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Review Article

Emerging strategies in cancer immunotherapy: Expanding horizons and future perspectives

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ABSTRACT

Cancer immunotherapy has revolutionized oncology by harnessing the body's immune system to target and eradicate malignant cells. This review delves into emerging strategies in cancer immunotherapy, focusing on novel approaches and future directions of this rapidly evolving field. Key areas of exploration include immune checkpoint inhibitors (ICIs), chimeric antigen receptor (CAR) T-cell therapy, and cancer vaccines. ICIs, which target proteins such as cytotoxic T-lymphocyte antigen-4 and programmed cell death-1/programmed cell death ligand 1, have shown significant efficacy in various cancers, transforming clinical outcomes. CAR-T cell therapy, with its ability to genetically modify T-cells to attack cancer cells, has demonstrated remarkable success in hematologic malignancies and is being adapted for solid tumors. Cancer vaccines designed to stimulate an immune response against specific tumor antigens are also advancing with promising clinical results. Despite these advances, challenges such as immunogenicity, side effects, and treatment resistance remain. This review provides a comprehensive overview of the latest developments, clinical trials, and future perspectives in cancer immunotherapy, highlighting the potential for these strategies to redefine cancer treatment.

Keywords: Cancer immunotherapy, Immune checkpoint inhibitors, CAR-T-cell therapy, Cancer vaccines, Oncology

INTRODUCTION

Cancer is a complex disease characterized by genomic instability, leading to the production of tumor antigens (TAs) recognized by the immune system.[1] The immune system, encompassing both innate and adaptive immunity, is essential for immunosurveillance, distinguishing between self and non-self, infiltrating the tumor microenvironment (TME), and influencing tumor progression[1,2] [Table 1]. While innate immune cells contribute to tumor suppression through direct killing or initiation of adaptive responses,[3,4] the adaptive immune system is essential for humoral and cell-mediated responses.[5,6]

Conventional cancer treatments such as surgery, chemotherapy, and radiation therapy, while effective to some degree, often carry significant side effects and limitations. This has led researchers to explore newer, safer, and more effective approaches, with immunotherapy emerging as a promising alternative. Immunotherapy leverages the body's natural immune system to target cancer cells, representing a major advancement in oncology.[7] Unlike conventional methods, immunotherapy enhances the body's defenses, offering a more targeted and less invasive treatment option. Over recent decades, immunotherapy has achieved notable success in treating cancer, revolutionizing the field.[7]

Table 1: Comparative analysis of immune system attributes: innate vs. adaptive Immunity

Characteristic	Innate immunity	Adaptive immunity
Specificity	Non-specific; responds to a broad range of pathogens	Highly specific; targets specific antigens on pathogens
Response time	Immediate; rapid response within minutes to hours	Delayed; takes days to weeks to develop after initial exposure
Memory cells	No memory cells; does not retain memory of pathogens	Possesses memory cells; retains memory of specific pathogens for faster response
Cell components	Includes macrophages, neutrophils, dendritic cells, NK cells	Includes B cells, T cells (CD4+ helper T cells, CD8+ cytotoxic T cells)
Recognition mechanism (receptors)	Uses pattern recognition receptors (PRRs) to detect common pathogen-associated patterns	Uses specific receptors on B and T cells to recognize unique antigens
Function	Provides initial defence, activates adaptive immunity	Provides long-term defence and immunological memory
Types of responses	Inflammatory response, phagocytosis, activation of complement system	Humoral immunity (B cells and antibodies) and cell-mediated immunity (T cells)
Duration of response	Short-lived; responds quickly but does not provide long-term protection	Long-lasting; can provide long-term immunity through memory cells
Evolutionary aspect	Evolutionarily older, found in all multicellular organisms	More recent, found in vertebrate

Advances in scientific research have enhanced our understanding of the immune system's interaction with cancer cells.[8] The development of cancer immunotherapies, based on insights into tumor escape mechanisms, represents a crucial breakthrough. These therapies work by reactivating the immune system's antitumor response, providing new hope for cancer patients.[9,10] Immunotherapies aim to target and eliminate cancer cells selectively, sparing healthy tissue. Key strategies include immune checkpoint blockade, adoptive cell therapy, and cancer vaccines, all of which have demonstrated the potential to improve patient outcomes significantly.[10]

In recent years, cancer treatment has been transformed by immunotherapy. The evolution of this field, from non-specific immune stimulation to precision medicine, has been driven by continuous innovation. Landmark developments such as immune checkpoint inhibitors (ICIs), adoptive cell therapies, and personalized cancer vaccines have reshaped the approach to cancer care. Early treatments using cytokines such as recombinant Interferon-gamma (IFN-γ) and interleukin 2 (IL 2) were notable milestones, especially in treating melanoma and renal cell carcinoma (RCC). However, the limited efficacy of interferon (IFN) alpha-2 led to its replacement by purine analogues in treating hairy cell leukemia.[11]

Monoclonal antibodies (mAbs) have become essential tools in cancer therapy, with their effectiveness in various cancers being continuously evaluated in adjuvant and initial treatments.[8] Today, more than a dozen immunotherapies are approved for cancer treatment [Table 2], including ICIs, lymphocyte-activating cytokines, chimeric antigen receptor (CAR) T-cell therapy, cancer vaccines, bispecific antibodies (BsAbs), oncolytic viruses, agonistic antibodies targeting co-stimulatory receptors, and other cellular therapies.

Despite these advancements, cancer immunotherapy faces several challenges. These include the low immunogenicity of cancer vaccines, unintended side effects of immunotherapeutics, and suboptimal outcomes from adoptive T-cell transfer therapies.[10] An emerging solution lies in the field of nanomaterials. Nanoparticle-based approaches are showing significant promise in enhancing the targeted delivery of TAs and therapeutics, improving immune activation, and increasing the efficacy of adoptive cell therapies.[12] One innovative development is a biomimetic nanoparticle platform that can directly stimulate T-cells without the need for professional antigen-presenting cells (APCs).[12]

The future of cancer immunotherapy appears promising as ongoing research focuses on refining immune response strategies against cancer.[12] This review aims to explore and analyze emerging strategies in immunotherapy, providing insights into recent advancements that are transforming cancer treatment. By reviewing the latest research, clinical trials, and technological innovations, this paper seeks to deepen the understanding of current immunotherapy practices and their potential to revolutionize cancer care. The review will critically assess novel approaches, address existing challenges, and explore future directions, contributing to the ongoing scientific dialogue and providing valuable perspectives for clinicians, researchers, and policymakers.

ICIS

ICIs represent a category of medications that have significantly transformed cancer therapy in recent times. Their application

Table 2: List of some of the approved immunotherapies for cancer

Therapy	Cancer treated	Year approved
Immune checkpoint inhibitors	Melanoma, Lung, Bladder, Kidney, Hodgkin's Lymphoma, etc.	2011 (ipilimumab), 2014 (pembrolizumab), 2014 (nivolumab), 2016 (atezolizumab), 2016 (avelumab), 2018 (durvalumab)
CAR-T cell therapy	B cell acute lymphocytic leukaemia, large B cell lymphoma and non-hodgkin lymphoma	2017 (tisagenlecleucel) 2018 (axicabtagene ciloleucel)
Oncolytic virus therapies	Melanoma	2015 (talimogene laherparepvec)
Cancer vaccines	Prostate, Bladder, Cervical, etc.	2010 (sipuleucel-T)
Cytokine therapies	Renal cell carcinoma, Melanoma, AIDS-related Kaposi sarcoma, follicular lymphoma and chronic myelogenous leukaemia	1992(Aldesleukin) 1986 (Roferon-A) 2004 (Imiquimod)
Bispecific antibodies	B cell acute lymphocytic leukaemia	2014 (Blinatumomab)
Targeted antibodies	Breast, Colon, Lung, etc.	1998 (Trastuzumab), 2004 (Bevacizumab) 2004 (Cetuximab)

in tumor treatment has been underlined by their extensive efficacy across diverse histological tumors, their consistent response stability, and their notable therapeutic impact even in cases of metastatic and chemotherapy-resistant malignancies.[13] These drugs function by targeting specific proteins found on either immune or cancer cells, which serve as "checkpoints" regulating the immune response. Immune checkpoints consist of cell-surface proteins, such as cytotoxic T-lymphocyte antigen-4 (CTLA4) and programmed cell death-1 (PD-1), which are pivotal in governing the initiation, duration, and intensity of immune responses.[14] On activation, these checkpoints can impede the effective attack of cancer cells by immune cells. By inhibiting these checkpoints, ICIs enable the immune system to better identify and eliminate cancer cells.

ICIs have revolutionized cancer treatment, demonstrating broad efficacy across various tumor types, including metastatic and chemotherapy-resistant cancers, due to their consistent response and therapeutic impact.[13] These inhibitors work by targeting immune checkpoint proteins, either on immune cells or tumor cells, that regulate the immune response. Key immune checkpoints, such as CTLA-4 and PD-1, play essential roles in controlling immune activation, persistence, and intensity.[14] When these checkpoints are activated, they can impair the immune system's ability to attack cancer cells effectively. ICIs block these inhibitory checkpoints, enabling the immune system to better recognize and destroy tumor cells.

The main strategies for immune checkpoint inhibition focus on CTLA-4 or cluster of differentiation (CD)*152*, and the interaction between PD-1 (CD279) and its ligand, programmed cell death ligand 1 (PD-L1) (CD274 or B7 homolog 1).[13] CTLA-4, located on the surface of T-lymphocytes, competes with CD28 for binding to the B7 ligand, regulating cytokine production, including Interleukin 1 (IL-1).[15] When *CTLA-4* is activated, it reduces the production of pro-inflammatory cytokines, limiting T-lymphocyte survival.[15] PD-1, present on activated T-cells, halts antigen recognition and signaling, with its ligands PD-L1 and programmed cell death ligand 2 (PD-L2*)* playing crucial roles. While PD-L2 is predominantly found on APCs, PD-L1 is expressed across a wide range of cell types, including tumor cells.[13] Activation of PD-1 inhibits T-lymphocyte proliferation, cytokine production and reduces T-lymphocyte survivability, allowing tumor cells to evade immune detection by expressing PD-L1.[15]

In recent years, significant attention has been devoted to comprehending cancer immunobiology and immunotherapy, fuelled by the clinical triumph of inhibitors to the immune checkpoint PD-1 and its ligands PD-L1 and PD-L2. This concentration has been steered by the clinical triumph of ICIs, such as those targeting the PD-1 pathway. These inhibitors, including those aimed at PD-1 and its ligands PD-L1 and PD-L2, have displayed immense potential in treating various cancer types.[16] They have proven particularly effective in cancers characterized by high levels of immune cell infiltration, PD-L1 expression on tumor cells, and elevated production of IFN and IFN-γ-induced genes.

