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Review Article

Advancements in vaccines against cancer stem cells (CSCs)

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Abstract: Cancer stem cells (CSCs) are a significant problem in cancer treatment due to their ability to self-renew, differentiate, and migrate, thus initiating and maintaining extensive support for metastasis. One way to eliminate CSCs is by using CSC-specific targeting vaccines, which mainly utilise their unique CSC-associated antigens. The current potential vaccines against the CSCs are the Dendritic Cell (DC)-based vaccine, Nanoparticle (NP)-based vaccine, and iPSC (Induced Pluripotent Stem Cell)-based vaccine. Potential vaccines/immunotherapies, such as GM-CSF Surface-Modified vaccines and VLP-based xCT immunotherapy, are also discussed. This review aims to highlight the progress in vaccinology against CSCs and the current limitations of these vaccines, such as the tumour microenvironment (TME) and a higher proliferation rate responsible for the low efficacy of anti-CSC vaccines.

Keywords: Cancer stem cells, therapy, immunotherapy, dendritic cell (DC)-based vaccine, nanoparticle (NP)-based vaccine, iPSC (Induced Pluripotent Stem Cell)-based vaccine

Introduction

The primary problem in modern oncology is not the removal of the primary tumour, but the complete eradication of a persistent, adaptive subset of cells that contribute to treatment failure and metastatic recurrence: the Cancer Stem Cells (CSCs). These cells, referred to as tumour-initiating cells, facilitate tumour proliferation, exhibit resistance to standard therapies such as chemotherapy and radiation, and propagate new tumours in remote locations, contributing significantly to cancer-related mortality [1]. The development of therapeutic strategies that can specifically and effectively eliminate this cellular reservoir represents one of the most critical unmet needs in cancer medicine [2].

This review addresses the primary immunological challenge presented by cancer stem cells: how can the immune system be conditioned to eradicate a target that continually alters its phenotype while concurrently establishing its own protective barrier, the immunosuppressive tumour microenvironment? Although the notion of cancer vaccines is well-established, with preventive vaccinations for oncogenic viruses such as HPV (Human Papillomavirus Virus) representing a significant public health achievement, therapeutic vaccines aimed at treating existing tumours encounter considerably larger challenges. We will rigorously assess the principal vaccine platforms: Dendritic Cell (DC), Nanoparticle (NP), and induced Pluripotent Stem Cell (iPSC)-based methodologies not as a descriptive summary, but through an analytical lens focused on how each strategy attempts to solve the fundamental problems of CSC biology. We contend that the future of efficacious anti-CSC immunotherapy necessitates a paradigm shift from singular "magic bullet" approaches to strategically designed, multi-modal strategies that combine personalised, multi-

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antigenic vaccines with therapies that actively restructure the immunosuppressive tumour microenvironment.

Resistance to cancer therapy

Cancer stem cells are highly resistant to traditional cancer therapies, such as radiation and chemotherapy. Due to their resilience, CSCs may survive therapy by developing resistance through several mechanisms. The surviving drug-resistant CSCs repopulate the tumour, leading to recurrence and progression even when the bulk of tumour cells are killed. Production of drug efflux pumps, the initiation of anti-apoptotic pathways, and the capacity to live in protected niches within the tumour microenvironment are a few of the many diverse resistance mechanisms of CSCs. Moreover, CSCs can modify their metabolism in response to environmental changes, such as in hypoxia, alternating between mitochondrial and glycolytic metabolism to meet their energy requirements and ensure their survival [3]. Because of their flexible metabolism, CSCs are more challenging to target with medications that interfere with their ability to produce energy. They often exist in a quiescent state, which makes them less susceptible to chemotherapy that targets rapidly dividing cells [4]. They also possess superior DNA repair capabilities compared to non-stem cancer cells. Many CSCs express high levels of drug efflux pumps, such as P-glycoprotein, which actively transport chemotherapeutic agents out of the cell [5]. This mechanism reduces the intracellular concentration of drugs, diminishing their efficacy and allowing CSCs to survive treatment.

Cancer stem cells utilise autophagy and mitophagy signalling during tumour formation to survive and gain resistance through several mechanisms. Autophagy and mitophagy are cellular processes that help maintain cellular homeostasis by recycling damaged or dysfunctional cellular components. In CSCs, these processes are exploited to promote survival and resistance to therapy. Autophagy helps preserve the integrity of the CSC population by recycling damaged cellular components and maintaining the cellular energy balance. Specifically, mitophagy helps maintain the mitochondrial function and integrity of CSCs, which are crucial for their survival and proliferation. Additionally, autophagy and mitophagy can help CSCs evade the immune system by reducing the expression of surface antigens and promoting the degradation of immune-related proteins [6-8]. Metabolic rewiring is a crucial aspect of CSC biology, as it allows these cells to adapt to changing microenvironmental conditions and maintain their stemness [2]. For example, lactate dehydrogenase (LDHA) has been demonstrated to play an essential role in maintaining the stemness of breast cancer stem cells (BCSCs) by enabling glycolytic metabolism [9]. High levels of ALDHA in BCSCs correspond with increased stemness markers and higher tumorigenic potential. This suggests that the metabolic pathways directly assist the maintenance of stem cell-like features in tumours. Therefore, given their heterogeneity and the possibility that acquired stemness is a fluid condition, it can be challenging to identify and target CSCs [10] using the traditional cancer therapies.

