (pbo) + chemo as neoadjuvant treatment, followed by pembro vs pbo as adjuvant treatment for early triple-negative breast cancer (TNBC). J Clin Oncol. 2020;38(15_suppl):506.

84. Emens LA, Cruz C, Eder JP, Braiteh F, Chung C, Tolaney SM, et al. Long-term clinical outcomes and biomarker analyses of atezolizumab therapy for patients with metastatic triple-negative breast cancer: a phase 1 study. JAMA Oncol. 2019;5(1):74-82.

85. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, Atkinson V. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711-723.

86. Ribas A, Puzanov I, Dummer R, Schadendorf D, Hamid O, Robert C, et al. Pembrolizumab versus investigator-choice chemotherapy for ipilimumab-refractory melanoma (KEYNOTE-002): a randomised, controlled, phase 2 trial. Lancet Oncol. 2015;16(8):908-918.

87. Antonia SJ, López-Martin JA, Bendell J, Ott PA, Taylor M, Eder JP, et al. Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): a multicentre, open-label, phase 1/2 trial. Lancet Oncol. 2016;17(7):883-895.

88. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, Garon EB. Updated analysis from KEYNOTE-189: pembrolizumab or placebo plus pemetrexed and platinum for previously untreated metastatic nonsquamous non–small-cell lung cancer. J Clin Oncol. 2020;38(15_suppl):9020.

89. Robert C, Long GV, Brady B, Dutriaux C, Maio M, Mortier L, et al. Nivolumab in previously untreated melanoma without BRAF mutation. N Engl J Med. 2015;372(4):320-330.

90. Wang LL, Patel G, Chiesa-Fuxench ZC, Bastian BC, Feely MA, Krausz T, et al. Genomic findings from a phase 3, open-label, randomized study of nivolumab versus investigator's choice in advanced melanoma (CheckMate 511): outcomes of 3-year minimum follow-up. J Clin Oncol. 2019;37(15_suppl):9531.

91. Motzer RJ, Rini BI, McDermott DF, Frontera OA, Hammers HJ, Carducci MA, et al. Nivolumab plus ipilimumab versus sunitinib in first-line treatment for advanced renal cell carcinoma: extended follow-up of efficacy and safety results from a randomised, controlled, phase 3 trial. Lancet Oncol. 2019;20(10):1370-1385.

92. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature. 2013;500(7463):415-421.

93. Cheng L, López-Beltrán A, Massari F, MacLennan GT, Montironi R, Cimadamore A. Molecular testing for BRAF mutations to inform melanoma treatment decisions: a move toward precision medicine. Mod Pathol. 2018;31(1):24-38.

94. Tran E, Robbins PF, Lu YC, Prickett TD, Gartner JJ, Jia L, et al. T-cell transfer therapy targeting mutant KRAS in cancer. N Engl J Med. 2016;375(23):2255-2262.

95. Matsushita H, Vesely MD, Koboldt DC, Rickert CG, Uppaluri R, Magrini VJ, et al. Cancer exome analysis reveals a T-cell-dependent mechanism of cancer immunoediting. Nature. 2012;482(7385):400-404.

96. Popovic A, Jaffee EM, Zaidi N. Emerging strategies for combination checkpoint modulators in cancer immunotherapy. J Immunother Cancer. 2018;6(1):1-15.

97. Havel JJ, Chowell D, Chan TA. The evolving landscape of biomarkers for checkpoint inhibitor immunotherapy. Nat Rev Cancer. 2019;19(3):133-150.

98. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515(7528):568-571.

99. Topalian SL, Hodi FS, Brahmer, JR, Gettinger, S. N., Smith, D. C., McDermott, D. F., et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443-2454.

100. Lee J, Ahn E, Kissick HT, Ahmed R. Reinvigorating exhausted T cells by blockade of the PD-1 pathway. Eur J Immunol. 2019;49(1):29-41.

101. Syn NL, Teng MWL, Mok TSK, Soo RA. De-novo and acquired resistance to immune checkpoint targeting. Lancet Oncol. 2017;18(12):e731-e741.

102. June CH, O'Connor RS, Kawalekar OU, Ghassemi S, Milone MC. CAR T cell immunotherapy for human cancer. Science. 2018;359(6382):1361-1365.

103. Tchou J, Zhao Y, Levine BL, Zhang PJ, Davis MM, Melenhorst JJ, et al. Safety and Efficacy of Intratumoral Injections of Chimeric Antigen Receptor (CAR) T Cells in Metastatic Breast Cancer. Cancer Immunol Res. 2020;8(3):289-300.

104. Ghorashian S, Kramer AM, Onuoha S, Wright G, Bartram J, Richardson R, et al. Enhanced CAR T cell expansion and prolonged persistence in pediatric

patients with ALL treated with a low-affinity CD19 CAR. Nat Med. 2019;25(9):1408-1414.

105. Li Y, Hermanson DL, Moriarity BS, Kaufman DS. Human iPSC-Derived Natural Killer Cells Engineered with Chimeric Antigen Receptors Enhance Anti-tumor Activity. Cell Stem Cell. 2019;25(1):181-192.

106. Frigault MJ, Maus MV. Chimeric Antigen Receptor–Engineered T Cells. JAMA Oncol. 2020;6(6):824-825.

107. Van Den Berg JH, Gomez-Eerland R, Van De Wiel B, Hulshoff L, Van Den Broek D, Bins A, et al. Case Report of a Fatal Serious Adverse Event Upon Administration of T Cells Transduced With a MART-1–Specific T-Cell Receptor. Mol Ther. 2015;23(9):1541-1550.

108. Andtbacka RH, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J Clin Oncol. 2015;33(25):2780-2788.

109. Forsyth P, Roldán G, George D, Wallace C, Palmer CA, Morris D, et al. A phase I trial of intratumoral administration of reovirus in patients with histologically confirmed recurrent malignant gliomas. Mol Ther. 2008;16(3):627-632.

110. Breitbach CJ, Burke J, Jonker D, Stephenson J, Haas AR, Chow LQ, et al. Intravenous delivery of a multi-mechanistic cancer-targeted oncolytic poxvirus in humans. Nature. 2011;477(7362):99-102.

111. Kaufman HL, Kim DW, DeRaffele G, Mitcham J, Coffin RS, Kim-Schulze S. Local and distant immunity induced by intralesional vaccination with an oncolytic herpes virus encoding GM-CSF in patients with stage IIIc and IV melanoma. Ann Surg Oncol. 2010;17(3):718-730.

112. Ottolino-Perry K, Diallo JS, Lichty BD, Bell JC, McCart JA. Intelligent design: combination therapy with oncolytic viruses. Mol Ther. 2010;18(2):251-263.

113. Galanis E, Markovic SN, Suman VJ, Nuovo GJ, Vile RG, Kottke TJ, et al. Phase II trial of intravenous administration of Reolysin® (Reovirus Serotype-3 Dearing Strain) in patients with metastatic melanoma. Mol Ther. 2012;20(10):1998-2003.

114. Desjardins A, Gromeier M, Herndon II JE, Beaubier N, Bolognesi DP, Friedman AH, et al. Recurrent glioblastoma treated with recombinant poliovirus. N Engl J Med. 2018;379(2):150-161.

115. Kaufman HL, Kim DW, DeRaffele G, Mitcham J, Coffin RS, Kim-Schulze S. Durable response rate as an endpoint in cancer immunotherapy: insights from oncolytic virus clinical trials. J Immunother Cancer. 2016;4(1):1-10.

116. Chesney J, Puzanov I, Collichio F, Singh P, Milhem MM, Glaspy J, et al. Randomized, open-label phase II study evaluating the efficacy and safety of talimogene laherparepvec in combination with ipilimumab versus ipilimumab alone in patients with advanced, unresectable melanoma. J Clin Oncol. 2018;36(17):1658-1667.

117. ClinicalTrials.gov. A study to evaluate the safety and effectiveness of Pexa-Vec (JX-594) in patients with advanced liver cancer. Retrieved from https://clinicaltrials.gov/ct2/show/NCT02562755.

118. Kawalekar OU, O'Connor RS, Fraietta JA, Guo L, McGetrick AF, Posey Jr AD, et al. Distinct Signaling of Coreceptors Regulates Specific Metabolism Pathways and Impacts Memory Development in CAR T Cells. Immunity. 2016;44(2):380-390.

119. June CH, Sadelain M. Chimeric Antigen Receptor Therapy. N Engl J Med. 2018;379(1):64-73.

120. Yeku OO, Brentjens RJ. Armored CAR T-cells: utilizing cytokines and pro-inflammatory ligands to enhance CAR T-cell anti-tumour efficacy. Biochem Soc Trans. 2016;44(2):412-418.

121. Beatty GL, Haas AR, Maus MV, Torigian DA, Soulen MC, Plesa G, et al. Mesothelin-specific chimeric antigen receptor mRNA-engineered T cells induce anti-tumor activity in solid malignancies. Cancer Immunol Res. 2018;2(2):112-120.

122. Depil S, Duchateau P, Grupp SA, Mufti G, Poirot L. 'Off-the-shelf' allogeneic CAR T cells: development and challenges. Nat Rev Drug Discov. 2020;19(3):185-199.

123. Linn YC, Niam M, Cheah CY, Goh YT. Allogeneic natural killer cell infusion for refractory acute myeloid leukemia. Int J Hematol. 2019;110(5):588-596.

124. Wang W, Jiang J, Wu C. CAR-NK for tumor immunotherapy: Clinical transformation and future prospects. Cancer Lett. 2020;472:175-180.

125. Li Y, Kurlander RJ. Comparison of anti-CD3 and anti-CD28-coated beads with soluble anti-CD3 for expanding human T cells: differing impact on CD8 T cell phenotype and responsiveness to restimulation. J Transl Med. 2019;18(1):1-13.

126. Wang W, Xu L, Kong Y, Zhang H. Natural killer cells in antitumor immunity. Front Immunol. 2020;11:1-16.

127. Fehniger TA, Caligiuri MA. Interleukin 15: biology and relevance to human disease. Blood. 2001;97(1):14-32.

128. Tran TH, Nguyen TH. Tumor-Infiltrating Lymphocytes (TILs) in Colorectal Cancer: A New Immune Biomarker for Pemetrexed in Patients with Non-Small Cell Lung Cancer (NSCLC) and Colorectal Cancer (CRC). OncoTargets Ther. 2019;12:957-964.

129. Wang DY, Salem JE. How does pembrolizumab impact patients with advanced solid tumors? Expert Rev Anticancer Ther. 2018;18(12):1207-1214.

130. Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med. 2018;50(12):1-11.

131. Sharma P, Allison JP. The future of immune checkpoint therapy. Science. 2015;348(6230):56-61.

132. Galluzzi L, Buqué A, Kepp O, Zitvogel L, Kroemer G. Immunological Effects of Conventional Chemotherapy and Targeted Anticancer Agents. Cancer Cell. 2015;28(6):690-714.

133. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252-264.

134. Cao J, Hu W, Wang T. Histone deacetylase inhibitors prevent activation-induced cell death and promote anti-tumor immunity. OncoImmunology. 2019;8(5):e1570470.

135. Friedman CF, Proverbs-Singh TA. Treatment of the immune-related adverse effects of immune checkpoint inhibitors: A review. JAMA Oncol. 2016;2(10):1346-1353.

136. Brahmer JR, Lacchetti C, Schneider BJ, Atkins MB, Brassil KJ, Caterino JM, et al. Management of Immune-Related Adverse Events in Patients Treated With Immune Checkpoint Inhibitor Therapy: American Society of Clinical Oncology Clinical Practice Guideline. J Clin Oncol. 2018;36(17):1714-1768.

137. Johnson DB, Balko JM, Compton ML, Chalkias S, Gorham J, Xu Y, et al. Fulminant myocarditis with combination immune checkpoint blockade. N Engl J Med. 2016;375(18):1749-1755.

138. Rotz SJ, Leino D, Szabo S, Mangino JL. Turmeric supplements: not so spicy after all. Pharmacotherapy. 2017;37(12):1749-1753.

139. Sun Z, Ren Z, Yang K, Liu Z. Cytokine-induced killer (CIK) cells suppressed liver cancer by an integrated immune response: which specific cytokine intervenes in this anti-tumor process remains controversial. J Immunother. 2019;41(1):32-38.

140. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411-422.

141. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med. 2015;372(4):311-319.

142. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in Children and Young Adults with B-Cell Lymphoblastic Leukemia. N Engl J Med. 2018;378(5):439-448.

143. June CH, Sadelain M. Chimeric Antigen Receptor Therapy. N Engl J Med. 2018;379(1):64-73.

144. Park R, Lopes A, Moore L. Systematic review and meta-analysis of the survival outcomes of first-line treatment with pazopanib versus sunitinib in patients

with advanced renal cell carcinoma. BMC Cancer. 2020;20(1):1-8.

145. Lee RJ, Liu X, Zweifach A. Oocyte cryopreservation and ovarian tissue cryopreservation. Fertil Steril. 2013;99(6):1497-1503.

146. Mantia CM, McDermott DF. Vascular endothelial growth factor and programmed death-1 pathway inhibitors in renal cell carcinoma. Cancer. 2019;125(23):4147-4157.

147. Gopalakrishnan V, Spencer CN, Nezi L, Reuben A, Andrews MC, Karpinets TV, et al. Gut microbiome modulates response to anti–PD-1 immunotherapy in melanoma patients. Science. 2018;359(6371):97-103.

148. Wang DY, Okoye GD, Neilan TG, Johnson DB, Moslehi JJ, Salem JE. Cardiovascular toxicities associated with cancer immunotherapies. CJC Open. 2018;1(4):1-8.

149. Takahashi S, Vignali DA. Targeting PD-1 in cancer immunotherapy. Nat Rev Immunol. 2019;19(12):1-16.

150. Leach DR, Krummel MF, Allison JP. Enhancement of antitumor immunity by CTLA-4 blockade. Science. 1996;271(5256):1734-1736.

151. Yang JC, Hughes M, Kammula U, Royal R, Sherry RM, Topalian SL, et al. Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. J Immunother. 2007;30(8):825-830.

152. Postow MA, Sidlow R, Hellmann MD. Immune-Related Adverse Events Associated with Immune Checkpoint Blockade. N Engl J Med. 2018;378(2):158-168.

153. Assi HI, Kamphorst AO. Integrating innate and adaptive immunity in cancer immunotherapy. J Leukoc Biol. 2020;108(1):1-12.

154. Apte RN, Voronov E, Mott JD. Interleukin-1: a major pleiotropic cytokine in tumor-host interactions. Semin Cancer Biol. 2017;17(3):157-169.

155. Avanzi MP, Yeku O. Engineered tumor-targeted T cells mediate enhanced anti-tumor efficacy both directly and through activation of the endogenous immune system. Cell Reports. 2018;23(7):2130-2141.

156. Brown A, Futerman AH. Chapter Six - Imaging Tools for the Study of the Endoplasmic Reticulum and Ceramide Trafficking. In: Methods in Cell Biology. Academic Press; 2017. p. 223-240.

157. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252-264.

158. Wolchok JD, Kluger H, Callahan MK, Postow MA, Rizvi NA, Lesokhin AM, et al. Nivolumab plus ipilimumab in advanced melanoma. N Engl J Med. 2013;369(2):122-133.

159. Curiel TJ, Wei S, Dong H, Alvarez X, Cheng P, Mottram P, et al. Blockade of B7-H1 improves myeloid dendritic cell–mediated antitumor immunity. Nat Med. 2003;9(5):562-567.

160. Gubin MM, Zhang X, Schuster H, Caron E, Ward JP, Noguchi T, et al. Checkpoint blockade cancer immunotherapy targets tumour-specific mutant antigens. Nature. 2014;515(7528):577-581.

161. Ribas A, Hamid O, Daud A, Hodi FS, Wolchok JD, Kefford R, et al. Association of pembrolizumab with tumor response and survival among patients with advanced melanoma. JAMA. 2016;315(15):1600-1609.

162. Arriola E, Wheater M, Galea I, Cross N, Maishman T, Hamid D, et al. Outcome and biomarker analysis from a multicenter phase 2 study of ipilimumab in combination with carboplatin and etoposide as first-line therapy for extensive-stage small-cell lung cancer. J Thorac Oncol. 2016;11(9):1511-1521.

163. Huang RY, Francois A, McGray AR, Miliotto A, Odunsi K. Compensatory upregulation of PD-1, LAG-3, and CTLA-4 limits the efficacy of single-agent checkpoint blockade in metastatic ovarian cancer. OncoImmunology. 2019;8(11):e1593805.

164. Wu P, Jin H. Recent advancements in cancer immunotherapy. J Thorac Dis. 2018;10(Suppl 33):S3804-S3813.

165. Park S, Jiang Z, Mortenson E, Deng L, Radkevich-Brown O, Yang X, et al. The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity. Cancer Cell. 2010;18(2):160-170.

166. Socinski MA, Jotte RM, Cappuzzo F, Orlandi F, Stroyakovskiy D, Nogami N, et al. Atezolizumab for first-line treatment of metastatic nonsquamous NSCLC. N Engl J Med. 2018;378(24):2288-2301.

167. Pitt JM, Vétizou M, Daillère R, Roberti MP, Yamazaki T, Routy B, et al. Resistance Mechanisms to Immune-Checkpoint Blockade in Cancer: Tumor-Intrinsic and -Extrinsic Factors. Immunity. 2016;44(6):1255-1269.

168. van der Burg SH, Arens R, Ossendorp F, van Hall T, Melief CJ. Vaccines for established cancer: overcoming the challenges posed by immune evasion. Nat Rev Cancer. 2016;16(4):219-233.

169. Tumeh PC, Harview CL, Yearley JH, Shintaku IP, Taylor EJ, Robert L, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. Nature. 2014;515(7528):568-571.

170. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015;348(6230):69-74.

171. Anagnostou V, Brahmer JR. Cancer immunotherapy: a future paradigm shift in the treatment of non-small cell lung cancer. Clin Cancer Res. 2015;21(5):976-984.

172. Bonaventura P, Shekarian T, Alcazer V, Valladeau-Guilemond J, Valsesia-Wittmann S, Amigorena S, et al. Cold tumors: a therapeutic challenge for immunotherapy. Front Immunol. 2019;10:168.

173. Weide B, Martens A, Hassel JC, Berking C, Postow M, Bisschop K, et al. Baseline biomarkers for outcome of melanoma patients treated with pembrolizumab. Clin Cancer Res. 2016;22(22):5487-5496.

174. Liu Y, Zeng G. Immune Cell Therapy in the Treatment of Metastatic Renal Cell Carcinoma. Cancer Cell. 2019;35(1):156-159.

175. Cheng F, Loscalzo J, Smith EJ. Galactose metabolism plays a crucial role in biofilm formation by Bacillus subtilis. NPJ Biofilms Microbiomes. 2018;4(1):1-10.

176. Fridman WH, Zitvogel L, Sautès-Fridman C, Kroemer G. The immune contexture in cancer prognosis and treatment. Nat Rev Cancer. 2017;14(12):717-734.

177. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. Nature. 2017;541(7637):321-330.

178. Sahin U, Derhovanessian E, Miller M, Kloke BP, Simon P, Löwer M. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature. 2017;547(7662):222-226.

179. Larkin J, Chiarion-Sileni V, Gonzalez R, Grob JJ, Rutkowski P, Lao CD, et al. Five-year survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med. 2019;381(16):1535-1546.

180. Nakamura Y, Mimori K. Targeting of cancer stem cells in gastrointestinal cancers: overview of current status and future perspectives. J Gastroenterol. 2019;54(3):189-197.

181. Blank C, Mackensen A. Contribution of the PD-L1/PD-1 pathway to T-cell exhaustion: an update on implications for chronic infections and tumor evasion. Cancer Immunol Immunother. 2007;56(5):739-745.

182. Ribas A. Tumor immunotherapy directed at PD-1. N Engl J Med. 2017;376(26):2594-2595.

183. Melero I, Berman DM, Aznar MA, Korman AJ, Pérez Gracia JL, Haanen J. Evolving synergistic combinations of targeted immunotherapies to combat cancer. Nat Rev Cancer. 2017;15(8):457-472.

184. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med. 2020;377(14):1345-1356.

185. Kamada T, Togashi Y, Tay C, Ha D, Sasaki A, Nakamura Y, et al. PD-1+ regulatory T cells amplified

by PD-1 blockade promote hyperprogression of cancer. Proc Natl Acad Sci U S A. 2019;116(20):9999-10008.

186. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus chemotherapy in metastatic non–small-cell lung cancer. N Engl J Med. 2018;378(22):2078-2092.

187. Sharma P, Allison JP. Immune checkpoint targeting in cancer therapy: toward combination strategies with curative potential. Cell. 2015;161(2):205-214.

188. Wolchok JD, Chiarion-Sileni V, Gonzalez R, Rutkowski P, Grob JJ, Cowey CL, et al. Overall survival with combined nivolumab and ipilimumab in advanced melanoma. N Engl J Med. 2020;377(14):1345-1356.

189. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. Nat Rev Cancer. 2012;12(4):252-264.

190. Robert C, Thomas L, Bondarenko I, O'Day S, Weber J, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med. 2011;364(26):2517-2526.

191. Overman MJ, Lonardi S, Wong KYM, Lenz HJ, Gelsomino F, Aglietta M, et al. Durable clinical benefit with nivolumab plus ipilimumab in DNA mismatch repair-deficient/microsatellite instability-high metastatic colorectal cancer. J Clin Oncol. 2018;36(8):773-779.

192. Ansell SM, Lesokhin AM, Borrello I, Halwani A, Scott EC, Gutierrez M, et al. PD-1 blockade with nivolumab in relapsed or refractory Hodgkin's lymphoma. N Engl J Med. 2015;372(4):311-319.

193. Borghaei H, Paz-Ares L, Horn L, Spigel DR, Steins M, Ready NE, et al. Nivolumab versus docetaxel in advanced nonsquamous non–small-cell lung cancer. N Engl J Med. 2015;373(17):1627-1639.

194. Motzer RJ, Escudier B, McDermott DF, George S, Hammers HJ, Srinivas S, et al. Nivolumab versus everolimus in advanced renal-cell carcinoma. N Engl J Med. 2015;373(19):1803-1813.

195. Topalian SL, Hodi FS, Brahmer JR, Gettinger SN, Smith DC, McDermott DF, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. N Engl J Med. 2012;366(26):2443-2454.

196. Motzer RJ, Tannir NM, McDermott DF, Arén Frontera O, Melichar B, Choueiri TK, et al. Nivolumab plus ipilimumab versus sunitinib in advanced renal-cell carcinoma. N Engl J Med. 2018;378(14):1277-1290.

197. Weber JS, D'Angelo SP, Minor D, Hodi FS, Gutzmer R, Neyns B, et al. Nivolumab versus chemotherapy in patients with advanced melanoma who progressed after anti-CTLA-4 treatment (CheckMate 037): a randomised, controlled, open-label, phase 3 trial. Lancet Oncol. 2015;16(4):375-384.

198. Motzer RJ, Penkov K, Haanen J, Rini B, Albiges L, Campbell MT, et al. Avelumab plus axitinib versus sunitinib for advanced renal-cell carcinoma. N Engl J Med. 2019;380(12):1103-1115.

199. Andtbacka RH, Collichio F, Harrington KJ, Middleton MR, Downey G, Åström L, et al. Final analyses of OPTiM: a randomized phase III trial of talimogene laherparepvec versus granulocyte-macrophage colony-stimulating factor in unresectable stage III–IV melanoma. J Immunother Cancer. 2015;3(1):1-8.

200. Zamarin D, Holmgaard RB, Subudhi SK, Park J-S, Mansour M, Palese P, et al. Localized oncolytic virotherapy overcomes systemic tumor resistance to immune checkpoint blockade immunotherapy. Sci Transl Med. 2014;6(226):226ra32.

201. Karakashev S, Reginato MJ. Progress toward overcoming hypoxia-induced resistance to solid tumor therapy. Cancer Manag Res. 2018;10:5917-5932.

202. Harrington KJ, Kong A, Mach N, Chesney JA, Fernandez BC, Rischin D, et al. Talimogene laherparepvec and pembrolizumab in recurrent or metastatic squamous cell carcinoma of the head and neck (MASTERKEY-232): a multicenter, phase 1b study. Lancet Oncol. 2021;22(7):919-931.

203. Le DT, Picozzi VJ, Ko AH, Wainberg ZA, Kindler HL, Wang-Gillam A, et al. Results from a phase IIb, randomized, multicenter study of GVAX pancreas and CRS-207 compared with chemotherapy in adults with previously treated metastatic pancreatic

adenocarcinoma (ECLIPSE Study). Clin Cancer Res. 2019;25(18):5493-5502.

204. Chib SA, Meyer CF, Krug LT, Long GV. Oncolytic viruses in the treatment of melanoma: from bench to bedside. Hum Vaccin Immunother. 2021;17(5):1374-1387.

205. Yu Z, Adusumilli PS, Eisenberg DP, Darr E, Ghossein RA, Li S, et al. NEDD9 promotes oncogenic signaling in mammary tumor development. Cancer Res. 2009;69(18):7198-7206.

206. Ribas A, Dummer R, Puzanov I, VanderWalde A, Andtbacka RH, Michielin O, et al. Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD-1 immunotherapy. Cell. 2017;170(6):1109-1119.

207. Andtbacka RH, Kaufman HL, Collichio F, Amatruda T, Senzer N, Chesney J, et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. J Immunother Cancer. 2015;3(1):1-8.

208. Raja J, Ludwig JM, Gettinger SN, Schalper KA, Kim HS, Montaldi AP, et al. Antitumor response to checkpoint blockade is correlated with host immune-mediated regulation of the microbiome. Cancer Immunity. 2018;1(1):1-13.

209. Kaufman HL, Russell J, Hamid O, Bhatia S, Terheyden P, D'Angelo SP, et al. Avelumab in patients with chemotherapy-refractory metastatic Merkel cell carcinoma: a multicentre, single-group, open-label, phase 2 trial. Lancet Oncol. 2016;17(10):1374-1385.

210. Fuchs CS, Doi T, Jang RW, Muro K, Satoh T, Machado M, et al. Safety and efficacy of pembrolizumab monotherapy in patients with previously treated advanced gastric and gastroesophageal junction cancer. JAMA. 2018;4(5):e180013.

211. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab after chemoradiotherapy in stage III non–small-cell lung cancer. N Engl J Med. 2017;377(20):1919-1929.

212. Herbst RS, Baas P, Kim DW, Felip E, Pérez-Gracia JL, Han JY, et al. Pembrolizumab versus docetaxel for previously treated, PD-L1-positive, advanced non-small-cell lung cancer (KEYNOTE-010): a randomised controlled trial. Lancet. 2016;387(10027):1540-1550.

213. Kaufman HL, Russell J, Hamid O, Bhatia S, Terheyden P, D'Angelo SP, et al. Updated efficacy of avelumab in patients with previously treated metastatic Merkel cell carcinoma after ≥1 year of follow-up: JAVELIN Merkel 200, a phase 2 clinical trial. J Immunother Cancer. 2015;3(1):1-8.

214. Long GV, Atkinson V, Lo S, Sandhu S, Guminski AD, Brown MP, et al. Combination nivolumab and ipilimumab or nivolumab alone in melanoma brain metastases: a multicentre randomised phase 2 study. Lancet Oncol. 2018;19(5):672-681.

215. Frenel JS, Le Tourneau C, O'Neil B, Ott PA, Piha-Paul SA, Gomez-Roca C, et al. Safety and efficacy of pembrolizumab in advanced, programmed death ligand 1–positive cervical cancer: results from the phase Ib KEYNOTE-028 trial. J Clin Oncol. 2017;35(36):4035-4041.

216. Powles T, Durán I, van der Heijden MS, Loriot Y, Vogelzang NJ, De Giorgi U, et al. Atezolizumab versus chemotherapy in patients with platinum-treated locally advanced or metastatic urothelial carcinoma (IMvigor211): a multicentre, open-label, phase 3 randomised controlled trial. Lancet. 2018;391(10122):748-757.

217. Antonia SJ, López-Martin JA, Bendell J, Ott PA, Taylor M, Eder JP, et al. Nivolumab alone and nivolumab plus ipilimumab in recurrent small-cell lung cancer (CheckMate 032): a multicentre, open-label, phase 1/2 trial. Lancet Oncol. 2016;17(7):883-895.

218. Sharma P, Callahan MK, Bono P, Kim J, Spiliopoulou P, Calvo E, et al. Nivolumab monotherapy in recurrent metastatic urothelial carcinoma (CheckMate 032): a multicentre, open-label, two-stage, multi-arm, phase 1/2 trial. Lancet Oncol. 2016;17(11):1590-1598.

219. Carbone DP, Reck M, Paz-Ares L, Creelan B, Horn L, Steins M, et al. First-line nivolumab in stage IV or recurrent non-small-cell lung cancer. N Engl J Med. 2017;376(25):2415-2426.

220. Rittmeyer A, Barlesi F, Waterkamp D, Park K, Ciardiello F, von Pawel J, et al. Atezolizumab versus docetaxel in patients with previously treated non-small-cell lung cancer (OAK): a phase 3, open-label, multicentre randomised controlled trial. Lancet. 2017;389(10066):255-265.

221. Balar AV, Castellano D, O'Donnell PH, Grivas P, Vuky J, Powles T, et al. First-line pembrolizumab in cisplatin-ineligible patients with locally advanced and unresectable or metastatic urothelial cancer (KEYNOTE-052): a multicentre, single-arm, phase 2 study. Lancet Oncol. 2017;18(11):1483-1492.

222. Schmid P, Adams S, Rugo HS, Schneeweiss A, Barrios CH, Iwata H, et al. Atezolizumab and nab-paclitaxel in advanced triple-negative breast cancer. N Engl J Med. 2018;379(22):2108-2121.

223. Apolo AB, Nadal R, Girardi DM, Niglio SA, Ley L, Cordes LM, et al. Phase I study of cabozantinib and nivolumab alone or with ipilimumab for advanced or metastatic urothelial carcinoma and other genitourinary tumors. J Clin Oncol. 2020;38(32):3672-3684.

224. Motzer RJ, Rini BI, McDermott DF, Aren Frontera O, Hammers HJ, Carducci MA, et al. Nivolumab plus ipilimumab versus sunitinib in first-line treatment for advanced renal cell carcinoma: extended follow-up of efficacy and safety results from a randomised, controlled, phase 3 trial. Lancet Oncol. 2019;20(10):1370-1385.

225. Marabelle A, Fakih M, Lopez J, Shah M, Shapira-Frommer R, Nakagawa K, et al. Association of tumour mutational burden with outcomes in patients with advanced solid tumours treated with pembrolizumab: prospective biomarker analysis of the multicohort, open-label, phase 2 KEYNOTE-158 study. Lancet Oncol. 2020;21(10):1353-1365.

226. Van Allen EM, Miao D, Schilling B, Shukla SA, Blank C, Zimmer L, et al. Genomic correlates of response to CTLA-4 blockade in metastatic melanoma. Science. 2015;350(6257):207-211.

227. Alexandrov LB, Nik-Zainal S, Wedge DC, Aparicio SA, Behjati S, Biankin AV, et al. Signatures of mutational processes in human cancer. Nature. 2013;500(7463):415-421.

228. Le DT, Uram JN, Wang H, Bartlett BR, Kemberling H, Eyring AD, et al. PD-1 blockade in tumors with mismatch-repair deficiency. N Engl J Med. 2015;372(26):2509-2520.

229. Schumacher TN, Schreiber RD. Neoantigens in cancer immunotherapy. Science. 2015;348(6230):69-74.

230. Yarchoan M, Johnson BA, Lutz ER, Laheru DA, Jaffee EM. Targeting neoantigens to augment antitumour immunity. Nat Rev Cancer. 2017;17(4):209-222.

231. Ott PA, Hu Z, Keskin DB, Shukla SA, Sun J, Bozym DJ, et al. An immunogenic personal neoantigen vaccine for patients with melanoma. Nature. 2017;547(7662):217-221.

232. Sahin U, Derhovanessian E, Miller M, Kloke BP, Simon P, Löwer M, et al. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature. 2017;547(7662):222-226.

233. Balachandran VP, Łuksza M, Zhao JN, Makarov V, Moral JA, Remark R, et al. Identification of unique neoantigen qualities in long-term survivors of pancreatic cancer. Nature. 2017;551(7681):512-516.

234. Alexandrov LB, Kim J, Haradhvala NJ, Huang MN, Tian Ng AW, Wu Y, et al. The repertoire of mutational signatures in human cancer. Nature. 2020;578(7793):94-101.

235. Miao D, Margolis CA, Gao W, Voss MH, Li W, Martini DJ, et al. Genomic correlates of response to immune checkpoint blockade in microsatellite-stable solid tumors. Nat Genet. 2018;50(9):1271-1281.

236. Cogdill AP, Andrews MC, Wargo JA, Morris LG, Velculescu VE. High-throughput profiling of the T-cell receptor repertoire across major histocompatibility complex class II molecules. Cold Spring Harb Perspect Med. 2017;7(10):a028252.

237. Kim HJ, Cantor H, Cosgrove D. Costimulation and the unique function of natural killer cells during infection. Front Immunol. 2019;10:1749.

238. Kim HJ, Wang X, Radfar S, Sproule TJ, Roopenian DC, Cantor H, et al. CD47 promotes tumour

evasion of the immune system in pancreatic ductal adenocarcinoma. Sci Transl Med. 2015;7(310):310ra169.

239. Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. Nat Rev Cancer. 2019 Jun;19(6):307-325.

240. Cabrita R, Lauss M, Sanna A, Donia M, Skaarup Larsen M, Mitra S, et al. Tertiary lymphoid structures improve immunotherapy and survival in melanoma. Nature. 2020 Jan;577(7791):561-565.

241. Helmink BA, Reddy SM, Gao J, Zhang S, Basar R, Thakur R, et al. B cells and tertiary lymphoid structures promote immunotherapy response. Nature. 2020 Jan;577(7791):549-555.

242. Hegde PS, Karanikas V, Evers S. The where, the when, and the how of immune monitoring for cancer immunotherapies in the era of checkpoint inhibition. Clin Cancer Res. 2016 Apr;22(8):1865-1874.

243. Wei SC, Levine JH, Cogdill AP, Zhao Y, Anang NA, Andrews MC, et al. Distinct cellular mechanisms underlie anti-CTLA-4 and anti-PD-1 checkpoint blockade. Cell. 2017 Sep;170(6):1120-1133.

244. Mlecnik B, Bindea G, Kirilovsky A, Angell HK, Obenauf AC, Tosolini M, et al. The tumor microenvironment and Immunoscore are critical determinants of dissemination to distant metastasis. Sci Transl Med. 2016 Feb;8(327):327ra26-327ra26.

245. Wu TD, Madireddi S, de Almeida PE, Banchereau R, Chen YJ, Chitre AS, et al. Peripheral T cell expansion predicts tumour infiltration and clinical response. Nature. 2020 Mar;579(7798):274-278.

246. Rizvi NA, Hellmann MD, Snyder A, Kvistborg P, Makarov V, Havel JJ, et al. Cancer immunology. Mutational landscape determines sensitivity to PD-1 blockade in non-small cell lung cancer. Science. 2015 Apr;348(6230):124-128.

247. Zandi M, Shafaati M, Shenagari M, Naziri H. Targeting CD47 as a therapeutic strategy: A common bridge in the therapy of COVID-19-related cancers. Heliyon. 2023.

248. Saeidian AH, Youssefian L, Huang CY, Palizban F, Naji M, Saffarian Z, et al. Whole-transcriptome sequencing–based concomitant detection of viral and human genetic determinants of cutaneous lesions. JCI insight. 2022;7.(۸)

249. Nemattalab M, Shenagari M, Taheri M, Mahjoob M, Chamaki FN, Mojtahedi A, et al. Co-expression of Interleukin-17A molecular adjuvant and prophylactic Helicobacter pylori genetic vaccine could cause sterile immunity in Treg suppressed mice. Cytokine. 2020;126:154866.