Recent research highlights the synergy between ICIs and other treatment modalities. For example, studies have explored combining low-dose immunotherapy with conventional or metronomic chemotherapy, which involves frequent administration of low-dose chemotherapeutic agents without extended breaks.[17] Kallolli *et al.* (2023) demonstrated that low-dose capecitabine (500 mg twice a day) combined with ICIs (nivolumab 240 mg every 2 weeks) enhanced antitumor activity while minimizing systemic

toxicity.[18] This aligns with global evidence supporting low-dose strategies to prime the immune microenvironment and improve the efficacy of immunotherapy. These findings underscore the evolving integration of ICIs into multimodal cancer treatment strategies, driving advancements in cancer immunotherapy.

Clinical indications and mechanism

CTLA-4 inhibitors

CTLA-4 inhibitors work by blocking the interaction between CTLA-4 and its ligands CD80 and CD86, thereby enhancing T-cell activation and triggering a stronger immune response against tumors. These inhibitors have been clinically approved for treating various cancers, including melanoma, RCC, bladder cancer, and advanced non-small-cell lung cancer.

Ipilimumab, the first anti-CTLA-4 antibody, was approved by the United States (US) Federal Drug Administration (FDA) in 2011 in 2011 for treating advanced melanoma.[15] Since then, six additional ICIs have gained approval, including three anti-PD-1 and three anti-PD-L1 antibodies [Table 3]. Ipilimumab has significantly improved overall survival (OS) in patients with advanced melanoma. Phase III trials demonstrated its superiority over chemotherapy as a first-line treatment and over a less effective vaccine in second-line therapy.[14] Ipilimumab works by lifting T-cell suppression, facilitating T-cell activation and proliferation, and increasing the diversity of T-cell populations by blocking the CD28-B7 costimulatory pathway, thereby broadening the T-cell repertoire.[19]

PD-1 inhibitors and PD-L1 inhibitors

PD-1 inhibitors belong to a group of immunotherapy drugs that have demonstrated significant clinical advantages in treating different forms of cancer. These drugs function by obstructing the PD-1 receptor found on activated T-cells, thereby preventing its interaction with its ligands, PD-L1 and PD-L2. On the other hand, PD-L1 inhibitors represent another category of immunotherapy agents that target the PD-L1 protein. These inhibitors operate by impeding the connection between PD-L1 and its receptor, PD-1, on immune cells. This interaction between PD-1 and its ligands causes a suppression of T-cell activity, enabling cancer cells to evade detection by the immune system. Through the inhibition of the PD-1 pathway, PD-1 inhibitors not only boost the immune response against tumors but also reinstate the functionality of T-cells. The approved clinical

Table 3: List of CTLA-4 inhibitors, Anti-PD-1, and Anti-PD-L1 agents

Drug name	Year of approval	Indications	Treatment regimen
CTLA-4 inhibitors			
Ipilimumab	2011	Advanced melanoma, renal cell carcinoma, bladder cancer, non-small cell lung cancer	Monotherapy: 3 mg/kg every 3 weeks for 4 doses, followed by 3 mg/kg every 12 weeks
Tremelimumab	2020	Metastatic melanoma, mesothelioma	Monotherapy: 15 mg/kg/90 days
Tocilizumab	2021	Advanced melanoma, bladder cancer, lung cancer, renal cell carcinoma	Monotherapy: 8 mg/kg every 4 weeks or 4 mg/kg every 2 weeks
Anti-PD-1			
Pembrolizumab	2014	Melanoma, non-small cell lung cancer, head and neck squamous cell carcinoma, classical Hodgkin lymphoma, urothelial carcinoma, microsatellite instability-high or mismatch repair deficient cancers, gastric cancer, cervical cancer	Monotherapy: 200 mg every 3 weeks
Nivolumab	2014	Melanoma, non-small cell lung cancer, renal cell carcinoma, Hodgkin lymphoma, head and neck squamous cell carcinoma, urothelial carcinoma, microsatellite instability-high or mismatch repair deficient cancers, gastric cancer, hepatocellular carcinoma, colorectal cancer	Monotherapy: 240 mg every 2 weeks
Cemiplimab	2018	Cutaneous squamous cell carcinoma (CSCC), advanced basal cell carcinoma (BCC)	Monotherapy: 350 mg every 3 weeks
Anti-PD-L1			
Atezolizumab	2016	Urothelial carcinoma, non-small cell lung cancer, triple-negative breast cancer, small cell lung cancer, hepatocellular carcinoma, gastric or gastroesophageal junction adenocarcinoma	Monotherapy: 1200 mg every 3 weeks
Durvalumab	2016	Urothelial carcinoma, non-small cell lung cancer	Monotherapy: 10 mg/kg every 2 weeks
Avelumab	2015	Merkel cell carcinoma, urothelial carcinoma, gastric cancer, non-small cell lung cancer, renal cell carcinoma, ovarian cancer	Monotherapy: 10 mg/kg every 2 weeks

uses for PD-1 inhibitors and PD-L1 inhibitors cover various cancer types, including melanoma, non-small-cell lung cancer, RCC, and Hodgkin's lymphoma (HL). Furthermore, ongoing investigations explore their potential efficacy in addressing other malignancies, such as head-and-neck squamous cell carcinoma (HNSCC) and various solid tumors.

Nivolumab, a PD-1 inhibitor, was approved by the FDA in 2014 for treating metastatic or unresectable melanoma, particularly in patients who failed ipilimumab treatment or had *B-Raf proto-oncogene* (*BRAF*) V600 mutation-positive tumors or disease progression post-*BRAF* inhibitor therapy.[20] Early trials of nivolumab showed durable tumor shrinkage in patients with advanced solid tumors, including melanoma.[19] Building on these results, subsequent investigations into a multi-dose regimen of nivolumab revealed that around 20–30% of patients with advanced, treatment-resistant melanoma, non-small-cell lung cancer, or kidney cancer experienced objective tumor shrinkage.[21] In India, efforts to optimize nivolumab's therapeutic benefits and cost-effectiveness have garnered attention. John *et al*. (2023) highlighted the advantages of weight-based dosing, showing that it maintains clinical efficacy while significantly reducing financial toxicity, thereby improving treatment accessibility.[22] Similarly, Patel *et al*. (2024) conducted a retrospective analysis that found weight-based dosing achieved comparable outcomes to fixed-dose regimens but at a lower cost, making immunotherapy more feasible for patients in low- and middle-income countries.[23] Further advancements in treatment strategies include combining low-dose nivolumab with metronomic chemotherapy. A study by Kate *et al*. (2024) demonstrated this combination improved OS in an Indian cohort of patients with advanced head-and-neck cancer, a population significantly affected by tobacco-related malignancies.[24] Similarly, clinical trial results of comparing the OS of patients treated with traditional metronomic chemotherapy combined with a low dose (20 mg) of nivolumab in HNSCC demonstrated superiority in OS compared to the traditional metronomic chemotherapy.[25] These findings underscore the potential of tailored dosing and combination therapies to expand access and improve outcomes for patients in resource-constrained settings**.** Pembrolizumab, a humanized monoclonal IgG4 kappa antibody that blocks the interaction between PD-1 and its ligands, allowing cytotoxic T cells to recognize and attack tumour cells more effectively.[26] It received FDA approval in 2016 for treating recurrent or metastatic HNSCC in patients whose disease has progressed following platinum-containing chemotherapy.[20] Initially administered as a weight-based dose of 2 mg/kg – since higher doses did not improve efficacy – Pembrolizumab later shifted to a flat dosing regimen of 200 mg every 3 weeks. While this fixed dosing approach was implemented for simplicity, it has faced criticism for potential cost inefficiencies without offering advantages in controlling pharmacokinetic (PK) variability. Current evidence indicates that both weight-based and fixed-dose regimens are viable options for pembrolizumab.[27] However, PK modeling suggests that reverting to weight-based dosing of pembrolizumab could preserve therapeutic efficacy while significantly reducing overall treatment costs.

Pidilizumab (CT-011), a humanized IgG-1κ mAb targeting PD-1, has shown preclinical efficacy in inhibiting tumor growth in various cancers, including melanoma, lymphoma, lung, colon, and breast cancers, as well as prolonging survival in mouse models.[28]

Atezolizumab, a modified humanized monoclonal immunoglobulin G1 antibody, specifically binds to PD-L1, preventing its interaction with PD-1 and B7-1, while leaving the interaction between PD-L2 and PD-1 intact.[29] This alteration eliminates antibody-dependent cell-mediated cytotoxicity, thus avoiding potential loss of PD-L1-expressing T-effector cells and reducing anticancer immunity.[23] In clinical trials, atezolizumab has demonstrated enduring responses in a group of patients with metastatic bladder cancer, particularly in those with higher levels of PD-L1 expression on tumor-infiltrating immune cells, as observed in a Phase I study. Moreover, in Phase II trials, atezolizumab has elicited persistent anti-tumor responses in patients with advanced urothelial carcinoma whose tumors progressed during or after treatment with platinum-based chemotherapy.[30] Atezolizumab, combined with chemotherapy, represents a significant breakthrough as the first immunotherapy approved for the first-line treatment of extensive small-cell lung cancer (SCLC).[31] Real-world studies from countries such as Japan, Canada, and Turkey have validated its effectiveness in SCLC. In addition, clinical trials such as IMpower110 and IMpower132 demonstrated the efficacy of atezolizumab, either as monotherapy or in combination with chemotherapy, for non-squamous non-SCLC (NSCLC). Notably, IMpower110 revealed a significantly longer OS with atezolizumab compared to chemotherapy in NSCLC patients with high PD-L1 expression, regardless of histological type.[31]

Durvalumab, a fully human IgG1k mAb against PD-L1, is under development by AstraZeneca for the treatment of advanced or metastatic urothelial cancer, non-small-cell lung cancer, and extensive-stage small cell lung cancer.[32] By blocking the PD-L1/PD-1 and PD-L1/CD80 interactions, durvalumab prevents the inhibition of T-cell activity, leading to enhanced recognition and destruction of tumor cells without causing antibody-dependent cytotoxicity.[33]

CAR-T-CELL THERAPY

CAR-T-cell therapy is a ground-breaking immunotherapy approach that leverages the body's immune system to combat cancer more effectively. This innovative technique

involves extracting T lymphocytes from the patient's blood, genetically engineering them to express chimeric antigen receptors (CARs) on their surface and reinfusing them into the patient to stimulate anti-tumor immune responses and eradicate cancer cells.[34]

The first generation of CARs was designed with an extracellular antigen-binding domain (typically a single-chain variable fragment [scFv] of an antibody), a transmembrane domain, and an intracellular signaling domain from cluster of Differentiation 3 zeta chain (CD3ζ).[34] This configuration enabled T-cell activation via the CD3ζ chain's tyrosine activation motif, triggering "signal I" for T-cell activation, cytolysis, and regulation of Interleukin 2 (IL-2) secretion.[35] However, these first-generation CARs exhibited limited anti-tumor efficacy, leading to reduced T-cell proliferation and eventual apoptosis.