Identification and characterization of CSCs

Surface marker identification

The primary approach to identifying CSCs involves analysing cell surface markers and conducting functional and gene expression analyses. Cell surface markers (Cluster of Differentiation (CD) markers) are used to identify and categorise different cell types within the immune system. These markers are proteins expressed on the cell surface, and their expression patterns can help distinguish cell types and determine their stage [11]. These markers are associated with stem cell potential and uncontrolled tumour growth, making them valuable for CSC detection and targeted therapy. Commonly used CD markers for identifying CSC include CD24, CD44, and CD133. While no single marker can identify all CSCs across all cancers, several cancers express similar markers [12]. Overexpression of CD markers such as CD44, an adhesion molecule, is often associated with CSCs and linked to increased tumour aggression, invasion, adhesion, metastasis, and poor prognosis in various tumours, including renal cell carcinoma [13]. Similarly, overexpression of CD133 or Prominin-1, a five-transmembrane glycoprotein, has been linked to CSCs isolated from brain, breasts, lungs, and colorectal cancers [14].

These cells exhibited high resistance to chemotherapy, radiation, and other conventional treatments [15]. Other relevant CD markers associated with CSCs include CD13 and CD171, which are involved in growth, differentiation, and survival. The core cells of spheres produced by liver cancer were shown to highly express CD13 and CD90 [16]. Several CD markers are expressed on the surface of leukaemia. These include CD15, CD34, CD38, and CD123. CD38 is often used in combination with CD34 to identify and characterise leukaemia and CD26 is expressed in leukaemia and lymphoma [17]. CD29 is overexpressed in some cancers associated with metastasis. CD47 and CD49 are expressed in various cancers that evade the immune system. CD26 is correlated with CSC properties such as sphere formation, tumour initiation, and chemotherapy resistance [18] and has been proposed as an additional marker for CSCs. In addition to the major markers discussed above, several other markers have been identified (**Table 1**).

CSC characterization methods

Functional assays, such as xenotransplantation, spheroid formation, and colony formation, can evaluate CSC properties like self-renewal and tumorigenic potential [19].

Xenotransplantation: Xenotransplantation is the gold standard functional assay for CSCs involving transplanting cancer cells into immunodeficient mice to assess their tumorigenic potential in a microenvironment resembling the human tumour microenvironment (TME) [20]. This assay enables the study of cancer biochemical and physiological processes, drug screening, gene therapy exploration, effective cancer treatment sites, real-time monitoring of signalling pathways, drug treatment, and the enhancement of early cancer detection and prevention strategies.

Colony formation assay: Another functional assay is the colony formation assay, which measures the ability of single cells to form colonies, reflecting the clonogenic potential of CSCs. Cancer stem cells are expected to initiate tumour formation at lower cell numbers than non-CSCs.

Spheroid formation assay: The spheroid formation assay, a widely recognised method for evaluating self-renewal and differentiation, involves culturing three-dimensional spheroid in serum-free and low-attachment conditions that closely mimic the *in vivo* TME of a tumour. This assay enhances the population of CSCs due to hypoxia-induced dedifferentiation of cancer cells at the core of the spheroids [21].

While informative, functional assays can be time-consuming and may require specialised expertise. Additionally, xenotransplantation raises ethical concerns and may not fully replicate the human TME [22].

Molecular marker assays (Gene expression and transcription factors profiling): Molecular marker assays, such as gene expression profiling and transcription factors, can identify genes and specific transcription factors commonly expressed by CSCs [23]. Gene profiling can reveal genes uniquely expressed in CSCs, serving as markers to identify and isolate these cells from the tumour population. Transcription factors like Oct 4, Sox 2, and Nanog, known as “stemness genes”, are frequently highly expressed in CSCs, contributing to their self-renewal and tumour-initiating capacities [24]. Gene expression profiling provides crucial data for predicting patient prognosis [25].