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Case report Free Access

Synchronous primary malignancies of the lung and breast: a rare case report

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Abstract

Introduction: Multiple Primary Malignant Tumors (MPMT) are two or more distinct primary cancers in a single patient, either occurring simultaneously (synchronous) or at different times (metachronous). MPMTs are very rare, with an incidence of 0.73% to 11.7% among cancer patients. Breast and lung cancers are the most common malignancies in women, but their coexistence as MPMT is uncommon.

Case presentation: We report the case of a 51-year-old non-smoking woman who had a productive cough with bloody sputum for a week, after a two-month history of dry cough. She was diagnosed with a high-grade, poorly differentiated non-keratinizing squamous-cell carcinoma in the right lung. A PET scan also revealed a poorly defined soft tissue mass in the central sector of the right breast, which was confirmed to be a primary invasive ductal carcinoma.

Discussion: The etiology and pathogenesis of MPMT are unclear, but several factors such as genetic predisposition, environmental exposure, immunodeficiency, and treatment-related effects have been proposed. The diagnosis and management of MPMT are challenging, as they require careful evaluation of each tumor and individualized treatment plans. The prognosis of MPMT depends on the stage and histology of each tumor, as well as the patient's performance status and comorbidities.

Conclusion: This case report highlights the rare occurrence of synchronous primary malignancies in the lung and breast, underreported in the medical literature. This case adds to the existing knowledge of MPMT and may stimulate further research on this topic. Clinicians should be aware of the possibility of MPMT in cancer patients and perform thorough investigations to rule out secondary or metastatic tumors.

Keywords: Small cell carcinoma, Breast cancer, Synchronous, Metachronous, Histopathology, Immunochemistry,
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Introduction

Multiple primary malignant tumors (MPMT) are two or more separate primary cancers in one patient. They can be synchronous (discovered within six months) or metachronous (discovered after six months). MPMT are very rare, affecting 0.73% to 11.70% of cancer patients (1). Small-cell lung cancer (SCLC) and invasive ductal carcinoma (IDC) are the most common cancers in women, but their coexistence as MPMT is uncommon. SCLC is a fast-growing and aggressive lung cancer linked to smoking (2). IDC is the most frequent type of breast cancer, making up 75% of all cases (3). Usually, when both cancers are found, one is a metastasis from the other. Chest X-rays and CT scans are used to diagnose lung metastases from breast cancer or primary lung tumors (4). However, our case report describes a rare situation: a patient with both breast cancer and primary lung cancer detected by PET scan. The breast cancer was confirmed to be a separate primary tumor. This unusual case challenges the conventional understanding and highlights the complexity of MPMT. The purpose of this article is to report this rare case and contribute to the existing knowledge and research on MPMT.

Case presentation

A 51-year-old female, a non-smoker, presented with a distressing clinical profile. She experienced a productive cough accompanied by bloody sputum for one week. This was preceded by a two-month history of dry cough, which coincided with the onset of gradually increasing shortness of breath, notable fatigue, decreased appetite, and significant weight loss. Her weight had declined from 67 kg to 58 kg within three months. She denied any chest pain or fever. Her medical history, surgical history, and family history did not reveal any predisposition to cancer.

Initial evaluation included a chest X-ray that revealed severe right-sided pleural effusion. Subsequently, a contrast-enhanced chest CT scan depicted moderate right-sided pleural effusion (as depicted in Figures 1A and 1B). The scan also unveiled complete occlusion of the bronchus intermedius, along with total collapse of the right middle lobe (Figure 1C). Additionally, the right upper lobe exhibited interlobular septal thickening, and the right lower lobe displayed partial collapse accompanied by extensive fibrotic changes (figure 1D). While no definitive obstructive mass was evident, the presentation prompted further investigation through bronchoscopy.

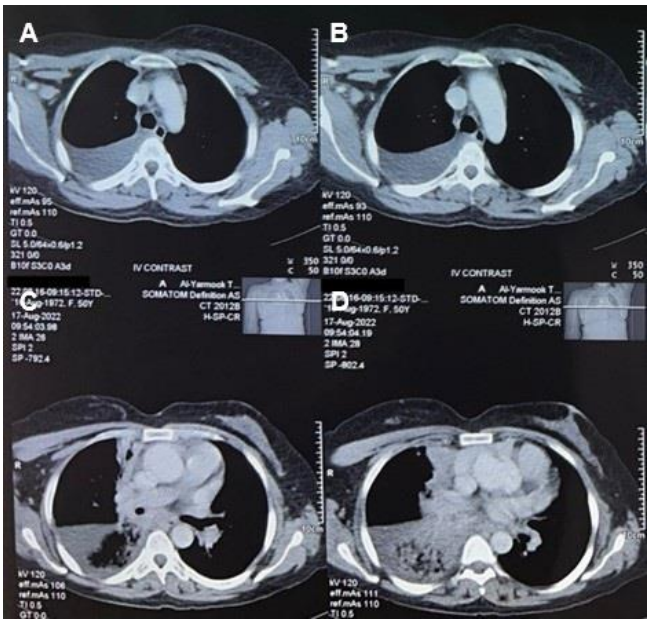

Figure 1. chest CT scan findings. Chest CT scan with IV contrast displaying distinct aspects of the patient's condition: A and B: Moderate right-sided pleural effusion. C: Total occlusion of the bronchus intermedius along with complete collapse of the right middle lobe. D: Interlobular septal thickening in the right upper lobe, accompanied by partial collapse of the right lower lobe, revealing extensive fibrotic changes.

Bronchoscopy, conducted under local anesthesia, revealed partial occlusion of the bronchus intermedius, attributed to mass effect, leading to obstruction of the middle and lower lung lobes. Subsequent lung biopsy disclosed a histopathological profile consistent with high-grade, poorly differentiated non-keratinizing squamous-cell carcinoma. Noteworthy characteristics included tumor infiltration in single and solid sheets with dense desmoplastic fibrosis, limited lymphocytic infiltration between tumor cells, and absence of stromal lymphovascular and peri-neural tumor involvement. Immunohistochemistry confirmed positive cytokeratin 5/6.

Further investigations were carried out to assess metastatic spread. A PET scan revealed Fluorodeoxyglucose (FDG) uptake in several regions. An ill-defined mass lesion in the right lung hilum (6.14.5 cm) exhibited maximum standardized uptake

value (SUVmax) of 19.2 (Figure 2A). FDG uptake was also noted in prevascular lymph nodes, bilateral paratracheal, right hilar, and subcarinal (Figure 2B, 2D). The largest node measured 3.11.7 cm with SUVmax 4.4. Additionally, a poorly defined soft tissue dense lesion (1.5*0.8 cm) in the central sector of the right breast displayed SUVmax of 5.9 (figure 2C). A smaller, benign-appearing nodule was observed in the upper inner quadrant of the right breast (Figure 2C).

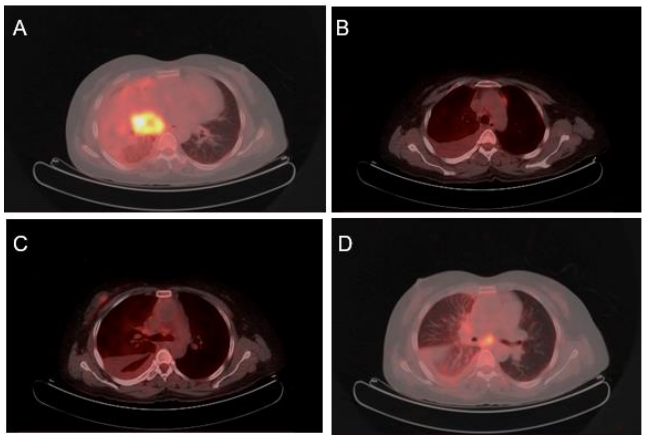

Figure 2. FDG-PET scan results. FDG-PET scan images showcasing notable observations: A: Reveals FDG uptake in an ill-defined mass lesion located in the hilar region of the right lung, involving bilateral paratracheal and right hilar lymph nodes. B: Illustrates FDG uptake in the prevascular lymph nodes. C: Demonstrates FDG uptake in an unwell-defined, dense soft tissue mass lesion in the central sector of the right breast. Additionally, it reveals the absence of significant FDG uptake in a smaller, dense soft tissue nodule located in the upper inner quadrant of the right breast. D: Displays FDG uptake in the subcarinal lymph nodes.

Ultrasonography of the right breast revealed an irregular ill-defined hypoechoic mass (16.6*10mm) within the subareolar region, along with internal echogenic foci (calcifications) classifying it as BI-RAD score four (Figure 3).

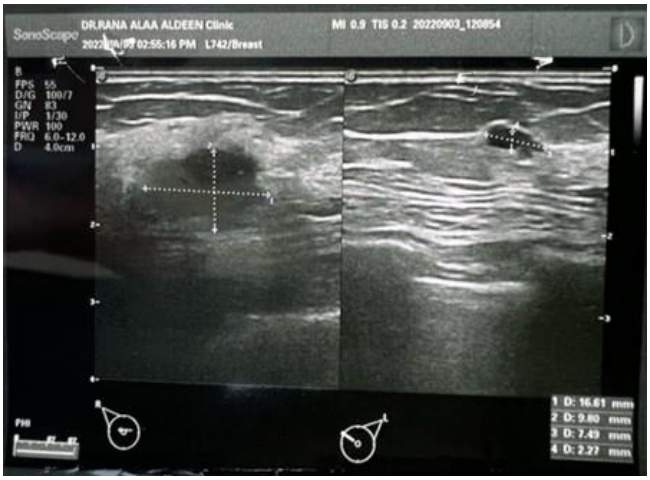

Figure 3. Breast ultrasonography findings. Breast ultrasonography image presenting specific features of interest: This image demonstrates: An irregular and ill-defined hypoechoic mass in the right breast, measuring 16.6 mm by 10 mm. The presence of internal tiny echogenic foci, indicative of calcifications. Notably, the mass is situated within the subareolar region.

Subsequent fine-needle aspiration (FNA) confirmed malignant features, displaying hyperchromatic irregular nuclei in clusters and scattered epithelial cells within a necrotic background (Figures 4A and 4B).

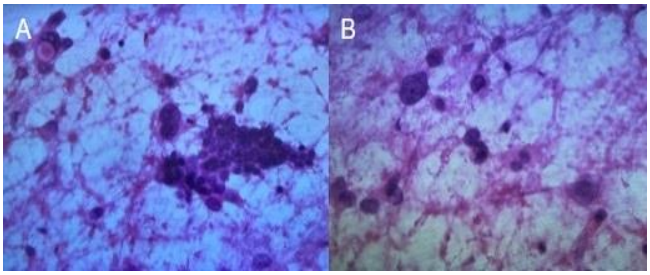

Figure 4. Cytological smears with H&E staining. Cytological smears stained with H&E, highlighting specific cellular characteristics: A and B: Depict clusters and scattered malignant epithelial cells, featuring hyperchromatic irregular nuclear borders and pleomorphic nuclei. These cells are set against a necrotic background.

Utilizing ultrasound guidance, a cell-block preparation of the right breast mass exhibited hyperchromatic and pleomorphic malignant cells with intermediate-grade nuclear atypia. Focal tubular differentiation and infiltration into fatty tissue were evident (Figures 5A and 5B). These findings corresponded to invasive ductal carcinoma (not otherwise specified), supported by immunohistochemistry results including positive CK7 and negative p63.

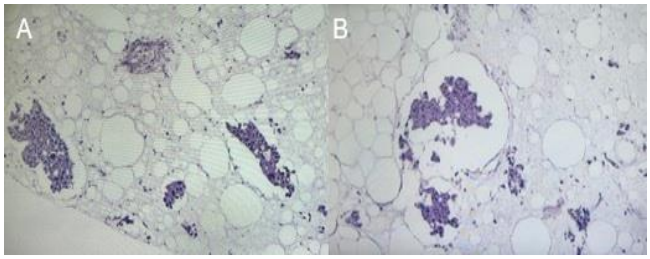

Figure 5. Cell-block preparation with H&E staining.

Cell-block preparation stained with H&E, emphasizing distinctive cellular attributes: A and B: Display hyperchromatic and pleomorphic malignant cells exhibiting intermediate-grade nuclear atypia. Notably, a focal region of tubular differentiation is also evident within the preparation.

Slide review and immunohistochemistry of the lung biopsy highlighted scattered malignant cells intermingled with inflammatory cells. Positive CK7 and absence of p63 confirmed epithelial origin, excluding lymphoma and small-cell carcinoma. The overall histopathology and immunohistochemistry aligned with poorly differentiated carcinoma exhibiting positive ER (3+5 8/8) and HER2 (score +3).

Collectively, these findings indicated the presence of two distinct cancerous lesions, each originating in different tissue types, with no evidence of metastasis between sites. The patient was eligible for palliative chemotherapy with trastuzumab and carboplatin, targeting both lung and breast cancers. Trastuzumab is a monoclonal antibody that binds to the HER2 protein, which is overexpressed in some cancers, and blocks its activity and triggers immune reactions that kill cancer cells. Carboplatin is a platinum-based drug that damages the DNA of cancer cells and prevents them from dividing. This combination is effective and well-tolerated in patients with HER2-positive breast cancer and may also have activity in HER2-positive lung cancer. The patient received an 8 mg/kg loading dose of trastuzumab followed by 6 mg/kg every three weeks, along with 5 mg/kg carboplatin every three weeks for at least six cycles. The expected outcomes of this treatment were to control the disease progression, reduce the tumor burden, and improve the quality of life. The potential side effects of this treatment included nausea, vomiting, fatigue, hair loss, low blood counts, infection, allergic reaction, kidney damage, nerve damage, and heart damage. The patient was monitored for these side effects and received supportive care as needed.

Discussion

Multiple primary malignant tumors (MPMT) are becoming more recognized due to improved diagnostic methods. However, diagnosing multiple primary lung cancers is still challenging, especially when they have the same histology. Gene mutation analysis can help to differentiate between primary and metastatic tumors (5). Synchronous breast and lung cancers are very rare, accounting for less than 0.5% of breast cancer cases. A study by Burstein et al. showed that 55% of lung lesions in women with breast cancer were primary lung cancers, 37% were metastases, and 8% were benign (6). This highlights the need for accurate histological diagnosis of lung lesions, as some of them may be treatable. This also follows the criteria by Warren and Gates for diagnosing MPMT, which require biopsy confirmation, distinct pathology, and exclusion of metastasis (6). De Luca et al. reported a case of synchronous skin and breast cancer and discussed the frequent co-occurrence of dual primary breast and lung cancers. They attributed this to three factors: the high prevalence of breast cancer in women, the good prognosis of early-detected breast cancer leading to an increased risk of secondary tumors, and the increased susceptibility of breast cancer survivors to develop primary lung tumors (7). Jin et al. described another rare case of a woman with lesions in the left breast and both lower lung lobes. They found that the lung lesions had different EGFR gene mutations, indicating genetic heterogeneity among primary malignancies (8). Hu et al. also studied the relationship between breast and lung cancers and found a strong correlation between EGFR mutation in lung cancer and hormone receptor expression in lung tissue. However, they did not find any association between EGFR mutation and HER2 expression, suggesting a possible role of sex hormones in lung cancer development in these patients (9). Besides breast and lung cancers, patients with breast cancer may also develop primary tumors in other organs, such as the ovaries, uterus/endometrium, colorectum, kidneys, pancreas, and thyroid. These occurrences may manifest synchronously or metachronously, often influenced by factors such as hormonal treatment for the primary breast tumor

(notably, a strong link exists between endometrial cancer and tamoxifen), genetic predispositions (e.g., BRCA1 and BRCA2 mutations), and obesity. Incidence rates fluctuate, with reported figures ranging from 4.1% by Kim and Song in a study tracking 108 breast cancer patients, to 16.4% by Weir et al., who followed a larger cohort of 301,963 patients (10). However, the convergence of multiple distinct cancer types, exemplified by the case presented here, remains a rare phenomenon.

This case report presents a rare occurrence of synchronous primary malignancies in both the lung and breast, which is underreported in the medical literature. This case adds to the existing knowledge of MPMT and may stimulate further research on this topic. Future directions for research may include genetic profiling, targeted therapies, or novel treatment approaches that could improve our understanding and management of these rare synchronous malignancies.

Our study has some limitations that should be considered when interpreting our results. First, our sample size was small, consisting of only one patient with synchronous primary malignancies in both the lung and breast. Therefore, our findings may not be generalizable to other patients with similar conditions. Second, our study was a case report, which is a descriptive and observational type of study that does not provide causal evidence or test hypotheses. Therefore, our study cannot establish the etiology, pathogenesis, or prognosis of these rare synchronous malignancies. Further studies with larger and more diverse samples are needed to confirm and expand our findings.

Author contribution

MKK, **MJI**, and **AMA** contributed to the conception and design of the manuscript. **MKK**, **MJI**, **HTH**, and **AMA** supervised the project. **MKK**, **MJI**, **MNA**, **HTH**, and **ADA** provided the materials and contributed to data collection and processing. **ADA**, **MJI**, **MKK**, **MNA**, **HTH** and **AMA** contributed to the interpretation and analysis of the project. **ADA**, **HTH**, and **MNA** contributed to the literature review and writing of the manuscript respectively. **ADA**, **MNA** and **AMA** critically revised the manuscript.

IRB approval

The case report was approved for publication by the University of Baghdad's Institutional Review Board under the ethics code UB/2023/022.

Ethics Statement

The manuscript complies with the ethical recommendations of the Declaration of Helsinki of the World Medical Association

Conflict of interest

The authors declare no conflict of interest.

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References

1. Jin B, Zhang S, Chuang X, Yu P, Chen Y, Teng Y, et al. Breast cancer and synchronous multiple primary lung adenocarcinomas with heterogeneous mutations: A case report. BMC Cancer. 2018;18(1):2–7.
2. Holly A. Swartz, Jessica C. Levenson and EFU. HHS Public Access Small-cell lung cancer. Physiol Behav. 2012;43(2):145–53.
3. Waks AG, Winer EP. Breast Cancer Treatment: A Review. JAMA. 2019;321(3):288-300. doi:10.1001/jama.2018.19323
4. Paul Spiteri Meilak B. The relationship between primary breast and primary lung cancer: A case series. Int J Case Reports Images. 2020;11(July):1.
5. Lekos A, Glantz MJ. Diagnosis in oncology. J Clin Oncol. 1997;15(8):3019–20.
6. De Luca A, Frusone F, Vergine M, Cocchiara R, Torre G La, Ballesio L, et al. Breast cancer and multiple primary malignant tumors: Case report and review of the literature. In Vivo (Brooklyn). 2019;33(4):1313–24.
7. Hu Z, Zou X, Qin S, Li Y, Wang H, Yu H, et al. Hormone receptor expression correlates with EGFR gene mutation in lung cancer in patients with simultaneous primary breast cancer. Transl Lung Cancer Res. 2020;9(2):325–36.
8. Vogt A, Schmid S, Heinimann K, Frick H, Herrmann C, Cerny T, et al. Multiple primary tumours:

Challenges and approaches, a review. ESMO Open. 2017;2(2):1–12.
9. Kim JY, Song HS. Metachronous double primary cancer after treatment of breast cancer. Cancer Res Treat. 2015;47(1):64–71.
10 . Weir HK, Johnson CJ, Thompson TD. The effect of multiple primary rules on population-based cancer survival. Cancer Causes Control. 2013;24(6):1231–42.

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Anti-Mullerian hormone level in relation to physical activity and reproductive determinants in North Iranian infertile women

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Abstract

Introduction: Female infertility is responsible for approximately half of all cases of infertility and one of the causes of infertility in women is related to ovarian disorders. Anti-Müllerian Hormone (AMH) is one of the clinical markers of ovarian reserve. Physical activity may affect the reproductive system and AMH concentration in serum. We aim to evaluate the relationship between physical activity and reproductive determining fertility and anti-mullerin hormone (AMH) in infertile women in northern Iran.

Materials and Methods: This cross-sectional study included 234 women aged 18–45 referred to the Infertility Clinic of the Al-Zahra Hospital, Rasht, Iran. The reproductive characteristics and the amount of physical activity of the patients were recorded. Exclusion criteria included menopause, cancer, underlying endocrine diseases, use of hormonal drugs, diagnosis of PCOS based on Rotterdam criteria, any ovarian and uterine surgery, and endometriosis.

Results: As expected, we observed significantly lower AMH concentrations in older participants. There was no association between reproductive determinants and AMH level (P> 0.05). We observed lack of physical activity as well as vigorous physical activity, is associated with lower AMH concentration (P= 0.025, and P= 0.039 respectively).

Conclusion: In this study, AMH levels appear to be significantly lower in patients with a lack of physical activity as well as vigorous physical activity. The results of this study showed that by improving lifestyle, including the appropriate amount of physical activity, it may be possible to improve the results of infertility treatments. However, a larger study is needed to verify the findings of this study.

Keywords: Anti-Mullerian hormone, AMH, Female infertility, Physical activity, Lifestyle

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Introduction

Female infertility is responsible for approximately half of all cases of infertility and one of the causes of infertility in women is related to ovarian disorders. anti-mullerin hormone (AMH) is produced by the granulosa cells of pre-antral and small antral ovarian follicles and is widely accepted as a clinical marker of ovarian reserve (1). It is a member of the transforming growth factor-β superfamily, and there is a strong positive correlation between circulating AMH concentrations and the number of follicles in the ovary (2). Since the number of follicles is well correlated with the level of AMH (3), it can reflect the number of dormant follicles in adult women. AMH suppresses the cyclic recruitment of primordial follicles into the pool of growing follicles and its levels decrease with age (4,5), thus serving as a marker of female reproductive aging (6). AMH level is highly variable among women, even measured on the same day in the menstrual cycle. Serum AMH level has been reported to be a highly accurate tool for the diagnosis of polycystic ovary syndrome (PCOS) (7) and premature ovarian insufficiency (8). In addition, AMH is used for the prediction of ovarian response during in vitro fertilization (IVF) treatment, and prediction of age at menopause (9,10).

The fact that AMH can't predict the probability of a woman conceiving within a given period may be related partly to variation of circulating AMH even within the same age in different women (11) due to various lifestyle and reproductive characteristics. Konishi et al. (2014) examined the association between AMH levels and menstrual cycle and lifestyle characteristics among young Japanese women. They reported that circulating AMH concentration was significantly lower among young women who had more severe menstrual pain (12). Lower AMH concentration has been found in using oral contraceptives (13), mild/ minimal endometriosis (14), obesity (15), smoking (16), and a regular and shorter menstrual cycle (13).

Physical activity plays an important role in maintaining energy balance which may affect the reproductive system (17). Weight loss via physical activity may protect ovarian function by increasing insulin resistance and changing the hormonal profile (18). It has been reported that an increased risk of infertility was found for the group of women reporting the highest levels of intensity and frequency of physical activity (19). Thus the possible risks of infertility should be highlighted among women who do heavy exercise. Steiner et al. (2010) reported that serum AMH levels do not fluctuate during oral contraceptive use in reproductive-aged women and AMH levels are significantly lower in obese women (1). It has been reported among premenopausal women, that lower AMH levels are associated with older age, younger age at menarche, and currently using oral contraceptives, suggesting these factors are related to decreased ovarian follicles (20). Bernardi et al. (2017) reported a significant association between obesity and lower AMH levels, suggesting that obesity may compromise ovarian reserve(21) through decreased responses to fertility medications, fewer oocytes retrieved (22), and lower pregnancy and live birth rates (23). However, there are contradictions in the literature regarding the association between obesity and AMH levels, so further investigation into this relationship is warranted.

On the other hand, ethnicity is an independent predictor for AMH (18) and the association between AMH and lifestyle factors like body mass index (BMI), smoking, and physical activity may vary across ethnic groups (13). Understanding the factors associated with individual variation of AMH levels among infertile women may help their infertility management. To our knowledge, no study has targeted in North Iranian between infertile women to examine such associations. Therefore, the present study aimed to evaluate the association between age, BMI, reproductive history, and physical activity with serum AMH concentration in North Iranian women with primary/secondary infertility.

Materials and Methods

Subjects

This cross-sectional study included 234 women aged 18–45 from April 2019 to March 2020. Patients participating in the study were selected from women candidates for assisted reproductive treatment and referred to the Infertility Clinic of the Al-Zahra Hospital, Rasht, Iran. Exclusion criteria included menopause, cancer, underlying endocrine diseases, use

of hormonal drugs, diagnosis of PCOS based on Rotterdam criteria, any ovarian and uterine surgery, and endometriosis. Approval was obtained from the Research Deputy and Ethics Committee of Guilan University of Medical Sciences (Approval ID: IR.GUMS.REC.1398.375). All the participants signed a written informed consent before sample collection and acknowledged that they had been fully anonymized. The reproductive characteristics included age at menarche, cycle regularity status, pregnancy, parity, breastfeeding history, and age at menarche, maternal menopause age. The amount of physical activity of the patients was also recorded. IPAQ (International Physical Activity Questionnaire) (24) was used to determine the amount of physical activity.

AMH assay

At the time of enrollment up to 5 mL of venous blood was drawn from each participant. Blood samples were centrifuged at 1400g/10min to separate the serum. Serum samples were stored at -20 °C until AMH concentration measurement. Serum AMH was measured using the Beckman Coulter AMH ELISA kit (cat no: B13127) according to the manufacturer's instructions.

Statistical analysis

Statistical analyses were performed using SPSS Software (v21; SPSS Inc; Chicago, Illinois, USA), and P-values less than 0.05 were considered significant and Chi-square tests, fisher exact test, and independent T-test were used to examine the relationship between variables

Results

This cross-sectional analysis included 234 women aged 18–45 years old referred to the Infertility Clinic of the Al-Zahra Hospital, Rasht, Iran. Table 1 includes information on the demographics and reproductive history of the women who participated in the study. As expected, we observed significantly lower AMH concentrations in older participants. The risk of infertility is increased for the group of women who report the highest intensity and frequency of physical activity. There was no significant association between BMI and AMH concentrations (P= 0.37). There was no association between reproductive determinants and AMH level (Table 1).

Table 1. Demographic characteristics and reproductive history of two groups of study.

Variables	AMH≤1.10	AMH≥1.11	P value
Age (years)			
18-30	9(9.1%)	37(27.4%)	**0.0001***
30-40	37(37.4%)	85(63%)	
>40	53(53.3%)	13(9.6%)	
BMI (kg/m2)			
<25	33(33.3%)	42(31.1%)	**0.371***
25-30	44(44.4%)	52(38.5%)	
≥30	22(22.2%)	41(30.4%)	
Breastfeeding history			
No	90(90.9%)	120(88.9%)	**0.615***
Yes	9(9.1%)	15(11.1%)	
Menstrual cycle pattern			
Regular	75(75.8%)	96(71.1%)	**0.429***
Irregular	24(24.2%)	39(28.9%)	
Gravidity			
0	72(72.2%)	99(73.3%)	**0.918***
≥1	27(27.3%)	36(26.7%)	

Abortion			**0.115***
0	79(79.8%)	118(87.4%)	
≥1	20(20.2%)	17(12.6%)	
Live birth			**0.295***
0	92(92.9%)	120(88.9%)	
≥1	7(7.1%)	15(11.1%)	
Still birth			**0.700****
0	96(97%)	132(97.8%)	
≥1	3(3%)	3(2.2%)	
Age at menarche	12.86±1.31	12.93±1.33	**0.70*****
Maternal menopausal age	50.54±2.47	50.85±2.86	**0.93*****

*** Chi-squared test** **** fisher's exact test** ***** independent t-test**

Physical activity is classified into fourepresentsr groups vigorous physical activity, moderate physical activity, low physical activity (walking), and inactive (sitting). As shown in Figure 1, lack of physical activity, as well as vigorous physical activity, is associated with lower AMH concentration (P= 0.025, and P= 0.039 respectively).

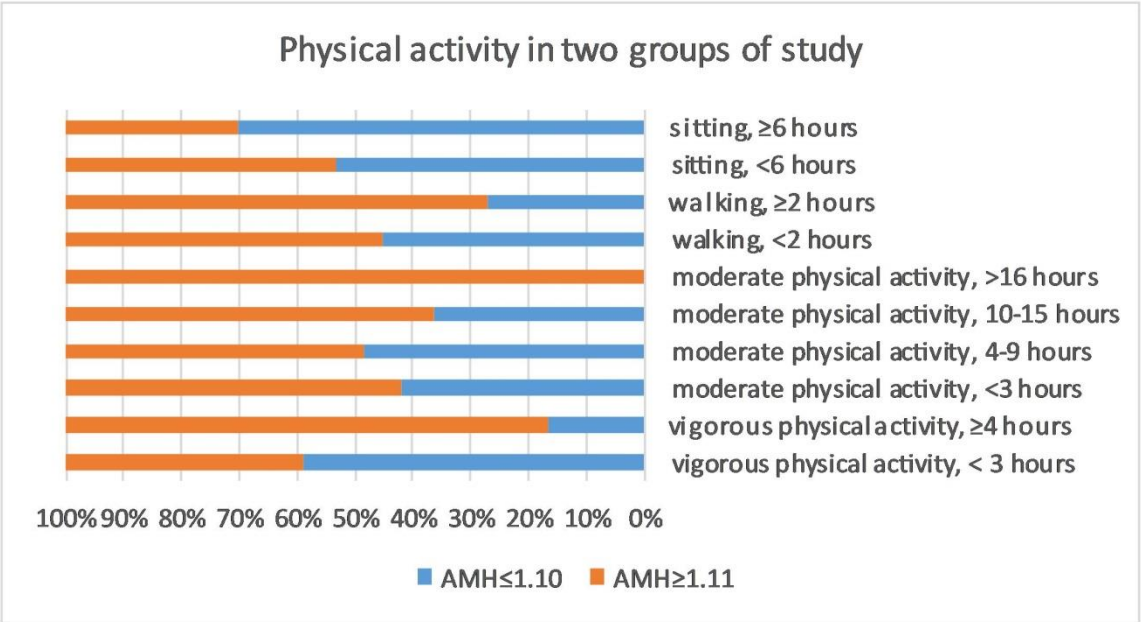

Figure 1. The chart representing physical activity in two groups of study. As shown in the chart, lack of physical activity as well as vigorous physical activity is associated with AMH ≤1.10.

Discussion

This study demonstrated that AMH levels are influenced by physical activity. More specifically, we found lack of physical activity, as well as vigorous physical activity, is associated with lower AMH concentration. Improvement of AMH levels and oxidative stress through regular exercise has been

reported in Chinese women with PCOS (25). So, improvement of oxidative stress might be an effective method for improvement of AMH level, which deserves further research. It has been reported that the level of AMH in women over 40 years of age was significantly lower than in women less than 35 years of age. Jung et al.(2017) reported higher AMH concentrations in women with older compared to younger ages at menarche (20) while our finding is consistent with other study reported no associations (26). We also observed no association between other reproductive determinants (Table 1) which may be due to the small sample size of the present study or ethnicity variations. So, future large studies are warranted to validate our findings. We observed no association between parity and AMH level that is consistent with earlier studies (20). The decrease of AMH levels with increasing age in adult premenopausal women is well established (26,27) as we observed in this present study.

Regular exercise causes weight loss and improves metabolic function and hormonal profile. It has been reported that the exercises also usually lead to a significant increase in fertility (28). Physical activity improves the quality of life in the general population but there is insufficient evidence for the effect of physical activity and quality of life on improving fertility in infertile women (29). Cicek et al. (2019) reported strength exercise decreases serum AMH levels and increases serum FSH levels (30). Therefore, excessive exercise practices have negative consequences for women's fertility, especially for those with lower ovarian reserve. It has been reported that moderate physical activity is associated with improved age-specific levels of ovarian reserve markers (31).

Physical activity through regulation of energy balance and insulin sensitivity can improve reproductive system function. Vigorous physical activity was associated with reduced fecundity in all women with normal BMI, but not in overweight and obese women (32). However, it has been demonstrated physical activity is unlikely to have a deleterious effect on IVF success and certain forms of vigorous activity may be beneficial (33). AMH can predict the ovarian response to hyperstimulation (34) and a low AMH test result has a negative psychological impact (35). On the other hand, maternal lifestyle during pregnancy may be associated with reproductive health and ovarian reserve in adult offspring (36). So, finding an association between lifestyle parameters such as physical activity and the level of AMH, and changing this lifestyle can affect the health of the next generation.

Conclusions

In this study, AMH levels appear to be significantly lower in patients with a lack of physical activity as well as vigorous physical activity. The results of the present study showed that by improving lifestyle, including the appropriate amount of physical activity, it may be possible to improve hormone levels and thus improve the results of infertility treatments. However, a larger clinical study is indicated to study the association between AMH and physical activity in reproductive-age women.

Author contribution

In this manuscript, the role of each of the authors, conceptualization with **RK**, conceptualization with **FM**, data collection with **ME**, formal Analysis with **AA**, writing, review and editing with **NGhG**, and writing an original draft with **SHSh**.

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Conflict of interest

The authors report no conflict of interest.