To overcome these limitations, the development of second-generation CARs, incorporating a new costimulatory signal to enhance the original "signal I" provided by the T-cell receptor (TCR)/CD3 complex.[35] These second-generation CARs demonstrated improved T-cell proliferation, enhanced cytokine secretion, increased expression of anti-apoptotic proteins, and delayed cell death compared to the first generation. Commonly used costimulatory molecules, such as CD28, have been increasingly replaced by 4-1BB (CD137), tumor necrosis factor receptor superfamily 9) due to their superior efficacy.[35] Recent advancements have also explored the use of the NK cell receptor CD244 to promote prolonged CAR-T-cell activation and proliferation.[35]

The third generation of CARs integrated multiple costimulatory molecules, such as CD28 and 4-1BB, further improving CAR-T-cell survival and functionality *in vivo*.[34] In addition, innovations in CAR-T therapy now include cytokine secretion, such as IL-12, to boost T-cell viability, recruit other immune cells, and enhance overall potency and safety. Ongoing research and clinical trials continue to refine CAR-T therapy, with a focus on optimizing its efficacy and safety for treating solid tumors. These advances highlight CAR-T cell therapy's growing potential in the field of cancer treatment.

Advances in CAR-T-cell therapy for hematological and solid tumors

Anti-CD19 CAR-T-cells have shown exceptional results in treating relapsed or refractory (R/R) B-cell malignancies, including B-cell non-HL (NHL), acute lymphoblastic leukemia (ALL), and chronic lymphocytic leukemia (CLL) in both pediatric and adult patients.[36] Clinical trials have reported complete remission (CR) rates ranging from 70% to 94%.[37] Following the success of CD19 CAR-T therapy, subsequent trials have been conducted on larger patient cohorts with conditions such as follicular lymphoma (FL), CLL, and ALL. Due to its effectiveness in patients unresponsive to conventional treatments, the US FDA has approved six CAR-T products for these patients.[37] These FDA-approved CAR-T products are summarized in Table 4.

Table 4: FDA approved chimeric antigen receptor T (CAR-T) cells products

Generic name	Year of approval	Target	Target domain type	Generation	Indications	Line of treatment
Tisagenlecleucel	2017	CD19	Single-chain variable fragment (scFv)	Second	Relapsed or Refractory B-cell precursor acute lymphoblastic leukaemia (ALL) Relapsed or refractory large B-cell lymphoma Diffuse Large B-Cell Lymphoma (DLBCL) Follicular Lymphoma (FL)	Third-line or later
Axicabtagene ciloleucel	2017	CD19	scFv	Second	Diffuse Large B-Cell Lymphoma (DLBCL) Follicular Lymphoma (FL)	Second line or Third-line
Brexucabtagene autoleucel	2020	CD19	scFv	Second	R/R mantle cell lymphoma B-cell Acute Lymphoblastic Leukemia (B-ALL)	Third-line
Lisocabtagene maraleucel	2021	CD19	scFv	Second	Diffuse Large B-Cell Lymphoma (DLBCL)	Third-line
Idecabtagene vicleucel	2021	BCMA	scFv	Second	R/R multiple myeloma	Fourth-line or later
Ciltacabtagene autoleucel	2022	BCMA	scFv	Second	R/R multiple myeloma	Fourth-line or later

Breast cancer

CAR-T-cell therapy has demonstrated promising outcomes in treating triple-negative breast cancer (TNBC), particularly by targeting Tumor-associated *MUC 1* with high specificity. *Mucin-28* CD3 zeta chain (MUC28z) CAR-T-cells, which incorporate CD3ζ and CD28 signaling domains, have been shown to increase cytokine production, including IFN-γ and Granzyme B, leading to the suppression of TNBC cell proliferation and improved survival in xenograft models.[38]

In addition, researchers have explored natural killer (NK) NK group 2D ligands (NKG2DL) as potential immunotherapy targets for TNBC. CAR-T-cells engineered by combining full-length NKG2DL with the CD3ζ cytoplasmic domain, along with endogenous DNAX-activating protein 10 (DAP10) costimulation, have demonstrated cytokine secretion, cytotoxicity, and *in vivo* tumor suppression. A Phase I clinical trial (NCT04107142) is currently investigating the safety and tolerability of these CAR-T-cells in patients with R/R solid tumors, including TNBC.[39]

Human epidermal growth factor receptor 2 (HER2) is overexpressed in a significant percentage of breast cancers and represents a promising target for CAR-T therapy. Preclinical studies with HER2-targeted CAR-T cells have shown tumor growth inhibition, metastasis regression, and potential efficacy against trastuzumab resistance.[40]

Moreover, mesothelin (MSLN) is gaining attention as a biomarker in breast cancers, particularly TNBC. CAR-T therapies directed toward *MSLN* have demonstrated potent antitumor activity in preclinical models. Ongoing clinical trials (NCT02792114 and NCT02414269) are assessing the safety and efficacy of these therapies in patients with *MSLN*-positive breast cancers.[30-32]

Pancreatic tumor

CAR-T-cell therapy has shown promise in treating pancreatic cancer, with significant efficacy observed *in vitro* and in xenograft models. Enhancing CAR-T-cells with the chemokine receptor CXC chemokine receptor 2 (CXCR2) has improved their migration toward Interleukin *8* (IL-8), resulting in notable antitumor activity against αvβ6-expressing pancreatic tumors in animal models.[38]

Dual-targeting strategies have emerged as a potential approach to improve CAR-T-cell efficacy in pancreatic cancer. By targeting both carcinoembryonic antigen (CEA) and *MSLN*, researchers have achieved precise tumor targeting and a reduced tumor burden in pancreatic cancer models.[33] In addition, modifying IL-8 receptors in CARs, in combination with CD7*0* enhancement, has been shown to enhance CAR T-cell efficacy in pancreatic cancer therapy. Moreover, the production of IL-7 and chemokine (C-C motif ligand 19) in 7 × 19 CAR T-cells demonstrated superior antitumor activity against pancreatic cancer compared to standard CAR-T cell therapy, as reported in preclinical studies.[41]

Thyroid cancer

Thyroid cancer incidence is rising rapidly, and the thyroid-stimulating hormone receptor (TSHR) is a highly expressed glycoprotein receptor in most thyroid cancers.[34] Consequently, CAR T-cells engineered with two co-stimulatory domains and targeting the TSHR antigen have shown both safety and potent efficacy in treating differentiated thyroid cancer, releasing elevated levels of IL-2, IFN-γ, tumor necrosis factor-alpha (TNF-α), and Granzyme-B compared to conventional T-cells.[42]

In addition, there exists a positive correlation between the expression levels of Intercellular Adhesion Molecule 1 (ICAM-1) RNA and the aggressiveness of papillary thyroid cancer, often driven by mutually exclusive somatic mutations, *BRAF*V600E or mutated Rat Sarcoma (RAS) is a promising therapeutic target in thyroid cancer.[43] To specifically target ICAM-1, a scFv derived from the ICAM-1–specific R6.5 mAb was integrated into a third-generation CAR construct comprising intracellular CD3ζ, CD28, and 4–1BB (CD13*7*) signal transduction domains. This approach significantly reduced tumor burden in metastatic and aggressive thyroid cancer, prolonging OS in animal models xenografted with autologous anaplastic thyroid carcinomas (ATC) tumors.[43] AIC100, a CAR-T therapy developed by AffyImmune Therapeutics, is being tested in Phase I clinical trials (NCT04420754) for treating ATC and poorly differentiated thyroid cancers.[44]

Brain cancer

Epidermal growth factor receptor variant III (*EGFRvIII*), a mutated variant of the *epidermal growth factor receptor* (*EGFR*) resulting from an in-frame deletion spanning exons 2–7, is prevalent in various cancers. In glioblastomas (GBMs), approximately 40% of newly diagnosed patients exhibit *EGFR* gene amplification, with around 50% of *EGFR*-amplified GBM cases featuring the constitutively oncogenic *EGFRvIII*.[37] The mutation-induced alteration in the extracellular domain structure presents a unique epitope targeted by specific mAbs with minimal risk of on-target/off-tumor toxicity.[45] Consequently, both vaccine and CAR-T cell therapies directed at *EGFRvIII* have been meticulously developed. In preclinical studies, *EGFRvIII* CAR T-cells demonstrated significant efficacy in reducing tumor growth. However, translating this success to GBM patients has been somewhat limited, with *EGFRvIII*-specific CAR-T-cells showing restricted efficacy.