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References

1. Steiner AZ, Stanczyk FZ, Patel S, Edelman A. Antimullerian hormone and obesity: insights in oral contraceptive users. Contraception. 2010;81(3):245–8.
2. Karkanaki A, Vosnakis C, Panidis D. The clinical significance of anti-Müllerian hormone evaluation in gynecological endocrinology. Hormones. 2011;10(2):95–103.
3. Toner JP, Seifer DB. Why we may abandon basal follicle-stimulating hormone testing: a sea change

in determining ovarian reserve using antimüllerian hormone. Fertil Steril. 2013;99(7):1825–30.
4. Dolleman M, Verschuren WMM, Eijkemans MJC, Dollé MET, Jansen E, Broekmans FJM, et al. Reproductive and lifestyle determinants of anti-Müllerian hormone in a large population-based study. J Clin Endocrinol Metab. 2013;98(5):2106–15.
5. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WHB. A validated model of serum anti-Müllerian hormone from conception to menopause. PLoS One. 2011;6(7):e22024.
6. Sowers MFR, Eyvazzadeh AD, McConnell D, Yosef M, Jannausch ML, Zhang D, et al. Anti-mullerian hormone and inhibin B in the definition of ovarian aging and the menopause transition. J Clin Endocrinol Metab. 2015;93(9):3478–83.
7. Iliodromiti S, Kelsey TW, Anderson RA, Nelson SM. Can anti-Müllerian hormone predict the diagnosis of polycystic ovary syndrome? A systematic review and meta-analysis of extracted data. J Clin Endocrinol Metab. 2013;98(8):3332–40.
8. Knauff EAH, Eijkemans MJC, Lambalk CB, ten Kate-Booij MJ, Hoek A, Beerendonk CCM, et al. Anti-Mullerian hormone, inhibin B, and antral follicle count in young women with ovarian failure. J Clin Endocrinol Metab. 2011;94(3):786–92.
9. Broer SL, Mol BWJ, Hendriks D, Broekmans FJM. The role of antimullerian hormone in prediction of outcome after IVF: comparison with the antral follicle count. Fertil Steril. 2011;91(3):705–14.
10. Broer SL, Eijkemans MJC, Scheffer GJ, Van Rooij IAJ, De Vet A, Themmen APN, et al. Anti-Müllerian hormone predicts menopause: a long-term follow-up study in normoovulatory women. J Clin Endocrinol Metab. 2011;96(8):2532–9.
11. Nelson SM, Messow MC, Wallace AM, Fleming R, McConnachie A. Nomogram for the decline in serum antimüllerian hormone: a population study of 9,601 infertility patients. Fertil Steril. 2011;95(2):736–41.
12. Konishi S, Nishihama Y, Iida A, Yoshinaga J, Imai H. Association of antimüllerian hormone levels with menstrual-cycle type and dysmenorrhea in young asymptomatic women. Fertil Steril. 2014;102(5):1439–43.
13. Kristensen SL, Ramlau-Hansen CH, Andersen CY, Ernst E, Olsen SF, Bonde JP, et al. The association between circulating levels of antimüllerian hormone and follicle number, androgens, and menstrual cycle characteristics in young women. Fertil Steril. 2012;97(3):779–85.
14. Lemos NA, Arbo E, Scalco R, Weiler E, Rosa V, Cunha-Filho JS. Decreased anti-Müllerian hormone and altered ovarian follicular cohort in infertile patients with mild/minimal endometriosis. Fertil Steril. 2011;89(5):1064–8.
15. Su HI, Sammel MD, Freeman EW, Lin H, DeBlasis T, Gracia CR. Body size affects measures of ovarian reserve in late reproductive age women. Menopause (New York, NY). 2015;15(5):857.
16. Sowers MR, McConnell D, Yosef M, Jannausch ML, Harlow SD, Randolph Jr JF. Relating smoking, obesity, insulin resistance and ovarian biomarker changes to the final menstrual period (FMP). Ann N Y Acad Sci. 2010;1204:95.
17. Redman LM. Physical activity and its effects on reproduction. Reprod Biomed Online. 201112(5):579–86.
18. Norman RJ, Noakes M, Wu R, Davies MJ, Moran L, Wang JX. Improving reproductive performance in overweight/obese women with effective weight management. Hum Reprod Update. 2014;10(3):267–80.
19. Gudmundsdottir SL, Flanders WD, Augestad LB. Physical activity and fertility in women: The North-Trøndelag Health Study. Hum Reprod. 2015;24(12):3196–204.
20. Jung S, Allen N, Arslan AA, Baglietto L, Brinton LA, Egleston BL, et al. Demographic, lifestyle, and other factors in relation to antimüllerian hormone levels in mostly late premenopausal women. Fertil Steril. 2017;107(4):1012–22.
21. Bernardi LA, Carnethon MR, de Chavez PJ, Ikhena DE, Neff LM, Baird DD, et al. Relationship between obesity and anti-Müllerian hormone in reproductive-aged African American women. obesity. 2017;25(1):229–35.
22. Pinborg A, Gaarslev C, Hougaard CO, Andersen AN, Andersen PK, Boivin J, et al. Influence of female bodyweight on IVF outcome: a longitudinal multicentre cohort study of 487 infertile couples. Reprod Biomed Online. 2011;23(4):490–9.
23. Shah DK, Missmer SA, Berry KF, Racowsky C, Ginsburg ES. Effect of obesity on oocyte and embryo quality in women undergoing in vitro fertilization. Obstet Gynecol. 2011;118(1):63–70.

24. Tomioka K, Iwamoto J, Saeki K, Okamoto N. Reliability and validity of the International Physical Activity Questionnaire (IPAQ) in elderly adults: the Fujiwara-kyo Study. J Epidemiol. 2011;1109210254.
25. Wu X, Wu H, Sun W, Wang C. Improvement of anti-Müllerian hormone and oxidative stress through regular exercise in Chinese women with polycystic ovary syndrome. Hormones. 2020;1–7.
26. Shaw CM, Stanczyk FZ, Egleston BL, Kahle LL, Spittle CS, Godwin AK, et al. Serum antimüllerian hormone in healthy premenopausal women. Fertil Steril. 2011;95(8):2718–21.
27. van Rooij IAJ, Broekmans FJM, Scheffer GJ, Looman CWN, Habbema JDF, de Jong FH, et al. Serum antimüllerian hormone levels best reflect the reproductive decline with age in normal women with proven fertility: a longitudinal study. Fertil Steril. 2015;83(4):979–87.
28. Al-Eisa E, Gabr SA, Alghadir AH. Effects of supervised aerobic training on the levels of anti-Mullerian hormone and adiposity measures in women with normo-ovulatory and polycystic ovary syndrome. J Pak Med Assoc. 2017;67(4):499–507.
29. Orio F, Muscogiuri G, Ascione A, Marciano F, Volpe A, La Sala G, et al. Effects of physical exercise on the female reproductive system. Minerva Endocrinol. 2013;38(3):305–19.
30. Cicek G, Gorkem U, Yamaner F, Gullu A, Gullu E. Adverse Effect of Different Exercise Types on Ovarian Reserve. J Educ Train Stud. 2019;7(1):115–20.
31. Kiranmayee D, Praveena T, Himabindu Y, Sriharibabu M, Kavya K, Mahalakshmi M. The effect of moderate physical activity on ovarian reserve markers in reproductive age women below and above 30 years. J Hum Reprod Sci. 2017;10(1):44.
32. Wise LA, Rothman KJ, Mikkelsen EM, Sørensen HT, Riis AH, Hatch EE. A prospective cohort study of physical activity and time to pregnancy. Fertil Steril. 2012;97(5):1136–42.
33. Gaskins AJ, Williams PL, Keller MG, Souter I, Hauser R, Chavarro JE, et al. Maternal physical and sedentary activities in relation to reproductive outcomes following IVF. Reprod Biomed Online. 2016;33(4):513–21.
34. Dewailly D, Andersen CY, Balen A, Broekmans F, Dilaver N, Fanchin R, et al. The physiology and clinical utility of anti-Müllerian hormone in women. Hum Reprod Update. 2014;20(3):370–85.
35. O'Brien Y, Kelleher C, Wingfield M. "So what happens next?" exploring the psychological and emotional impact of anti-Mullerian hormone testing. J Psychosom Obstet Gynecol. 2020;41(1):30–7.
36. Eubanks AA, Nobles CJ, Hill MJ, DeCherney AH, Kim K, Sjaarda LA, et al. Recalled maternal lifestyle behaviors associated with anti-müllerian hormone of adult female offspring. Reprod Toxicol. 2020;98:75–81.

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Biological properties and therapeutic effects of apigenin and its evaluation on several types of cancer

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Abstract

Apigenin is a member of the flavonoid family that has been used in medicine for a long time. Apigenin is one of the compounds that has been used for a long time to treat various disorders and diseases. Apigenin is chemically known as 4',5,7, trihydroxyflavone and belongs to the family of flavones. Apigenin has many pharmacological activities such as anti-inflammatory, anti-viral, anti-bacterial, etc. Various studies have shown that apigenin plays an important role in suppressing diseases such as Parkinson's, Alzheimer's, inflammatory diseases, and different types of cancers. In the present study, various therapeutic properties, biological effects, and the effect of apigenin on different cancers are discussed. Different studies have been conducted on the anti-cancer effect of apigenin. It has been proven that apigenin has inhibitory effects on various cancers including lung, stomach, neuroblastoma, thyroid, liver, skin, and prostate cancer through different signaling pathways. In general, it can be mentioned that the anti-cancer properties of apigenin are due to its effects in various signaling pathways such as angiogenesis, tumor suppressor genes, apoptosis, cell cycle and nuclear factor kappa B (NF-κB), Janus kinase/signal transducer and activator of transcription (JAK/STAT3), phosphoinositide 3-kinase /protein kinase B /mammalian target of rapamycin (PI3K/AKT/mTOR), mitogen-activated protein kinase/ estrogen receptor 2 (MAPK/ER2), Wnt/B-catenin pathways.

Keywords: Inflammation, Apigenin, Cancer, Cell cycle, Apoptosis

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Introduction

Polyphenol compounds are described by phenolic structures. These biological molecules are used in the treatment of various diseases. They are a large family of natural compounds that have many biological, pharmacological, and physiological advantages for human health. They are known as protectors against oxidative stress, ultraviolet and other pathogens. Polyphenols can play their role in cell protection against oxidative stress and inflammation by activation of the transcription factor nuclear factor erythroid-2 related factor (Nrf2) (1-5). In addition, these compounds can modulate some of the most important cellular processes such as proliferation, cell growth, differentiation, etc. (6). Various studies have shown that polyphenols are effective in radiation protection. The main mechanisms are neutralizing free radicals caused by radiation, reducing inflammatory responses, repairing hematopoietic cells, and repairing deoxyribonucleic acid (DNA) (7). So far, more than 8000 polyphenolic compounds have been known in different plants. Polyphenols have different chemical structures, the most prominent of which are flavonoids, stilbenes, and phenolic acids. One of the most important polyphenolic compounds is flavonoids (1, 3, 8, 9). The first studies on flavonoid compounds were done in 1936. Flavonoids are low molecular weight compounds. Although flavonoids are not made by humans and animals, they are considered essential compounds in the human diet. The compounds are abundant in our diet, including nuts, fruits, flowers, seeds, stem, wine, and tea (10, 11). Flavonoids are divided into different classes according to their molecular structures such as flavanones, flavones, flavanols, isoflavones, flavanonols, neoflavanes, flavanes, and flavonols (10, 12). Flavonoids are almost 5000 compounds that chemically have a prevalent phenylchromanone structure (C6-C3-C6). The general structure of flavonoid is based on two benzene rings (A and B ring) that are connected by a heterocyclic pyran (C ring) that contains oxygen. They have indicated various biological effects such as anti-inflammatory, antiviral, anti-mutagenic, and free radical scavenging (11, 13, 14). One of the flavonoids that have attracted a lot of attention is apigenin (15). Apigenin is one of the sub-classes of flavones, the unique properties of flavones are non-toxic and non-mutagenic (Figure1). Apigenin is mainly found in fruits (oranges), vegetables (onion, parsley, celery), herbs (basil, oregano, thyme, chamomile), and in some seasonings (13, 16, 17). Table 1 demonstrates common plants contain the highest amount of apigenin. Apigenin is chemically represented as 4',5,7, trihydroxyflavone. It's a low molecular weight flavonoid (270.24=KDa). In general, apigenin is insoluble in water, but the best solvents for this substance are dilute dimethyl sulfoxide (DMSO), potassium hydroxide (KOH), dimethylformamide (DMF), and ethanol (12, 13, 20, 21). Its melting point is reported as 347.5. The pure form of apigenin is unstable and is usually recommended to be kept at -20oC (13). For a long time, apigenin has been used to treat various diseases, including insomnia, Parkinson's, asthma, nervous system disorders, indigestion, gastritis, cancers, and cardiovascular diseases (12, 13, 22). Apigenin can also modulate different intercellular and extracellular signaling pathways to prevent abnormal tissue growth. For this reason, the administration of apigenin can be one of the effective factors in cancer treatment (22). Although previous studies have indicated that flavonoid compounds cannot have a good effect on blood lipid metabolism, apigenin plays a considerable role in regulating blood lipid and reduces triglyceride, cholesterol, and low-density lipoprotein cholesterol in the serum of mice (23). As a result, apigenin has attracted a lot of attention due to its low toxicity and significant impacts on natural versus cancer cells compared to other flavonoids (24). In this article, the biological effects of apigenin are discussed first, and then the effect of this flavonoid compound on several cancers is investigated. It has been reported that apigenin and other medicinal herbs can have remarkable effects in preventing various diseases and cancers. And also, it has been shown that different phytochemicals including flavonoids are responsible for the therapeutic impacts of these plants (25). Various studies have demonstrated that apigenin has different biological effects such as anti-inflammatory, anti-carcinogenic, anti-mutagenic, antioxidant, anti-viral, anti-allergic cardioprotective, neuroprotective, and antibacterial. In general, it can be said that apigenin has attracted more attention due to its considerable effects on cancers and low toxicity compared to other flavonoids (13, 15, 24, 26, 27). The biological properties mentioned above are caused by the

functional mechanisms of flavonoid compounds such as apoptosis induction, stimulation of the immune system, improvement of the enzymatic detoxification activity, reduction of oxidative stress, and cell cycle inhibition (15, 28). Some of these biological effects of apigenin are discussed below.

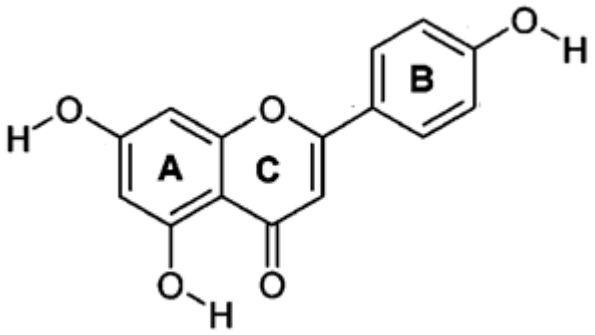

Figure 1. The basic structure of apigenin.

Table 1. Plants with the highest level of apigenin.

Scientific name	Commonly known
Achillea millefolium	Yarrow
Apium graveolens	Celery
Artemisia dracunculus	Tarragon
Chamaemelum nobile	Perennial chammomile
Coriandrum sativum	Cilantro
Digitalis purpurea	Purple foxglove
Echinacea spp	Coneflower
Gingko biloba	Biloba
Glycyrrhiza glabra	Licorice
Linum usitatissimum	Flax
Marrubium vulgare	Horehound
Matricaria retcutita	Annual chamomile
Mentha spicata	Spearmint
Ocimum basilicum	Basil
Origanum vulgare	Oregano

Anti-inflammatory effects of apigenin

Inflammation is a critical immune response to maintain tissue homeostasis. Two different types of inflammation are acute and chronic inflammation. Acute inflammation is a protective and essential response of therapeutic processes that initiates rapidly and its symptoms last for a short period up to a few days. In general, this response should be local and limited. Although acute inflammation tries to restore homeostasis, if it is not resolved, it leads to chronic inflammation (18). And also, Inflammation is one of the most important characteristics that confirm tumor progression and increase the risk of cancer. Flavonoids such as apigenin have been shown to suppress the activation of different cytokines and immune cells, so they may be considered natural inhibitors that can stop the activation of an adaptive and innate immune system. Apigenin can diminish inflammation by inhibiting tumor necrosis factor-alpha (TNF-α), C-C motif chemokine ligand 2 (CCL-2), granulocyte-macrophage colony-stimulating factor (GMCSF), interleukin 1-alpha (IL-1α) and IL-6 (19, 20). Many studies have indicated that apigenin can enhance various anti-inflammatory pathways, such as phosphatidylinositol 3-kinase/ protein kinase B (PI3k/Akt) and p38 mitogen-activated protein kinase (P38/MAPK). The inflammatory and antioxidant pathways of apigenin in cell lines are shown in figure 2. In addition, it reduces the activity of nitric oxide synthase-2 and ccyclooxygenase-2 (Cox-2). And also, apigenin prevents TNF-α-induced nuclear factor kappa B (NF-κB) activation and IkappaB kinase degradation (21). Apigenin can exert a wide range of molecular signaling effects (22). It has been reported that apigenin inhibits mitogen-activated protein kinase (MAPK) and the activity of protein kinase-C (23, 24). On the other hand, apigenin is a famous protein-tyrosine kinase inhibitor. In addition, it has been indicated that it can inhibit extracellular signal-regulated kinases (ERK) (25). Inactivation of NF-κB by apigenin in human cells culture medium is through suppression of the phosphorylation of the p65 subunit (26).

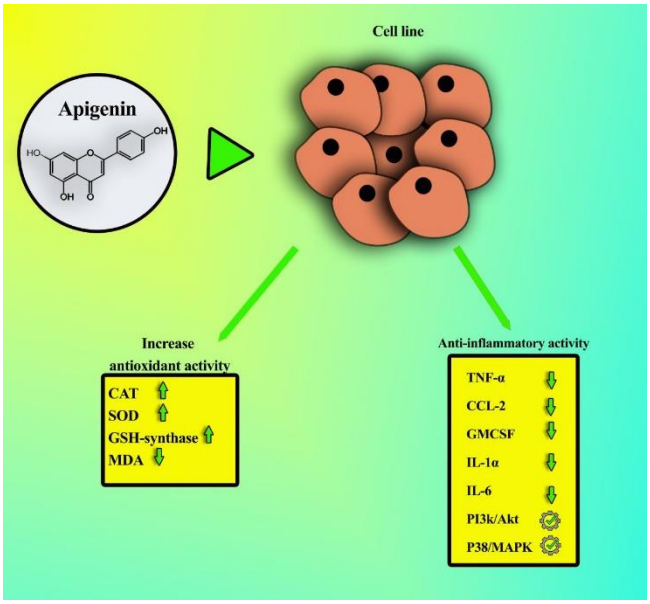

Figure 2. Anti-inflammatory and antioxidant effects of apigenin. Apigenin decreases malondialdehyde (MDA), increases antioxidants enzymes such as catalase (CAT), superoxide dismutase (SOD), glutathione synthetase (GSH-synthase), reduces the activity of anti-inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), c-c motif chemokine ligand 2 (CCL-2), granulocyte-macrophage colony-stimulating factor (GMCSF), interleukin 1-alpha (IL-1α), interleukin 6 (IL-6), and also it can can promote different anti-inflammatory pathways, such as phosphatidylinositol 3-kinase/ protein kinase B (pI3k/Akt) and p38 mitogen-activated protein kinase (p38/MAPK).

The effect of apigenin on the cell cycle

Cell division activates cell proliferation and distributes the exact genetic copies to daughter cells which is essential for the reproduction of life (27). Studies have demonstrated that medicinal plants can have a considerable role in cell cycle arrest, which is done by inhibiting G0/G1 or G2/M checkpoints. Several biochemical events cause cells to progress through the cell cycle. Before cells enter the S phase, a cascade of events must occur, including in the level of D-type cyclins and cyclin E at the beginning and the end of G phase. In general, with the formation of D-type cyclins complex with cyclin-dependent kinase 2 and CDK 4, cell cycle progress occurs through phosphorylation tumor suppressor protein retinoblastoma (Rb), which is necessary to enter the S phase. Apigenin is known as an effective inhibitor of some protein tyrosine kinases such as steroid receptor coactivator (Src) tyrosine kinase and epidermal growth factor receptor. Apigenin also can suppress the activation of protein kinase B/AKt, phosphatidylinositol 3-kinase, and casein kinase-2, which can play an important role in the development of cancer. In fact, apigenin has been indicated to inhibit cyclin-dependent kinases (CDKS) and cyclins in vitro. In addition, apigenin can enhance CDK inhibitors such as KIP1/p17 and WAF1/p21, which reduces the activity of G1 CDK, p53 stabilization, and Rb dephosphorylation (39). In one of the studies, the inhibitory effect of apigenin on the growth of human prostate tumor cells was evaluated in nude mice. In this study, apigenin was administrated orally. The consumption of apigenin increased the expression of WAF1/p21, KIP1/p27, INK4c/p18 and INK4a/p16, decreased the expression of cyclins D1, D2, E; and cyclin-dependent kinase (CDK), including CDK2 and CDK4 (Figure 3). With the decrease of cyclin D1, the inhibitor of WAF1/p21 increases. On the other hand, CDK4 can be partially reduced while cyclin E remains unchanged. These findings show that the inhibitory effect of apigenin on the proliferation of cells in the G1 phase is due to its decrease of cyclins D1 and the increase of WAF1/p21.Another mechanism of cell growth inhibition by apigenin has also been investigated. When cells are exposed to apigenin, the amount of protein p53 and its downstream proteins, such as Protein p21(Cip1/Waf1), which is a potential CDK inhibitor in G1 and G2/M phases, increases and leads to the inhibition of the cell cycle (28, 29). As mentioned above, apigenin causes cell cycle arrest in different phases such as G1/S or G2/M, which is done by modulating the expression of CDKs and other related genes (30). It has been indicated that exposure to a broad range of malignant cells such as fibroblast and epidermal cells with apigenin causes a reversible G0/G1 and G2/M arrest through the inhibition of p53 (CDK2) kinase activity along with enhancement of the stability of the p53 protein (31, 32).

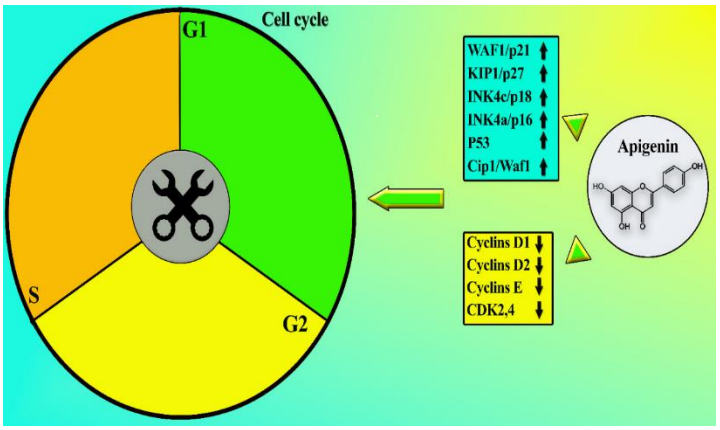

Figure 3. The above image shows the effects of apigenin on influencing factors in cell cycle. Apigenin can inhibit the cell cycle by increasing the expression of WAF1/p21, KIP1/p27, INK4c/p18 and INK4a/p16, (Cip1/Waf1), p53, and decreasing the expression of cyclins D1, D2, E; and cyclin-dependent kinase, including CDK2 and CDK4.

The effect of apigenin on apoptosis

Different types of cell death are necrosis, apoptosis, pyroptosis, and autophagy. Programmed cell death is an essential process in multicellular organisms that removes hazardous cells and keeps tissue homeostasis. Apoptosis is one of the types of regulated cell death, which is divided into two pathways, intrinsic and

extrinsic. Both pathways result in the activation of a group of caspases and proteases that are responsible for cell death. In addition, these pathways regulate apoptosis through proteins such as the B-cell lymphoma 2 (BCL-2) family (33). Apigenin plays an important role in apoptosis and its administration reduces cell survival. The function of apigenin is intensified by the reduction of BCL-2 and B-cell lymphoma-extra Large (BCL-XL) as well as the increase of Bcl-2-associated X (BAX) protein (34, 35). Studies have indicated apigenin causes apoptosis and cell growth inhibition in various tumors, including lung, skin, blood, liver, breast, stomach, colon, and prostate, by modulating different signaling pathways (36). Apigenin activates both intrinsic and extrinsic pathways of apoptosis. In general, in the process of internal pathway regulation, the mitochondrial membrane potential changes and leads to the secretion of cytochrome C in the cytoplasm, which activates caspase 3 with the formation of apoptotic protease activating factor (APAF), and as a result, apoptosis occurs (37). And also, apigenin regulates the extrinsic pathways of apoptosis by increasing the expression of mRNA of TNF-α, caspase-3, and caspase 8 (36-38). In cancer cells, apigenin induces apoptosis by regulating the expression of Bax, Bcl-2, Akt, and signal transducer and activator of transcription 3 (STAT-3) proteins (37, 38).

The effect of apigenin on oxidative stress

Oxidative stress is related to the imbalance between the antioxidant system and the production of free radicals. In general, reactive oxygen species (ROS) are essential in a limited amount for redox signaling and homeostasis of cells. Excessive production of reactive oxygen species/ reactive nitrogen species (ROS/RNS) neutralizes the body's defense system, which is called oxidative stress. Oxidative stress can be related to cancer, cardiovascular diseases, eye diseases, kidney disease, and diabetes. In addition, oxidative stress causes oxidative changes including protein carbonylation, nitration, sulfoxidation, lipid peroxidation, and DNA breaks such as single-strand breaks (SSB) and double-strand breaks (DSB) (39, 40). Various diseases, including cardiovascular diseases, diabetes, cancer, etc., are related to excessive production of free oxygen species and oxidative stress. Apigenin has significant antioxidant properties, such as enhancing enzymatic and non-enzymatic antioxidants, free radical scavenging, and modulating signaling pathways such as PI3/Akt, Nfr2, MAPK, and NF-KB. (41). Studies show that apigenin reduces adhesion molecules expression, which can be a useful strategy against oxidative stress, such as free-radical scavenging (42). Apigenin can also increase the activity of antioxidant enzymes such as catalase (CAT), superoxide dismutase (SOD), and glutathione synthetase (GSH-synthase) and also decreased the level of malondialdehyde (MDA) to counteract oxidative stress (43). In one of the studies to investigate the antioxidant effect of apigenin, 25 mg/kg of apigenin was administrated for two weeks. It was demonstrated that apigenin was able to reduce the amount of lipid peroxidation product (malondialdehyde). On the other hand, it increased the activities of antioxidant enzymes, including CAT, GPX, and SOD as well as non-enzymatic antioxidants, such as vitamins C and E, which led to a reduction in oxidative stress (52).

The effect of apigenin on cancer cells

The anti-cancer property of apigenin is due to its ability to modulate various signaling pathways including angiogenesis, apoptosis, tumor suppressor genes, cell cycle, inflammation, and NF-κB, JAK/STAT3, PI3K/AKT/mTOR, MAPK/ER2, Wnt/B-catenin pathways. Evidence shows that reactive oxygen species are of great importance in the anti-tumor properties of apigenin (44). Apigenin can inhibit the invasion and metabolism of cancer cells by regulating the production of protease (45). Studies indicate that apigenin suppresses lung melanoma metastasis by eliminating the interaction of cancer cells with the endothelium (46). Moreover, the exposure of endothelial cells to apigenin can lead to the suppression of vascular endothelial growth factors (VEGF) expression, which is an essential factor in angiogenesis through the degradation of hypoxia-inducible factor 1-α (HIF-1a) protein (Figure 4) (47). Apigenin can also inhibit the expression of VEGF and HIF-1a through human double minute 2 (HDM2)/P53 and PI3K/AKT/P70s6K1 pathways in ovarian cancer cells (48).

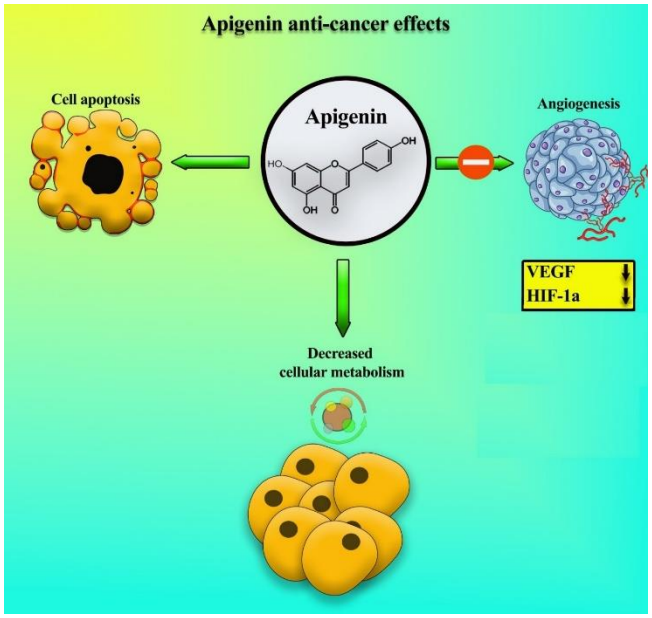

Figure 4. Anti-cancer effects of apigenin, including inhibition of angiogenesis through vascular endothelial growth factors (VEGF) suppression and protein hypoxia-inducible factor 1-α (HIF-1a) degradation, reduction of metabolism, and activation of apoptosis.

The effect of apigenin on different cancers

Despite, the significant progress made in cancer diagnosis and treatment in recent years, it is still considered the second main cause of death in the world. There are various modalities for cancer therapy, including hormone therapy, radiation therapy, chemotherapy, and target therapy. Some of the main challenges in cancer treatment, especially in the advanced stages, are the side effects of drugs, chemical resistance, the killing of normal cells, and treatment costs. Therefore, finding a treatment method with the least side effects is very important and is in the preliminary stages. Using natural products with strong therapeutic and preventive properties is of great value and importance. It should be noted that their importance is because of reducing the resistance of cancer cells to treatment and having fewer side effects (49, 50). Different studies have demonstrated that high consumption of polyphenolic compounds such as flavonoids can diminish the incidence of various cancers (51). In this study, we have tried to show the effect of apigenin on several types of cancers.

Lung cancer and apigenin

Lung cancer is one of the leading causes of death in the world (27). Biologically and histologically, lung cancer is considered a complex neoplasm. The four main histological kinds of lung cancer are small cell carcinoma, large cell carcinoma, adenocarcinoma, and squamous cell carcinoma (62). Knekt et al (63), investigated the relationship between the consumption of flavonoids such as apigenin, quercetin, luteolin, and myricetin and lung cancer. They have found that there is an inverse relationship between the occurrence of cancer and flavonoid consumption. They have concluded that onion and apple, as two sources rich in apigenin, can play a protective role against lung cancer. The relationship between the consumption of flavonoids and their protective role in the occurrence of various cancers, including breast cancer, ovarian cancer, and colorectal cancer, has also been investigated (13, 52). Lui et al (64), have suggested that apigenin could diminish the risk of lung cancer by inhibiting vascular endothelial growth factor (VEGF) transcription and proliferation of A549 lung cancer cells.

Gastric cancer and apigenin

Gastric cancer is one of the most common types of cancer around the world (53). There is compelling evidence that Helicobacter pylori infection can be associated with gastric cancer. Therefore, one of the preventive measures for gastric cancer is to eradicate the infection of Heliobacter pylori (H.pylori). In addition, another strategy to reduce the progress of gastric cancer is to use different flavonoid compounds such as apigenin, which have significant antioxidant properties. In one of the conducted studies, the effectiveness of apigenin on the progression of gastric cancer and atrophic gastric caused by helicobacter pylori was investigated. And the result showed that apigenin therapy significantly reduces the rates of histological changes of neutrophils and monocyte infiltration as well as H.pilori colonization in both gastric cancer and gastritis. In addition, apigenin could dramatically increase the expression of IKBa. Therefore, it could reduce the activation of NF-KB and inflammatory cytokines expression. Moreover, the level of ROS diminished due to the scavenging characteristic of apigenin (65, 66). Wu et al (67), evaluated the effect of apoptosis induction and cell cycle inhibition of apigenin on SGC-7910 gastric

carcinoma cells. They observed that apigenin inhibits clone formation and growth of these cells through apoptosis.

Neuroblastoma and apigenin

Neuroblastoma causes approximately 15% of childhood cancer-related deaths (53). Neuroblastoma is one of the most common extracranial solid tumors in children that originate from neural progenitor cells. These tumors can occur in the central nervous system, pelvic and thoracic regions. But they mainly appear in the abdominal region. Many factors play a role in the occurrence of this disease, such as inflammation, patient age, protein aggregation, tumor metastasis, etc. One of the important risk factors of neuroblastoma is MYCN Proto-Oncogene amplification, which can intensify neuroblastoma tumorigenesis. The age of the patient and elimination of a protein from chromosome 11 (11q aberration) are other risk factors (54, 55). It's notable for the wide range of clinical behavior. Some neuroblastoma tumors can differentiate into benign types (benign ganglioneuromas) and some undergo sudden regression (56, 57). Therapeutic modalities for neuroblastoma include surgery, chemotherapy, and radiotherapy (58). Stages 1 and 2 of the disease can only be treated by surgery (59). But in higher stages, favorable results are obtained with surgery and chemotherapy (60). Natural compounds have been proven to have valuable anti-cancer properties. Some of these compounds with few side effects can help prevent or even treat cancer. Flavonoids can suppress cancer by epithelial-mesenchymal transition (EMT) inhibition, extracellular matrix (ECM) protein modulation, and inhibiting the metabolism of cancer cells (61). Torkin et al (62), evaluated the effect of apigenin on human neuroblastoma cell lines. They found that apigenin inhibits the ability of colony formation and survival, and stimulates apoptosis in these cell lines. Apigenin elevated p53 protein level and products derived from p53, including Bax, p21WAF1/CIP1 gene. In addition, apigenin could increase the activity of caspase-3 and cause cell death.

Thyroid cancer and apigenin

Thyroid cancer, as an unusual cancer, can account for about 1% of all malignancies (53). Thyroid cancer is known as the fifth most prevalent cancer among women in the united states. The prevalence of this cancer is rising around the world. Treatment modalities for thyroid cancer in most patients are surgery combined with radioiodine therapy (63). Studies have shown that malignant thyroid cancer is divided into different types, including follicular thyroid cancer (FTC), papillary thyroid cancer (PTC), Hurthle cell cancer (HCC), and anaplastic thyroid cancer (ATC), all of which are derived from epithelial cancer cells. Other types include medullary thyroid cancer derived from parafollicular and non-epithelial types such as teratoma, sarcoma, and lymphoma. Among the different types of thyroid cancer, FTC, PTC, and HCC are called differentiated thyroid cancer. While ATC is considered a very malignant neoplasm. PTC is one of the most prevalent malignancies of thyroid cancer (64, 65). A study conducted on PCCL3 rat thyroid cells showed that apigenin was able to increase iodide influx by inhibiting AKT under thyrotropin stimulation (66). In addition, in the BCPAP cell line, apigenin caused a considerable cell accumulation in the G2/M phase through the reduction of cell division cycle 25 (Cdc25c) expression. Also, apigenin suppressed the viability of PTC cells through the stimulation of ROS production, which caused DNA damage and eventually resulted in autophagy cell death (67). Yin et al (68), assessed the impact of some flavonoid compounds such as apigenin on thyroid carcinoma cell lines, including UCLA Ro-w-1(WRO) (follicular carcinoma), UCLA RO-81A-1(ARO) (anaplastic carcinoma), and UClA NPA-87-1(NPA) (papillary carcinoma). Of all the flavonoids used, apigenin has been the most effective proliferation inhibitor of cell lines. Yin et al (69), in another study, showed that the inhibitory impact of apigenin on the proliferation of ARO cells was related to both phosphorylation of down-stream effector (MAPK) and epidermal growth factor receptor (EGFR) tyrosine autophosphorylation.

Elst et al (70), investigated the efficiency of flavonoid compounds on the growth of follicular cell lines and iodine transport. It was found that apigenin could suppress the expression of Sodium-iodide symporter (NIS) mRNA, and this finding can have useful therapeutic consequences for the treatment of thyroid cancer.

Liver cancer and apigenin

Liver cancer, more precisely hepatocellular carcinoma, can be considered as the second main reason for cancer deaths and its prevalence is rising worldwide (71). This disease occurs more in men than women, and it is also more common in West and Middle Africa, South and East Asia, and Melanesia (72). The consumption of flavonoids can be effective in preventing Hepatocellular carcinoma (73). Flavonoids such as apigenin reduced the survival of hepatocellular carcinoma HePG2 cells and induced apoptosis by diminishing the expression of low-density lipoprotein receptor-related protein (LRP6) and S-phase kinase-associated protein-2 (SKP2). Further studies on apigenin against liver cancer cells indicated that this anticancer agent suppressed cell proliferation and increased cell death. In addition, apigenin caused autophagy and apoptosis by inhibiting the phosphatidylinositol-3-kinase (PI3K/Akt) and mechanistic target of rapamycin (mTOR) pathways. It has been indicated that apigenin therapy caused G1 arrest in HepG2 cells. Also, the cells that were exposed to apigenin experienced an increase in the amount of cyclin D1 and a decrease in cyclin 4, which indicates that the cell cycle can be stopped by regulating the expression of CD1 and CDK4 (74, 75). Yee et al (76), studied the inhibitory efficiency of two flavonoid compounds named apigenin and luteolin on Hepatocellular carcinoma HepG2 cells. The results showed that both of these flavonoids had an effective role in inhibiting cell growth, which was caused by diminishing the expression of CDK4 and cell cycle arrest by inducing P21 and p53, respectively.

Skin cancer and apigenin

Skin cancer is one of the most prevalent types of cancer in the united kingdom (UK) and the united states (US) (77). The two most common types of skin cancer are melanoma and non-melanoma skin cancer. Most skin cancers are related to non-melanoma and result from keratinized epithelial cells. These types of cancers can be divided into squamous cell carcinoma (SCC) and basal cell carcinoma (BCC). BCC is the most common form and progresses slowly. Melanoma accounts for approximately 2% of malignancies and causes the most mortality (77). It has been shown that ultraviolet B (UVB) radiation is the main cause of this disease. Various studies have indicated that apigenin can be remarkably effective in preventing skin carcinogenesis caused by ultraviolet A/B in SKH-1 mice (78). Caltagirone et al (79), investigated the combined impacts of apigenin and quercetin on suppressing the metastatic, invasiveness, and melanoma growth potential. They showed that the administration of quercetin and apigenin under in vivo conditions inhibited the metastatic potential of melanoma lung tumors in a BL6-BL6 murine model. This effect can be due to demolishing the interaction between malignant and endothelial cells.

Prostate cancer and apigenin

In addition to skin cancer, another common cancer in men is prostate cancer (53). This cancer is one of the multifactorial diseases. Prostate cancer is the second most common cancer and the fifth main cause of death in the world. The prevalence and mortality rate of prostate cancer is related to factors such as age, and the highest prevalence can be seen in older men. The most prevalent therapeutic modalities are surgery, radiotherapy, and/ or chemotherapy. It should be mentioned that these options are efficient in the early stages and become ineffective in the higher stages. This cancer can be reduced by increasing the consumption of fruits and vegetables as well as reducing the consumption of fatty foods. (80, 81). Flavonoids can be well tolerated by prostate cells, but it should be noted that these natural compounds act as mutagens, inhibitors of key regulatory enzymes, or pro-oxidant molecules in case of excessive consumption. Various types of polyphenols have been studied to kill prostate cancer cells(82, 83). In one of the studies, 22Rv1, PCa, and PC3 cells were exposed to different concentrations of apigenin (20 and 40μM) for 24 hours. The results indicated that the activity of histone deacetylation (HDAC) was reduced compared to that obtained from the famous HDAC inhibitor trichostatin A (TSA). Also, apigenin decreased the regulation of HDAC1 and HDAC3 at both protein and mRNA levels along with the simultaneous increase in H3 and H4 acetylation. As a result, this causes the DNA promoter to have more access to transcription factors and also, increases synthesis of cell cycle regulating protein p21/waf1 in prostate cancer cells. P21/waf1 can control cell cycle progression through cyclin-dependent kinase 2 (CDK2) inhibition (84). Prostate cancer cells showed induction of apoptotic pathways and cell cycle arrest 24 hours after apigenin administration. In one of the in-

vivo studies performed on PC3 xenografts in athymic nude mice, the antitumor effect of apigenin was investigated. Oral administration of apigenin (20 and 50 mg/mouse/d) during eight weeks caused a significant decrease in HDAC1 and HDAC3 protein expression, HDAC activity, and also a decrease in tumor growth. Mice were exposed to apigenin, the expression of P21/waf1 was higher than the control group, and the change in the amount of bax/bcl2 led to apoptosis induction (84). Knowles et al (85), evaluated the effectiveness of apigenin on prostate cancer PC3 cell proliferation, and it was demonstrated that when these cells are exposed to apigenin, their growth rate is delayed. Hessenauer et al (86), indicated the relationship between the growth of prostate cancer cells and casein kinase 2 (CK2) activity. They found that apigenin was able to suppress the activity of CK2 in both hormone-refractory PC3 and hormone-sensitive lymph node carcinoma of the prostate (LNCap), but only the latter underwent apoptosis. This result indicates that high activity of CK2 is not necessary for the proliferation and protection of PC3 cells against apoptosis. A summary of the effectiveness of apigenin on the mentioned cancers is indicated in Table 2.

Table 2. Summary of the several studies conducted on the effect of apigenin on the mentioned cancers.

Name	Year	Cancer	Result
Liu et al	2005	Lung	They have indicated that apigenin can diminish the risk of lung cancer by suppressing vascular endothelial growth factor transcription and the proliferation of A549 lung cancer
Wue et al	2005	Gastric	They found that apigenin inhibited the growth of SGC-7910 gastric carcinoma cells
Torkin et al	2005	Neuroblastoma	The result shows that apigenin can suppress growth, survival, and induce apoptosis in neuroblastoma cells
Elst et al	2004	Thyroid	They evaluated that apigenin plays an effective role in the treatment of thyroid cancer by inhibiting sodium- iodide symporter mRNA
Yee et al	2003	Liver	In this study, the results showed that apigenin and luteolin can inhibit the growth of Hepatocellular carcinoma HepG2cells by reducing the expression of CDK4 and stopping the cell cycle arrest by inducing P21 and p53, respectively
Caltagirone et al	2000	Skin	It has been observed that the combined effects of two flavonoid compounds, such as apigenin and quercetin inhibit the metastasis, invasiveness, and growth potential of melanoma
Knowles et al	2000	Prostate	They indicated that apigenin can have a beneficial effect on the proliferation of prostate cancer PC3 cells and also delay the growth of these cells

Conclusion

All the evidence obtained so far demonstrates that apigenin as a flavonoid compound has significant effectiveness against inflammation, oxidative stress, cancer, and various other diseases. Apigenin, due to its inherent low toxicity, non-mutagenicity, and its abundant presence in various vegetables, fruits, and herbal drinks, can be used as a preventive and reducing factor against various ailments, including inflammatory diseases and cancers. The useful functional mechanism of this compound, which play important roles in the prevention and treatment of different diseases, can be mentioned as induction of apoptosis, cell cycle arrest, stimulation of detoxification enzymes, neutralization of free radicals, and alterations in cellular signaling pathways. Based on the obtained results, apigenin has a significant effect in suppressing inflammation by reducing TNF-α, CCL-2, GMCSF, IL-1α, and IL-6 and increasing various anti-inflammatory pathways, including PI3K/Akt and P38/MAPK. It's worth mentioning that apigenin can reduce apoptosis by its effect on proteins such as BCL-2, BCL-XL, and BAX. Another beneficial effect of apigenin is on oxidative stress, which can inhibit oxidative stress by increasing enzymatic and non-enzymatic antioxidants and also by modulating different signaling pathways such as PI3/Akt, Nfr2, MAPK, and NF-κB. In recent years, a lot of progress has been made regarding the effect of apigenin in the treatment and prevention of various

types of disease such as cancer (in vivo- in vitro), but continuous efforts are still needed to confirm the impact of apigenin in human ailments by stimulating different diseases in animal models

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Conflict of Interests

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Author Contributions

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References

1. Hussain T, Tan B, Yin Y, Blachier F, Tossou MC, Rahu N. Oxidative stress and inflammation: what polyphenols can do for us? Oxidative medicine and cellular longevity. 2016;2016.
2. Khalil A, Tazeddinova D. The upshot of Polyphenolic compounds on immunity amid COVID-19 pandemic and other emerging communicable diseases: An appraisal. Natural Products and Bioprospecting. 2020;10(6):411-29.
3. Lee U-J, Sohng JK, Kim B-G, Choi K-Y. Recent trends in the modification of polyphenolic compounds using hydroxylation and glycosylation. Current Opinion in Biotechnology. 2023;80:102914.
4. Caponio GR, Noviello M, Calabrese FM, Gambacorta G, Giannelli G, De Angelis M. Effects of grape pomace polyphenols and in vitro gastrointestinal digestion on antimicrobial activity: Recovery of bioactive compounds. Antioxidants. 2022;11(3):567.
5. Lippolis T, Cofano M, Caponio GR, De Nunzio V, Notarnicola M. Bioaccessibility and Bioavailability of Diet Polyphenols and Their Modulation of Gut Microbiota. International Journal of Molecular Sciences. 2023;24(4):3813.
6. Rahman I, Biswas SK, Kirkham PA. Regulation of inflammation and redox signaling by dietary polyphenols. Biochemical pharmacology. 2006;72(11):1439-52.
7. Adnan M, Rasul A, Shah MA, Hussain G, Asrar M, Riaz A, et al. Radioprotective role of natural polyphenols: From sources to mechanisms. Anti-Cancer Agents in Medicinal Chemistry (Formerly Current Medicinal Chemistry-Anti-Cancer Agents). 2022;22(1):30-9.
8. Aravind SM, Wichienchot S, Tsao R, Ramakrishnan S, Chakkaravarthi S. Role of dietary polyphenols on gut microbiota, their metabolites and health benefits. Food Research International. 2021;142:110189.
9. Crozier A, Del Rio D, Clifford MN. Bioavailability of dietary flavonoids and phenolic compounds. Molecular aspects of medicine. 2010;31(6):446-67.
10. Banjarnahor SD, Artanti N. Antioxidant properties of flavonoids. Medical Journal of Indonesia. 2014;23(4):239-44.
11. Panche AN, Diwan AD, Chandra SR. Flavonoids: an overview. Journal of nutritional science. 2016;5:e47.
12. Ginwala R, Bhavsar R, Chigbu DGI, Jain P, Khan ZK. Potential role of flavonoids in treating chronic inflammatory diseases with a special focus on the anti-inflammatory activity of apigenin. Antioxidants. 2019;8(2):35.
13. Patel D, Shukla S, Gupta S. Apigenin and cancer chemoprevention: progress, potential and promise. International journal of oncology. 2007;30(1):233-45.
14. Kumar S, Pandey AK. Chemistry and biological activities of flavonoids: an overview. The scientific world journal. 2013;2013.
15. Rithidech KN, Tungjai M, Reungpatthanaphong P, Honikel L, Simon SR. Attenuation of oxidative damage and inflammatory responses by apigenin given to mice after irradiation. Mutation Research/Genetic Toxicology and Environmental Mutagenesis. 2012;749(1-2):29-38.
16. Salehi B, Venditti A, Sharifi-Rad M, Kręgiel D, Sharifi-Rad J, Durazzo A, et al. The therapeutic potential of apigenin. International journal of molecular sciences. 2019;20(6):1305.
17. Chen L, Zhao W. Apigenin protects against bleomycin-induced lung fibrosis in rats. Experimental and therapeutic medicine. 2016;11(1):230-4.
18. Pérez-Pérez A, Sánchez-Jiménez F, Vilariño-García T, Sánchez-Margalet V. Role of leptin in inflammation and vice versa. International journal of molecular sciences. 2020;21(16):5887.
19. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. nature. 2008;454(7203):436-44.
20. Bauer D, Redmon N, Mazzio E, Soliman KF. Apigenin inhibits TNFα/IL-1α-induced CCL2 release through IKBK-epsilon signaling in MDA-MB-231

human breast cancer cells. PloS one. 2017;12(4):e0175558.
21. Lee J-H, Zhou HY, Cho SY, Kim YS, Lee YS, Jeong CS. Anti-inflammatory mechanisms of apigenin: inhibition of cyclooxygenase-2 expression, adhesion of monocytes to human umbilical vein endothelial cells, and expression of cellular adhesion molecules. Archives of pharmacal research. 2007;30:1318-27.
22. Williams RJ, Spencer JP, Rice-Evans C. Flavonoids: antioxidants or signalling molecules? Free radical biology and medicine. 2004;36(7):838-49.
23. Lee SF, Lin JK. Inhibitory effects of phytopolyphenols on TPA-induced transformation, PKC activation, and c-jun expression in mouse fibroblast cells. 1997.
24. Lin JK, Chen YC, Huang YT, Lin-Shiau SY. Suppression of protein kinase C and nuclear oncogene expression as possible molecular mechanisms of cancer chemoprevention by apigenin and curcumin. Journal of cellular biochemistry. 1997;67(S28–29):39-48.
25. Mounho BJ, Thrall BD. The extracellular signal-regulated kinase pathway contributes to mitogenic and antiapoptotic effects of peroxisome proliferators in vitro. Toxicology and applied pharmacology. 1999;159(2):125-33.
26. Nicholas C, Batra S, Vargo MA, Voss OH, Gavrilin MA, Wewers MD, et al. Apigenin blocks lipopolysaccharide-induced lethality in vivo and proinflammatory cytokines expression by inactivating NF-κB through the suppression of p65 phosphorylation. The Journal of Immunology. 2007;179(10):7121-7.
27. Jones MC, Zha J, Humphries MJ. Connections between the cell cycle, cell adhesion and the cytoskeleton. Philosophical Transactions of the Royal Society B. 2019;374(1779):20180227.
28. Shukla S, Gupta S. Molecular targets for apigenin-induced cell cycle arrest and apoptosis in prostate cancer cell xenograft. Molecular cancer therapeutics. 2006;5(4):843-52.
29. Lee Y, Sung B, Kang YJ, Kim DH, Jang J-Y, Hwang SY, et al. Apigenin-induced apoptosis is enhanced by inhibition of autophagy formation in HCT116 human colon cancer cells. International journal of oncology. 2014;44(5):1599-606.
30. Takagaki N, Sowa Y, Oki T, Nakanishi R, Yogosawa S, Sakai T. Apigenin induces cell cycle arrest and p21/WAF1 expression in a p53-independent pathway. International journal of oncology. 2005;26(1):185-9.
31. Plaumann B, Fritsche M, Rimpler H, Brandner G, Hess RD. Flavonoids activate wild-type p53. Oncogene. 1996;13(8):1605-14.
32. Lepley DM, Pelling JC. Induction of p21/WAF1 and G1 cell-cycle arrest by the chemopreventive agent apigenin. Molecular Carcinogenesis: Published in cooperation with the University of Texas MD Anderson Cancer Center. 1997;19(2):74-82.
33. Singh P, Lim B. Targeting apoptosis in cancer. Current oncology reports. 2022;24(3):273-84.
34. Shukla S, Fu P, Gupta S. Apigenin induces apoptosis by targeting inhibitor of apoptosis proteins and Ku70–Bax interaction in prostate cancer. Apoptosis. 2014;19:883-94.
35. Wang W, Liu X, Zhang Z, Yin M, Chen X, Zhao S, et al. Apigenin Induced Apoptosis by Downregulating Sulfiredoxin Expression in Cutaneous Squamous Cell Carcinoma. Oxidative Medicine and Cellular Longevity. 2022;2022.
36. Imran M, Aslam Gondal T, Atif M, Shahbaz M, Batool Qaisarani T, Hanif Mughal M, et al. Apigenin as an anticancer agent. Phytotherapy Research. 2020;34(8):1812-28.
37. Shukla S, Gupta S. Molecular mechanisms for apigenin-induced cell-cycle arrest and apoptosis of hormone refractory human prostate carcinoma DU145 cells. Molecular Carcinogenesis: Published in cooperation with the University of Texas MD Anderson Cancer Center. 2004;39(2):114-26.
38. Seo H-S, Choi H-S, Kim S-R, Choi YK, Woo S-M, Shin I, et al. Apigenin induces apoptosis via extrinsic pathway, inducing p53 and inhibiting STAT3 and NFκB signaling in HER2-overexpressing breast cancer cells. Molecular and cellular biochemistry. 2012;366:319-34.
39. Pisoschi AM, Pop A, Iordache F, Stanca L, Predoi G, Serban AI. Oxidative stress mitigation by antioxidants-an overview on their chemistry and influences on health status. European Journal of Medicinal Chemistry. 2021;209:112891.
40. Yaribeygi H, Sathyapalan T, Atkin SL, Sahebkar A. Molecular mechanisms linking oxidative stress and diabetes mellitus. Oxidative medicine and cellular longevity. 2020;2020.
41. Kashyap P, Shikha D, Thakur M, Aneja A. Functionality of apigenin as a potent antioxidant with emphasis on bioavailability, metabolism, action mechanism and in vitro and in vivo studies: A review. Journal of food biochemistry. 2022;46(4):e13950.
42. Myhrstad MC, Carlsen H, Nordström O, Blomhoff R, Moskaug JØ. Flavonoids increase the intracellular glutathione level by transactivation of the γ-glutamylcysteine synthetase catalytical subunit promoter. Free Radical Biology and Medicine. 2002;32(5):386-93.
43. Rahimi A, Alimohammadi M, Faramarzi F, Alizadeh-Navaei R, Rafiei A. The effects of apigenin administration on the inhibition of inflammatory responses and oxidative stress in the lung injury

models: a systematic review and meta-analysis of preclinical evidence. Inflammopharmacology. 2022;30(4):1259-76.
44. Zhou Y, Yu Y, Lv H, Zhang H, Liang T, Zhou G, et al. Apigenin in cancer therapy: From mechanism of action to nano-therapeutic agent. Food and Chemical Toxicology. 2022:113385.
45. Lindenmeyer F, Li H, Menashi S, Soria C, Lu H. Apigenin acts on the tumor cell invasion process and regulates protease production. Nutrition and cancer. 2001;39(1):139-47.
46. Piantelli M, Rossi C, Iezzi M, La Sorda R, Iacobelli S, Alberti S, et al. Flavonoids inhibit melanoma lung metastasis by impairing tumor cells endothelium interactions. Journal of Cellular Physiology. 2006;207(1):23-9.
47. Osada M, Imaoka S, Funae Y. Apigenin suppresses the expression of VEGF, an important factor for angiogenesis, in endothelial cells via degradation of HIF-1α protein. FEBS letters. 2004;575(1-3):59-63.
48. Fang J, Xia C, Cao Z, Zheng JZ, Reed E, Jiang B-H. Apigenin inhibits VEGF and HIF-1 expression via PI3K/AKT/p70S6K1 and HDM2/p53 pathways. The FASEB Journal. 2005;19(3):342-53.
49. Rahmani AH, Alsahli MA, Almatroudi A, Almogbel MA, Khan AA, Anwar S, et al. The potential role of apigenin in cancer prevention and treatment. Molecules. 2022;27(18):6051.
50. Ahmed SA, Parama D, Daimari E, Girisa S, Banik K, Harsha C, et al. Rationalizing the therapeutic potential of apigenin against cancer. Life Sciences. 2021;267:118814.
51. Abbaszadeh H, Keikhaei B, Mottaghi S. A review of molecular mechanisms involved in anticancer and antiangiogenic effects of natural polyphenolic compounds. Phytotherapy Research. 2019;33(8):2002-14.
52. Knekt P, Järvinen R, Seppänen R, Heliövaara M, Teppo L, Pukkala E, et al. Dietary flavonoids and the risk of lung cancer and other malignant neoplasms. American journal of epidemiology. 1997;146(3):223-30.
53. Jemal A, Murray T, Ward E, Samuels A, Tiwari RC, Ghafoor A, et al. Cancer statistics, 2005. 2005.
54. Maris JM, Matthay KK. Molecular biology of neuroblastoma. Journal of clinical oncology. 1999;17(7):2264-.
55. Zhai K, Brockmüller A, Kubatka P, Shakibaei M, Büsselberg D. Curcumin's beneficial effects on neuroblastoma: Mechanisms, challenges, and potential solutions. Biomolecules. 2020;10(11):1469.
56. Draper G, Kroll M, Stiller C. Childhood cancer. Cancer surveys. 1994;19: 493-517.
57. Weinstein JL, Katzenstein HM, Cohn SL. Advances in the diagnosis and treatment of neuroblastoma. The oncologist. 2003;8(3):278-92.
58. Strother DR, London WB, Schmidt ML, Brodeur GM, Shimada H, Thorner P, et al. Outcome after surgery alone or with restricted use of chemotherapy for patients with low-risk neuroblastoma: results of Children's Oncology Group study P9641. Journal of clinical oncology. 2012;30(15):1842.
59. Alvarado CS, London WB, Look AT, Brodeur GM, Altmiller DH, Thorner PS, et al. Natural history and biology of stage A neuroblastoma: a Pediatric Oncology Group Study. Journal of pediatric hematology/oncology. 2000;22(3):197-205.
60. Schmidt ML, Lukens JN, Seeger RC, Brodeur GM, Shimada H, Gerbing RB, et al. Biologic factors determine prognosis in infants with stage IV neuroblastoma: a prospective Children's Cancer Group study. Journal of Clinical Oncology. 2000;18(6):1260-8.
61. Liskova A, Koklesova L, Samec M, Smejkal K, Samuel SM, Varghese E, et al. Flavonoids in cancer metastasis. Cancers. 2020;12(6):1498.
62. Torkin R, Lavoie J-F, Kaplan DR, Yeger H. Induction of caspase-dependent, p53-mediated apoptosis by apigenin in human neuroblastoma. Molecular cancer therapeutics. 2005;4(1):1-11.
63. Cabanillas ME, McFadden DG, Durante C. Thyroid cancer. The Lancet. 2016;388(10061):2783-95.
64. Brown RL, de Souza JA, Cohen EE. Thyroid cancer: burden of illness and management of disease. Journal of Cancer. 2011;2:193.
65. Gosain R, Alexander JS, Gill A, Perez C. Radioactive iodine-refractory differentiated thyroid cancer in the elderly. Current Oncology Reports. 2018;20:1-6.
66. Lakshmanan A, Doseff AI, Ringel MD, Saji M, Rousset B, Zhang X, et al. Apigenin in combination with Akt inhibition significantly enhances thyrotropin-stimulated radioiodide accumulation in thyroid cells. Thyroid. 2014;24(5):878-87.
67. Zhang L, Cheng X, Gao Y, Zheng J, Xu Q, Sun Y, et al. Apigenin induces autophagic cell death in human papillary thyroid carcinoma BCPAP cells. Food & function. 2015;6(11):3464-72.
68. Yin F, Giuliano A, Van Herle A. Signal pathways involved in apigenin inhibition of growth and induction of apoptosis of human anaplastic thyroid cancer cells (ARO). Anticancer Research. 1999;19(5B):4297-303.
69. Hirano T, Oka K, Akiba M. Antiproliferative effects of synthetic and naturally occurring flavonoids on tumor cells of the human breast carcinoma cell line,

ZR-75-1. Research communications in chemical pathology and pharmacology. 1989;64(1):69-78.
70. Schröder-van der Elst JP, van der Heide D, Romijn JA, Smit J. Differential effects of natural flavonoids on growth and iodide content in a human Na*/I-symporter-transfected follicular thyroid carcinoma cell line. European journal of endocrinology. 2004;150(4):557-64.
71. Llovet JM, Castet F, Heikenwalder M, Maini MK, Mazzaferro V, Pinato DJ, et al. Immunotherapies for hepatocellular carcinoma. Nature reviews Clinical oncology. 2022;19(3):151-72.
72. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. International journal of cancer. 2010;127(12):2893-917.
73. Eaton EA, Walle UK, Lewis AJ, Hudson T, Wilson AA, Walle T. Flavonoids, potent inhibitors of the human P-form phenolsulfotransferase. Potential role in drug metabolism and chemoprevention. Drug Metabolism and Disposition. 1996;24(2):232-7.
74. Yang J, Pi C, Wang G. Inhibition of PI3K/Akt/mTOR pathway by apigenin induces apoptosis and autophagy in hepatocellular carcinoma cells. Biomedicine & Pharmacotherapy. 2018;103:699-707.
75. Li Y, Cheng X, Chen C, Huijuan W, Zhao H, Liu W, et al. Apigenin, a flavonoid constituent derived from P. villosa, inhibits hepatocellular carcinoma cell growth by CyclinD1/CDK4 regulation via p38 MAPK-p21 signaling. Pathology-Research and Practice. 2020;216(1):152701.
76. Yee S-B, Lee J-H, Chung H-Y, Im K-S, Bae S-J, Choi J-S, et al. Inhibitory effects of luteolin isolated from Ixeris sonchifolia Hance on the proliferation of HepG2 human hepatocellular carcinoma cells. Archives of pharmacal research. 2003;26(2):151-6.
77. Linares MA, Zakaria A, Nizran P. Skin cancer. Primary care: Clinics in office practice. 2015;42(4):645-59.
78. Birt DF, Mitchell D, Gold B, Pour P, Pinch HC. Inhibition of ultraviolet light induced skin carcinogenesis in SKH-1 mice by apigenin, a plant flavonoid. Anticancer research. 1997;17(1A):85-91.
79. Caltagirone S, Rossi C, Poggi A, Ranelletti FO, Natali PG, Brunetti M, et al. Flavonoids apigenin and quercetin inhibit melanoma growth and metastatic potential. International journal of Cancer. 2000;87(4):595-600.
80. Rawla P. Epidemiology of prostate cancer. World journal of oncology. 2019;10(2):63.
81. Pandey KB, Rizvi SI. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative medicine and cellular longevity. 2009;2:270-8.
82. Caporali A, Davalli P, Astancolle S, D'Arca D, Brausi M, Bettuzzi S, et al. The chemopreventive action of catechins in the TRAMP mouse model of prostate carcinogenesis is accompanied by clusterin over-expression. Carcinogenesis. 2004;25(11):2217-24.
83. Skibola CF, Smith MT. Potential health impacts of excessive flavonoid intake. Free radical biology and medicine. 2000;29(3-4):375-83.
84. Pandey M, Kaur P, Shukla S, Abbas A, Fu P, Gupta S. Plant flavone apigenin inhibits HDAC and remodels chromatin to induce growth arrest and apoptosis in human prostate cancer cells: in vitro and in vivo study. Molecular carcinogenesis. 2012;51(12):952-62.
85. Knowles LM, Zigrossi DA, Tauber RA, Hightower C, Milner JA. Flavonoids suppress androgen-independent human prostate tumor proliferation. Nutrition and cancer. 2000;38(1):116-22.
86. Hessenauer A, Montenarh M, Götz C. Inhibition of CK2 activity provokes different responses in hormone-sensitive and hormone-refractory prostate cancer cells. International journal of oncology. 2003;22(6):1263-70.

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Case report Free Access

Papillary thyroid carcinoma arising from mature cystic teratoma ovary: a case report

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Abstract

Introduction: Mature cystic teratoma is a kind of ovarian germ cell tumour. Malignant transformation in it is uncommon with thyroid cancer being rarely found. Given its rarity and nonspecific symptoms, misdiagnosis and indifference when compared to other ovarian lesions is very common.

Case presentation: Herein we report a case of a 58-year-old post-menopausal female who presented with a history of abdominal distension and loss of appetite. She was found to have an abdominopelvic mass on examination and a raised CA125 levels for which she underwent an MRI pelvis which was suggestive of an O-RADS 5 lesion for which she underwent a staging laparotomy. The final histopathology and immunohistochemistry were suggestive of papillary thyroid carcinoma arising from a mature ovarian teratoma. After a multidisciplinary tumour board analysis, she was planned to be kept under follow–up with regular serum thyroglobulin monitoring. She has no signs of disease recurrence to date.

Discussion: Struma ovarii is one type of monodermal ovarian teratoma in which the tumour contains more than 50 % thyroid tissue. Diagnosis in such cases is difficult due to the lack of typical symptoms. In most of the cases, the diagnosis is incidental. Optimal treatment is still unclear given the rarity of the disease. In a few cases, thyroidectomy was done whereas in a few others it was omitted. Further therapy may include radioiodine treatment if needed..

Conclusion: To the best of our knowledge there is very scant information available on the natural history, prognosis and management of papillary thyroid carcinoma arising from mature cystic teratoma ovary. Hence, a multidisciplinary treatment approach may be needed for the same.

Keywords: Papillary thyroid carcinoma, Mature cystic teratoma, Germ cell tumour

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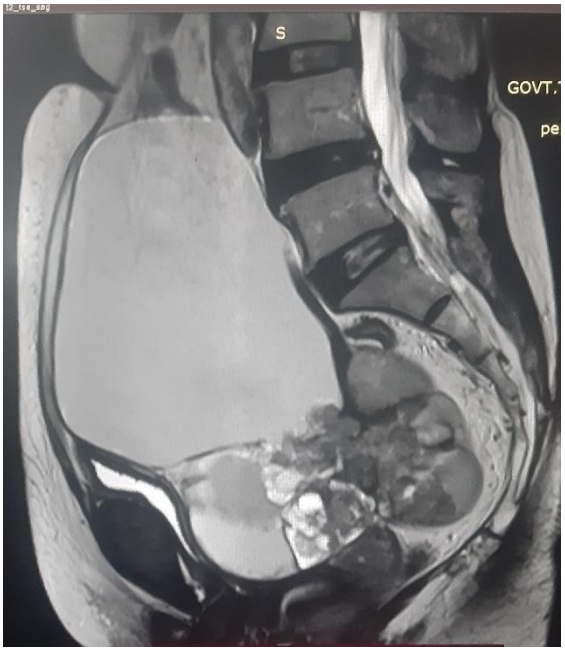
Introduction

Mature cystic teratomas comprise 20% of all ovarian neoplasms and are considered to be the most common type of germ cell tumors of the ovary (1). They can be either unilateral or bilateral and commonly appear in reproductive age, but have also been reported in postmenopausal women and children (2). Malignant transformation is uncommon, with an estimated risk of 0.17% to 2% (3). When malignant transformation occurs, in most cases (80%) it is squamous cell carcinoma as histology (4). Less common ones include sarcomas, adenocarcinomas, malignant melanomas, basal cell carcinomas, carcinoid tumors, and thyroid carcinomas (5). Struma ovarii is a rare ovarian lesion that is characterized by the presence of thyroid tissue in at least half of the overall ovarian mass. This mass comprises less than 1 % of ovarian tumors and also upto 2 to 5 % of all ovarian teratomas. The patients usually are asymptomatic with pelvic mass and pain being the common presenting symptoms, making it usually diagnosed post-operatively based on histopathology (6). A small proportion of struma ovarii may undergo malignant transformation, with papillary carcinoma the most common type of malignancy seen. The criteria used to identify a malignant change in struma ovarii are identical to those used to evaluate the thyroid gland (7). Only 5–8 % of these patients usually have clinical hyperthyroidism (8). Owing to the rarity of the tumor, there are no specific clinical, radiological, or serum markers that distinguish struma ovarii in the absence of thyroid hormone abnormalities. Thus, a definitive diagnosis is made by histopathological examination (7). Herein we present a case of papillary thyroid carcinoma arising within a mature cystic ovarian teratoma in a 58 year old post menopausal female.

Case presentation

A 58 year old post menopausal female presented with a two months history of abdominal distension and loss of appetite. On examination she had a palpable mass per abdomen which was of 18 week size felt more towards the left side . An ultrasonography of the abdomen was done which revealed a large abdominopelvic multilocular cystic lesion which was likely of pelvic origin. An MRI pelvis followed which revealed a large abdominopelvic cystic lesion of 12.8 x11.7x 15.2 cm with multiple internal septation (Ovarian-Adnexal Reporting and Data System - O-RADS 5) with mild ascites and no evidence of pelvic lymphadenopathy (Figure1).



Figire 1. MRI pelvis showed an abdominopelvic cystic lesion.

Her serum tumour markers showed a raised CA – 125(Cancer Antigen 125) level (157.8 U/L). Serum CEA(Carcinoembryonic Antigen) and CA19.9 (Cancer Antigen 19.9) were within the normal limits. She then underwent a total abdominal hysterectomy with bilateral salpingoophorectomy with omental biopsy. Intraoperatively she was found to have a large cystic lesion of around 15x 15 cm replacing the whole of the left ovary with smooth surface and no papillary excruscences. The right ovary was adherent to the mass and uterus was found to be atrophic . The omentum was found to have nodularity and a biopsy was taken from it and ascitic fluid was sent for cytology. The pathology findings showed an ovarian mass of size 19x13x 8 cm with the bosselated surface which was solid and cystic with multiple rents on the surface. The microscopic examination showed papillary carcinoma with no immature component (Figure 2).

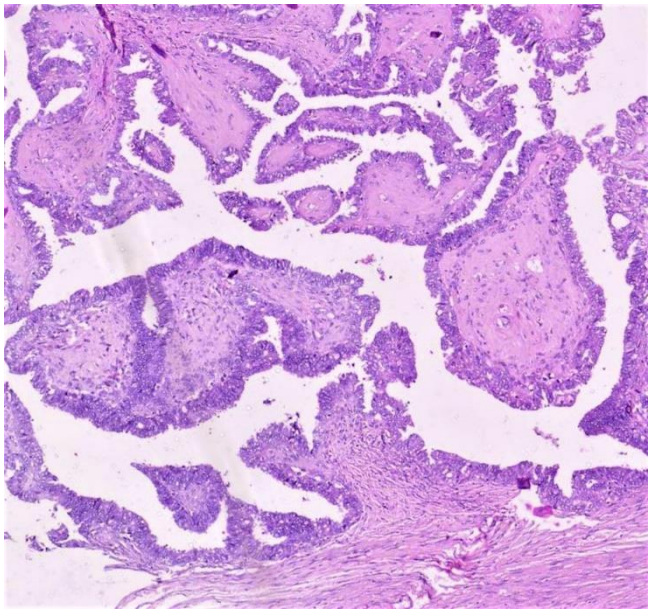


Figure 2. Histopathology image [40 X magnification] showing papillary thyroid carcinoma in a mature cystic ovarian teratoma.

Immunohistochemistry showed diffuse strong positivity for PAX8 (Paired Box 8), TTF 1(Transcription Termination Factor 1), thyroglobulin (Figure 3) and negativity for WT1 (Wilms' tumour gene 1) thus confirming the final diagnosis of papillary thyroid carcinoma in a mature cystic ovarian teratoma. The omental biopsy was suggestive of congestion only. Ascitic fluid cytology was doe which was found to be negative. Thyroid function tests were done which were found to be normal. Serum alpha-fetoprotein [AFP] was found to be 7.46 and beta HCG (human chorionic gonadotrophin) to be 5.55. Post-operative CA-125 was 13.9 U/L. An ultrasound neck was done which showed a small solid nodule measuring 2.7 x 1.7 mm in the midpole of right lobe with no calcification. A fine needle aspiration was done which was negative for malignancy. She was planned to be kept on follow–up with serum thyroglobulin check every 6 months. At present, she has completed about 6 months of follow-up with no signs of disease recurrence anywhere.

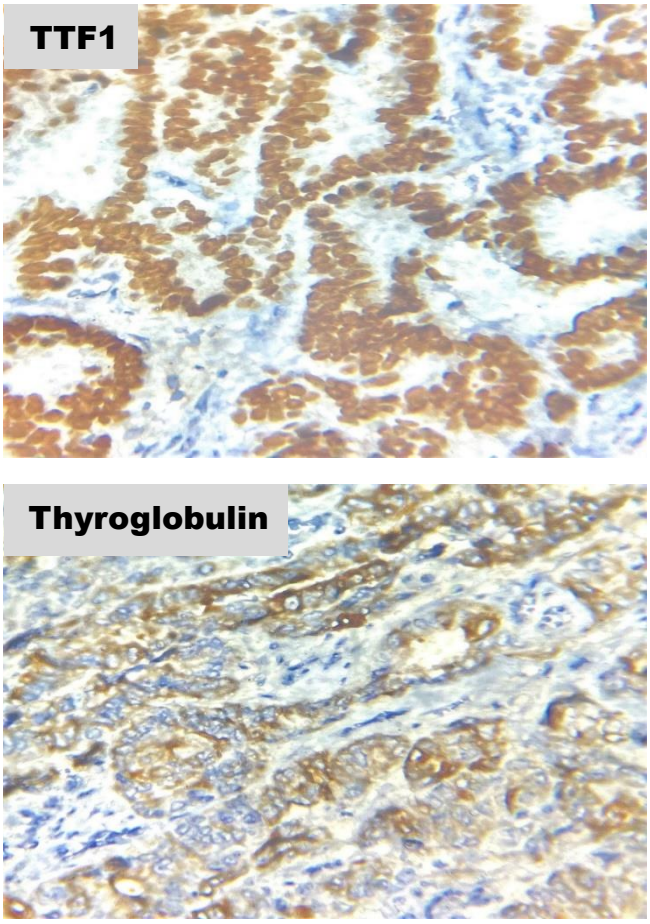


Figure 3. Immunohistochemistry images showing TTF-1 and thyroglobulin positivity.