Advancing CAR-T-cell therapy requires identifying a stable

and specific tumor-associated antigen (TAA) exhibiting heterogeneity throughout the tumor region. Meeting these criteria, a suitable target has been identified. A study demonstrated the *in vivo* therapeutic effects of intracranial delivery of chondroitin sulfate proteoglycan 4 (CSPG4)-CAR-T-cells in nude mice transplanted with *CSPG4*-expressing glioma cells or GBM neurosphere models.[46] This research marks a significant step forward in the pursuit of effective CAR-T-cell therapies in the complex landscape of brain cancer.

Interleukin 13 receptor alpha 2 (IL13Rα2), a receptor involved in regulating inflammation, binds to IL-13 and is found in more than 75% of GBMs, which correlates with the aggressiveness of the tumor and poor prognosis. Its limited presence in normal brain tissue suggests that IL13Rα2 could be a promising target for CAR-T-cell therapy in treating GBM.[47] In a pioneering human trial, the intracranial administration of IL13Rα2 CAR–T-cells demonstrated acceptable tolerance, remarkable anti-tumor responses in approximately two-thirds of treated patients, and manageable side effects.[47] Meanwhile, HER2, which is overexpressed in 80% of GBMs, initially showed potential with third-generation CAR-T-cells. However, safety concerns prompted a modified approach using second-generation CAR-T-cells, which exhibited persistence for up to 1 year without adverse effects.

Current clinical and preclinical investigations are exploring CAR-T-cells targeting TAAs, including B7-H3, CD147, and Ganglioside D2 (GD2). B7-H3 CAR-T-cells, which are highly prevalent in solid cancers, aim to disrupt the stroma and hinder neo-angiogenesis. CD147, associated with tumor progression, invasion, and metastasis, is being evaluated as a target in a dose-escalation clinical study for recurrent GBM patients. In addition, GD2, abundantly expressed in various cancers, is the subject of investigation in a Phase I clinical study for CAR T therapy against brain tumors, including GBM. These studies represent significant progress in the development of effective CAR-T-cell therapies targeting diverse TAAs in the complex landscape of brain cancer treatment.[45]

Hematologic malignancies

Hematologic cancers, or blood cancers, account for approximately 10% of all cancer cases in the US, resulting from uncontrolled growth of abnormal blood cells.[38] The introduction of CAR-T-cell therapy has revolutionized the treatment of these cancers, particularly in cases of R/R B-cell acute lymphocytic leukemia NHL, and multiple myeloma (MM), showing impressive outcomes in recent years

B Cell Lymphoblastic Leukemia

The first FDA-approved Chimeric Antigen Receptor T-cell (CAR-T) therapy, Tisagenlecleucel (tisa-cel), targets CD19 and was developed for the treatment of R/R B-cell ALL and diffuse large B-cell lymphoma (DLBCL), the most common subtype of NHL, comprising about 40% of cases.[48] In the Phase II study of efficacy and safety of *CTL019* in Pediatric ALL Patients (ELIANA) trial involving 79 pediatric and young adult patients with CD19 + R/R B-cell ALL, 82% achieved complete remission (CR) or CR with incomplete blood count recovery, with all patients achieving minimal residual disease (MRD) negativity.[49] Six-month event-free survival and OS rates were 73% and 90%, respectively, though cytokine release syndrome (CRS) occurred in 77% of patients, with 49% experiencing grade 3 or higher, and neurological toxicity observed in 39%.[50] This led to FDA approval of tisa-cel in August 2017 for patients aged 25 years or younger with R/R B-ALL or in second or later relapse.[49]

In the Phase II JULIET trial, 167 patients with R/R DLBCL were treated with tisa-cel. The overall response rate (ORR) was 53%, with 39% achieving CR. Notably, grade 3 or higher CRS was reported in 26% of patients.[51] In addition, the ZUMA study demonstrated that CD19-targeted CAR T-cells (Yescarta) induced complete remission in 58% and partial remission in 25% of patients with refractory large B-cell lymphomas, with responses lasting over 2 years, leading to approval of Yescarta (axicabtagene ciloleucel) in 2017.[48] More recently, in March 2021, the FDA approved Breyanzi (lisocabtagene marleucel), another CAR-T therapy for refractory large B-cell lymphomas, including DLBCL, high-grade B-cell lymphoma, primary mediastinal large B-cell lymphoma, and FL.[48]

T Cell Lymphoblastic Leukemia

Treating R/R T-cell acute lymphoblastic leukemia and T-cell lymphomas has proven challenging due to antigen overlap between malignant and normal T-cells, resulting in CAR-T therapy depleting both cancerous and healthy T-cells. This often leads to severe immunosuppression, raising the risk of life-threatening infections and mortality.[51] Unlike the success seen with anti-CD19 CAR-T therapy in B-cell cancers, the development of CAR-T therapies for T-cell malignancies remains complex and is still being explored.[52] At present, treatment options for T-cell lymphomas are primarily limited to allogeneic hematopoietic cell transplantation.[37]

The development of CAR-T cell therapies for T-cell malignancies holds promise but necessitates the identification of antigen markers exclusively present on malignant T-cells.[38] An example is targeting CD3, a pan-T surface antigen that forms a complex with the TCR, enabling the recognition of target antigens and subsequent T-cell activation signaling.[51] The specific expression of CD3 on all mature T-cells makes it an attractive target for immunotherapy in T-cell malignancies.[51]

Acute myeloid leukemia (AML)

AML is a severe hematologic malignancy characterized by an accumulation of immature myeloid cells, with a 27.4% 5-year survival rate from 2008 to 2014.[52,53] Allogeneic hematopoietic stem cell transplantation remains the most effective curative option for intermediate and high-risk AML, though it carries significant risks of non-relapse mortality and relapse.[53] CAR-T therapy for AML has not achieved the success seen in ALL due to challenges in targeting antigens uniquely expressed on malignant cells without affecting healthy tissues. CAR-T approaches have focused on antigens such as CD33 and CD123, but these have shown limited success due to their expression on normal cells.[54,55]

Numerous *in vitro* and *in vivo* studies have demonstrated that CAR-T-cells targeting surface proteins such as CD33, CD123, C-type lectin-like receptor 1 (CLL-1), CD13, CD7, NKG2DL, CD38, CD70, and T-cell immunoglobulin and mucin-domain protein 3 can effectively eliminate AML cells.[52] One of the initial efforts involved using CAR-T cell therapy targeting CD33 in a patient with R/R AML, which resulted in a significant reduction of tumor burden in the bone marrow.[54] Subsequently, CD123 was introduced as a potential antigen target. However, anti-CD123 CAR-T-cell therapy showed low efficiency due to the expression of CD123 on normal cells such as monocytes and endothelial cells, although at lower levels than on AML cells. Given these disappointing results, additional preclinical studies were conducted, exploring various antigens as new targets to achieve effective treatment with minor and tolerable toxicities for patients, such as Lewis-Y antigen (LeY) and C-type lectin domain family 12A (CLEC12A).[38,54]

A Phase I clinical trial involving LeY CAR-T-cells with a CD28 costimulatory domain demonstrated modest responses in two patients who had received prior fludarabine chemotherapy. The CAR-T-cells exhibited durable persistence in patients and led to mild toxicity.[38] Other potential CAR-T-cell therapy targets, including *CD47*, CD96, and CD44v6, are currently under investigation in preclinical models.[38] Siglec-6, expressed in approximately 60% of AML patients and absent on normal hematopoietic stem and progenitor cells, has emerged as a promising target. In preclinical studies, Siglec-6 CAR-T-cells effectively eliminated AML blasts in an AML mouse xenotransplantation model, suggesting its potential as a well-validated target for CAR-T cell therapy in AML.[56]

MM

MM, the second most common hematologic cancer, accounts for 2% of cancer-related deaths in the US and about 10% of hematologic malignancies.[57,58] CD19-targeted CAR-T therapies have shown limited efficacy in MM due to low CD19 expression.[48] Consequently, research has shifted toward targeting B-cell maturation antigen (BCMA), which is highly expressed on plasma cells and mature B-cells, making it a promising target for CAR-T-cell therapy in MM.[48]

The first studies of BCMA-targeted CAR-T-cells in MM began in 2013. BCMA is a member of the TNF receptor superfamily found predominantly on plasma cells.[59] Its overexpression, induced by its ligand April, promotes MM progression and survival through activation of the *Protein kinase B* (*PKB*), also known as AKT, mitogen-activated protein kinase, and nuclear factor-kappa B signaling pathways.[59] BCMA is shed from MM cells by γ-secretase, releasing a soluble form Soluble B-cell maturation antigen (sBCMA) that serves as a biomarker of MM tumor burden but can also limit the efficacy of membrane-bound BCMA-targeted therapies.[60]

At present, there are two FDA-approved CAR-T-cell products for the treatment of R/R MM (RRMM): Idecabtagene vicleucel (ide-cel) and ciltacabtagene autoleucel (cilta-cel).[58] The first-in-human Phase I clinical trial (NCT02215967) testing BCMA-targeted CAR-T-cells in RRMM achieved an ORR of 81%, with 63% of patients showing a very good partial response or complete response.[61] Anti-BCMA CAR-T-cell therapy has also proven effective in R/R MM patients with extramedullary disease.[37]

Despite these successes, some MM patients relapse after anti-BCMA CAR-T-cell therapy due to downregulated BCMA expression under therapeutic pressure. This has spurred efforts to develop novel anti-BCMA CARs and refine existing ones, as well as to identify new target antigens.[37,61] At present, over a hundred studies are exploring new targets for CAR-T-cell therapy in MM therapy.[40]

HL

It is a unique B-cell malignancy characterized by a small number of Hodgkin and Reed-Sternberg cells within a large immune cell population.[62] At present, CD30 CAR-T-cells are the most commonly tested CAR-T-cells for HL in clinical trials. In a Phase I clinical trial involving 17 heavily pre-treated patients with R/R HL, including 4 (24%) who had previously received brentuximab vedotin, patients were given one of three lymphodepletion regimens (fludarabine + cyclophosphamide, gemcitabine + cyclophosphamide + chlormethine, or nab-paclitaxel + cyclophosphamide) followed by a CD30-BBz CAR-T-cell product at a median dose of 1.56×10^7 cells/kg.[63] The trial reported the safety and tolerability of the treatment, along with the potential of anti-CD30 CAR-T-cell therapy for patients with R/R HL, showing durable antitumor responses in HL cell lines and mouse models.[4,38] The most common treatment-related side effects were nausea and vomiting (27.8%) and a rash resembling urticaria (11.1%).[54]