Discussion

Struma ovarii is an ovarian germ cell tumour. It comprises of more than 50% thyroid tissue and can be differentiated from a mature teratoma, which contains only a small component (less than 50%) of benign thyroid tissue. Struma ovarii typically arises unilaterally, with 5% of cases seen bilaterally. A small proportion of struma ovarii may undergo malignant transformation (7). Malignant struma ovarii was first described by Wetteland in 1956 (9). Malignancy in struma ovarii is diagnosed based on histopathological criteria and guidelines for primary thyroid gland disease. Papillary and follicular carcinoma are the common histologies seen (10)0 Differentiated thyroid carcinoma arising from an MCT is rare with an estimated incidence being that of 0.1% to 0.2% (8). It is typically found incidentally in histopathology (5).

Multiple molecular abnormalities have been reported in thyroid cancer arising from ovarian teratomas, primarily in malignant struma ovarii. In thyroid

carcinomas arising within MCT without struma ovarii, no molecular markers have been reported. Molecular genetics may help to differentiate benign from malignant lesions. However, it is uncertain if they have a significant impact on cancer prognosis in this type (5).

Struma ovarii may mimic the clinical symptoms of ovarian malignancy, presenting with ascites, a complex ovarian cyst, and an elevation of CA-125 (7). A case of pseudo-Meigs syndrome which includes ascites in the setting of hydrothorax, and elevated CA 125 levels has been described in malignant struma ovarii. The associated symptoms disappear, and the elevated CA 125 levels return to normal postoperatively usually without adjuvant therapy (11). Metastasis of malignant struma ovarii is seen in approximately 5 to 23 % of cases and is mainly intra-abdominal, although blood-borne metastasis can occur in the liver, lung, brain, bone, vertebra, and the contralateral ovary (8). Follicular carcinoma is more likely to metastasize to the lung, liver, and central nervous system whereas papillary carcinoma is said to involve the abdominal cavity and lymph nodes and occasionally the liver (12).

Dane *et al* (13) reviewed 15 cases of differentiated thyroid carcinoma arising in a mature ovarian teratoma and since then, 4 additional cases have been reported. (14 -17) Most patients, as in our case, presented with abdominal pain, only 2 patients did not report any symptoms. Papillary thyroid carcinoma (PTC) was the most common histopathologic type (53%), followed by follicular variant of PTC (42%) and follicular carcinoma (5%). Only 2 cases presented with thyroid tumor size $\leqslant$1 cm (5).

Ryder *et al* (18) reported a 0.9-cm follicular variant PTC within a 4.6-cm mature cystic teratoma (MCT). Thyroid ultrasound and ^{131}I diagnostic whole body scan were normal. No further treatment was performed on this patient. Dias *et al* (17) reported 2 foci of follicular variant PTC (the largest of 3 mm) within a 4.5-cm mature ovarian teratoma. Thyroid ultrasound was also normal and no additional treatment was done.

The optimal treatment of thyroid carcinoma arising within MCT is unclear because of the rarity of the disease. Moreover, no data on recurrence are available. In some of the reported cases, thyroidectomy was performed (5). whereas in some others, no thyroidectomy was performed (19-21). In these cases, no primary thyroid carcinoma was clinically apparent in further follow-up.

Differentiated thyroid carcinomas seen in struma ovarii can rarely present as a locally invasive or metastatic disease (22). Ovarian metastases from a primary thyroid carcinoma may occasionally occur and in such cases, the ovarian mass does not present with teratomatous characteristics (23).

After surgical resection subsequent therapy depends on the extent of the primary lesion and disease stratification. There is no consensus on the optimal treatment of malignant struma ovarii. Treatment recommendations are based on either single case reports or case series. Further therapy may include total thyroidectomy and radioiodine ablation which needs thyroglobulin monitoring, as well as radioiodine treatment if needed (24).

Conclusion

To our best knowledge, there is very scant information on the natural history and prognosis of papillary thyroid carcinoma arising on a mature cystic ovarian teratoma. Currently, there is no management consensus on this entity. It is important to have a multidisciplinary approach in such cases with an individualized approach toward treatment. We believe that a long-term follow-up is needed to comment on the natural course and prognosis of this disease.

Author contribution

PJN, **NN**, and **VSH** contributed to the conception, design, and definition of intellectual content, literature search, data acquisition, data analysis, statistical analysis, manuscript preparation, editing and review.

Conflict of interest

The authors declare no conflict of interest.

References

1. Yassa L, Sadow P, Marqusee E. Malignant struma ovarii. Nat Clin Pract Endocrinol Metab 2008;4:469–72.

2. Hasleton PS, Kelehan P, Whittaker JS, et al. Benign and malignant struma ovarii. Arch Pathol Lab Med 1978;102:180–4.

3. Curling OM, Potsides PN, Hudson CN. Malignant change in benign cystic teratoma of the ovary. *Br J Obstet Gynaecol.* 1979;86:399–402

4. Sakuma M, Otsuki T, Yoshinaga K, et al. Malignant transformation arising from mature cystic teratoma of the ovary: a retrospective study of 20 cases. *Int J Gynecol Cancer.* 2010;20:766–771.

5. Pineyro, M. M., Pereda, J., Schou, P., de Los Santos, K., de la Peña, S., Caserta, B., & Pisabarro, R. (2017). Papillary Thyroid Microcarcinoma Arising Within a Mature Ovarian Teratoma: Case Report and Review of the Literature. *Clinical medicine insights. Endocrinology and diabetes*, *10*, 1179551417712521.

6. Zamani, F., Abdolrazaghnejad, A., Ameli, F., GHashghaee, S., Nassiri, S., & Zamani, N. (2022). Struma ovarii: A case report and review the literature. *International journal of surgery case reports*, *96*, 107318.

7. Rahma, A., Mardiyana, L., & Fauziah, D. (2022). Malignant struma ovarii: Case report of an unusual ovarian tumor with CT imaging. *Radiology case reports*, *17*(5), 1705–1708.

8. Makani K. Gaba. Struma ovarii with a focus of papillary thyroid. *Gynecol Oncol.* 2004;94(3):835–839. doi: 10.1016/j.ygyno.2004.06.003.

9. Wetteland. Malignant struma ovarii [in Norwegian]. Nord Med.56(43). 1956; 56(43): p. 1568–1570.

10. Kumar SS, Rema P, R AK, Varghese BT. Thyroid type papillary carcinoma arising in a mature teratoma. Indian J Surg Oncol. 2014 Sep;5(3):168-70.

11. Zannoni , Gallotta , Legge , Tarquini , Scambia , Ferrandina. Pseudo-Meigs' syndrome associated with malignant struma ovarii: a case report. Gynecol Oncol. 2004; 94(1).

12. Roth K. Highly differentiated follicular carcinoma arising. *Int J Gynecol Pathol.* 2008;27(2):213–222.

13. Dane C, Ekmez M, Karaca A, Ak A, Dane B. Follicular variant of papillary thyroid carcinoma arising from a dermoid cyst: a rare malignancy in young women and review of the literature. *Taiwan J Obstet Gynecol.* 2012;51:421–425.

14. Cymbaluk-Ploska A, Chudecka-Glaz A, Chosia M, Ashuryk O, Menkiszak J. Conservative treatment of a young patient with thyroid carcinoma in adult ovarian teratoma—case report. *Gynecol Endocrinol.* 2014;30:187–191

15. Uzum AK, Iyibozkurt C, Canbaz B, et al. Management and follow-up results of an incidental thyroid carcinoma in a young woman with ovarian teratoma. *Gynecol Endocrinol.* 2013;29:724–726.

16. Souaf I, El Fatemi H, Bennani A, et al. Papillary carcinoma derived from ovarian mature cystic teratoma: a new case report and literature review. *Case Reports Clin Med.* 2014;3:197–202.

17. Dias G, Diniz da Costa T, Pedro A, Silva Pereira J. A case of follicular variant of papillary thyroid carcinoma in a mature cystic teratoma in a young woman. *Acta Obs Ginecol Port.* 2015;9:302–304.

18. Ryder M, Nikiforov YE, Fagin JA. Follicular variant papillary thyroid carcinoma arising within an ovarian teratoma. *Thyroid.* 2007;17:179–180.

19. Doldi N, Taccagni GL, Bassan M, et al. Hashimoto's disease in a papillary carcinoma of the thyroid originating in a teratoma of the ovary (malignant struma ovarii) *Gynecol Endocrinol.* 1998;12:41–42.

20. Lee JM, Kim JW, Song JY, et al. Adenocarcinoma arising in mature cystic teratoma: a case report. *J Gynecol Oncol.* 2008;19:199–201

21. Lataifeh I, Abdel-Hadi M, Morcos B, Sughayer M, Barahmeh S. Papillary thyroid carcinoma arising from mature cystic teratoma of

the ovary. *J Obstet Gynaecol (Lahore)* 2010;30:884–886.

22. Zhu Y, Wang C, Zhang G-N, et al. Papillary thyroid cancer located in malignant struma ovarii with omentum metastasis: a case report and review of the literature. *World J Surg Oncol*. 2016;14:17

23. Brogioni S, Viacava P, Tomisti L, Martino E, Macchia E. A special case of bilateral ovarian metastases in a woman with papillary carcinoma of the thyroid. *Exp Clin Endocrinol Diabetes.* 2007;115:397–400

24. Goffredo P, Sawka AM, Pura J, Adam MA, Roman SA, Sosa JA. Malignant struma ovarii: a population-level analysis of a large series of 68 patients. *Thyroid*. 2015;25: 211–215.

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Investigating the relationship between anxiety and perceived stress with coping strategies adopted in pregnant women during the COVID-19 pandemic

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Abstract

Introduction: The COVID-19 pandemic has led to mental problems, including stress and anxiety, for people, especially pregnant women. Identifying strategies to deal with stress is important and can help pregnant mothers to adapt to stressful life factors such as the conditions of the COVID-19 pandemic. The present study was designed and implemented with the aim of investigating the relationship between anxiety and perceived- stress with the coping strategies of pregnant women referring to Al-Zahra Hospital in Rasht.

Methods: The current study was conducted on 221 pregnant women using a cross-sectional analysis method. The required information was collected by the self-report method through demographic questionnaires, Corona disease anxiety (CDAS), Cohen's perceived stress, and Endler and Parker's coping strategies questionnaire. Data were analyzed using SPSS version 22 software using Spearman's correlation coefficient and linear regression tests. The significance level of the tests was considered as $P < 0.05$.

Results: 53.4% of women had moderate anxiety and 60.6% of pregnant women had high levels of perceived stress. There was a direct and significant correlation between anxiety-perceived stress and emotion-focused strategy ($P<0.001$).

Conclusion: The present study showed high perceived stress and moderate anxiety in pregnant women during the COVID-19 pandemic and their relationship with emotion-focused coping strategies.

Keywords: Coping strategies, Anxiety, Perceived stress, Self-care, Coronavirus

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Introduction

COVID-19 is a new respiratory disease that is spreading rapidly worldwide the world and was declared a pandemic by the World Health Organization on March 11, 2020 (1). In addition to physical complications (2) and mortality, the COVID-19 pandemic also causes psychological disorders in members of society (3) and especially in pregnant women (4). The mental health of women, especially pregnant women, is crucial due to their role in the family. Studies have shown that during the COVID-19 pandemic, women are experiencing higher rates of anxiety, depression, and stress compared to men (5-7). Due to physiological and psychological changes during pregnancy, this period is one of the most sensitive stages of a woman's life. These changes in pregnant women lead to the induction of great changes, including physiological and psychological changes, which cause the emergence of psychopathological disorders, including stress and anxiety (8).

Mood and anxiety disorders are among the most common problems during pregnancy, which is why half of pregnant women experience pregnancy-specific anxiety (9). With the Prevalence of infectious diseases, such as the stressful conditions during the COVID-19 pandemic and the changes created due to the existing conditions, widespread anxiety disorders during pregnancy have intensified so that in pregnant women, symptoms of anxiety (57%) and depression (37%) compared to the period before Corona shows an increase (10, 11). Despite the prevalence of corona disease, fear and stress in pregnant women due to the fear of infection and transmission to the fetus have caused excessive and obvious anxiety with negative psychological effects in this vulnerable group (12). Due to physiological changes, these worries increase in the first and third trimester compared to the second trimester (13, 14). During the COVID-19 pandemic, pregnant women in the first trimester reported increased stress at work, increased stress from home, and greater feelings of anxiety than pregnant women in the second and third trimesters. In addition, pregnant women in the second trimester of pregnancy felt more helpless than pregnant women in the first and third trimesters of pregnancy (13). The stress hormone cortisol, along with the release of inflammatory markers like cytokines, can lead to negative consequences for both mother and fetus due to elevated levels of these chemicals (15).

The negative effects of maternal anxiety and stress during pregnancy lead to complications such as postpartum depression and mood disorders (16), preeclampsia, pregnancy-related nausea and vomiting, increased blood pressure, and increased number of unplanned cesarean section. Furthermore, due to the increase of glucocorticoids, their negative effects on the fetus include weight loss, increased fetal birth defects, infant mortality (17, 18), as well as fetal and neonatal complications such as premature delivery (19, 20), low birth weight, low Apgar score, neonatal abnormalities such as cleft palate, hospitalization, and developmental delay. These babies often have symptoms such as severe bloating and heart pain, insomnia at night, and constant crying (21-23). Although studies show that fear and anxiety caused by the illness can increase preventive behaviors in a person, fear and anxiety related to the disease are are directly related to psychological problems (9, 24). The World Health Organization announced in 2014 that mental disorders in women not only affect the individual, but also their children and other family members, and thus the society, as well as future generations in economic planning (25).

Coping is a person's first reaction to stressful events (26). Interestingly, some research suggests that coping can also moderate the effects of stress on mental health (27, 28). But many studies indicate the relationship of coping strategies with mental health consequences during the COVID-19 pandemic (9, 11, 23, 29, 30). Therefore, it is important to identify stress coping strategies and it can help pregnant mothers to adapt to the stressful factors of life, especially the existing conditions affecting the COVID-19 disease. There are three types of coping strategies: Problem-focused strategy, emotional-focused strategy, and avoidance coping strategy (31, 32). In Problem-focused strategy, the person tries to manage or modify the stressful situation, and this type of coping is useful when faced with a controllable stressor (33). People who use problem-based coping reduce their stress levels by gathering available information to deal with the stressor (18, 34). The more problem-focused coping strategies a person uses, the better their mental health

and the less anxiety and worry they display, and vice versa. Problem-focused coping strategies are associated with more coping, and emotion-focused coping strategies are associated with less coping (35, 36). Avoidant and emotion-focused coping strategies act as mediators through which experiences of COVID-19 is indirectly related to mental health during pregnancy (9, 23).

Prior to the COVID-19 pandemic, a study was conducted on a group of pregnant women which revealed that avoidant coping strategies such as refusal, non-involvement, and self-blame were associated with an increased risk of mental health issues. On the other hand, emotion-focused coping strategies were found to be less associated with mental health issues, while problem-focused coping strategies were not found to be related to mental wellbeing issues. In a recent study conducted on non-pregnant women prior to the outbreak, it was found that maladaptive coping strategies such as avoidance were associated with increased levels of stress and anxiety. During outbreaks, these maladaptive coping strategies were found to be associated with even higher levels of stress and anxiety (8, 9, 29). It seems that when faced with stressors that are beyond our control, utilizing emotion-focused coping strategies proves to be more effective. On the other hand, when dealing with situations that we have some level of control over, employing problem-focused coping strategies tends to yield better results (37). The mental health of pregnant women is a high-risk concern in society, especially during stressful conditions such as the coronavirus pandemic. Effective interventions can be taken to reduce stress by adopting coping strategies and eliminating inappropriate solutions. By understanding the coping strategies adopted by pregnant women in the face of perceived anxiety and stress, necessary interventions can be implemented to improve their mental health. Due to the scarcity of studies related to coping strategies during pregnancy, this study aims to investigate the relationship between perceived anxiety and stress and coping strategies adopted by pregnant women, highlighting the importance and necessity of this topic.

Methods

This cross-sectional analytical study was conducted after receiving the code of Guilan University of Medical Sciences from June to September 2022 and with a random sampling of 221 pregnant women referred to the educational-therapeutic center of Al-Zahra Hospital in Rasht. To be considered for the study, patients must have singleton pregnancies, have ultrasound confirmation at 8 weeks, basic literacy level or above, know the Persian language, consent to participate in the application process, and meet certain conditions such as substance abuse risk factors. Patients who have had physical illness, undergone medical consultations or had experienced significant stress in the last six months (such as a loved one's divorce or death), were not willing to cooperate with others, and completed the questionnaire unfinished..

Method of determining sample size

The sample size was obtained using the study of Basharpoor et al (38) and the study of Masjoudi et al (24) The initial sample size was obtained from the following formula, but the questionnaires were given to 256 pregnant women in this study.

$$N = \frac{\left(z_{1-\alpha} + z_{1-\beta}\right)^2}{\left(\frac{1}{2} Ln \frac{1+\rho}{1-\rho}\right)^2} + 3 = 98$$

$$\alpha = 0.05 \quad \beta = 0.05 \quad \rho = 0.25$$

Measures

1. **Demographic information questionnaire**: personal, social, midwifery profile questionnaire which is a questionnaire of 23 questions made by the researcher, 12 questions about age, education, occupation, level of education of spouse, occupation of a spouse, number of pregnancies, history of abortion, amount of income Household, residence status, covered by health insurance, current week of pregnancy and additionally, there are 11 questions addressing potential risk factors in the individual, including contact with a COVID-19 patient, smoking, and hookah usage, among others.

2. **COVID-19 Anxiety Scale (CDAS**): This questionnaire was prepared and validated to measure anxiety during the Corona era in Iran and has 18 items and 2 components (factors) regarding anxiety. Items 1 to 9 measure psychological symptoms and items 10 to 18 measure physical symptoms. The instrument is rated

on a 4-degree Likert scale (never = 0, sometimes = 1, most of the time = 2, and always = 3). Therefore, the highest and lowest scores that respondents get in this questionnaire are between 0 and 54. High scores indicate a high level of anxiety in individuals. The total CDAS score was divided into 0–16 (mild), 17–29 (moderate), and 30–54 (severe). The reliability of this tool was obtained using Cronbach's alpha method for the cause of psychological symptoms (0.879) and physical symptoms (0.861) of the total questionnaire (0.919) (39).

3. Cohen's Perceived Stress Scale (PSS): 14-item version was used in this research. This scale is a self-report tool consisting of 14 items that was developed by Cohen, Kamarck & and Mermelstein in 1983 in order to know how individuals evaluate their difficult and exhausting experiences. In this scale, individuals are asked to indicate on a five-point scale from 0 (never) to 4 (very much) how they often felt during the last 10 weeks. In this scale, after reverse scoring the items 4, 5, 6, 7, 9, 10, and 13, a total score is obtained by summing up the scores of all items for each person. On this scale, the minimum and maximum scores are 0 to 56. The higher the score, the higher the score. It means more perceived stress. In the study of Cohen et al. (1983), the internal consistency coefficients for each of the subscales and the overall score were between 0.84 and 0.86 (40). This questionnaire was developed in Iran by Safaei and Shokri. , with the translation and construct validity and convergent validity being confirmed. Furthermore, the reliability of the survey was assessed and found to be appropriate, with a value of 0.84 (41).

"4. "Endler" and "Parker" Coping Strategies Questionnaire: The Coping Strategies Questionnaire developed by Endler and Parker (1990) is comprised of 45 items that utilize the Likert method to determine responses ranging from never (1) to always (5). The questionnaire is divided into three main areas of coping behaviors, with each area containing 15 questions. These areas include problem-focused coping, emotion-focused coping, and avoidant coping. Problem-focused coping involves actively managing and solving the problem, while emotion-focused coping focuses on emotional responses to the problem, and avoidant coping involves running away from the problem. The scoring system for this questionnaire is based on a 5-point Likert scale, with a maximum score of 5 and a minimum score of 1 for each subject. The score for each of the three coping behaviors ranges from 15 to 75, with the behavior that receives the highest score being considered the person's primary coping strategy. The total score for the coping strategy ranges from 45 to 225 (42). Qureshi Rad et al. conducted the validation of this scale, yielding a correlation coefficient of 0.84 and Cronbach's alpha of 0.83 for the overall scale. Additionally, the subscales of problem-focused, emotion-focused, avoidance, and social orientation demonstrated correlation coefficients of 0.86, 0.81, 0.79, and 0.69, respectively. The coping strategy in this study was operationally defined as the total score obtained by individuals participating in the study, based on their responses to the Andler and Parker coping strategies questionnaire (43).

Data analysis

In this research, a total of 256 pregnant women were selected to participate by completing questionnaires. However, three individuals declined to continue their cooperation, resulting in a final sample size of 253 participants. Among the remaining participants, 23 reported having an underlying disease, and nine experienced significant stressful events within the past six months. These individuals were excluded from the study, leaving a final analysis sample of 221 pregnant women. For data analysis, the researchers utilized SPSS-22 software. Descriptive statistics methods were employed to analyze the data, including the use of frequency and percentage distribution tables for qualitative variables. Additionally, quantitative variables were analyzed using measures such as standard deviation, average, minimum, and maximum. To examine the relationship between variables, Spearman's correlation coefficient tests were conducted. Furthermore, to account for any confounding factors, the researchers employed the multivariable linear regression method. The significance level for all tests was set at 5%.

Results

Table 1 presents the demographic characteristics information of the participants. Based on the data provided, the average age of pregnant women was 30.96 years, with a standard deviation of 11.64. The age

range varied from 18 to 44 years. The gestational age ranged from 8 to 39 weeks. The number of pregnancies for women ranged from 1 to 5, and the average gestational age was 26.62 with a standard deviation of 8.87. A majority of the women (57.5%) held a diploma, while 86% were housewives. Additionally, 67.4% of the participants had an average household income between 2 and 5 million Tomans (Table 1).

Table 1. Participants' demographic and obstetrics characteristics (Frequency distribution of quantitative and qualitative variables).

variables	M±SD	Maximum-minimum
Age	30.96±11.64	18-14
Gravida	1.95±1.21	1-5
number of children	0.57±0.75	0-3
Number of abortions	0.35±0.75	0-5
Gestational age (weeks)	26.62±8.87	8-39
variables		**Frequency(%)**
Mother's Educational status		
Secondary school		33(14.9)
Diploma		127(57.5)
University		61(27.6)
Mother's Employment status		
Housewife		190(86)
Self-employed		12(5.4)
Employed		19(8.6)
Spouse's Educational status		
Secondary school		42(19)
Diploma		119(53.8)
University		60(27.2)
Spouse's Employment status		
Self-employed		147(66.5)
Worker		35(15.8)
Employed		30(13.6)
Farmer		9(4.1)
Income		
≥ 20000000 rail		36(16.3)
20000000-50000000 rail		149(67.4)
≥50000000 rail		36(16.3)

The mean (standard deviation) of the anxiety score and perceived stress score were (16.57±7.16) and (31.06±8.64), respectively. The mean (standard deviation) score of Problem-focused strategy, Emotional-focused strategy, and avoidant coping strategy were (49.95±9.32), (44.53±12.41) and (43.06±8.99) respectively. The minimum and maximum anxiety score was 5-44, and the minimum and maximum perceived stress score was 13-56. In addition, the minimum and maximum score of the total coping strategy was 59-192, the minimum and maximum score of the Problem-focused strategy was 21-70, the Emotional-focused strategy was 17-67, and the Avoidant coping strategy was 21-68 (Table 2).

Table 2. Mean and standard deviation of different dimensions of anxiety, perceived stress and adopted coping strategies.

Variable	kurtosis	Skewness	SD	mean	Min-max
Anxiety	1.222	1.009	7.16	16.57	5-44
Perceived Stress	-0.239	0.191	8.64	31.06	13-56
Coping strategy	0.111	-0.105	21.36	137.55	59-192
Problem-focused strategy	-0.375	-0.169	9.32	49.95	21-70
Emotional-focused strategy	-0.869	-0.142	12.41	44.53	17-67
Avoidant coping strategy	0.176	0.320	8.99	43.06	21-68

Initial findings additionally indicated that 118 individuals (60.6%) experienced mild anxiety, while 89 participants (40.3%) reported moderate anxiety, and 14 individuals (6.3%) suffered from severe anxiety as a result of the COVID-19 pandemic. Moreover, the assessment of perceived stress revealed that 134 pregnant women (60.6%) exhibited elevated levels of stress. In terms of coping strategies, 121 individuals (54.8%) employed problem-focused coping, 79 individuals (35.7%) utilized emotion-focused coping, and 21 individuals (9.5%) resorted to avoidance coping (Table 3).

Table 3. Frequency of anxiety, perceived stress and stress and adopted coping strategies.

Variable	Level	Frequency	%
Anxiety	mild	118	**53.4%**
	moderate	89	**40.3%**
	severe	14	**6/3%**
Perceived stress			
	low	87	**39/4%**
	high	134	**60/6%**
Coping strategy			
	Problem-focused strategies	121	**54/8%**
	emotional-focused strategies	79	**35/7%**
	Avoidance strategies	21	**9/5%**

The results show that there is a direct and significant linear correlation between anxiety and the adopted coping strategies (r=0.263); also the perceived stress and the adopted coping strategies (r=0.309) (P-value=0.001>) in Meanwhile, there is a direct and significant linear correlation between anxiety and emotion-focused coping strategy (r=0.413) and between perceived stress and emotion-focused coping strategy (r=0.408) (P-value=0.001). However, there is a direct linear correlation between anxiety with avoidance coping strategy (r=0.183) (P-value=0.006) and between perceived stress with avoidance coping strategy (r=0.169) (P-value=0.012). Also, there is no direct and significant linear correlation between anxiety with problem-focused strategies (r=-0.119) (P-value=0.078) and There is no direct and significant linear correlation between perceived stress and problem-focused strategies (r=-0.008) (P-value=0.906) (Table 4).

Table 4. Correlation between anxiety and perceived stress with adopted coping strategies.

Statistical tests	coping strategy	Problem-focused strategies	emotional-focused strategies	Avoidance strategies
Anxiety				
Spearman correlation coefficient	0.263	-0.119	0.413	0.183
P-value	<0001	0.078	<0.001	0.006
Perceived stress				
Spearman correlation coefficient	0.309	-0.008	0.408	0.169
P-value	<0.001	0.906	<0.001	0.012

The results of linear regression show that with the increase of each unit in the emotion- focused strategy score, the anxiety score increases by 0.4 or 40%, provided that other factors are constant. In the variable of anxiety, the squared multiple correlation coefficient (R2 variable coefficient) equal to 0.167 shows that the predicting variables of triple strategies predict 16.7% of the variance of anxiety scores of pregnant women. Also, the results of multiple linear regression show that with the increase of each unit in the emotion-focused strategy score, the perceived stress score increases by 0.39 or 39%, provided that other factors are constant. In the stress variable, the squared multiple correlation coefficient (R2 variable coefficient) equal to 0.147 shows that the predicting variables of the triple strategies predict 14.7% of the variance of the stress scores of pregnant women (Table 5).

Table 5. Results of linear regression analysis of anxiety and perceived stress based on coping strategies.

Collinearity assumption		P-value	t	Beta	B	SE	Sig	F	R2	Predictor variables	Criterion variable
VIF	Tolerance										

1.108	0.903	0.164	-1.397	-0.091	-0.070	0.050	۰/۰۰۱<	14.514	0.167	Problem-focused strategies	**Anxiety**
1.241	0.806	۰/۰۰< ۱	5.819	0.402	0.232	0.040				emotional-focused strategies	
1.341	0.745	0.840	0.202	-0.015	-0.012	0.057				Avoidance strategies	
1.108	0.903	0.752	0.317	0.021	0.019	0.061	۰/۰۰۱<	12.479	0.147	Problem-focused strategies	**Perceived stress**
1.241	0.806	۰/۰۰< ۱	5.663	0.393	0.274	0.049				emotional-focused strategies	
1.341	0.745	0.736	-0.338	-0.025	-0.024	0.070				Avoidance strategies	

Discussion

The present study was conducted to investigate the relationship between perceived anxiety and stress and the coping strategies adopted by pregnant women. The results of our study show that there is a direct and significant linear correlation between perceived anxiety and stress caused by COVID-19 and the coping strategies adopted. In addition, there is a direct and significant linear correlation between perceived anxiety and stress with the emotion-oriented strategy subscale (P-value=0.001). The mean (standard deviation) of the anxiety score (7.16) was 16.57 and the level of moderate to high anxiety in our study was 46.6%, while in the study of Alipour et al., which was conducted on the general population consisting of men and women, the average The anxiety score (11.05) is reported to be 17.74, which is almost consistent with our study (39). but, the average score of the total anxiety of COVID-19 in Masjoudi et al.'s study (10/45) is 18.20 and the level of moderate to high anxiety is 49.3% slightly higher than our study. But the level of perceived stress was high in our study (60.6 %), which is higher than Masjoudi et al.'s study (49.3%) (24).

It seems that with the passage of time and the increase of sufficient information about COVID-19 and vaccination, the level of anxiety caused by COVID-19 in pregnant women has decreased. Because one of the factors causing anxiety can be not having enough information about this disease, as was done in the study of Rah Nejat et al. Anxiety and stress were not having enough information in this field (44). However, the results of Kazemi et al.'s study showed that the more pregnant women know about COVID-19, the more worried and stressed they are. There was a positive correlation between the amount of knowledge of the studied pregnant mothers about the coronavirus disease, with the perceived stress and worry of the pregnant mothers about the coronavirus disease (r=0.126) and (r=0.141), respectively. Furthermore, Masjoudi's research revealed a significant association between the apprehension and unease caused by the COVID-19 pandemic and the level of perceived stress (r = 50; indicating a moderate effect; P < 0.001). Similarly, there was a noteworthy correlation between fear and anxiety related to COVID-19 and perceived stress (r = 0.48; indicating a moderate effect; P < 0.001). These findings highlight the meaningful impact

of fear and anxiety on individuals' stress levels during the pandemic (45).

Considering the role of perceived anxiety and stress on coping strategies and considering that during the COVID-19 pandemic, no study has been conducted on anxiety and stress on coping strategies using the desired tool. Discussion, from the studies conducted on pregnant women under stressful conditions, less related articles, and articles before the outbreak of corona disease were also used. For example, in Berhl et al.'s 2021 study in a non-pregnant sample, the use of maladaptive coping strategies was associated with increased stress and anxiety during the COVID-19 pandemic (46). Wheeler et al.'s study conducted both before and during the COVID-19 pandemic showed that greater use of avoidant coping was associated with higher levels of perceived stress (47). In The study of Sarani et al. in 2015, to examin the relationship between coping strategies in pregnancy and the level of perceived stress of pregnant mothers, which was conducted before COVID-19, between perceived stress and planned preparation strategy (r=.69) and spiritual strategy. There was a positive (r=.68) inverse and significant linear correlation, and also there was a direct and significant linear correlation between perceived stress and avoidance strategy with pregnancy stress (r=.75) (18). Therefore, considering that pregnancy itself creates stressful conditions for pregnant women and despite the double stressful conditions during the COVID-19 pandemic, the results of our study showed that there is a significant relationship between anxiety and perceived stress and coping strategies. (P-value=0.001) because in our study there was a direct and significant linear correlation between perceived stress and emotion-focused strategy. The results of our study show that there is a direct and significant linear correlation between anxiety and the adopted coping strategies and also between the perceived stress and the adopted coping strategies (P-value=0.001).

Emotion-focused coping was associated with decreased mental health due to the uncontrollable nature of the COVID-19 pandemic. Ineffective (avoidant) coping and emotion-focused coping were related to mental health problems, while problem-focused coping was not related to mental health problems (9, 48, 49). In this case, it can be said that the mentioned studies are consistent with our study because the findings of Khoury and others show that coping strategies are directly related to mental health outcomes, and ineffective coping and emotion-focused coping (maladaptive and emotion-focused coping strategies) between the experiences of COVID-19. and related mental health outcomes in pregnancy.

Conclusion

Coping strategies play a crucial role in maintaining the mental well-being of pregnant women, particularly when faced with stressful situations. Therefore, it is imperative to identify effective strategies that can help pregnant women adapt to the various stressors in their lives. The findings of the study revealed that a significant proportion (45.2%) of the coping strategies employed during the COVID-19 pandemic were emotion-oriented. However, these strategies were found to be ineffective as they were associated with higher levels of pregnancy anxiety and inverse (50). Training Basharpoor pregnant mothers to use an efficient and appropriate coping strategy with the stress created during pregnancy, especially in special and critical situations, including the critical period of COVID-19, can improve their mental health. Interventions are suggested to improve coping strategies in pregnant women.

Limitation

In the present study, sampling was done in an educational-therapeutic center, which does not include a wider range of women referring to health centers and private clinics.

Suggestion

Based on the current research, it is recommended that midwives and healthcare providers who work with pregnant women should assist in reducing anxiety levels by educating them on coping mechanisms that focus on problem-solving. By encouraging the use of effective coping strategies and minimizing the use of ineffective ones, it is possible to enhance the physical and mental well-being of expectant mothers and reduce the negative outcomes associated with anxiety and stress, such as prenatal and postpartum depression, as well as maternal and fetal complications.

Ethics approval and consent to participate

The study was approved by the ethics committee of Guilan University of Medical Sciences (IR.GUMS.REC.1401.115). The Helsinki Declaration was adhered to throughout all phases of this research. The participants, who met the necessary inclusion criteria, were provided with comprehensive explanations of all study procedures. Additionally, before their involvement in the study, all participants willingly completed a written informed consent form. They were given the freedom to make their own decisions regarding their participation and had the option to withdraw from the study at any point, for any reason, without any impact on their medical care.

Availability of data and materials

supporting data are available in the Reproductive Health Research Center, Department of Obstetrics & Gynecology, Al-Zahra Hospital, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

Competing interests

The authors declare that they have no competing interests.

Authors contributions

FSH, **SHSh** and **RF** contributed to the concept and design of the study. **FSH** collected the data. H.E performed the data analysis and **AN** contributed to the interpretation of the data. **FSH** drafted the manuscript and prepared the final version, while **SHSh** and **RF** read and revised the manuscript critically for important intellectual content. Finally, all authors approved the final version of the manuscript for publication.

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References

1. Wang C, Pan R, Wan X, Tan Y, Xu L, McIntyre RS, et al. A longitudinal study on the mental health of general population during the COVID-19 epidemic in China. Brain, behavior, and immunity. 2020;87:40-8.
2. Gualano MR, Lo Moro G, Voglino G, Bert F, Siliquini R. Effects of COVID-19 lockdown on mental health and sleep disturbances in Italy. International journal of environmental research and public health. 2020;17(13):4779.
3. Huang Y, Zhao N. Generalized anxiety disorder, depressive symptoms and sleep quality during COVID-19 outbreak in China: a web-based cross-sectional survey. Psychiatry research. 2020;288:112954.
4. Saccone G, Florio A, Aiello F, Venturella R, De Angelis MC, Locci M, et al. Psychological impact of coronavirus disease 2019 in pregnant women. American Journal of Obstetrics & Gynecology. 2020;223(2):293-5.
5. Salari N, Hosseinian-Far A, Jalali R, Vaisi-Raygani A, Rasoulpoor S, Mohammadi M, et al. Prevalence of stress, anxiety, depression among the general population during the COVID-19 pandemic: a systematic review and meta-analysis. Globalization and health. 2020;16(1):1-11.
6. Wang Y, Di Y, Ye J, Wei W. Study on the public psychological states and its related factors during the outbreak of coronavirus disease 2019 (COVID-19) in some regions of China. Psychology, health & medicine. 2021;26(1):13-22.
7. Liu D, Ren Y, Yan F, Li Y, Xu X, Yu X, et al. Psychological impact and predisposing factors of the coronavirus disease 2019 (COVID-19) pandemic on general public in China. 2020.
8. Dolatian M, Mirabzadeh A, Forouzan AS, Sajjadi H, Majd HA, Moafi F, et al. Correlation between self-esteem and perceived stress in pregnancy and ways to coping with stress. Pajoohandeh Journal. 2013;18(3):148-55.
9. Khoury JE, Atkinson L, Bennett T, Jack SM, Gonzalez A. Coping strategies mediate the associations between COVID-19 experiences and mental health outcomes in pregnancy. Archives of Women's Mental Health. 2021:1-11.
10. Komeil MS, Mirghafourvand M, Pourmehr HS, Shamsaeii F, Malakouti J. Maternal anxiety and its relationship with the coping strategies in iranian pregnant women. SN Comprehensive Clinical Medicine. 2021;3(5):1088-95.

11. Thurkkada AP, Joseph NE, Manoj G, Ravindran GC. Prenatal Anxiety, Perceived Stress, and Coping Behaviour regarding COVID-19 among Pregnant Women at a selected Hospital, Kochi, South India. Africa Journal of Nursing and Midwifery. 2022;24(2):1-10.
12. Akgor U, Fadıloglu E, Soyak B, Unal C, Cagan M, Temiz BE, et al. Anxiety, depression and concerns of pregnant women during the COVID-19 pandemic. Archives of gynecology and obstetrics. 2021;304(1):125-30.
13. Zhang Y, Ma ZF. Psychological responses and lifestyle changes among pregnant women with respect to the early stages of COVID-19 pandemic. International Journal of Social Psychiatry. 2021;67(4):344-50.
14. Qiao J. What are the risks of COVID-19 infection in pregnant women? The Lancet. 2020;395(10226):760-2.
15. Coussons-Read ME, Okun ML, Nettles CD. Psychosocial stress increases inflammatory markers and alters cytokine production across pregnancy. Brain, behavior, and immunity. 2007;21(3):343-50.
16. BAEZZAT F, AHMADI GHOZLOJEG A, MARZBANI Y, KARIMI A, AZARNIOSHAN B. A STUDY OF PSYCHOMETRIC PROPERTIES OF PERSIAN VERSION OF ATTITUDES TOWARD FERTILITY AND CHILDBEARING SCALE. JOURNAL OF URMIA NURSING AND MIDWIFERY FACULTY. 2017;15(1 (90)):-.
17. Luo Z, Shen Y, Yuan J, Zhao Y, Liu Z, Shangguan F. Perceived Stress, Resilience, and Anxiety Among Pregnant Chinese Women During the COVID-19 Pandemic: Latent Profile Analysis and Mediation Analysis. Frontiers in Psychology. 2021;12:2851.
18. Sarani A, Azhari S, Mazlom SR, Aghamohammadian Sherbaf H. The relationship between psychological hardiness and coping strategies during pregnancy. Journal of midwifery and reproductive health. 2015;3(3):408-17.
19. Lebel C, MacKinnon A, Bagshawe M, Tomfohr-Madsen L, Giesbrecht G. Elevated depression and anxiety symptoms among pregnant individuals during the COVID-19 pandemic. Journal of affective disorders. 2020;277:5-13.
20. Crespi EJ, Denver RJ. Ancient origins of human developmental plasticity. American Journal of Human Biology. 2005;17(1):44-54.
21. Liu X, Chen M, Wang Y, Sun L, Zhang J, Shi Y, et al. Prenatal anxiety and obstetric decisions among pregnant women in Wuhan and Chongqing during the COVID-19 outbreak: a cross-sectional study. BJOG: An International Journal of Obstetrics & Gynaecology. 2020;127(10):1229-40.
22. Shangguan F, Wang R, Quan X, Zhou C, Zhang C, Qian W, et al. Association of Stress-Related Factors With Anxiety Among Chinese Pregnant Participants in an Online Crisis Intervention During COVID-19 Epidemic. Frontiers in Psychology. 2021;12:1171.
23. Werchan DM, Hendrix CL, Ablow JC, Amstadter AB, Austin AC, Babineau V, et al. Behavioral coping phenotypes and associated psychosocial outcomes of pregnant and postpartum women during the COVID-19 pandemic. Scientific reports. 2022;12(1):1-12.
24. Masjoudi M, Aslani A, Seifi M, Khazaeian S, Fathnezhad-Kazemi A. Association between perceived stress, fear and anxiety of COVID 19 with self-care in pregnant women: a cross-sectional study. Psychology, Health & Medicine. 2021:1-12.
25. WHO CO. World health organization. Responding to Community Spread of COVID-19 Reference WHO/COVID-19/Community_Transmission/20201. 2020.
26. Folkman S, Lazarus RS. An analysis of coping in a middle-aged community sample. Journal of health and social behavior. 1980:219-39.
27. Chen T, Laplante D, Elgbeili G, Brunet A, Simcock G, Kildea S, et al. Coping during pregnancy following exposure to a natural disaster: The QF2011 Queensland Flood Study. Journal of affective disorders. 2020;273:341-9.
28. Lau Y, Wang Y, Kwong DHK, Wang Y. Testing direct and moderating effects of coping styles on the relationship between perceived stress and antenatal anxiety symptoms. Journal of Psychosomatic Obstetrics & Gynecology. 2015;36(1):29-35.
29. Firouzbakht M, Rahmani N, Sharif Nia H, Omidvar S. Coping strategies and depression during the COVID-19 pandemic in pregnant women: a cross sectional study. BMC psychiatry. 2022;22(1):1-8.

30. Abedzadeh-Kalahroudi M, Karimian Z, Nasiri S, Sadat Khorshidifard M. Anxiety and perceived stress of pregnant women towards COVID-19 disease and its related factors in Kashan (2020). Obstetrics, Gynecology and Infertility (Iranian Journal) 2021;24(5):8-18.
31. Lazarus RS, Folkman S. Stress, appraisal, and coping: Springer publishing company; 1984.
32. Sadock BJ, Sadock VA, Ruiz P. Kaplan and Sadock's Comprehensive Textbook of Psychiatry: Wolters Kluwer Health; 2017.
33. Coon D, Mitterer JO. Introduction to psychology: Gateways to mind and behavior with concept maps and reviews: Cengage Learning; 2012.
34. van Berkel HK. The relationship between personality, coping styles and stress, anxiety and depression. 2009.
35. Clark LA, Cuthbert B, Lewis-Fernández R, Narrow WE, Reed GM. Three approaches to understanding and classifying mental disorder: ICD-11, DSM-5, and the National Institute of Mental Health's Research Domain Criteria (RDoC). Psychological Science in the Public Interest. 2017;18(2):72-145.
36. Agha-yousefi A, Choubsaz F, Shaghaghi F, Motiei G. The effect of coping techniques training on coping strategies of infertile women in Kermanshah. Journal of Kermanshah University of Medical Sciences. 2012;16(2).
37. Folkman S, Moskowitz JT. Coping: Pitfalls and promise. Annu Rev Psychol. 2004;55:745-74.
38. Basharpoor S, Heydarirad H, Daryadel SJ, Heydari F, Ghamari Givi H, Kishore J. The role of perceived stress and social support among predicting anxiety in pregnant women. Journal of Holistic Nursing And Midwifery. 2017;27(2):9-16.
39. Alipour A, Ghadami A, Alipour Z, Abdollahzadeh H. Preliminary validation of the Corona Disease Anxiety Scale (CDAS) in the Iranian sample. 2020.
40. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. Journal of health and social behavior. 1983:385-96.
41. Safaei M, Shokri O. Assessing stress in cancer patients: Factorial validity of the perceived stress scale in Iran. 2014.
42. Parker JD, Endler NS. Coping with coping assessment: A critical review. European Journal of personality. 1992;6(5):321-44.
43. Ghoreyshi Rad F. Validation of Endler & Parker coping scale of stressful situations. International Journal of Behavioral Sciences. 2010;4(1):1-7.
44. Shahed Haghghadam H, | Fathi Ashtiani A, | Rahenejat AM. Psychological Consequences and Interventions during the COVID-19 Pandemic: Narrative Review. 2020;2(1):1-11.
45. Kazemi Aski S, Alizadeh S, Ghafourian Abadi S, Yaseri Gilvaei F, Kiai SM. Awareness of Coronavirus Disease and Perceived Stress in Pregnant Women. Journal of Obstetrics, Gynecology and Cancer Research (JOGCR). 2022;7(3):237-44.
46. Brehl A-K, Schene A, Kohn N, Fernández G. Maladaptive emotion regulation strategies in a vulnerable population predict increased anxiety during the COVID-19 pandemic: A pseudo-prospective study. Journal of Affective Disorders Reports. 2021;4:100113.
47. Wheeler JM, Misra DP, Giurgescu C. Stress and coping among pregnant black women during the COVID-19 pandemic. Public Health Nursing. 2021.
48. Guardino CM, Dunkel Schetter C. Coping during pregnancy: a systematic review and recommendations. Health psychology review. 2014;8(1):70-94.
49. Lau Y, Wang Y, Kwong D. Are different coping styles mitigating perceived stress associated with depressive symptoms among pregnant women? Perspectives in Psychiatric Care. 2015;52(2):102-12.
50. Lazarus RS. Stress and emotion: A new synthesis: Springer publishing company; 2006.

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A practical general review of lung cancer

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Abstract

Lung cancer, also known as lung carcinoma, is a malignant tumor that begins in the lung. Lung cancer is caused by genetic damage to the DNA of cells in the airways and is often caused by cigarette smoking or inhalation of harmful chemicals. Damaged airway cells gain the ability to multiply unchecked, causing tumor growth. Without treatment, tumors spread throughout the lungs, damaging lung function. Eventually, the lung tumors metastasize and spread to other body parts. On the other hand, lung cancer or bronchogenic carcinoma refers to tumors originating in the lung parenchyma or within the bronchi. It ranks among the primary causes of cancer-related mortality globally. It is estimated that there is an increasing rate of new cases of lung cancer worldwide annually, with an approximately high mortality rate because of lung cancer. It is worth mentioning that lung cancer was a relatively uncommon condition at the beginning of the 20th century. Its dramatic rise in later decades is primarily attributable to the increase in smoking among both males and females. Treatments include surgery, chemotherapy, immunotherapy, radiation, and targeted drugs. This review article describes lung cancer's causes, pathophysiology, and presentation.

Keywords: Lung cancer, Etiology, Diagnosis, Treatment

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Introduction

Lung cancer, also known as bronchogenic carcinoma, denotes the development of tumors within the lung parenchyma or bronchi. It stands as a prominent contributor to cancer-related mortality in the United States. Since 1987, lung cancer has surpassed breast cancer as the leading cause of death among women. Annually, an estimated 225,000 new cases of lung cancer are diagnosed in the United States, resulting in approximately 160,000 fatalities. Notably, lung cancer was a relatively uncommon ailment at the onset of the 20th century, with its substantial escalation in subsequent decades largely attributed to the heightened prevalence of smoking among both genders **(Figure 1)** (1, 2).

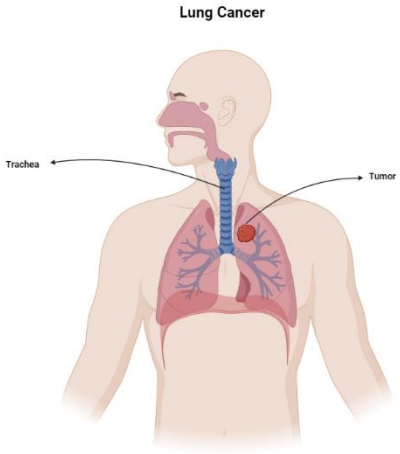

Figure 1. A schematic picture of the location of lung cancer.

Etiology

The predominant factor contributing to the development of lung cancer is smoking. It is approximated that smoking accounts for 90% of lung cancer cases (3). The highest risk of developing lung cancer is observed in male individuals who engage in smoking. This risk is further exacerbated by exposure to additional carcinogens, such as asbestos. The relationship between the incidence of lung cancer and the quantity of cigarette packs smoked annually is not directly correlated, owing to the intricate interaction between smoking habits and various environmental and genetic influences. Additionally, the risk of developing lung cancer as a result of passive smoking is augmented by 20 to 30% (3). Additional factors to consider are the use of radiation therapy for the treatment of cancers other than lung cancer, particularly non-Hodgkin's lymphoma and breast cancer (4). Exposure to certain metals, including chromium, nickel, arsenic, and polycyclic aromatic hydrocarbons, has been linked to an increased risk of lung cancer. Additionally, lung diseases such as idiopathic pulmonary fibrosis can independently raise the risk of lung cancer, regardless of smoking habits. Asbestos and radon are well-established risk factors for lung cancer (5). The risk of lung cancer associated with asbestos exposure, particularly in occupational settings, increases proportionally with the dose and varies based on the type of asbestos fiber. The risk from nonoccupational asbestos exposure is less clearly defined. However, the United States Environmental Protection Agency (EPA) has established standards for acceptable low-level nonoccupational asbestos exposure. The EPA states that if asbestos is undisturbed and does not release respirable particles, the health risk to occupants of a building is not significant (6). Radon exposure in uranium miners was associated with a small but significant risk of lung cancer (7). Radon has been demonstrated to build up in residential environments as a byproduct of the decay of uranium and radium. A comprehensive analysis of studies conducted in Europe revealed significant risks associated with residential radon exposure, particularly for individuals who smoke. This exposure was found to be accountable for approximately 2% of all lung cancer-related deaths in Europe (8).

Epidemiology

Lung cancer is the most frequently identified form of cancer on a global scale, constituting around 12.4% of all cancer diagnoses worldwide, and stands as the primary contributor to cancer-related mortality (9). The American Cancer Society projects that there will be more than 234,000 new cases of lung cancer and over 154,000 deaths associated with lung cancer in the United States annually (9). Based on the 2020 Global Cancer Statistics report, it was found that lung cancer continued to be the primary contributor to global cancer-related mortality, resulting in approximately 1.8 million deaths (10). In the past, the prevalence of lung cancer appeared to primarily affect developed nations. However, recent evidence indicates a significant increase in lung cancer incidence, with nearly half of

new cases, 49.9%, being diagnosed in underdeveloped regions(11). In the United States, there is a higher mortality rate among men compared to women. While there is no racial disparity in the occurrence of lung cancer overall, the age-adjusted mortality rate is elevated in African-American males in comparison to Caucasian males. This distinction is not observed among women (3).

Pathophysiology

The pathophysiology of lung cancer is a multifaceted and not fully elucidated process. It is postulated that recurrent exposure to carcinogens, particularly from cigarette smoke, results in the development of dysplasia in the lung epithelium. Prolonged exposure further leads to genetic mutations and disrupts protein synthesis (12). This consequently interrupts the process of cell division and encourages the formation of cancer. The prevalent genetic alterations associated with the onset of lung cancer include MYC, BCL2, and p53 for small cell lung cancer (SCLC), and EGFR, KRAS, and p16 for non-small cell lung cancer (NSCLC) (13, 14). The histopathological categorization of lung cancers is crucial for their diagnosis and management, and is based on cellular and molecular subtypes. The 2021 World Health Organization (WHO) classification system divides lung cancers into various categories, including precursor glandular lesions, adenocarcinomas, adenosquamous carcinomas, squamous precursor lesions, squamous cell carcinomas, large cell carcinomas, sarcomatoid

carcinomas, lung neuroendocrine neoplasms, salivary gland-type tumors, neuroendocrine tumors, neuroendocrine carcinomas, and other epithelial tumors. The WHO emphasizes the identification of histologic features, measurement of invasion depth, and mode of spread for prognostic purposes. For instance, the presence of tumor spread through air spaces is associated with a higher recurrence rate after limited resections and should be documented in pathological evaluations. Additionally, the WHO has discontinued the clear cell, rhabdoid, and signet ring subtypes in the most recent classification, as they are considered to be cytologic features that can occur in any adenocarcinoma. The WHO classification system places significant emphasis on the use of immunohistochemical staining to classify cancers that may not exhibit typical cytologic features under light microscopy **(Figure 2).** In the 2015 classification system established by the World Health Organization (WHO), poorly differentiated carcinomas underwent reclassification based on specific biomarker expressions. Those exhibiting p40 expression were reclassified as squamous cell carcinomas, while those demonstrating thyroid transcription factor 1 expression were categorized as adenocarcinomas with solid subtype. Additionally, carcinomas showing positivity for chromogranin and synaptophysin were reclassified as neuroendocrine carcinomas.

Precursor Glandular Lesions

These lesions encompass atypical adenomatous hyperplasia (AAH) and adenocarcinoma in situ. AAH serves as a precursor to lung adenocarcinoma and typically presents as a lesion measuring $\leq$ 5 mm. Adenocarcinoma in situ can manifest as either mucinous or nonmucinous and is generally a localized lesion of 3 cm or less. It exhibits a "lepidic" growth pattern, characterized by growth confined along the alveolar structures. This type of lesion is non-invasive and demonstrates intact alveolar septa.

Adenocarcinoma

The pathology of adenocarcinoma involves the development of neoplastic gland formation and the expression of pneumocyte markers such as thyroid transcription factor 1 (TTF-1) with or without napsin expression, or intracytoplasmic mucin. It is further categorized based on the extent and structure of neoplastic gland formation as either mucinous or non-mucinous. The non-mucinous subtypes include acinar, papillary, micropapillary, lepidic, and solid subtypes. Accurate pathological identification of these subtypes is crucial for determining prognosis. Specifically, the solid, micropapillary, and cribriform (a subtype of acinar non-mucinous adenocarcinoma) patterns are associated with unfavorable prognostic implications (15). Mucinous adenocarcinomas may exhibit various architectural patterns such as papillary, micropapillary, solid, and cribriform. However, the World Health Organization (WHO) does not provide grading recommendations for mucinous carcinomas based on these growth patterns. Other less common forms of adenocarcinoma include colloid, enteric-like,

lymphoepithelial, and fetal forms. Minimally invasive adenocarcinoma (MIA) is characterized by a small, solitary adenocarcinoma measuring ≤ 3 cm with minimal invasion (less than 5 mm) and a predominant lepidic growth pattern, resembling similar precursor glandular lesions. If the invasion exceeds 5 mm, it is classified as lepidic-predominant adenocarcinoma. Invasive mucinous adenocarcinoma, previously known as mucinous bronchioloalveolar carcinoma, encompasses mucinous lesions that do not meet the criteria for MIA. Lesions with more than 10% of mucinous and non-mucinous growth patterns should be classified as mixed adenocarcinoma.

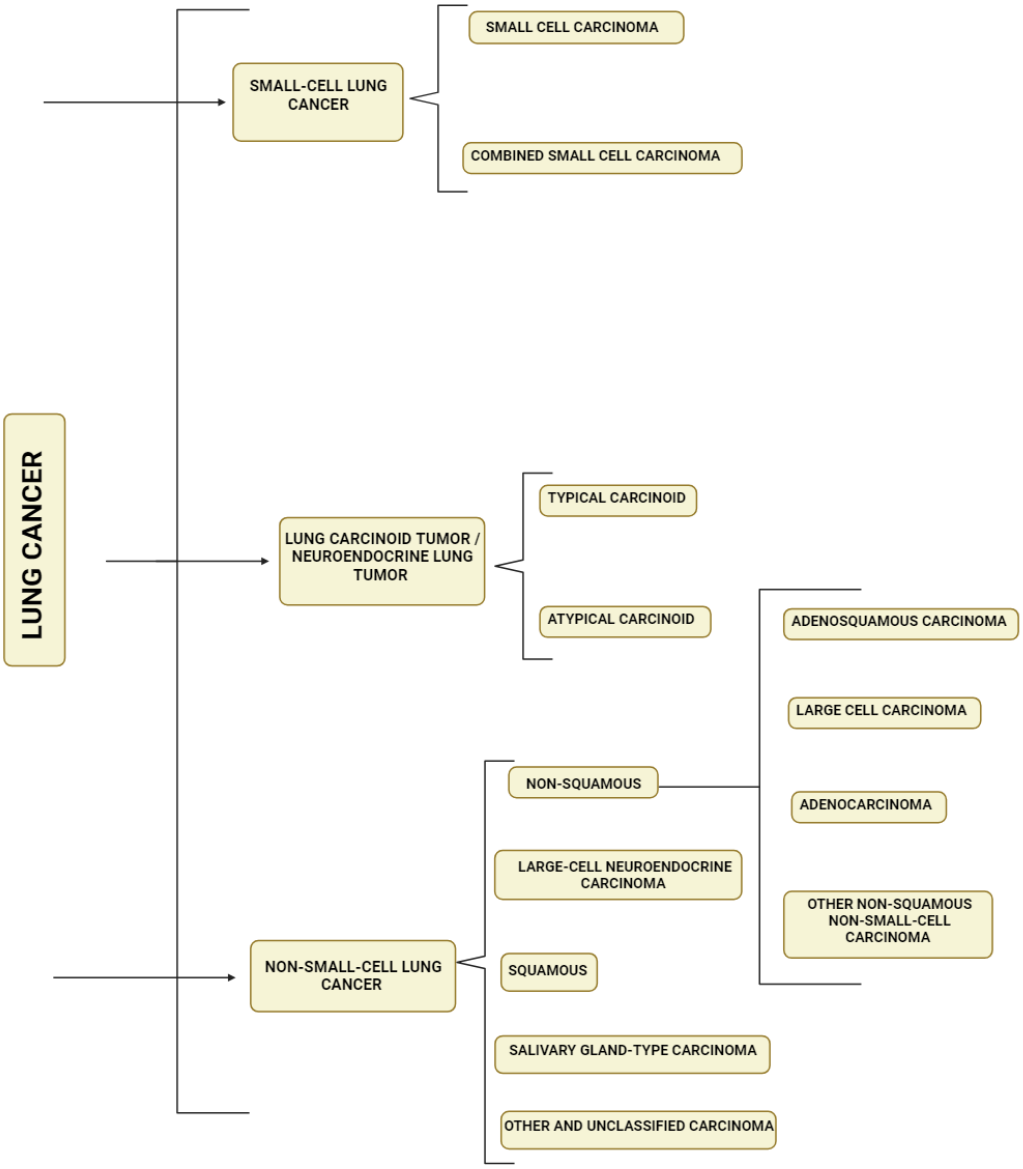

Figure 2. Small cell lung cancer, Lung carcinoid tumor/neuroendocrine lung tumor and non-small cell lung cancer.

Adenosquamous Carcinoma

Adenosquamous carcinomas are a type of lung tumor characterized by the presence of more than 10% glandular and squamous components. This subtype of lung cancer is rare and known for its aggressive nature. Current guidelines suggest the use of adjuvant chemotherapy, even in cases of Stage I tumors that have been completely removed through surgery, along with postoperative prophylactic radiotherapy to the entire brain. This approach is recommended due to the elevated likelihood of recurrence and the development of brain metastases associated with adenosquamous carcinomas (16).

Squamous Cell Carcinoma

Squamous cell pathology is characterized by the presence of keratin and/or intercellular desmosomes on cytology or by immunohistochemical (IHC) evidence of p40, p63, CK5, CK5/6, or desmoglein expression. The subtypes of squamous cell carcinoma encompass non-keratinizing, keratinizing, and basaloid cancers. Squamous cell carcinomas exhibit extensive central necrosis leading to cavitation. These cancers may manifest as coastal tumors and hypercalcemia. Pancoast tumors, located in the superior sulcus of the lung, are a specific type of squamous cell carcinoma. Postoperative recurrence in patients with Pancoast tumors most commonly occurs in the brain.

Large Cell Carcinoma

Large cell carcinoma (LCC) is an aggressive epithelial tumor characterized by the absence of cytological characteristics associated with glandular, squamous, or neuroendocrine malignancies. Immunohistochemical analysis typically reveals negative expression of p40 and TTF-1, and lacks cytological features indicative of small cell carcinoma. LCCs are typically comprised of round to polygonal cells with prominent nucleoli, exhibiting large size, abundant cytoplasm, and a lack of defining features. The diagnosis of LCC is primarily based on the exclusion of other specific tumor types (17).

Sarcomatoid Carcinoma

These are uncommon types of carcinomas characterized by the presence of malignant epithelial elements and characteristics resembling sarcomas. These subtypes encompass pleomorphic carcinomas, carcinosarcomas, and pulmonary blastomas.

Small Cell Carcinoma

Small cell carcinoma (SCLC) is characterized by the presence of round, oval, or angulated cells with minimal cytoplasm, similar in size to a resting lymphocyte, and lacking distinct nucleoli. SCLCs exhibit extensive necrosis and typically demonstrate positive staining for chromogranin and synaptophysin. The World Health Organization (WHO) has previously categorized SCLC into three cell subtypes: oat cells, intermediate cells, and combined cells (SCLC with non-small cell lung cancer component, squamous, or adenocarcinoma). However, research indicates that these classifications lack significant clinical relevance or prognostic value (18).

History and Physical

Lung cancer typically does not exhibit specific signs or symptoms, and many patients are diagnosed with advanced disease upon presentation. Symptoms of lung cancer manifest as a result of the localized impact of the tumor, including coughing due to bronchial compression, stroke-like symptoms from brain metastasis, paraneoplastic syndrome, and kidney stones caused by persistent hypercalcemia (19). Cough is observed in 50 to 75 percent of individuals diagnosed with lung cancer (2). Mucinous adenocarcinoma is characterized by the production of copious amounts of thin mucoid secretions, often leading to coughing. In cases where there are exophytic bronchial masses, coughing may indicate the development of secondary post-obstructive pneumonia. Additionally, hemoptysis, or coughing up blood, is reported in 15–30% of patients with lung cancer (2). Chest pain is reported in around 20-40% of individuals diagnosed with lung cancer, while dyspnea may be present in as high as 25-40% of cases at the time of diagnosis (2). Nevertheless, these indications may be predominantly attributed to lung cancer or underlying bronchopulmonary ailment, and pleural engagement in lung cancer can present as pleural thickening/nodules or malignant pleural effusion. Throughout the progression of their condition, around 10-15% of individuals with lung cancer will experience malignant pleural effusion, with certain cases exhibiting unilateral pleural effusion as the sole initial manifestation (20). Bronchogenic carcinoma accompanied by malignant pleural effusion on the same side is typically deemed inoperable. It is important to acknowledge that not all pleural effusions in individuals with lung cancer are of a malignant nature (21). Non-cancerous accumulation of fluid in the pleural cavity can result from lymphatic blockage, postobstructive pneumonitis, or atelectasis. In cases where two successive cytology samples yield negative results for malignancy in individuals with bronchogenic carcinoma, it is advisable to conduct surgical thoracoscopy or medical pleuroscopy to assess the pleural space prior to surgical removal of the primary lesion (22). Pleuroscopy in the medical field demonstrates a sensitivity exceeding 90% in the identification of malignancy among individuals with

bronchogenic carcinomas (23). The manifestation of small cell lung cancer often includes superior vena cava syndrome, characterized by the presence of dilated neck veins, facial and upper extremity edema, and a plethoric appearance. These symptoms may serve as the initial indication of the disease. Chest radiography typically reveals mediastinal widening or a mass in the right hilar region (24). As mentioned previously, lung cancers located in the superior sulcus are associated with PanCoast syndrome, characterized by shoulder pain, Horner syndrome, and signs of bony destruction, along with muscle atrophy in the hand. The metastasis of lung cancer to the bone often manifests with symptoms, such as bone pain at the metastatic site, accompanied by elevated serum alkaline phosphatase and hypercalcemia. Approximately 20% of patients with non-small cell lung cancer may initially (25), experience bone pain due to metastasis, while the percentage rises to 30-40% in patients with small-cell lung cancer (26). Imaging typically identifies osteolytic lesions, with the vertebral bodies being the most prevalent location for metastasis. Adrenal metastases are also present in lung cancer, although they are seldom symptomatic and are generally detected during staging. Nevertheless, not all adrenal lesions are cancerous, and positron emission tomography (PET) scanning is advised for distinguishing between benign and malignant adrenal lesions (27). Brain metastasis is a prevalent characteristic of both small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). In SCLC, the occurrence of brain metastases may be observed in approximately 20 to 30% of patients at the time of diagnosis (28). Other common sites of metastasis in lung cancer include the liver, often manifesting symptoms only in the advanced stages of the illness.

Paraneoplastic Syndromes Associated with Lung Cancer

Symptomatic hypercalcemia resulting from lung cancer may arise from the production of parathyroid hormone-related proteins or widespread bone metastases **(Figure 3).** Patients typically exhibit anorexia, nausea, constipation, and lethargy as common manifestations of hypercalcemia, and they generally have a bleak prognosis due to their correlation with advanced disease (29). The syndrome of inappropriate antidiuretic hormone secretion (SIADH) is linked to small cell lung cancer (SCLC) and manifests with symptoms of low sodium levels. Neurologic paraneoplastic syndromes are immune-mediated conditions connected with SCLC, encompassing Lambert-Eaton myasthenic syndrome (LEMS), encephalomyelitis, limbic encephalitis, cerebellar ataxia, sensory neuropathy, and autonomic neuropathy (30). The production of adrenal corticotropin in an abnormal location, known as ectopic production, can lead to the development of Cushing syndrome. This condition is linked to small cell lung cancer (SCLC), large cell neuroendocrine carcinoma, and carcinoid tumors of the lung, and is indicative of a poorer prognosis (31). Additional non-pulmonary clinical presentations of lung cancers encompass hypertrophic pulmonary osteoarthropathy, dermatomyositis, and polymyositis.

Standard phase for surgery of Lung Cancer

The standard treatment for patients with stage I and II, as well as some patients with stage IIIA, non-small-cell lung cancer (NSCLC) involves surgical removal of the tumor. After the surgery, patients may be recommended to undergo adjuvant systemic therapy. In the Lung Adjuvant Cisplatin Evaluation (LACE) meta-analysis, patients with completely removed NSCLC received adjuvant systemic therapy with a cisplatin-based doublet regimen. The benefit of adjuvant therapy varied depending on the stage of cancer, with stage IB (tumor ≥ 4 cm) patients having a 3% decrease in the risk of death at 5 years. It is important to note that the benefit of adjuvant chemotherapy was only significant for stage IB patients who had a high risk of recurrence. The benefit of adjuvant chemotherapy increased to 13% for stage III lung cancers when compared to no chemotherapy (32). Adjuvant chemotherapy usually comprises four cycles of a cisplatin-based combination and is recommended for patients with completely resected stage IB (high-risk) to IIIA non-small-cell lung cancers (33). Despite administering post-operative chemotherapy, approximately half of stage IB lung cancer patients (tumor size ≥ 4 cm) and three-quarters of stage IIIA lung cancer patients experience recurrence of metastatic disease (32). Until 2020, no additional systemic therapy was recommended after adjuvant chemotherapy. However, recent data has shown that further adjuvant treatment with

immunotherapy or oral tyrosine kinase inhibitor (TKI) therapy may be necessary and challenge the current standard of care. Osimertinib, a third-generation oral EGFR–TKI, can selectively bind to both EGFR driver mutations and EGFR resistance mutations T790M (34). It has been approved for adjuvant therapy of stage II and III NSCLC after complete resection. The ADAURA trial studied adjuvant osimertinib therapy vs placebo for up to 3 years (35). The study enrolled patients who had undergone complete surgical removal of stage IB (tumor > 3 cm) to IIIA non-small cell lung cancer (NSCLC) and had EGFR exon 19 deletion or exon 21 L858R driver mutations. The study reported a 37% increase in two-year disease-free survival (DFS) and an 80% relative improvement in two-year DFS(35). There is increasing interest in using immunotherapy in the adjuvant setting due to its effectiveness in treating stage III and IV disease. The IMpower010 study showed that adjuvant atezolizumab is effective in treating patients with stage IB (tumors ≥ 4 cm) to IIIA NSCLC who have undergone surgery and up to four cycles of adjuvant chemotherapy(36). In the primary analysis of patients with stage II-IIIA NSCLC and PD-L1 expression on at least 1% of tumor cells, 16 cycles of atezolizumab resulted in a 44% relative improvement in 3-year DFS compared to best supportive care (36). Several additional trials are currently underway to evaluate adjuvant oral TKI or immunotherapy. The most highly-anticipated Canadian study is BR31, which is a phase III placebo-controlled trial investigating adjuvant durvalumab in completely resected NSCLC(37). The overall survival data for both ADAURA and IMpower010 are not yet available. However, chemotherapy as the only adjuvant therapy for completely resected stage IB (tumors ≥ 4 cm) to IIIA NSCLC may soon be outdated. To treat curative lung cancer, clinicians should aim to enroll patients in clinical trials that assess the role of additional adjuvant therapy with immunotherapy or oral TKI.

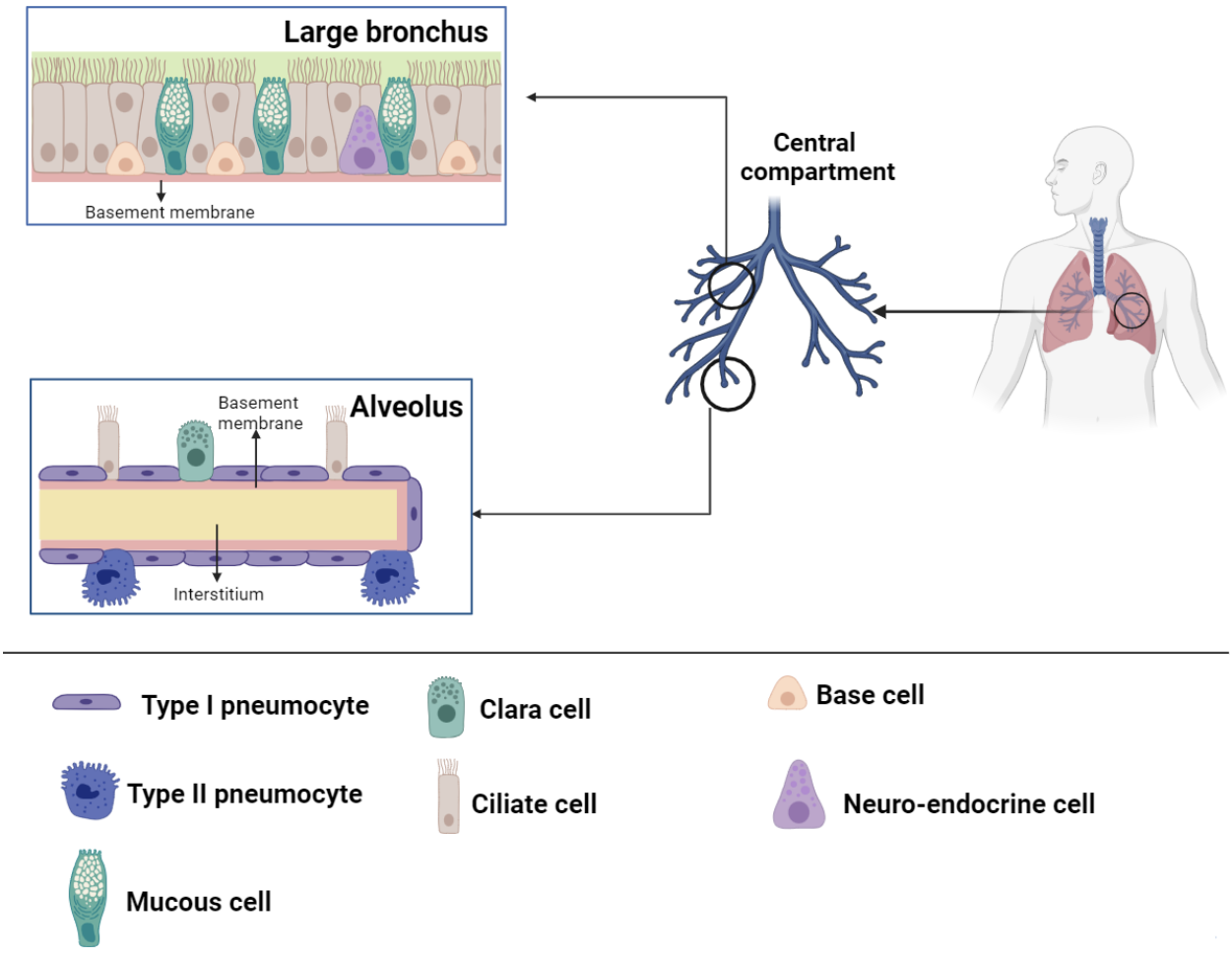

Figure 3. Histological combinations of lung cancer.