CANCER VACCINES

A significant challenge in cancer immunotherapy is overcoming the lack of immune response in patients with advanced tumors.[64] Cancer vaccines offer a potential solution by harnessing the body's immune system to combat the disease. Despite decades of research, cancer vaccines have not achieved the same success as vaccines for infectious diseases due to the complexity of cancer and its interactions with the immune system.[65]

Effective cancer immunotherapy requires cytolytic effectors, such as T-cells and antibodies, to specifically recognize TAAs or tumor-specific antigens (TSAs).[66] Although some cancer patients naturally develop antigen-specific T-cells capable of mounting a strong anti-tumor response, the majority do not. One strategy to ensure sufficient levels and functionality of immune effectors is therapeutic cancer vaccination. Therapeutic cancer vaccines aim to address this by inducing robust anti-tumor immune responses targeting TAAs or TSAs.[66] These vaccines trigger immune responses involving T-cells, B-cells, and other immune cells to attack and eliminate cancer cells.[67]

Li *et al*. reported that Mitchell and colleagues pioneered the development of the first cancer vaccine in 1988 by immunizing melanoma patients with allogeneic melanoma lysate, successfully triggering an anti-melanoma immune reaction in several patients. The discovery of TAs, which are overexpressed in tumor tissues and contribute to tumor progression, has since opened new avenues for cancer therapy. Over the past two decades, various antigen delivery methods have been developed, some demonstrating promising anti-tumor immune responses and clinical benefits.[68]

From a technological standpoint, cancer vaccines share similarities with vaccines for infectious diseases, including whole-cell vaccines, DNA and mRNA vaccines, antigen vaccines, and dendritic cell (DC) vaccines.[68] Hundreds of clinical trials have demonstrated both the promise and challenges of therapeutic vaccines [Table 5]. Unlike traditional treatments such as surgery, chemotherapy, or radiotherapy, therapeutic cancer vaccines specifically activate the immune system to target tumor cells, leading to higher response rates and improved quality of life.[68] The journey of cancer vaccines highlights the ongoing effort to unlock the full potential of immunotherapy in transforming cancer treatment.

Types of cancer vaccines

Cancer vaccines can be categorized based on their targets and mechanisms of action. Below are the main types:

Cancer-preventive and treatment vaccines

Cancer vaccines are generally divided into two types: Preventive and therapeutic. Preventive vaccines aim to stop cancer from developing in healthy individuals, while therapeutic vaccines are designed to treat existing cancers by enhancing the immune system's response to tumors.[69] In the U.S., there are currently three preventive vaccines and one therapeutic vaccine approved.[61]

Preventive cancer vaccines focus on stimulating the immune system to either prevent oncogenic viral infections or target pre-malignant and latent cancer cells that have not yet become clinically apparent.[70] For example, vaccines against human PAPillomavirus (HPV) are expected to greatly reduce the incidence and mortality of cervical and other HPV-related cancers.[71] Studies have shown that HPV vaccination can nearly eliminate persistent infections and precancerous cervical lesions caused by the HPV types included in the vaccine, though longer follow-up is necessary to confirm its effect on cancer incidence.[71]

Since their introduction in 2007–08, two HPV vaccines, Gardasil (by Merck) and Cervarix (by GlaxoSmithKline), have been widely used. Gardasil targets HPV types 16 and 18 (which cause cancer), as well as types 6 and 11 (linked to genital warts), while Cervarix targets types 16 and 18 only. Both vaccines have received FDA approval for preventing HPV infections, particularly types 16 and 18, which are responsible for about 70% of cervical cancers.[69,70] In addition, the FDA has approved a preventive vaccine against hepatitis B virus (HBV) infection, which can lead to hepatocellular carcinoma. Since 1981, HBV vaccines have evolved from plasma-derived to recombinant formulations, providing broad protection and significantly reducing HBV prevalence and hepatocellular carcinoma incidence, especially in infants.[70]

In contrast to preventive vaccines, therapeutic cancer vaccines are administered to individuals already diagnosed with cancer to stimulate the immune system to fight the disease.[72] These vaccines face the challenge of overcoming immune suppression mechanisms that cancers use to evade detection. Nevertheless, recent advancements show promising clinical outcomes for therapeutic vaccination.[65] Notable examples include sipuleucel-T (Provenge®) and rilimogene galvacirepvec/rilimogene glafolivec (PROSTVAC-VF), both of which have demonstrated survival benefits in patients with hormone-resistant prostate cancer.[73] Neoantigen-based vaccines, targeting mutation-derived epitopes, have shown promise in eliciting strong antitumor responses, with positive results in preclinical and clinical studies for various cancers, including melanoma. Initial trials of mRNA-based neoantigen vaccines and personalized cancer vaccines targeting predicted neo-epitopes have yielded encouraging results, leading to sustained progression-free survival and the induction of multifunctional antigen-specific T-cells in high-risk melanoma patients.[74] Furthermore, combining neoantigen vaccines with ICIs has enhanced their efficacy. For instance, Personalized neoantigen vaccine (NEO-PV-01),

Table 5: Types of cancer vaccines

Type of cancer vaccine	Examples	Mode of implementation	Significance
Preventive	Gardasil, Cervarix, HBV vaccine	Administered to healthy individuals to prevent infections with oncogenic viruses (e.g., HPV, HBV)	Significantly reduces incidence of cervical cancer (HPV), liver cancer (HBV) and other HPV-related cancers.
Therapeutic	Sipuleucel-T (Provenge®), PROSTVAC-VF, NEO-PV-01, Oncophage (HSPPC-96)	Administered to cancer patients to enhance immune response against established tumours	Prolongs survival in patients with advanced cancers like hormone-resistant prostate cancer
RNA	mRNA-4157 (Moderna, for melanoma), BNT111,BNT16b2	Injection of mRNA encoding TAAs or TSAs into muscle or skin	Elicits immune response by translating mRNA into antigen proteins that activate immune cells; shown promise in melanoma and ovarian cancer.
Viral	Talimogene laherparepvec (T-VEC), PROSTVAC-VF	Uses modified viruses to infect and kill cancer cells while stimulating an immune response	Directly lyses tumour cells and promotes systemic anti-tumour immunity. It is currently investigated for lung cancer and also used for respiratory syncytical virus. T-VEC also directly lyses tumor cells.
Peptide	NeuVax (Nelipepimut-S or E75), NY-ESO-1, IMA901 (renal cell carcinoma)	Uses specific peptides from tumour antigens to stimulate T-cell response	Highly specific, can be tailored to individual tumour profiles in breast, ovarian, pancreatic, colon, bladder and prostate cancers.
Dendritic Cell (DC)	Sipuleucel-T (Provenge®), DCVax-L	Uses patient's *dendritic cells* loaded with tumour antigens to activate the immune system	Personalizes treatment, has shown promise in glioblastoma and other cancers
DNA Vaccines	INO-5401 (for glioblastoma), VGX-3100 (for HPV-related cancers)	Injection of plasmid DNA encoding TAAs or TSAs into muscle or skin	Induces strong and long-lasting immune responses; used in clinical trials for cervical dysplasia.

This table provides an overview of various types of cancer vaccines, including examples, their mode of implementation, and their significance in cancer prevention and treatment. TAA: tumor-associated antigen, TSA: tumor-specific antigen, T-VEC: Talimogene laherparepvec.

a long peptide cancer vaccine combined with nivolumab, has induced cytotoxic neoantigen-specific T-cells in patients with NSCLC, melanoma, or bladder cancer.[74]

Peptide- and protein-based vaccines

Peptide-based vaccines use short chains of amino acids derived from cancer-associated proteins to stimulate an immune response. These vaccines target specific TAAs within the TME, encouraging the immune system to attack the cancer cells.[75] In contrast, protein-based vaccines use whole or modified proteins to stimulate a broad immune response, potentially providing greater efficacy by targeting multiple antigens. The rational design and clinical status of peptide- and protein-based cancer vaccines have been extensively studied but designing effective peptide-based vaccines remains challenging due to the complexity of the involved interactions.

While early clinical trials for peptide-based vaccines (such as NCT00088660, NCT00089856, and NCT00052130) showed limited success, advances in bioinformatics now allow researchers to better predict and design peptide vaccines.[66,76] Peptide vaccines offer benefits such as faster production, easier storage, lower cost, and fewer side effects compared to conventional vaccines. However, successfully designing a peptide-based vaccine involves several tasks: Identifying potential antigens, predicting T-cell and B-cell epitopes, analyzing epitope immunogenicity, antigenicity, allergenicity, and toxicity, selecting linkers and adjuvant peptides, constructing and optimizing the final vaccine, and analyzing the characteristics of the final vaccines.[76]

Peptides with medium-to-high major histocompatibility complex (MHC) binding affinity have typically been used in vaccinations but have shown limited success due to immune tolerance.[77] Recently, low-to-medium affinity peptides have been explored for their immunogenic potential. Enhancing their MHC binding affinity through "anchor" residue modification, while maintaining or improving TCR binding, has shown promise.[77] When peptide cancer vaccines are introduced into the TME, they are processed by APCs, such as dendritic cells (DCs). The APCs internalize the peptides and present them on their surface in complex with MHC molecules.[75] This presentation allows the peptides to be recognized by T-cells, specifically CD8 + cytotoxic T lymphocytes (CTLs), which can directly kill cancer cells

expressing the targeted TAAs.[75] The interaction between the presented peptide-MHC complex and the TCR activates the CTLs. This activation leads to the expansion of a population of tumor-specific CTLs that can specifically recognize and target cancer cells expressing the TAAs. These *CTLs* infiltrate the tumor and exert their cytotoxic effects, thereby combating the cancer cells within the TME.[75]

DNA and RNA vaccines

DNA vaccines

DNA vaccines introduce DNA fragments encoding TSAs, prompting the immune system to attack cancer cells. One example is an investigational DNA-based immunotherapy VGX 3100 vaccine, targeting high-grade cervical dysplasia caused by HPV types 16 and 18.[78] DNA vaccines offer benefits such as broad immune responses, easy production, low cost, and the fact that information about human leukocyte antigen class I and II genotypes is not required.[69] Similar to protein-based vaccines, DNA vaccines rely on antigen processing and presentation by APCs.[69]