Stage III Non-Small-Cell Lung Cancer (NSCLC)

At initial diagnosis, approximately 20% of cases are classified as Stage III NSCLC, which includes tumors that have metastasized to mediastinal lymph nodes (Any T stage, N2) or large tumors that may involve local lymph nodes (T3N1 and T4N0) (38). Stage III non-small cell lung cancer (NSCLC) is a complex disease, and the treatment approach varies depending on different factors such as the size of the tumor, the severity of symptoms, and patient-specific criteria. The role of surgery in treating stage III NSCLC is a matter of debate. In a specific group of patients with single station mediastinal lymph node involvement, a trimodality treatment approach consisting of neoadjuvant chemotherapy and radiation followed by surgery can be considered. In the Intergroup 0139 study, patients who were eligible for lobectomy showed a significant survival benefit with the addition of surgery after preoperative chemotherapy and radiation. However, patients needing pneumonectomy didn't demonstrate the same benefit due to the perioperative risks involved (39). The process of selecting suitable patients for surgery is of utmost importance and should ideally involve a multidisciplinary approach. A majority of patients diagnosed with stage III NSCLC are considered unsuitable for surgery due to reasons such as their own choice, high tumor burden or not being fit for surgery. The combination of chemotherapy and radiation therapy, given either concurrently or sequentially, has been proven to provide the best chances of long-term survival for such patients. It has been observed that survival rates are better when chemotherapy and radiation therapy are given concurrently rather than sequentially (40). Patients need to have a good performance status and be able to tolerate multimodality therapy. Some common side effects that patients may experience include esophagitis, hematological toxicity, and pneumonitis. For patients with a borderline performance status, an alternative treatment option is sequential treatment with chemotherapy, followed by radiation. With this approach, the approximate five-year survival rate is 10% (40). For many years, studies have attempted to improve combination chemotherapy and radiation for unresectable stage III NSCLC. Increasing the number of chemotherapy cycles and radiation doses has not improved overall survival in these patients (41, 42). It has been shown for the first time that the inclusion of immunotherapy after concurrent chemotherapy and radiation therapy has resulted in an improvement in overall survival for patients. The latest update of the PACIFIC trial revealed that consolidation therapy with durvalumab for one year reduced the risk of death by 29% when compared to the placebo. Patients who received immunotherapy had a four-year overall survival rate of 49.6%, while those who did not had a rate of 36.3%. (43). It is recommended to undergo a baseline CT scan after completing chemotherapy or radiation treatment in order to rule out radiation pneumonitis and disease progression. Treatment should commence within a period of 42 days following the completion of chemotherapy and radiation therapy. Immunotherapy poses a small but clinically significant risk of pneumonitis, as well as an increased risk of thyroid dysfunction(44). Durvalumab is usually administered every two weeks. However, some provinces have approved the agent's administration on a 28-day cycle to reduce the travel burden and potential exposure during the COVID-19 pandemic. There is renewed interest in neoadjuvant strategies due to the poor outcomes for stage III NSCLC and high rates of local relapse. For instance, patients with stage III NSCLC involving single or multiple mediastinal lymph nodes underwent neoadjuvant durvalumab immunotherapy and chemotherapy, followed by surgery. In this study, 62% of patients achieved a major pathological response, which was defined as having less than 10% of viable tumor cells at the time of surgery. An additional 10% of patients had a complete pathological response (45). These points have been linked to the patient's overall survival, and there are numerous ongoing studies investigating the use of immunotherapy in the pre-treatment setting. If patients are not eligible for multiple treatment approaches, definitive radiation or palliative radiotherapy can effectively manage symptoms.

Metastatic Non-Small-Cell Lung Cancer (NSCLC)

The majority of individuals with lung cancer are initially diagnosed with distant metastases, although some with early-stage or locally advanced disease may

subsequently develop metastasis. The primary goals in managing metastatic non-small cell lung cancer (NSCLC) are to improve or maintain quality of life and prolong overall survival. Early integration of palliative care has been demonstrated to improve quality of life, reduce depression, and extend overall survival(46). In systemic therapy, the available treatment modalities encompass chemotherapy, targeted therapy, and immunotherapy. It is recommended that all non-squamous tumors undergo testing for driver mutations, particularly in individuals with a limited or absent history of smoking. For squamous histology tumors in non-smokers, the consideration for driver mutation testing should be individualized. Targeted therapy is generally the preferred approach for patients with mutations in EGFR, ALK, or ROS1, as it offers greater efficacy and lower toxicity. The International Association for the Study of Lung Cancer advocates for testing for EGFR, ALK, and ROS1 as a minimum requirement, and more recent guidelines also suggest testing for BRAF, KRAS, MET, NTRK, and RET (14, 47). This review centers on prevalent driver mutations that have actionable targets. Patients lacking a driver mutation have treatment options such as single-agent immunotherapy, combination immunotherapy regimens, or chemotherapy alone. A comprehensive summary of the treatment for metastatic NSCLC can be found in Figure 1.

Immunotherapy

Various standard and specialized approaches are available for the treatment of lung cancer (Figure 4). Immunotherapy has brought about substantial changes in the management of patients with metastatic non-small cell lung cancer (NSCLC). In 2015, a pivotal study on immunotherapy showcasing its efficacy in NSCLC was published in the Phase II Checkmate 063 trial. Nivolumab exhibited significant efficacy and manageable toxicity in heavily treated patients (48). In the few years since then, several immunotherapy approaches have been created. Some patients with metastatic non-small cell lung cancer (NSCLC) have achieved prolonged survival, referred to as the "tail of the survival curve" (49). The effectiveness of immunotherapy is influenced by the level of tumor PDL-1 expression. PDL-1 expression is commonly classified into three groups: PDL-1 negative (less than 1% of tumor cells express PDL-1), PDL-1 low positive (1–49%), and PDL-1 positive (more than 50%). The duration of response and overall survival rates are positively correlated with higher PDL-1 expression levels. In patients with PDL-1 positive tumors, single-agent immunotherapy has consistently demonstrated superior outcomes compared to chemotherapy, with lower toxicity and improved survival rates. For instance, in the KEYNOTE-024 study, the median survival with pembrolizumab reached 26.3 months, and notably, 31.9% of patients achieved a five-year survival, which is the highest reported in a phase III study to date (50). Comparable research conducted on patients with PDL-1-positive status has demonstrated that immunotherapy agents such as atezolizumab and cemiplimab exhibit superior efficacy compared to chemotherapy (51, 52). These studies have shown, for the first time, that some patients with metastatic non-small cell lung cancer (NSCLC) may be able to avoid chemotherapy. For patients with PDL-1 negative (<1%) or PDL-1 (1–49%) tumors, newer combination strategies have become the standard of care in the initial treatment. In the KEYNOTE-189 study, patients with metastatic nonsquamous NSCLC were randomly assigned to receive either carboplatin and pemetrexed or the same regimen in combination with pembrolizumab. The combination of chemotherapy and immunotherapy resulted in an overall survival of 22 months and reduced the risk of death by 44% compared to chemotherapy alone (53). In the same vein, the concurrent administration of chemotherapy and pembrolizumab resulted in a 36% decrease in the mortality risk among individuals diagnosed with metastatic squamous non-small cell lung cancer, as demonstrated in the KEYNOTE-407 clinical trial. The chemotherapy regimen utilized in this study comprised carboplatin and a taxane(54). Clinical trials have also investigated the use of a combination of dual immunotherapy for metastatic non-small cell lung cancer (NSCLC). In the Checkmate-9LA study, patients with nonsquamous or squamous histology were randomly assigned to receive either a platinum doublet or a combination of ipilimumab and nivolumab, along with two cycles of a platinum doublet. The arm receiving the dual immunotherapy-chemotherapy combination demonstrated a median overall survival of 15.8 months and a 28% reduction in the risk of death compared to chemotherapy alone(50). The comparative efficacy of dual immunotherapy

combinations in relation to chemotherapy-immunotherapy combinations has not been established, and further investigation is required to determine potential benefits for specific subgroups with longer-term follow-up.

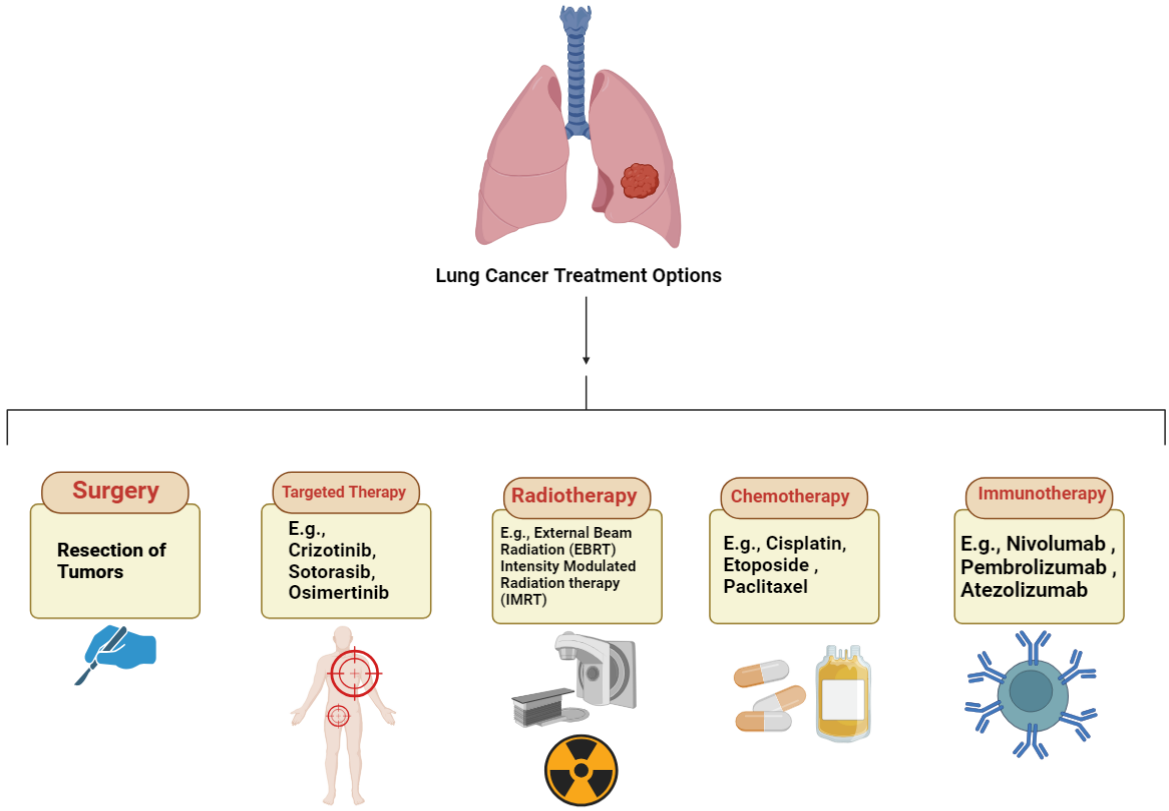

Figure 4. Details of standard ways of treatment for lung cancer including surgery, targeted therapy, radiotherapy, chemotherapy and immunotherapy.

Chemotherapy

Chemotherapy remains a primary treatment option for patients who are not suitable candidates for single-agent immunotherapy or combination immunotherapy regimens. These patients may have contraindications to immunotherapy, such as pre-existing autoimmune conditions, or there may be concerns about their performance status and the potential for toxicity with combination immunotherapy regimens. In such cases, platinum doublets are commonly utilized. For patients with nonsquamous metastatic NSCLC, a typical example would involve the use of carboplatin or cisplatin in combination with pemetrexed for 4–6 cycles, followed by maintenance pemetrexed until disease progression or unacceptable toxicity. In the case of squamous metastatic NSCLC, a platinum doublet may consist of carboplatin or cisplatin in combination with either paclitaxel or gemcitabine.

Biomarker testing

Tailoring medical treatment by focusing on specific molecular targets within tumors has led to enhanced survival rates for individuals with non-small cell lung cancer (NSCLC) (55). Various specific drugs have demonstrated efficacy in treating mutations in the epidermal growth factor receptor (EGFR) and anaplastic lymphoma kinase (ALK). Genomic testing has identified additional molecular alterations such as ROS1 and RET gene rearrangements, MET amplification, and activating mutations in BRAF, HER2, and KRAS genes. These findings suggest potential targets for future therapeutic interventions.

Epidermal growth factor receptor (EGFR) gene

The EGFR receptor is a tyrosine kinase receptor located on the surface of cells, capable of initiating signaling pathways related to cellular growth and

proliferation upon activation. In the context of cancer, mutations in the EGFR gene result in unregulated cell division due to continuous activation. These mutations are observed in 10-15% of lung cancer adenocarcinoma patients of European and Asian ancestry, particularly in individuals who have never smoked and in females (56-58). Although these traits are prevalent, mutation testing plays a crucial role in identifying individuals who would gain from targeted tyrosine kinase inhibitor treatment. Mutations in EGFR commonly arise in exons 18–21, which confer sensitivity to EGFR tyrosine kinase inhibitors; these exons encode a segment of the EGFR kinase domain. Roughly 90% of these mutations consist of exon 19 deletions and the L858R point mutation on exon 21, and are associated with a 70% response rate in patients undergoing erlotinib or gefitinib therapy (59).

KRAS

The KRAS oncogene is frequently mutated in non-small cell lung cancer (NSCLC) through missense mutations that result in the substitution of an amino acid at positions 12, 13, or 61. Mutations at residues G12 and G13 are particularly prevalent. These mutations are more commonly found in adenocarcinomas, individuals of Caucasian descent, and those with a history of smoking (60). Roughly 10 to 25% of individuals diagnosed with adenocarcinoma exhibit tumors that are associated with KRAS mutations (61). In the context of concurrent occurrence with other cancer-causing genetic mutations, KRAS has been primarily identified in tumor types that lack mutations in EGFR and ALK, indicating that these mutations represent a distinct molecular subset of non-small cell lung cancer (NSCLC). Recent evidence indicates that KRAS mutations may have potential prognostic significance, but their ability to predict the response to EGFR tyrosine kinase inhibitors or cytotoxic chemotherapy is limited (55, 59). A study has proposed the feasibility of specifically targeting a subset of KRAS mutations using small-molecule inhibitors designed to address the prevalent G12C mutation in lung cancer, which is more common in smokers than non-smokers. These potential new agents depend on binding to the mutant cysteine and do not impact the wild-type KRAS protein, demonstrating specificity for a particular subtype (62).

Anaplastic lymphoma kinase (ALK)

Roughly 3-7% of lung tumors exhibit ALK mutations, (63-65) which are frequently observed in younger patients. Koh et al. found that individuals with ALK mutations had a median age of 49, while those without ALK mutations had a median age of 61 ($P<0.001$; n=221) (66). ALK mutations are also prevalent in adenocarcinoma patients with acinar histology or signet ring cells, as well as in those who have no history of smoking (67, 68). The predominant ALK rearrangement observed in non-small cell lung cancer (NSCLC) patients is the EML-4-ALK rearrangement. This genetic alteration occurs on chromosome 2p23 and involves the fusion of the 5' end of the EML-4 gene with the 3' end of the ALK gene, resulting in at least nine distinct fusion variants. EML-4 mutations are frequently identified in adenocarcinomas of individuals with no history of smoking or light smoking, whose tumors do not exhibit mutations in either EGFR or KRAS genes (63, 68). ALK mutations do not overlap with other oncogenic mutations linked to non-small cell lung cancer, such as EGFR or RAS mutations (68, 69). Additional ALK mutations unrelated to EML-4, such as KIF5B-ALK and TFG-ALK, have been identified. Patients with EML4-ALK fusions or ALK rearrangements do not derive therapeutic benefits from EGFR-specific tyrosine kinase inhibitor therapy (70).

Presently, there exists an FDA-approved medication, crizotinib (Xalkori®, Pfizer), which is designed to target constitutively activated receptor tyrosine kinases resulting from EML4-ALK and other ALK fusions. A single arm study of ALK-positive metastatic NSCLC(71), demonstrated objective response rates of 50–61% in patients. In a trial involving previously untreated advanced non-squamous ALK-positive NSCLC, patients were randomly assigned to receive either crizotinib 250 mg orally twice daily (n=172) or intravenous chemotherapy (pemetrexed 500 mg/m2 plus either cisplatin 75 mg/m2 or carboplatin target area under the curve 5–6 mg/mL/min (PPC group); all administered intravenously every three weeks for ≤6 cycles, n=171). The primary endpoint of the study was progression-free survival, while secondary endpoints included overall response rate, overall survival, safety, and patient-reported outcomes. The study revealed that crizotinib extended progression-free survival to 10.9 months compared to 7 months in patients receiving

PPC. Additionally, the overall response rate was higher in patients receiving crizotinib at 74% compared to 45% in patients receiving PPC. Overall, crizotinib demonstrated significant improvements in progression-free survival and overall response rate compared to standard chemotherapy, and its safety profile was deemed acceptable (71). This landmark study solidified crizotinib as the recommended treatment for individuals with advanced ALK-positive non-squamous non-small cell lung cancer who have not received prior therapy.

BRAF

The BRAF gene is classified as a proto-oncogene, functioning as a controlled signal transduction serine/threonine protein kinase that has the capability to stimulate cell proliferation and viability (72). Somatic mutations in the BRAF gene have been identified in 1–4% of non-small cell lung cancer (NSCLC) cases, with the highest prevalence observed in patients diagnosed with adenocarcinomas (61, 73-77). These mutations are frequently associated with individuals who have a history of smoking, either currently or in the past (76, 77). The localization of BRAF mutations within the kinase domain varies between lung cancer and breast cancer patients. A study involving 697 individuals diagnosed with lung adenocarcinoma revealed that 3% of the patients harbored BRAF mutations, with the identified mutations being V600E (50%), G469A (39%), and D594G (11%) (76). The majority of BRAF mutations in non-small cell lung cancer (NSCLC) have been identified as distinct from other oncogenic mutations, such as EGFR mutations and ALK rearrangements.

Conclusion

Lung cancer is the primary contributor to cancer-related fatalities on a global scale, resulting in the highest mortality rates for both genders. Approximately 85% of lung cancer cases are attributed to smoking. Diagnosis of lung cancer frequently occurs at advanced stages, limiting treatment options. Screening individuals at high risk has the potential to facilitate early detection and significantly enhance survival rates. Implementing primary prevention strategies, such as tobacco control measures and minimizing exposure to environmental risk factors, has the potential to decrease the occurrence of lung cancer and ultimately save lives. Considerable progress has been achieved in mitigating occupational health risks related to lung cancer, particularly in the context of smoking, and in the prevention of diverse disorders. In recent years, targeted therapy and immunotherapy have significantly contributed to the enhanced management of lung cancer. Furthermore, genetic and biomarker testing are aiding in the personalized management of different types of lung cancer. Through personalized management of non-small cell lung cancer (NSCLC), treatments are tailored to individual patients and can specifically target mutations with greater precision, aiming to prolong progression-free survival. Immunotherapy involves the concept of enhancing and directing the body's own immune defenses to combat cancer cells. Ongoing clinical trials are exploring the use of vaccines for treating NSCLC. Given that lung cancer is the leading cause of cancer-related deaths in the United States, ongoing research efforts are focused on developing innovative treatments.

In the past ten years, the landscape of lung cancer treatment in Canada has experienced rapid changes. New targets have been identified, leading to significant benefits for patients with metastatic non-small cell lung cancer (NSCLC), particularly those without a history of smoking. The integration of immunotherapy has altered the standard of care for patients with metastatic NSCLC and is now being incorporated into earlier stages of treatment. Physicians treating lung cancer must now be able to identify and manage the specific toxicities associated with immunotherapy. This review has only addressed some of the complexities involved in treating NSCLC and has not delved into the details of therapy sequencing. Despite these advancements, lung cancer continues to impose a substantial burden of morbidity and mortality on the Canadian population. Smoking cessation and screening high-risk individuals are crucial strategies for alleviating this burden.

Author contribution

MP conceptualized and wrote the manuscript. **TYK** edited the final version of the manuscript. **RAE** accompanied in writing of some sections of the paper. All authors have read and confirmed the final revised version of the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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References

1. Miller KD, Siegel RL, Lin CC, Mariotto AB, Kramer JL, Rowland JH, et al. Cancer treatment and survivorship statistics, 2016. CA: a cancer journal for clinicians. 2016;66(4):271-89.
2. Kocher F, Hilbe W, Seeber A, Pircher A, Schmid T, Greil R, et al. Longitudinal analysis of 2293 NSCLC patients: a comprehensive study from the TYROL registry. Lung Cancer. 2015;87(2):193-200.
3. Alberg AJ, Samet JM. Epidemiology of lung cancer. Chest. 2003;123(1):21S-49S.
4. Lorigan P, Radford J, Howell A, Thatcher N. Lung cancer after treatment for Hodgkin's lymphoma: a systematic review. The lancet oncology. 2005;6(10):773-9.
5. Burns DM. Primary prevention, smoking, and smoking cessation: implications for future trends in lung cancer prevention. Cancer. 2000;89(S11):2506-9.
6. Wagner GR. Asbestosis and silicosis. The Lancet. 1997;349(9061):1311-5.
7. Grosche B, Kreuzer M, Kreisheimer M, Schnelzer M, Tschense A. Lung cancer risk among German male uranium miners: a cohort study, 1946–1998. British journal of cancer. 2006;95(9):1280-7.
8. Darby S, Hill D, Auvinen A, Barros-Dios J, Baysson H, Bochicchio F, et al. Radon in homes and risk of lung cancer: collaborative analysis of individual data from 13 European case-control studies. Bmj. 2005;330(7485):223.
9. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. CA: a cancer journal for clinicians. 2019;69(1):7-34.
10. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2021;71(3):209-49.
11. Barta JA, Powell CA, Wisnivesky JP. Global epidemiology of lung cancer. Annals of global health. 2019;85(1).
12. Cagle PT, Allen TC, Olsen RJ. Lung cancer biomarkers: present status and future developments. Archives of Pathology and Laboratory medicine. 2013;137(9):1191-8.
13. Lindeman NI, Cagle PT, Beasley MB, Chitale DA, Dacic S, Giaccone G, et al. Molecular testing guideline for selection of lung cancer patients for EGFR and ALK tyrosine kinase inhibitors: guideline from the College of American Pathologists, International Association for the Study of Lung Cancer, and Association for Molecular Pathology. Journal of Thoracic Oncology. 2013;8(7):823-59.
14. Lindeman NI, Cagle PT, Aisner DL, Arcila ME, Beasley MB, Bernicker EH, et al. Updated molecular testing guideline for the selection of lung cancer patients for treatment with targeted tyrosine kinase inhibitors: guideline from the College of American Pathologists, the International Association for the Study of Lung Cancer, and the Association for Molecular Pathology. Archives of pathology & laboratory medicine. 2018;142(3):321-46.
15. Kadota K, Yeh Y-C, Sima CS, Rusch VW, Moreira AL, Adusumilli PS, et al. The cribriform pattern identifies a subset of acinar predominant tumors with poor prognosis in patients with stage I lung adenocarcinoma: a conceptual proposal to classify cribriform predominant tumors as a distinct histologic subtype. Modern Pathology. 2014;27(5):690-700.
16. Filosso PL, Ruffini E, Asioli S, Giobbe R, Macri L, Bruna MC, et al. Adenosquamous lung carcinomas: a histologic subtype with poor prognosis. Lung cancer. 2011;74(1):25-9.
17. Rajdev K, Siddiqui AH, Ibrahim U, Patibandla P, Khan T, El-Sayegh D, et al. An unusually aggressive large cell carcinoma of the lung: undiagnosed until autopsy. Cureus. 2018;10(2).
18. Aisner SC, Finkelstein DM, Ettinger DS, Abeloff MD, Ruckdeschel JC, Eggleston JC. The clinical significance of variant-morphology small-cell carcinoma of the lung. Journal of clinical oncology. 1990;8(3):402-8.
19. Chute CG, Greenberg ER, Baron J, Korson R, Baker J, Yates J. Presenting conditions of 1539 population-based lung cancer patients by cell type and

stage in new hampshire and vermont. Cancer. 1985;56(8):2107-11.
20. Sahn SA. Malignancy metastatic to the pleura. Clinics in chest medicine. 1998;19(2):351-61.
21. Decker DA, Dines DE, Payne WS, Bernatz PE, Pairolero PC. The significance of a cytologically negative pleural effusion in bronchogenic carcinoma. Chest. 1978;74(6):640-2.
22. Maskell N, Butland R. BTS guidelines for the investigation of a unilateral pleural effusion in adults. Thorax. 2003;58(Suppl 2):ii8.
23. Rahman NM, Ali NJ, Brown G, Chapman SJ, Davies RJ, Downer NJ, et al. Local anaesthetic thoracoscopy: British Thoracic Society pleural disease guideline 2010. Thorax. 2010;65(Suppl 2):ii54-ii60.
24. Eren S, Karaman A, Okur A. The superior vena cava syndrome caused by malignant disease: imaging with multi-detector row CT. European journal of radiology. 2006;59(1):93-103.
25. Toloza EM, Harpole L, McCrory DC. Noninvasive staging of non-small cell lung cancer: a review of the current evidence. Chest. 2003;123(1):137S-46S.
26. Schumacher T, Brink I, Mix M, Reinhardt M, Herget G, Digel W, et al. FDG-PET imaging for the staging and follow-up of small cell lung cancer. European journal of nuclear medicine. 2001;28:483-8.
27. Erasmus JJ, Patz Jr E, McAdams HP, Murray JG, Herndon J, Coleman RE, et al. Evaluation of adrenal masses in patients with bronchogenic carcinoma using 18F-fluorodeoxyglucose positron emission tomography. AJR American journal of roentgenology. 1997;168(5):1357-60.
28. Quan AL, Videtic GM, Suh JH. Brain metastases in small cell lung cancer. ONCOLOGY-WILLISTON PARK THEN HUNTINGTON THE MELVILLE NEW YORK-. 2004;18:961-72.
29. Hiraki A, Ueoka H, Takata I, Gemba K, Bessho A, Segawa Y, et al. Hypercalcemia–leukocytosis syndrome associated with lung cancer. Lung Cancer. 2004;43(3):301-7.
30. Honnorat J, Antoine J-C. Paraneoplastic neurological syndromes. Orphanet journal of rare diseases. 2007;2(1):1-8.
31. Hayes AR, Grossman AB. Distinguishing Cushing's disease from the ectopic ACTH syndrome: Needles in a haystack or hiding in plain sight? Journal of Neuroendocrinology. 2022;34(8):e13137.
32. Pignon J-P, Tribodet H, Scagliotti GV, Douillard J-Y, Shepherd FA, Stephens RJ, et al. Lung adjuvant cisplatin evaluation: a pooled analysis by the LACE Collaborative Group. Database of Abstracts of Reviews of Effects (DARE): Quality-Assessed Reviews [Internet]: Centre for Reviews and Dissemination (UK); 2008.
33. Pisters KM, Evans WK, Azzoli CG, Kris MG, Smith CA, Desch CE, et al. Cancer Care Ontario and American Society of Clinical Oncology adjuvant chemotherapy and adjuvant radiation therapy for stages I-IIIA resectable non–small-cell lung cancer guideline. Journal of clinical oncology. 2007;25(34):5506-18.
34. Soria J-C, Ohe Y, Vansteenkiste J, Reungwetwattana T, Chewaskulyong B, Lee KH, et al. Osimertinib in untreated EGFR-mutated advanced non–small-cell lung cancer. New England journal of medicine. 2018;378(2):113-25.
35. Wu Y-L, Tsuboi M, He J, John T, Grohe C, Majem M, et al. Osimertinib in resected EGFR-mutated non–small-cell lung cancer. New England journal of medicine. 2020;383(18):1711-23.
36. Felip E, Altorki N, Zhou C, Csőszi T, Vynnychenko I, Goloborodko O, et al. Adjuvant atezolizumab after adjuvant chemotherapy in resected stage IB–IIIA non-small-cell lung cancer (IMpower010): a randomised, multicentre, open-label, phase 3 trial. The Lancet. 2021;398(10308):1344-57.
37. Wu Y, Chen K, Xing W, Chen Q, Liu L, Zhang Q, et al. 84P SHR-1316 vs placebo in combination with chemotherapy as perioperative treatment in patients with resectable stage II-III NSCLC: A randomized, double-blind, multicenter, phase Ib/III trial. Annals of Oncology. 2022;33:S72.
38. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2021. Ca Cancer J Clin. 2021;71(1):7-33.
39. Albain KS, Swann RS, Rusch VW, Turrisi AT, Shepherd FA, Smith C, et al. Radiotherapy plus chemotherapy with or without surgical resection for stage III non-small-cell lung cancer: a phase III randomised controlled trial. The Lancet. 2009;374(9687):379-86.
40. Aupérin A, Le Péchoux C, Rolland E, Curran WJ, Furuse K, Fournel P, et al. Meta-analysis of concomitant versus sequential radiochemotherapy in locally advanced non-small-cell lung cancer. J Clin Oncol. 2010;28(13):2181-90.

41. Vokes EE, Herndon JE, Kelley MJ, Cicchetti MG, Ramnath N, Neill H, et al. Induction chemotherapy followed by chemoradiotherapy compared with chemoradiotherapy alone for regionally advanced unresectable stage III non–small-cell lung cancer: Cancer and Leukemia Group B. Journal of clinical oncology. 2007;25(13):1698-704.
42. Bradley JD, Hu C, Komaki RR, Masters GA, Blumenschein GR, Schild SE, et al. Long-term results of NRG oncology RTOG 0617: standard-versus high-dose chemoradiotherapy with or without cetuximab for unresectable stage III non–small-cell lung cancer. Journal of Clinical Oncology. 2020;38(7):706.
43. Faivre-Finn C, Vicente D, Kurata T, Planchard D, Paz-Ares L, Vansteenkiste JF, et al. Four-year survival with durvalumab after chemoradiotherapy in stage III NSCLC—an update from the PACIFIC trial. Journal of Thoracic Oncology. 2021;16(5):860-7.
44. Antonia SJ, Villegas A, Daniel D, Vicente D, Murakami S, Hui R, et al. Durvalumab after chemoradiotherapy in stage III non–small-cell lung cancer. New England Journal of Medicine. 2017;377(20):1919-29.
45. Rothschild SI, Zippelius A, Eboulet EI, Savic Prince S, Betticher D, Bettini A, et al. SAKK 16/14: durvalumab in addition to neoadjuvant chemotherapy in patients with stage IIIA (N2) non–small-cell lung cancer—a multicenter single-arm phase II trial. Journal of clinical oncology. 2021;39(26):2872-80.
46. Temel JS, Greer JA, Muzikansky A, Gallagher ER, Admane S, Jackson VA, et al. Early palliative care for patients with metastatic non–small-cell lung cancer. New England Journal of Medicine. 2010;363(8):733-42.
47. Ettinger DS, Wood DE, Aisner DL, Akerley W, Bauman JR, Bharat A, et al. Non-small cell lung cancer, Version 2.2021 featured updates to the NCCN guidelines. JNCCN Journal of the National Comprehensive Cancer Network. 2021;19(3):254-66.
48. Rizvi NA, Mazières J, Planchard D, Stinchcombe TE, Dy GK, Antonia SJ, et al. Activity and safety of nivolumab, an anti-PD-1 immune checkpoint inhibitor, for patients with advanced, refractory squamous non-small-cell lung cancer (CheckMate 063): a phase 2, single-arm trial. The lancet oncology. 2015;16(3):257-65.
49. Borghaei H, Gettinger S, Vokes EE, Chow LQ, Burgio MA, de Castro Carpeno J, et al. Five-year outcomes from the randomized, phase III trials checkmate 017 and 057: nivolumab versus docetaxel in previously treated non–small-cell lung cancer. Journal of Clinical Oncology. 2021;39(7):723.
50. Reck M, Rodríguez-Abreu D, Robinson AG, Hui R, Csőszi T, Fülöp A, et al. Five-year outcomes with pembrolizumab versus chemotherapy for metastatic non–small-cell lung cancer with PD-L1 tumor proportion score≥ 50%. Journal of Clinical Oncology. 2021;39(21):2339.
51. Herbst RS, Giaccone G, de Marinis F, Reinmuth N, Vergnenegre A, Barrios CH, et al. Atezolizumab for first-line treatment of PD-L1–selected patients with NSCLC. New England Journal of Medicine. 2020;383(14):1328-39.
52. Sezer A, Kilickap S, Gümüş M, Bondarenko I, Özgüroğlu M, Gogishvili M, et al. Cemiplimab monotherapy for first-line treatment of advanced non-small-cell lung cancer with PD-L1 of at least 50%: a multicentre, open-label, global, phase 3, randomised, controlled trial. The Lancet. 2021;397(10274):592-604.
53. Gadgeel S, Rodríguez-Abreu D, Speranza G, Esteban E, Felip E, Dómine M, et al. Updated analysis from KEYNOTE-189: pembrolizumab or placebo plus pemetrexed and platinum for previously untreated metastatic nonsquamous non-small-cell lung cancer. Journal of clinical oncology. 2020;38(14):1505-17.
54. Gandhi L, Rodríguez-Abreu D, Gadgeel S, Esteban E, Felip E, De Angelis F, et al. Pembrolizumab plus chemotherapy in metastatic non–small-cell lung cancer. New England journal of medicine. 2018;378(22):2078-92.
55. Riely GJ, Marks J, Pao W. KRAS mutations in non–small cell lung cancer. Proceedings of the American Thoracic Society. 2009;6(2):201-5.
56. Lynch TJ, Bell DW, Sordella R, Gurubhagavatula S, Okimoto RA, Brannigan BW, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non–small-cell lung cancer to gefitinib. New England Journal of Medicine. 2004;350(21):2129-39.
57. Paez JG, Janne PA, Lee JC, Tracy S, Greulich H, Gabriel S, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. Science. 2004;304(5676):1497-500.
58. Pao W, Miller V, Zakowski M, Doherty J, Politi K, Sarkaria I, et al. EGF receptor gene mutations

are common in lung cancers from “never smokers” and are associated with sensitivity of tumors to gefitinib and erlotinib. Proceedings of the National Academy of Sciences. 2004;101(36):13306-11.
59. Riely GJ, Ladanyi M. KRAS mutations: an old oncogene becomes a new predictive biomarker. The Journal of Molecular Diagnostics. 2008;10(6):493-5.
60. Riely GJ, Kris MG, Rosenbaum D, Marks J, Li A, Chitale DA, et al. Frequency and distinctive spectrum of KRAS mutations in never smokers with lung adenocarcinoma. Clinical cancer research. 2008;14(18):5731-4.
61. Brose MS, Volpe P, Feldman M, Kumar M, Rishi I, Gerrero R, et al. BRAF and RAS mutations in human lung cancer and melanoma. Cancer research. 2002;62(23):6997-7000.
62. Ostrem JM, Peters U, Sos ML, Wells JA, Shokat KM. K-Ras (G12C) inhibitors allosterically control GTP affinity and effector interactions. Nature. 2013;503(7477):548-51.
63. Soda M, Choi YL, Enomoto M, Takada S, Yamashita Y, Ishikawa S, et al. Identification of the transforming EML4–ALK fusion gene in non-small-cell lung cancer. Nature. 2007;448(7153):561-6.
64. Takeuchi K, Choi YL, Soda M, Inamura K, Togashi Y, Hatano S, et al. Multiplex reverse transcription-PCR screening for EML4-ALK fusion transcripts. Clinical Cancer Research. 2008;14(20):6618-24.
65. Koivunen JP, Mermel C, Zejnullahu K, Murphy C, Lifshits E, Holmes AJ, et al. EML4-ALK fusion gene and efficacy of an ALK kinase inhibitor in lung cancer. Clinical cancer research. 2008;14(13):4275-83.
66. Koh Y, Kim D-W, Kim TM, Lee S-H, Jeon YK, Chung DH, et al. Clinicopathologic characteristics and outcomes of patients with anaplastic lymphoma kinase-positive advanced pulmonary adenocarcinoma: suggestion for an effective screening strategy for these tumors. Journal of Thoracic Oncology. 2011;6(5):905-12.
67. Inamura K, Takeuchi K, Togashi Y, Nomura K, Ninomiya H, Okui M, et al. EML4-ALK fusion is linked to histological characteristics in a subset of lung cancers. Journal of thoracic oncology. 2008;3(1):13-7.
68. Inamura K, Takeuchi K, Togashi Y, Hatano S, Ninomiya H, Motoi N, et al. EML4-ALK lung cancers are characterized by rare other mutations, a TTF-1 cell lineage, an acinar histology, and young onset. Modern Pathology. 2009;22(4):508-15.
69. Kwak EL, Bang Y-J, Camidge DR, Shaw AT, Solomon B, Maki RG, et al. Anaplastic lymphoma kinase inhibition in non–small-cell lung cancer. New England Journal of Medicine. 2010;363(18):1693-703.
70. Togashi Y, Soda M, Sakata S, Sugawara E, Hatano S, Asaka R, et al. KLC1-ALK: a novel fusion in lung cancer identified using a formalin-fixed paraffin-embedded tissue only. PLoS One. 2012;7(2):e31323.
71. Solomon BJ, Mok T, Kim D-W, Wu Y-L, Nakagawa K, Mekhail T, et al. First-line crizotinib versus chemotherapy in ALK-positive lung cancer. New England Journal of Medicine. 2014;371(23):2167-77.
72. Daum G, Eisenmann-Tappe I, Fries H-W, Troppmair J, Rapp UR. The ins and outs of Raf kinases. Trends in biochemical sciences. 1994;19(11):474-80.
73. Cardarella S, Ogino A, Nishino M, Butaney M, Shen J, Lydon C, et al. Clinical, pathologic, and biologic features associated with BRAF mutations in non–small cell lung cancer. Clinical cancer research. 2013;19(16):4532-40.
74. Davies H, Bignell GR, Cox C, Stephens P, Edkins S, Clegg S, et al. Mutations of the BRAF gene in human cancer. Nature. 2002;417(6892):949-54.
75. Naoki K, Chen T-H, Richards WG, Sugarbaker DJ, Meyerson M. Missense mutations of the BRAF gene in human lung adenocarcinoma. Cancer research. 2002;62(23):7001-3.
76. Paik PK, Arcila ME, Fara M, Sima CS, Miller VA, Kris MG, et al. Clinical characteristics of patients with lung adenocarcinomas harboring BRAF mutations. Journal of clinical oncology. 2011;29(15):2046.
77. Pratilas CA, Hanrahan AJ, Halilovic E, Persaud Y, Soh J, Chitale D, et al. Genetic predictors of MEK dependence in non–small cell lung cancer. Cancer research. 2008;68(22):9375-83.