Recently, several Phase I/II clinical trials using DNA-based vaccines targeting different TAAs (e.g., prostate-specific antigen [*PSA*], prostatic acid phosphatase [PAP], Glycoprotein 100 (gp100), CEA, and heat shock protein 65 [*hsp65*]) in prostate cancer, melanoma, colorectal cancer, and head and neck carcinomas.[79] In these trials, DNA-based vaccines were administered either as monotherapy or in combination with various delivery systems and adjuvants. Although most of these trials showed low immunogenicity of TAAs, the small sample size precluded achieving a statistical correlation between immune response development and clinical outcomes in vaccinated patients.[79]

RNA vaccines

RNA vaccines, which are rapidly manufactured and do not integrate into the host genome, offer another approach. Their single-stranded structure also provides an adjuvant function by stimulating immune receptors such as Toll-like receptor 7 and Toll-like receptor 8. However, RNA is highly susceptible to degradation within cells. To address this challenge, RNA has been administered directly into inguinal lymph nodes or delivered using nanoparticle systems during clinical trials. Delivery systems such as nanoparticles and liposomes are employed to enhance transfection efficiency and prevent degradation by RNases.[80]

RNA-based vaccines have gained attention, with the FDA approving Imlygic (talimogene laherparepvec) in 2020. Imlygic uses a modified HERpes simplex virus (HSV) to replicate within cancer cells, releasing immune-stimulating proteins like Granulocyte-macrophage colony-stimulating factor (GM-CSF) to trigger the immune system to attack tumor cells.[75] Clinical trials have explored mRNA-transfected *DCs* or direct mRNA injection in patients with diverse cancers, including prostate cancer, RCC, ovarian cancer, lung cancer, breast cancer, pediatric brain cancer, neuroblastoma, and melanoma.[79] A Phase I clinical trial using PSA-mRNA-transfected *DCs* in metastatic prostate cancer patients revealed that repeated vaccinations could enhance PSA-specific *CTL* responses.[79] At present, numerous human clinical trials are underway, aiming to induce broad T-cell responses through the simultaneous delivery of multiple antigens.

DCs

DCs play a crucial role in initiating immune responses and are being explored in cancer vaccine development. DCs can be loaded with various tumor-derived materials (such as lysates, RNA, DNA) to generate vaccines capable of triggering anti-tumor responses. Studies in animal models have demonstrated the immunogenicity of these preparations, showing potential for tumor rejection and are currently undergoing evaluation in clinical settings.[81]

Present clinical strategies for DC-based therapies involve harvesting DCs from patients, maturing them *in vitro*, and loading them with TAs before reinfusing them. Following injection, these DCs present TAs to specific T-cells, leading to their activation and subsequent expansion.[82] One notable example is sipuleucel-T (Provenge), the first FDA-approved DC vaccine for prostate cancer patients as of 2010. This vaccine involves collecting a patient's own *DCs*, exposing them to a prostate cancer-specific antigen, and then reinfusing them to stimulate an immune response against the cancer cells.[81]

BSABS

BsAbs have emerged as a highly promising class of therapeutics in cancer immunotherapy, offering a distinct approach to harnessing the immune system to target cancer cells. Unlike conventional mAbs, which bind to a single antigen, bsAbs are designed to bind simultaneously to two different epitopes.[83,84] This dual-targeting capability allows bsAbs to perform multiple functions, such as redirecting immune cells to tumor cells, blocking two separate signaling pathways, or delivering therapeutic agents to specific sites.[85,86]

A classical method of producing BsAbs is quadroma technology, which creates antibodies with two different binding sites targeting distinct antigens or epitopes on the same antigen. This dual binding enhances BsAbs' effectiveness compared to traditional mAbs in cancer immunotherapy, particularly by reducing resistance rates. The development of BsAbs is an active area of research, with numerous clinical trials underway to evaluate their potential in cancer treatment.[87]

BsAbs offer several advantages over mAbs, especially in terms of efficacy and safety. One key benefit is their ability to redirect cytotoxic effector cells, such as T-cells or NK cells, toward malignant cells expressing specific TAs.[88] In addition, BsAbs facilitate closer interactions between immune effector cells and tumor cells, a capability that mAbs alone cannot achieve.[89]

Since the first BsAb (Catumaxomab) was launched in 2009, over 86% of BsAbs are being developed for cancer treatment, with two therapies, catumaxomab and blinatumomab, already approved for clinical use. Recently, ten new BsAbs—eight of which target tumors – have been approved, with several expected to be released between 2021 and 2024 [Table 6]. The field continues to evolve, with researchers exploring innovative BsAb formats that are easier to produce, more stable, and capable of targeting clinically relevant cancer markers. BsAbs' ability to redirect T-cells to tumor cells, thereby enhancing the cytotoxic T-cell response, represents a significant advantage in cancer immunotherapy.[87,90]

Mechanism of action of BsAbs

BsAbs are highly effective in treating diseases such as cancer, autoimmune conditions, and inflammatory disorders due to their ability to selectively engage immune effector cells against disease-specific antigens. BsAbs act as linkers between target cells and immune effector cells. Their mechanisms of action include complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis, apoptosis, and modulation of cell surface receptors to either inhibit or activate signaling pathways.[91]

Catumaxomab (Removab®)

Catumaxomab, marketed as Removab®, is a trifunctional bsAb approved for treating malignant ascites, a common complication in cancer patients. It targets the TA *epithelial* cell adhesion molecule (EpCAM) (CD32*6*), found on many human adenocarcinomas, squamous cell carcinomas, retinoblastoma, and hepatocellular carcinoma. While EpCAM is also present on normal cells, its location in intercellular spaces of epithelial cells makes it less accessible to antibodies, unlike its homogenous distribution on cancer cell surfaces. In addition, EpCAM is often present on cancer stem cells, further establishing its value as a tumor marker.[92]

Catumaxomab destroys tumor cells primarily through T-cell-mediated lysis, ADCC, and phagocytosis. It binds to CD3 on T-cells and EpCAM on tumor cells, facilitating the recruitment of T-cells to kill EpCAM-positive cancer cells and treat malignant ascites.[93] The trifunctional nature of catumaxomab allows its Fc region to bind *Fcγ receptors* on immune accessory cells, activating effector cells to release perforins and granzymes that accelerate tumor cell destruction. In addition, cytokines such as IFN-γ and TNF-α, secreted by T-cells, enhance its anti-tumor activity by stimulating both innate and adaptive immune responses.[93]

Catumaxomab's pharmacology has been well-studied. A Phase II PK study showed that intraperitoneal administration resulted in both local and systemic antibody concentrations, enabling it to target the primary tumor and potential metastatic lesions.[94] Studies have demonstrated catumaxomab's ability to stimulate an immune response in patients, with the most common side effects being manageable symptoms such as fever, nausea, and vomiting.[95]

Blinatumomab (MT103)

Blinatumomab, also known as MT103 or bscCD19 × CD3, is a 55-kDa bispecific T-cell engager antibody that targets CD19 on B-cells and CD3 on T-cells. It is produced using a cDNA expression vector in Chinese hamster ovary cells.[90]

On administration, blinatumomab brings B-cells and T-cells into close proximity, forming a cytolytic immune synapse. This interaction prompts T-cells to release perforins and granzymes, leading to apoptosis in CD19-positive

Table 6: Bispecific antibodies endorsed for treating cancer

Name	Targets	Developer	Date approved	Medical conditions
Blinatumomab	CD19/CD3	Amgen	December 2014	Acute lymphoblastic leukaemia (ALL)
Amivantamab	*EGFR*/c-MET	Janssen	May 2021	Non-small cell lung cancer (NSCLC)
Tebentafusp	gp100/CD3	Immunocore	January 2022	Uveal melanoma
Tebotelimab	PD-1/LAG-3	MacroGenics	November 2022	Various solid tumours
Mosunetuzumab	CD20/CD3	Roche	December 2022	Relapsed or refractory follicular lymphoma
Faricimab	VEGF-A/Ang-2	Roche/Genentech	January 2022	Neovascular (wet) age-related macular degeneration
Talquetamab	GPRC5D/CD3	Janssen	August 2022	Relapsed or refractory multiple myeloma
Epcoritamab	CD20/CD3	Genmab/AbbVie	May 2023	Relapsed or refractory diffuse large B-cell lymphoma (DLBCL)
Teclistamab	BCMA/CD3	Janssen	October 2022	Relapsed or refractory multiple myeloma
Catumaxomab	CD3/EpCAM	Trion pharma	April 2009	Malignant Ascites (withdrawn in 2017)

cells such as NALM- 6 cells which is a human pre-B acute lymphoblastic leukemia (ALL) cell line. The drug's efficacy depends on the perforin-mediated killing of tumor cells, as CD19-negative malignancies show no T-cell activation. Notably, Blinatumomab's T-cell activation bypasses the need for MHC presentation, making it effective against tumors with downregulated MHC molecules. In addition, the drug enhances T-cell activation markers and adhesion molecules, boosting the immune response.[96,97]

At present, blinatumomab is being evaluated in a Phase II trial for patients with B-precursor acute lymphoblastic leukemia (B-ALL) who have minimal residual disease (MRD). Early findings indicate that the drug can locate and eliminate rare disseminated tumor cells, achieving MRD negativity in 81% of patients, with responses lasting up to 47 weeks. The trial also noted significantly reduced toxicity compared to NHL patients at the same dose, suggesting that toxicity may be related to the number of target cells. Moreover, a Phase III trial comparing blinatumomab to standard chemotherapy in heavily pre-treated B-ALL patients showed superior survival outcomes and remission rates with blinatumomab.[86,92]

Ertumaxomab (rexomun)

Ertumaxomab, marketed as Rexomun, is a trifunctional bsAb designed to treat cancers by linking T-lymphocytes and macrophages to cancer cells. Developed by Fresenius Biotech (Trion), it is currently used for metastatic breast cancer (MBC).[98]

Ertumaxomab has two recognition sites: one for CD3 on T-cells and another for HER2/neu, a TAA. By targeting both HER2 + tumor cells and CD3-expressing T-cells, Ertumaxomab facilitates T-cell activation and macrophage-mediated phagocytosis. Phase I trials demonstrated tumor responses even in patients with low HER2 antigen density, suggesting its potential for patients ineligible for trastuzumab.[98]

In a Phase I study involving 17 HER2 + MBC patients, ertumaxomab triggered strong immune responses, resulting in two partial responses, one complete response, and two cases of stable disease. The drug forms complexes between tumor cells, T-cells, and macrophages or *DCs*, leading to cytokine release and tumor cell phagocytosis, which may offer long-lasting anti-tumor immunity. Ertumaxomab is currently undergoing Phase II trials for MBC patients, irrespective of HER2 gene amplification.[92,99]

ONCOLYTIC VIRUSES

Oncolytic viruses represent an innovative and promising avenue in cancer therapy, utilizing the natural ability of certain viruses to selectively target and destroy cancer cells while sparing healthy tissues.[100-104] Several naturally occurring viruses, such as Newcastle disease virus, Reovirus, and Vaccinia virus, exhibit a preference for infecting tumor cells, leaving normal tissues unharmed. Other viruses, including Adenovirus and HSV type 1, have been engineered to act as potent oncolytic agents once attenuated.[105] These modifications make the viruses less harmful to healthy cells while enhancing their tumor specificity. This approach, known as oncolytic virotherapy (OVT), leverages cancer cells' unique characteristics – such as their rapid proliferation and altered cellular pathways – to enable viral replication and spread within the TME.[101,104]

The concept of using oncolytic viruses to combat cancer dates back to the 20th century, with early reports of patients experiencing temporary remission after viral infections.[100] However, the therapeutic application faced challenges related to safety and genetic engineering.[105] Significant progress has since been made, revealing how oncolytic viruses can induce both direct tumor cell lysis and a robust anti-tumor immune response. Genetic modifications often allow these viruses to selectively replicate in and destroy cancer cells, while leaving healthy cells largely unaffected. This selectivity is typically achieved by exploiting the distinct cellular environment of cancer cells, such as their altered signaling pathways and increased metabolic activity.[102,106]

Genetic modifications to enhance the potency of oncolytic viruses

Several strategies have been explored to increase the potency of oncolytic viruses, such as overcoming the extracellular matrix (ECM) barrier, enhancing viral replication in primary tumors and metastases, modifying tumor cell signaling, and targeting gene expression regulators such as signal Transducer and Activator of Transcription 3 (STAT3) and special AT-rich Sequence-binding Protein 1 (SATB1).[102]

The ECM plays a crucial role in tumor development, acting as a dense barrier of proteins and sugars that surrounds tumor cells. The ECM in tumors is stiffer and denser than in normal tissues, preventing therapeutic agents, including oncolytic viruses, from penetrating effectively.[107,108] In addition, interactions between the ECM and tumor cells can activate signaling pathways that promote cell survival and prevent cell cycle arrest.[108] By addressing these challenges, the effectiveness of oncolytic viruses can be significantly improved.

Successes of oncolytic viruses in preclinical and clinical studies

Adenovirus

Adenoviral vectors were among the first viral-based therapies developed for cancer treatment. One example is ONYX-

015 (CI-1042), designed to replicate selectively in tumor protein p53 (p53)-deficient cells by deleting the adenoviral *early region 1B 55-kDa protein* (*E1B-55K*) gene. This deletion reduces the virus's activity in healthy cells, allowing replication in cancer cells that lack *p53*.[101] While later studies suggested that the *E1B-55k* gene was not solely responsible for *p53* inactivation, ONYX-015 showed antitumor efficacy in preclinical models.[109]

In a Phase I trial, ONYX-015 was administered to 22 patients with recurrent HNSCC that was resistant to radiation or chemotherapy. The primary goal was to assess safety, and the trial reported mild flu-like symptoms as the most common side effect, with minimal toxicity. Some patients experienced moderate pain during injection, but it subsided quickly without additional treatment. The maximum injection dose reached 10^{11} pfu, with no major side effects, suggesting the possibility of higher doses in future studies.[110]

A Phase II trial evaluating multiple intratumoral injections of ONYX-015 in patients with recurrent squamous cell carcinoma of the head and neck demonstrated that approximately 14% of patients exhibited significant tumor destruction.[111] ONYX-015 mouthwash therapy also showed promise for treating oral dysplasia when applied topically.[112]

Tasadenoturev (DNX-2401), developed by DNAtrix, incorporates an Arginylglycylaspartic acid motif in its fiber knob HI-loop and features a 24 bp deletion in the early region 1A (E1A) region. A study showed that using convection-enhanced delivery of Delta24-RGD was safe for treating GBM.[113] Another adenovirus, ONCOS-102 (Ad5/3-D24-GMCSF), which expresses GM-CSF, demonstrated safety and the ability to trigger anti-tumor immune responses in Phase I trials involving patients with advanced melanoma and solid tumors.[114,115]

VCN-01(NCT05673811), an oncolytic adenovirus developed by VCN Biosciences, is armed with hyaluronidase and targets the Rb pathway. It is currently being evaluated in clinical trials for advanced solid tumors, refractory retinoblastoma, and in combination with durvalumab for HNSCC.[114] VCN-01 has shown cytotoxicity against glioma cells in both *in vitro* and *in vivo* models.[116] Adenovirus-based oncolytic therapies rely on the inactivation of *p53* in cancer cells to sustain viral replication, with various studies affirming their safety and feasibility.[114,117]

Oncolytic HERpes simplex virus (oHSV)

oHSV is engineered to specifically target and destroy tumor cells while also stimulating an anti-tumor immune response.[118] The well-characterized viral proteins of HSV allow for the deletion of multiple genes to prevent neurotoxicity and increase cancer specificity. Modifications often include the removal of genes essential for viral replication in normal cells, thus confining replication to cancer cells with dysregulated signaling pathways. Additional genetic changes can enhance cancer cell targeting or stimulate the immune system to attack tumors.[119]

Talimogene laherparepvec (T-VEC), or Imlygic, became the first FDA- and European medicines agency (EMA)-approved oncolytic virus. T-VEC expresses GM-CSF and has demonstrated notable efficacy in melanoma patients.[118] Administered intratumorally, T-VEC kills tumor cells and provides a source of antigens that stimulate the immune system to generate a systemic anticancer response.[120] Primed T-cells then generate a systemic polyclonal anticancer response, addressing intra- and intertumoral heterogeneity.[121] T-VEC enters cancer cells through herpes virus glycoproteins, proliferates, and promotes cell lysis.[122]

Clinical studies on T-VEC have demonstrated its safety and efficacy. A Phase I study reported that T-VEC monotherapy was well-tolerated, with mild toxicity characterized by fever, myalgia, shivering, and local responses.[123] In a Phase II study of 50 patients with locally advanced or metastatic melanoma, T-VEC achieved a 26% ORR, including eight complete responses and five partial responses, with most responses lasting nearly 3 years. The OS rates were 58% at 12 months and 52% at 24 months.[124] Real-world data further support T-VEC's efficacy. A multi-institutional study on 80 patients with stage IIIB-IV melanoma reported a 57% ORR, with 31 complete responses and 14 partial responses after a median follow-up of 9 months. Adverse events were mainly moderate, with flu-like symptoms occurring in 28% of participants.[125] Another single-institution study treated 26 patients with stage IIIB-IVM1a melanoma using the OPTiM protocol, achieving an ORR of 88.5% and a disease control rate of 92.3% without additional toxicity compared to the OPTiM trial results.[126]

NV1020 is another recombinant HSV virus with deletions of HBV, Immediate Early Protein 0 (ICP0), Immediate Early Protein 4 (ICP4), and latency-associated transcripts. It also includes an additional copy of the HSV-*1* thymidine kinase gene, making it highly sensitive to the antiviral medication acyclovir.[109] NV1020 was tested in a Phase I trial for intra-hepatic arterial infusion in patients with colorectal cancer and liver metastases.[127] Twelve participants received a single dose of NV1020 at four dose levels, followed by intra-arterial chemotherapy. The treatment was generally safe, with no significant adverse effects, and two patients showed an objective tumor response, although the impact of NV1020 was unclear due to concurrent chemotherapy.[109]

Oncolytic vaccinia virus (OVV)

Vaccinia virus, a double-stranded DNA virus originally used in smallpox vaccines, has emerged as a promising candidate for oncolytic therapy.[101] Its ability to replicate in the

cytoplasm and encapsulate itself within a host cell-derived envelope enables it to evade the immune system and circulate to metastatic tumor sites.[109]

OVV induces tumor destruction through direct oncolysis, disruption of tumor vasculature, and activation of anti-tumor immunity. Pexa-Vec and GL-ONC1 (olvimulogene nanivacirepvec), two prominent OVVs, are currently in Phase III clinical trials.[120] While Pexa-Vec's Phase III trial for hepatocellular carcinoma was unsuccessful in improving patient survival, ongoing research into combination therapies holds promise for future treatments.[128]

Research by Chen *et al.* highlighted the potential of vaccinia virus to target various hematologic cancers, with constructs from the lister institute virus prague (LIVP) strain showing the greatest efficacy in eliminating leukemic cells.[129] The LIVP-based constructs were the most successful at infecting leukemic cells, followed by WR-based constructs.[129] In another study investigating the effects of different vaccinia virus strains on murine mesothelioma cell lines, the Western Reserve virus strain genelux (GLV-0b347) was found to be the most effective in oncolysis. At 96 h' post-infection, GLV-0b347 significantly reduced cell death in AB12 cells, achieving an 80% reduction at a multiplicity of infection (MOI) of 0.1 and over 90% at an MOI of 1. Although no cell death was detected at lower MOIs, GLV-0b347 therapy significantly improved OS, reduced tumor load, and suppressed ascite development in mice with low tumor burden at the time of virus application.[130]

Challenges of OVT

OVT offers substantial promise as a novel cancer treatment; however, several obstacles must be overcome to unlock its full potential. These challenges include difficulties in viral spread and penetration, passive targeting inefficiencies, immune responses, hypoxic tumor environments, and patient selection issues.[131]

Spread and Penetration: For OVT to be effective, the virus must penetrate deep into the tumor and reach all cancer cells, not just those near the surface. Various factors, such as the tight intracellular junctions in epithelial cells, the ECM, host immune responses, tumor vascularization, and intratumoral pressure, can hinder the viral spread. Epithelial junctions, in particular, can act as a significant barrier, especially against adenoviruses that lack protein disulfide isomerase (Pt-Dd) production.[132-134] One potential solution involves modifying oncolytic viruses with agents that open these junctions or co-administering them with such agents to enhance penetration.