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Frequency of adult attention deficit hyperactivity disorder (ADHD) in outpatient psychiatric clinic, Babol University of Medical Sciences

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Abstract

Introduction: Attention Deficit Hyperactivity Disorder (ADHD) in adulthood is associated with significant impairment in occupational, academic, and social functioning. The aim of this study is to survey the frequency of ADHD in adults referred to psychiatric clinics.

Methods: The present cross-sectional descriptive study includes 300 patients referred to psychiatric clinics affiliated to Babol University of Medical Sciences with an age range of 18-45 years who were selected and included in the study. It is used the adults Attention Deficit Hyperactivity Disorder self-report scale (ASRS V1.1) to diagnose Adult ADHD in these individuals. Logistic regression and P-Paired test were used to analyze the data.

Results: The mean age of the subjects was 30.21 ± 7 7.94. Of these, 181 (60.3%) were men and 119 (39.7%) were women. The overall prevalence of Adult ADHD in the study samples was 39.3%. In the logistic regression analysis of crude and adjusted data of study variables, no significant relationship was seen between Adult ADHD and age, education, employment status and marital status ($P \geq 0.05$), but a significant relationship between Adult ADHD and consumption of Cigarettes, alcohol and drugs were observed ($P \leq 0.05$).

Conclusion: The findings of the present study show a relatively high prevalence of Adult ADHD among people with a history of psychiatric disorder, who are more likely to be exposed to smoking, alcohol and drug abuse.

Keywords: Attention Deficit Hyperactivity Disorder, Mental Disorder, Adult

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Introduction

Adult ADHD is a type of psychiatric disorder that often appears in childhood. It is characterized by a stable pattern of attention deficit, hyperactivity, and impulsive behaviors (1). Impulsive behaviors are performed momentarily, without thinking about their results and without analyzing and evaluating positive and negative achievements. Attention-deficit/hyperactivity disorder is known as a childhood disorder that can be improved with supervision and treatment, but many sources have shown that ADHD is stable in adulthood and difficult to treat (2,3). Executive functions that may be abnormal in adults with ADHD include working memory, task switching, self-monitoring, initiation, and self-control. These deficits are related to the attention deficit. Characterize Adult ADHD problems: concentrating on a specific task, especially for long periods, organizing activities, prioritizing tasks, following up and completing tasks, forgetfulness, time management (as example of missing an appointment) (4). Adults with Adult ADHD often report that things only get done at the last minute, often late or not at all. An increase in driving-related problems, including an increase in driving errors, traffic fines, and speeding, may be related to attention deficits (5).

Adults with ADHD have higher rates of employment problems, criminal activity, substance abuse problems, accidents, and vehicular referrals compared to adults without ADHD. It is believed that ADHD-related disorders from childhood such as academic problems, self-esteem problems significantly in family and peer relationships underlie these behavioral problems in adulthood (6). Mortality rates were higher than in people with ADHD compared to people without ADHD in a study in 2015 using Danish national registry data, with accidents being the most common cause of death in people with ADHD (7).

ADHD is a common disorder among young people worldwide. In a study in 2007, a meta-analysis of more than 100 studies estimated the prevalence of ADHD in children and adolescents worldwide at 5.3% (8). Simon et al., found the prevalence of ADHD to be 2.5% in adults based on a meta-analysis of six studies (9). Prospective longitudinal studies support the theory that approximately two-thirds of adolescents with ADHD retain symptoms of the disorder into adulthood (10). Recent changes in the new DSM-5 diagnostic criteria have increased the prevalence of ADHD, which is less significant for children but has likely had a significant impact on diagnosis rates in adults (11, 12). In a World Health Organization survey, among respondents aged 18 to 44 in ten countries in the Americas, Europe, and the Middle East, the current prevalence of ADHD in adults was assessed 3.4%, which was 1.9% in low-income countries and 4.2% in high-income countries (13).

ADHD is associated with a number of psychiatric illnesses. It has been reported that approximately 80% of adult ADHD patients have at least one lifelong psychiatric illness in adults. Major depressive disorder (MDD) is the most common comorbidity (prevalence 24.4 to 31%) (14). Anxiety disorders are also a common disease of adult ADHD patients. In the general population, the prevalence of any anxiety disorder approaches 20%, while this figure increases to 47% in adults with Adult ADHD (15). In addition, 65% of adult patients with adult ADHD and concurrent bipolar disorder have a history of at least one anxiety disorder during their lifetime (16). Substance abuse is another common comorbidity seen in adult ADHD patients, who may use alcohol, drugs, and nicotine as a form of self-medication (17). It has been estimated that approximately one-fourth of people with substance use disorder (SUD) have co-occurring ADHD, and in addition, they have a worse treatment prognosis compared to substance abusers without adult ADHD (18). There is evidence that ADHD treatment in childhood or adolescence may reduce the severity and course of substance use disorders in adulthood (19).

Perhaps the most serious aspect of ADHD lies in its tendency to be associated with disorders, some of which affect not only behavior but also personality. This not only endangers the well-being and life of ADHD sufferers but also their social environment. These comorbidities are seen in children and if not resolved in late puberty, they can turn into personality disorders such as antisocial personality disorder or continue as extroversion disorders until adulthood (20). Sleep disorders are another comorbidity that affects children with ADHD at a much higher level than developing children (21). In addition, these sleep

disturbances can exacerbate ADHD symptoms such as inattention and motor dysfunction even more (22).

Vnukova et al., in 2020 conducted a study in the Czech population to investigate the prevalence of ADHD among adults. It was observed that 119 (7.84%) of 1518 people were diagnosed with ADHD based on the ASRS questionnaire. Also, the rate of ADHD was higher in men than in women. The age of subjects was also related to ASRS score (23). In 2018, Valsecchi et al conducted a study to determine the prevalence and clinical correlates of Adult ADHD in a sample of psychiatric outpatients. Their study included 634 outpatients and they used the ASRS questionnaire and DIVA specialized calculator to diagnose ADHD. The findings of the study showed that 12.8% of people were considered ADHD-positive in the ASRS questionnaire and 6.9% of people based on the DIVA specialized interview (24). According to the mentioned issues, Adult ADHD has a great psychological and social burden for the individual and society. On the other hand, due to the relationship between Adult ADHD and various comorbidities, it is possible to reduce the psychological and social burden of the disease in people who go to the doctor because of other psychiatric disorders. In addition, due to its relatively high prevalence in adults and the fact that in many children, the symptoms continue until adulthood, and in the adult period, less importance is given to its diagnosis and treatment, and this issue can have many consequences for the affected person, his/her family and the community and cause problems such as job problems, marital problems and delinquency. The present study aims to investigate the frequency of adult attention deficit hyperactivity disorder (Adult ADHD) in outpatients of the psychiatric clinic of Babol University of Medical Sciences.

Methods

The current descriptive-cross-sectional study was conducted with the aim of investigating the frequency of adult attention deficit hyperactivity disorder (Adult ADHD) in the outpatients of the psychiatric clinic of Babol University of Medical Sciences. The research samples were selected using available sampling method, including 300 patients who visited the psychiatric clinics of Babol University of Medical Sciences as outpatients in the period of autumn 2019. Inclusion criteria include the age range of 18 to 45 years, absence of severe mental disability and psychotic disorder, cognitive impairment, willingness and consent to participate in the study. The only exclusion criterion of the study includes unwillingness to continue cooperating in the study.

Sample volume calculation formula:

n = sample size = 300

$$n = \frac{z_{1-\alpha/2} \times p(1-p)}{d^2}$$

(1)

In relationship (1)

α = 0.05

p =0.1

d =0.025

z = percentage of standard error of the acceptable confidence factor

p = proportion of the population with a given trait

q=1-p A proportion of the population without a certain trait

α= degree of confidence or desired possible accuracy

d= maximum sampling accuracy

Sampling and distribution of the questionnaire was done after obtaining permission from the Vice-Chancellor of Research and obtaining a research permit and code of ethics IR.MUBABOL.REC.1399.242 and obtaining permission from the responsible director and head of the psychiatric clinic of Babol University of Medical Sciences and explaining the research objectives to them. The questionnaires were completed by the researcher himself, and in order to preserve the confidentiality of the information of the research samples, the questionnaires were without names. The reason for using this method is that if the samples had problems in understanding the sentences of the questionnaire, sufficient explanations would be given to them. First, the research samples were talked to and the necessary explanations were given to these people about the research, its necessity and benefits.

Then, if they were satisfied and completed the written consent form, they answered the study questionnaires. In the present study, there are two questionnaires, in which form number 1 deals with the demographic characteristics of the individual, and in form number 2, all patients completed the self-report questionnaire of adult attention deficit hyperactivity disorder (ASRS-v.1.1).

Demographic information including age, sex, occupation, education, as well as clinical records such as physical illness records, psychiatric illness and hospitalization records, history of referral or treatment for ADHD in childhood, duration of drug use, type of drug used were collected from all patients. The Adult Attention Deficit Hyperactivity Disorder Self-Report Scale (ASRS-v.1.1) was developed by the World Health Organization (WHO) and a working group consisting of teams of psychiatrists and researchers from the World Health Organization. ASRS scale questions are consistent with DSM-5 criteria. This scale includes two dimensions and 18 questions, which are divided into two parts, A and B. There are 9 questions for the dimension of inattention and 9 questions for the dimension of hyperactivity/impulsivity. Research questions are scored on a 5-point Likert scale from never (1 point) to almost always (5 points). In a study conducted in Iran by Mokhtari et al., the reliability of the questionnaire using Cronbach's alpha method was 87%. Also, the sensitivity of this questionnaire with a cut-off point of 50 for diagnosing ADHD in adults is 70% and the specificity of this questionnaire is 99% (25).

After completing the questionnaires by the research samples, the score of the ASRS questionnaire is calculated and people with a score less than 50 are considered not suffering from Adult ADHD, and people with a score of 50 and above are considered suffering from Adult ADHD.

The resulting data were entered into SPSS statistical software version 24 and evaluated quantitatively and qualitatively. The significant level of the test will be less than 0.05 ($p<0.05$). P-Paired and Chi-square statistical tests were used to analyze the data.

Results

This cross-sectional study was conducted on 300 people who referred to psychiatric clinics affiliated to Babol University of Medical Sciences to determine the frequency of Adult ADHD in adults. All the study samples have answered the answer letters completely and are in accordance with the entry and exit criteria of the study. For this reason, we did not have a sample excluded from the study.

The average age of the subjects was 30.21 years with a standard deviation of 7.94. 181 cases (60.3%) were men and 119 cases (39.7%) were women. 43 cases (14.3%) had a bachelor's degree, 103 cases (34.3%) had diploma education and 154 cases (51.3%) had higher education. 214 cases (71.3%) were employed and the rest were unemployed, 157 cases (52.3%) were single and 143 cases (47.7%) were married. In addition, 51 cases (17%) had a history of using at least one of tobacco, alcohol or drugs. In connection with the use of the mentioned items, 49 cases (16.3%) used tobacco, 17 cases (5.7%) used alcohol, and 15 cases (5%) also used drugs.

Logistic regression analysis was used to investigate adult ADHD and risk factors affecting it. People with a score of 50 and above were considered to have Adult ADHD and people with a score below 50 were considered not to have Adult ADHD. Also, the average adult ADHD score of people in general was 46.7 with a standard deviation of 11.08 and 118 people (39.3%) had adult ADHD and 182 people (60.7%) did not have adult ADHD.

The findings related to data analysis and their relationship with adult ADHD are given in Table 1 and Figure 1.

Table 1. Review of risk factors related to Adult ADHD as raw data and adjusted data.

Variable		ADHD		Crude		Adjusted	
		+	-	OR CI 95%	P-value	OR CI 95%	P-value
Gender	Male	76 (42%)	105 (58%)	1	0.24	1	0.79
	Female	42 (35.3%)	77 (64.7%)	0.75 (0.1-46.21)		1.09 (0.2-55.15)	

Education	Diploma	42 (40.8%)	61 (59.2%)	1	-	1	-
	High school	19 (44.2%)	24 (55.8%)	0.87 (0.1-42.78)	0.70	0.57 (0.1-25.25)	0.16
	Above diploma	57 (37%)	97 (63%)	0.74 (0.1-37.47)	0.39	0.46 (0.1-21.01)	0.055
Employment	Unemployed	27 (31.4%)	59 (68.6%)	1	0.07	1	0.43
	Employed	91 (42.5%)	123 (57.5%)	1.61 (0.2-95.74)		1.36 (0.2-62.98)	
Marital Status	Single	71 (45.2%)	86 (54.8%)	1	0.02	1	0.20
	Married	47 (32.9%)	96 (67.1%)	0.59 (0.0-37.94)		0.65 (0.1-34.24)	
Substance abuse	+	29 (56.9%)	22 (43.1%)	2.371 (1.4-28.36)	0.005	2.37 (1.4-24.53)	0.009
	-	89 (35.7%)	160 (64.3%)				
Age		-	-	0.97 (0.1-94)	0.051	0.97 (0.1-94.02)	0.31

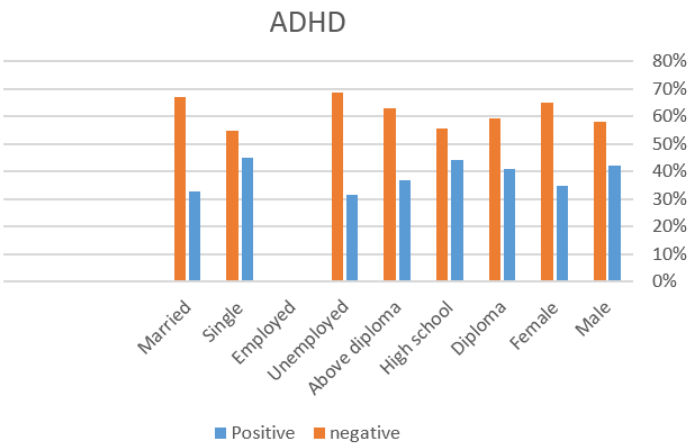

Figure 1: Review of risk factors related to Adult ADHD.

Discussion and Conclusion

The data obtained from the present study show the prevalence of Adult ADHD in psychiatric clinic outpatients at 39.3%. The prevalence rate obtained in the present study is higher than in similar studies. In a study conducted by Valsecchi et al. on Italian psychiatric outpatients, the overall prevalence of adult ADHD was reported as 6.9% (24). In order to evaluate patients with Adult ADHD, they subjected the patients who were diagnosed with Adult ADHD in the ASRS-V1.1 questionnaire to a specialized interview. So, patients were screened more intensively than using a questionnaire alone.

In other community-based studies, the observed prevalence is lower than in the present study. Polyzoi et al., and 2018 (26) On a Swedish population, the prevalence rate in people aged 18 years and older was estimated to be 3.54 per 1000 people. Vňuková et al., in 2021 (23). In the Czech Republic, the prevalence of Adult ADHD was assessed as 7.84% based on the 6-item ASRS questionnaire. On the other hand, the 6-item ASRS questionnaire shows that the reported rate is lower than the actual level (27), for this reason, the predicted prevalence in this study was evaluated as 14%. De Zwaan et al., in 2012, the raw prevalence rate of ADHD was evaluated as 4.7% (28). Ghoreishizadeh et al., in 2014, the prevalence rate in adults aged 18-45 years was evaluated as 3.8% (29).

The relatively high prevalence of observation in the present study can have various reasons. The people who entered the study were selected as available and from the population with psychiatric disorders. On the other hand, in a random sample of people who have a psychiatric illness, they may have a high percentage of Adult ADHD. According to the studies, Adult ADHD can be associated with other psychiatric diseases such as substance use disorder, mood disorders (such as depression and bipolar disorder) and anxiety disorders. ADHD and dysthymic disorder/depression are commonly associated, and the prevalence of depression in individuals with ADHD varies from 18.6% to 53.3%

in different studies (30, 31). Similarly, studies have reported comorbid ADHD in individuals with depression at rates of 9% to 16% (32), with an average incidence of 7.8% (33). The risk of anxiety disorders in people with ADHD is higher than in the general population, approaching 50% (28). Probably the most common comorbidity with ADHD is substance use disorder (SUD), especially alcohol or nicotine, cannabis, and cocaine (34).

Reports indicate that personality disorders are present in more than 50% of adults with Adult ADHD, usually cluster B and C personality disorders, and 25% of individuals have two or more personality disorders (35).

Also, there are studies showing the relationship between ADHD and bipolar disorder (36), sleep disorders (21), obesity (37), Internet addiction, virtual networks and video games (38). In other studies that have been conducted on adults with Adult ADHD, the age limit was 18 years and above, which also included the elderly, based on previous studies, with increasing age, especially in old age, the rate of adult ADHD decreases, while in the present study, the upper age limit of the people who entered the study was 45 years. Also, the average age of the people included in the present study was lower compared to the reviewed studies (20,21,39). The error in the answer should also be taken into account.

In the present study, the prevalence of adult ADHD was not statistically related to the age of the subjects. While in the study of Valsecchi et al., (24) in 2021, people with Adult ADHD were younger than people without Adult ADHD. Also, in a study, De zwaan et al. reported a decrease in the prevalence of ADHD with increasing age. However, De Zwaan et al., (29) observed a significant difference between the age groups of 18 to 24 years and 55 to 64 years, while the other age groups did not have a significant difference, which is similar to the age group of present study (28).

In the present study, there was no correlation between the prevalence of adult ADHD and gender. There is evidence that shows that the rate of ADHD in boys is 2 to 3 times higher than that of girls, but in adults, this ratio tends to equalize in the studies conducted (24). Also, in the study of Polyzoi et al., similar findings were observed with the present study, and no difference was observed in the incidence of Adult ADHD between men and women (26). On the other hand, a study conducted by Zetterqvist et al., (24) showed a higher prevalence in adult men. Because Zetterqvist's study was conducted between 2006 and 2009, it can be concluded that the proportion of women with adult ADHD has increased over time.

However, these results do not mean that the graduate education level is a protective factor against Adult ADHD. Various studies show a moderate association between IQ and attention deficits (40), and the diagnosis of ADHD has the same validity among children with high IQ and children with average IQ. It can be concluded that people diagnosed with Adult ADHD who have a high level of education compensate for their functional deficit due to ADHD with a lower IQ compared to their peers.

In the present study, a significant relationship between drug use and adult ADHD was observed, and 56.9% of people who use drugs have adult ADHD. This finding is expected, as ADHD is typically associated with risky behaviors and decision-making problems (41). The present finding has also been shown in various studies (24, 28, 42) In descriptive reports and demographic studies, adult ADHD patients have described marijuana as helpful in controlling inattention and impulsivity (43, 44). In a study conducted by Notzon et al. (45), the prevalence of marijuana use was estimated at 34-46%. ROMO et al. also observed a higher rate of marijuana use, alcohol use, and gambling in people with Adult ADHD (46).

In examining the relationship between marital status and adult ADHD, our findings did not show a significant relationship, which is consistent with the observations of Valsecchi et al. (24). But they observed that most people with Adult ADHD are single and less likely to have a partner. In some previous studies, no significant relationship between marital status and adult ADHD has been observed (2, 13, 29). Some studies show that the prevalence of Adult ADHD is higher in divorced people (15, 47). Also, in another study, the prevalence of Adult ADHD was higher in widowed, divorced and single people (48). But, considering the effect of Adult ADHD in adults and in their intimate relationships, which leads to less stability

and higher divorce rates, judging the impact of this disorder on marital status may require longer follow-ups. In the investigation of being employed, no significant relationship between being employed and Adult ADHD was observed, as some studies had similar results (24, 28). While other studies have shown a higher prevalence of Adult ADHD in unemployed people (15, 48).

The main limitation of the present study is the absence of a secondary follow-up for the additional examination of people who are diagnosed with Adult ADHD in the ASRS-v1.1 questionnaire due to the COVID-19 pandemic and quarantine restrictions, as well as the lack of cooperation of the patients in the circumstances. Considering the high prevalence that was observed, maybe a more detailed investigation by dedicated interviews could have brought us more accurate results. For example, Valsecchi et al. (24) subjected patients who scored above 50 in the ASRS-v1.1 questionnaire to a dedicated DIVA interview. The next limitation is the insufficient sample size, which may have caused the variables related to Adult ADHD to not be evaluated correctly. On the other hand, we did not have any information about the underlying disease and the history of ADHD or ADHD symptoms in the childhood of these people. The findings of the present study show that the prevalence of Adult ADHD is high among patients who refer to the Babol Psychiatric Clinic as an outpatient. This shows the importance of using appropriate screening methods in these people, early diagnosis and treatment. Also, people who have a history of using tobacco and/or alcohol and/or especially drugs, or are currently using them, should be screened for Adult ADHD so that we can prevent more problems for them with early diagnosis.

Authors contributions

SJ conceived and designed the analysis, **SMZ** collected the data, **MA** contributed data or analysis tools, **HGh** wrote the paper, **AM** performed the analysis.

References

1. Kaisari P., Dourish C.T., Higgs S. Attention Deficit Hyperactivity Disorder (ADHD) and disordered eating behaviour: A systematic review and a framework for future research. Clinical psychology review, 2017; 53:109-21.

2. De Graaf R., Kessler R.C., Fayyad J., Ten H. M., Alonso J., Angermeyer M. The prevalence and effects of adult attention-deficit/hyperactivity disorder (ADHD) on the performance of workers: results from the WHO World Mental Health Survey Initiative. Occupational and environmental medicine, 2008; 65(12):835-42.

3. Amira H., Mohammed Hala A., Abdel G., Ahmed M.M.M. Gross motor development in Egyptian preschool children with attention deficit hyperactivity disorder: Pilot study. Journal of Advanced Pharmacy Education & Research, 2017; 7(4): 438- 442.

4. Barkley, R. A. Executive functions: What they are, how they work, and why they evolved. The Guilford Press; 2012.

5. Barkley R.A., Fischer M., Smallish L., Fletcher K. Young adult outcome of hyperactive children: adaptive functioning in major life activities. Journal of the American academy of child & adolescent psychiatry. 2006; 45(2):192-202.

6. Barkley R.A., Cox D. A review of driving risks and impairments associated with attention-deficit/hyperactivity disorder and the effects of stimulant medication on driving performance. Journal of safety research. 2007; 38(1):113-28.

7. Dalsgaard S, Østergaard SD, Leckman JF, Mortensen PB, Pedersen MG. Mortality in children, adolescents, and adults with attention deficit hyperactivity disorder: a nationwide cohort study. The Lancet. 2015;385(9983):2190-6.

8. Polanczyk G., Maurício S.L., Bernardo L. H., Joseph B., Luis A. R. The Worldwide Prevalence of ADHD: A Systematic Review and Metaregression Analysis. American Journal of Psychiatry, 2007;164(6):942-8.

9. Simon V., Czobor P., Bálint S., Mészáros A., Bitter I. Prevalence and correlates of adult attention-deficit hyperactivity disorder: meta-analysis. The British Journal of Psychiatry. 2009;194(3):204-11.

10. Faraone S.V., Biederman J., Mick E. The age-dependent decline of attention deficit hyperactivity

disorder: a meta-analysis of follow-up studies. Psychological medicine, 2006; 36(2):159-65.

11. Polanczyk G., Caspi A., Houts R., Kollins S.H., Rohde L.A., Moffitt T.E. Implications of extending the ADHD age-of-onset criterion to age 12: results from a prospectively studied birth cohort. Journal of the American Academy of Child & Adolescent Psychiatry, 2010; 49(3):210-6.

12. Matte B., Anselmi L., Salum G., Kieling C., Gonçalves H., Menezes A. ADHD in DSM-5: a field trial in a large, representative sample of 18-to 19-year-old adults. Psychological medicine. 2015; 45 (2):361-73.

13. Fayyad J., De Graaf R., Kessler R., Alonso J., Angermeyer M., Demyttenaere K. Cross-national prevalence and correlates of adult attention-deficit hyperactivity disorder. The British Journal of Psychiatry, 2007;190(5):402-9.

14. Fischer A.G., Bau C.H., Grevet E.H., Salgado C.A., Victor M.M., Kalil K.L.. The role of comorbid major depressive disorder in the clinical presentation of adult ADHD. Journal of psychiatric research, 2007;41(12):991-6.

15. Kessler R.C., Adler L., Barkley R., Biederman J., Conners C.K., Demler O. The prevalence and correlates of adult ADHD in the United States: results from the National Comorbidity Survey Replication. American Journal of psychiatry, 2006; 163(4):716-23.

16. Tamam L., Karakus G., Ozpoyraz N. Comorbidity of adult attention-deficit hyperactivity disorder and bipolar disorder: prevalence and clinical correlates. European archives of psychiatry and clinical neuroscience, 2008; 258:385-93.

17. Ohlmeier M.D., Peters K., Kordon A., Seifert J., Wildt B.T., Wiese B. Nicotine and alcohol dependence in patients with comorbid attention-deficit/hyperactivity disorder (ADHD). Alcohol & Alcoholism, 2007; 42(6):539-43.

18. Dom G., Moggi F. Co-occurring addictive and psychiatric disorders: Springer; 2016.

19. Groenman A.P., Oosterlaan J., Rommelse N.N., Franke B., Greven C.U., Hoekstra P.J.Stimulant treatment for attention-deficit hyperactivity disorder and risk of developing substance use disorder. The British Journal of Psychiatry, 2013;203(2):112-9.

20. Silva D., Houghton S., Hagemann E., Jacoby P., Jongeling B., Bower C. Child attention deficit hyperactive disorder co morbidities on family stress: effect of medication. Community mental health journal, 2015;51:347-53.

21. Cortese S., Faraone S.V., Konofal E., Lecendreux M. Sleep in children with attention-deficit/hyperactivity disorder: meta-analysis of subjective and objective studies. Journal of the American Academy of Child & Adolescent Psychiatry, 2009; 48(9):894-908.

22. Schneider H.E., Lam J.C., Mahone E.M. Sleep disturbance and neuropsychological function in young children with ADHD. Child Neuropsychology, 2016; 22(4):493-506.

23. Vňuková M., Ptáček R., Děchtěrenko F., Weissenberger S., Ptáčková H., Braaten E. Prevalence of ADHD symptomatology in adult population in the Czech Republic–A National Study. Journal of Attention Disorders. 2021; 25(12):1657-64.

24. Valsecchi P., Nibbio G., Rosa J., Tamussi E., Turrina C., Sacchetti E. Adult ADHD: prevalence and clinical correlates in a sample of Italian psychiatric outpatients. Journal of Attention Disorders, 2021; 25(4):530-9.

25. Mokhtari H., Rabiei M., Salimi S.H. Psychometric properties of the persian version of adult attention-deficit/hyperactivity disorder self-report scale. Iranian Journal of Psychiatry and Clinical Psychology, 2015; 21(3):244-53.

26. Polyzoi M., Ahnemark E., Medin E., Ginsberg Y. Estimated prevalence and incidence of diagnosed ADHD and health care utilization in adults in Sweden–a longitudinal population-based register study. Neuropsychiatric disease and treatment, 2018:1149-61.

27. Kessler RC, Adler LA, Gruber MJ, Sarawate CA, Spencer T, Van Brunt DL. Validity of the World Health Organization Adult ADHD Self-Report Scale (ASRS) Screener in a representative sample of health plan members. International journal of methods in psychiatric research, 2007; 16(2):52-65.

28. De Zwaan M., Gruss B., Müller A., Graap H., Martin A., Glaesmer H. The estimated prevalence and correlates of adult ADHD in a German community sample. Eur Arch Psychiatry Clin Neurosci, 2012; 262(1):79-86.

29. Amiri S., Ghoreishizadeh M.A., Sadeghi-Bazargani H., Jonggoo M., Golmirzaei J., Abdi S. Prevalence of adult attention deficit hyperactivity disorder (Adult ADHD): tabriz. Iranian journal of psychiatry, 2014; 9(2):83.

30. Torgersen T., Gjervan B., Rasmussen K. ADHD in adults: a study of clinical characteristics, impairment and comorbidity. Nordic journal of psychiatry, 2006; 60(1):38-43.

31. Research APADo. Highlights of changes from dsm-iv to dsm-5: Somatic symptom and related disorders. Focus, 2013;11(4):525-7.

32. McIntosh D., Kutcher S., Binder C., Levitt A., Fallu A., Rosenbluth M. Adult ADHD and comorbid depression: a consensus-derived diagnostic algorithm for ADHD. Neuropsychiatric disease and treatment, 2009:137-50.

33. Bond D.J., Hadjipavlou G., Lam R.W., McIntyre R.S., Beaulieu S., Schaffer A. The Canadian Network for Mood and Anxiety Treatments (CANMAT) task force recommendations for the management of patients with mood disorders and comorbid attention-deficit/hyperactivity disorder. Ann Clin Psychiatry, 2012; 24(1):23-37.

34. Klassen L., Bilkey T., Katzman M., Chokka P. Comorbid attention deficit/hyperactivity disorder and substance use disorder: treatment considerations. Current drug abuse reviews, 2012; 5(3):190-8.

35. Olsen J.L., Reimherr F.W., Marchant B.K., Wender P.H., Robison R.J. The effect of personality disorder symptoms on response to treatment with methylphenidate transdermal system in adults with attention-deficit/hyperactivity disorder. The Primary Care Companion for CNS Disorders, 2012;14(5):26293.

36. Michelini G., Kitsune V., Vainieri I., Hosang G.M., Brandeis D., Asherson P. Shared and disorder-specific event-related brain oscillatory markers of attentional dysfunction in ADHD and bipolar disorder. Brain topography, 2018;31:672-89.

37. Cortese S., Moreira-Maia C.R., Fleur D., Morcillo-Peñalver C., Rohde L.A., Faraone S.V. Association between ADHD and obesity: a systematic review and meta-analysis. American Journal of psychiatry, 2016;173(1):34-43.

38. Romo L., Rémond J.J., Coeffec A., Kotbagi G., Plantey S., Boz F. Gambling and Attention Deficit Hyperactivity Disorders (ADHD) in a population of french students. Journal of Gambling Studies, 2015;31:1261-72.

39. Sobanski E., Banaschewski T., Asherson P., Buitelaar J., Chen W., Franke B. Emotional lability in children and adolescents with attention deficit/hyperactivity disorder (ADHD): clinical correlates and familial prevalence. Journal of Child Psychology and Psychiatry, 2010; 51(8):915-23.

40. Jepsen J.R.M., Fagerlund B., Mortensen E.L. Do attention deficits influence IQ assessment in children and adolescents with ADHD? Journal of Attention Disorders, 2009;12(6):551-62.

41. Dekkers T.J., Popma A., Rentergem J.A.A., Bexkens A., Huizenga H.M. Risky decision making in attention-deficit/hyperactivity disorder: A meta-regression analysis. Clinical psychology review, 2016;45:1-16.

42. Bachmann C.J., Philipsen A., Hoffmann F. ADHD in Germany: trends in diagnosis and pharmacotherapy: a country-wide analysis of health insurance data on attention-deficit/hyperactivity disorder (ADHD) in children, adolescents and adults from 2009–2014. Deutsches Ärzteblatt International, 2017;114(9):141.

43. Loflin M., Earleywine M., De Leo J., Hobkirk A. Subtypes of attention deficit-hyperactivity disorder (ADHD) and cannabis use. Substance use & misuse, 2014;49(4):427-34.

44. Wilens T.E. Impact of ADHD and its treatment on substance abuse in adults. Journal of Clinical Psychiatry, 2004;65:38-45.

45. Notzon D.P., Pavlicova M., Glass A., Mariani J.J., Mahony A.L., Brooks D.J. ADHD is highly

prevalent in patients seeking treatment for cannabis use disorders. Journal of attention disorders, 2020;24(11):1487-92.

46. Romo L., Ladner J., Kotbagi G., Morvan Y., Saleh D., Tavolacci M.P. Attention-deficit hyperactivity disorder and addictions (substance and behavioral): Prevalence and characteristics in a multicenter study in France. Journal of Behavioral Addictions, 2018;7(3):743-51.

47. Sobanski E., Brüggemann D., Alm B., Kern S., Deschner M., Schubert T. Psychiatric comorbidity and functional impairment in a clinically referred sample of adults with attention-deficit/hyperactivity disorder (ADHD). European archives of psychiatry and clinical neuroscience, 2007;257:371-7.

48. Park S., Cho M.J., Chang S.M., Jeon H.J., Cho S.J., Kim B.S. Prevalence, correlates, and comorbidities of adult ADHD symptoms in Korea: results of the Korean epidemiologic catchment area study. Psychiatry Res, 2011;186(2-3):378-83.