Passive targeting

Passive targeting relies on the virus's ability to naturally accumulate within tumors due to the tumor's leaky vasculature and reduced lymphatic drainage – hallmarks of malignant tissues. Despite this, issues such as poor tumor tropism and inefficient transduction have limited the success of oncolytic viruses in cancer therapy. For example, T-VEC has demonstrated therapeutic benefits in melanoma, but its utility is constrained by inadequate tumor cell tropism and transduction.[135] To address these challenges, viral modifications, such as introducing an RGD motif into the HI loop of the adenoviral fiber knob, have improved infection efficiency.[136] This enhancement has been particularly beneficial in cancer models that lack coxsackievirus and adenovirus receptor. Furthermore, combining oncolytic viruses with conventional therapies, such as chemotherapy or ICIs, is being explored as a way to boost efficacy and overcome resistance.[137]

Immune response

Pre-existing immunity, either from prior virus exposure or infection, and the body's innate immune response to viral infections present significant hurdles to OVT.[131,138] When injected into the body, the oncolytic virus triggers the immune system, which is designed to detect and eliminate viral infections.[139] This immune response may hinder the virus's ability to propagate and infect enough cancer cells, thereby reducing its efficacy.[140] In addition, some patients may have pre-existing immunity to the virus used in therapy, rendering the treatment ineffective from the start. Tumors often create immunosuppressive environments that further dampen both antiviral and anticancer immune responses.[141] Researchers are investigating methods to overcome these barriers, including "stealthing" viruses with polymers to evade immune detection and using cellular carriers such as Mesenchymal Stem Cells to protect viruses and enhance their tumor-targeting capabilities.[142-144]

Other challenges

Additional issues include the effects of hypoxic conditions within tumors and difficulties in selecting suitable patients for OVT. Delivery and safety concerns also pose significant obstacles, as highlighted by Chen *et al.*,[129] with ongoing research exploring improved methods for administration and risk mitigation.

Ongoing researches in OVT

Ongoing research in OVT is flourishing, with promising advancements aimed at overcoming hurdles and maximizing its potential for cancer treatment. Researchers are exploring alternative viral platforms, including adenoviruses, HSVs, and reoviruses, to enhance tumor selectivity and potency. In addition, investigations into next-generation viruses such as vaccinia and Newcastle disease virus are underway.

The identification of biomarkers predicting patient responses

to specific oncolytic viruses is underway to optimize patient selection and treatment techniques. Furthermore, the exploration of combination therapies aims to increase efficacy and overcome resistance mechanisms. Strategies to address pre-existing immunity are being developed to expand the potential patient pool.

Improvements in safety and delivery are being pursued through the development of "safety switches" and non-invasive delivery systems. Clinical trials are actively evaluating the efficacy and safety of oncolytic viruses across various cancer types and treatment settings. Regulatory bodies are also working to establish clear criteria and approval pathways for OVT.

BIOMARKERS AND PREDICTIVE TOOLS

Biomarkers are increasingly being investigated to identify individuals who are most likely to benefit from specific treatments.[145] According to the National Cancer Institute, a biomarker is a biological molecule found in blood, other body fluids, or tissues that signals a normal or abnormal process, or a condition such as cancer.[146] In cancer immunotherapy, key biomarkers include PD-L1, Tumor-infiltrating lymphocytes, tumor mutational burden, microsatellite instability, and mismatch repair deficiency. PD-L1 is a protein that enables tumor cells to evade immune attacks. While present in both tumor and non-tumor cells, it is predominantly found in malignant cells. The expression of PD-L1 in tumors is typically measured using immunohistochemistry.[93] Patients whose tumors exhibit high levels of PD-L1 often respond more favorably to immunotherapy targeting PD-L1 through ICIs.[147]

Emerging tools for patient's stratification in cancer

Emerging technologies for patient stratification offer new methods for identifying cancer patients based on their tumor's specific characteristics, enabling more tailored therapies and improved outcomes. These tools play a crucial role in precision oncology by identifying patient-specific biomarkers, personalizing treatments, and enhancing cancer care. Their integration into clinical practice holds significant promise for advancing personalized medicine and optimizing treatment strategies. Key tools include:

Next-generation sequencing (NGS)

NGS allows for rapid and comprehensive analysis of a patient's entire genome or select genes.[148] This technique helps identify mutations or genetic alterations that drive cancer progression,[149] enabling clinicians to tailor treatments to target the specific vulnerabilities of cancer cells.[150] NGS provides a detailed study of cancer genomes, facilitating more accurate diagnosis, prognosis, and the identification of drug-responsive mutations.[140] Pilot programs are currently assessing the clinical use of NGS for mutation-targeted therapies.[148]

Liquid biopsies

Liquid biopsies offer a non-invasive approach for monitoring disease progression, detecting therapy resistance, and identifying actionable mutations in real time. By analyzing circulating tumor DNA (ctDNA), they provide a dynamic snapshot of tumor evolution.[151] A decrease in ctDNA after treatment signals a positive response, whereas an increase may indicate resistance or recurrence, prompting early intervention.[152] Unlike single tissue biopsies, liquid biopsies capture tumor-derived components from the bloodstream, offering a comprehensive view of the tumor's genetic profile and guiding the selection of effective therapies.[153] Even when imaging fails to detect residual cancer, liquid biopsies can identify it, enabling early relapse detection.[154]

Machine learning and artificial intelligence

Machine learning algorithms analyze vast amounts of genetic, clinical, and imaging data to identify patterns related to treatment response, disease progression, and patient outcomes. These models can predict survival, stratify patients into risk categories, and inform treatment decisions. By analyzing individual tumor characteristics, machine learning can forecast a patient's response to specific therapies. Furthermore, it helps identify distinct tumor subtypes with unique molecular profiles,[155] supporting the development of tailored therapies for each subtype.

Multi-omics integration

Multi-omics integration combines data from multiple molecular layers, providing a holistic view of tumor biology. This approach enables the discovery of molecular subtypes, dysregulated pathways, and therapeutic vulnerabilities, ultimately driving personalized cancer treatments. Omics layers include genomics, proteomics, metabolomics, and transcriptomics. For instance, metabolomics investigates small molecules within cells, offering insights into cancer classification and patient prognosis through unique metabolic fingerprints.[156,157]

Future directions in biomarker discovery

Biomarker discovery for cancer treatment is a dynamic field that employs advanced technology, collaborative research, and personalized medicine to enhance patient outcomes and develop precision oncology. Researchers are focusing on several promising areas:

Multimodal biomarkers

Combining biomarkers from multiple sources, such as genomes, metabolomics, and proteomics, provides a comprehensive view of a patient's cancer. This approach can improve patient classification and guide treatment decisions. Multimodal biomarkers enhance accuracy by integrating data from genetics, metabolites, and proteins, offering a thorough picture of cancer for more accurate diagnoses, risk stratification, and treatment options. They address tumor heterogeneity, ensuring treatment targets the most relevant components of the malignancy, and identify resistance mechanisms, facilitating the development of strategies to overcome them. Researchers can develop targeted therapies addressing specific anomalies identified by multimodal biomarkers. For instance, Cancer antigen 19-9 (CA19-9) is a pancreatic cancer biomarker, but recent studies on non-coding RNAs such as microRNAs (miRNAs), circular RNAs (circRNAs), and long non-coding RNAs (lncRNAs) show significant promise as biomarkers and for understanding pancreatic regulatory network components.[158]

Liquid biopsy advancements

Liquid biopsies are relatively painless and yield a wealth of data. Ongoing research focuses on isolating and analyzing distinct subpopulations of circulating tumor cells (CTCs) or ctDNA to better understand tumor heterogeneity and treatment resistance. As liquid biopsy technology evolves, it holds the potential to revolutionize cancer diagnosis and treatment management, allowing real-time monitoring of tumor dynamics and facilitating personalized treatment strategies.[159]

Microbiome analysis

The gut microbiome plays a critical role in immune function and may influence cancer development.[160] Future research is expected to further explore the relationship between the microbiome and cancer, with the goal of identifying microbiome-based biomarkers that can guide treatment decisions and potentially enhance responses to immunotherapy.

Human and animal rights

This article does not contain any studies with human or animal subjects performed by any of the authors.

CONCLUSION

Cancer immunotherapy represents a paradigm shift in oncology, offering targeted and minimally invasive options compared to traditional therapies. ICIs, particularly those targeting CTLA-4 and PD-1/PD-L1 pathways, have revolutionized cancer treatment by enhancing the immune system's ability to recognize and destroy tumor cells, showing durable responses and extended survival in patients with previously refractory cancers. The success of CAR-T cell therapy in hematologic malignancies marks a significant milestone, with ongoing efforts to extend its benefits to solid tumors. Cancer vaccines, despite challenges in immunogenicity and efficacy, hold promise as personalized treatments, particularly when combined with other immunotherapeutic approaches. Despite these advancements, several challenges persist. Future research should focus on understanding the complex interactions within the tumor microenvironment, identifying biomarkers for patient selection, and developing combination therapies that enhance efficacy while minimizing adverse effects. Continued research, clinical trials, and interdisciplinary collaboration are essential to unlock the full potential of cancer immunotherapy, ultimately aiming for long-term remission and cure in cancer patients. This review underscores the importance of continued exploration and clinical translation of emerging strategies in cancer immunotherapy, aiming to improve patient outcomes and transform cancer care.

Ethical approval

Institutional Review Board approval is not required.

Declaration of patient consent

Patient's consent was not required as there are no patients in this study.

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Use of artificial intelligence (AI)-assisted technology for manuscript preparation

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