Side population and ALDH activity assay: Other methods, such as the side population assay and aldehyde dehydrogenase (ALDH) activity, can isolate CSCs based on their unique characteristics. The side population (SP) assay identifies and isolates cells with high efflux capacity, a characteristic associated with CSCs due to the overexpression of adenosine triphosphate (ATP) binding cassette (ABC) transporters like ABCB1 (P-glycoprotein) and ABCG2, linked to chemoresistance [26]. These transported actively pump out various substrates, including the fluorescent dye Hoechst 3342 [26]. SP cells have been identified in multiple types of cancers and exhibit enhanced self-renewal, tumorigenicity, stemness-related genes, drug resistance, and invasiveness compared to non-SP cells [27]. SP cells have been identified in cancers such as NSCLC (non-small-cell lung cancer), ovarian cancer, gastrointestinal system, bone sarcoma and breast [27]. Aldehyde dehydrogenases (ALDHs) detoxifying enzymes are highly expressed in stem cells,

Table 1. Cancer stem cell markers in various cancer types

Gene	Associated Cancer(s)	References
ABCB5	Melanoma	[30, 31]
AFP	Liver	[30, 32]
ALCAM/CD166	Colorectal, Melanoma, Prostate, Lung	[30-34]
ALDH1A1	Breast, Colorectal, Prostate, Ovarian, Lung, Liver, Head/Neck, Gastric, Leukaemia	[30, 32-34]
CD13	Breast, Liver, Cervix	[30, 31]
BMI1	Brain, Lung, Head/Neck, Leukaemia, Breast, Colorectal, Prostate, Pancreatic	[30, 32, 34]
CD15	Gastric, Brain	[31, 33, 34]
CD151	Prostate	[31, 34]
CD19	Blood	[31, 34]
CD34	Blood, Lung	[30, 31, 33-35]
CD38	Blood	[31, 33-35]
CD44	Breast, Pancreatic, Prostate, Colorectal, Ovarian, Lung, Liver, Head/Neck, Blood, Bladder, Gastric, Brain, Cervix	[30-35]
CD47	Leukaemia, Bladder	[34]
CD66c	Bladder	[34]
CXCR4	Pancreatic, Breast, Brain, Gastric	[30, 32, 34]
CD26	Colorectal, Blood	[31-34]
CD105	Ovarian, Cervix	[31, 34]
EPCAM	Pancreatic, Colorectal, Breast, Ovarian, Liver, Lung, Gastric, Brain	[30-35]
FUT4	Brain	[30, 34]
TIM3	Blood	[32, 34]
α2β1	Prostate	[30, 34]
CD123	Blood	[32-34]
CD49f	Liver, Breast, Brain, Prostate	[30, 32-35]
CD29	Colorectal, Breast, Cervix	[30-35]
CD61	Breast	[30, 32]
CD117	Blood, Ovarian, Lung, Liver	[30, 32-35]
KLF4	Breast, Brain, Colorectal, Leukemia, Prostate	[30, 34]
LETM1	Gastric, Colorectal	[32]
LGR5	Colorectal, Head/Neck, Breast, Gastric	[30, 32-34]
LINGO2	Gastric	[32]
CD20	Melanoma, Blood	[30, 31, 34, 35]
MSI1	Brain, Colorectal	[30, 34, 35]
MSI2	Gastric	[32]
NANOG	Breast, Brain, Ovarian, Lung, Colorectal, Prostate, Gastric, Leukemia	[30, 32, 34]
Nestin	Brain, Melanoma, Pancreatic	[30, 34]
CD271	Melanoma, Head/Neck	[30, 31, 35]
CD87	Lung	[30, 32]
POU5F1	Ovarian, Melanoma, Bladder, Pancreatic, Prostate, Lung, Breast, Liver, Leukemia	[30, 32, 34]
CD133	Pancreatic, Prostate, Brain, Liver, Colorectal, Ovarian, Melanoma, Lung, Head/Neck, Brain, Gastric, Renal	[30-35]
SALL4	Blood, Breast, Colorectal, Ovarian, Prostate	[32-34]
SOX2	Colorectal, Brain, Pancreatic, Prostate, Ovarian, Melanoma, Lung, Breast, Gastric, Leukemia	[30, 32, 34]
CD90	Blood, Liver, Breast, Brain, Lung, Head/Neck, Gastric	[30-35]

localised in the cytoplasm, mitochondria, and or nucleus, with activities linked to tumorigenicity, spheroid formation, chemoresistance, invasion, and poor prognosis [28]. ALDH is considered a marker for CSCs, playing a functional role in self-protection, differentiation, and expansion of CSC populations [28]. Overexpression of ALDH in cancer promotes tumour growth, metastasis, and chemoresistance [29].

CSC identification challenges

CSC populations' biological and molecular properties in different tumours vary, making it challenging to create a unified, efficient marker to characterise CSCs [36]. Moreover, the expression of particular surface markers such as ALDH1, CD133, and CD44 frequently used to identify and isolate CSCs are not unique across different tumours as these markers are also expressed by some normal cell types [37]. Although ALDH1, CD133, and CD44 are worse prognostic markers they are also expressed by normal intestinal mucosa cells [28], normal mammary stem cells and hematopoietic progenitors also express ALDH1 [38].

A fundamental challenge in developing CSC-targeted vaccines is the "marker problem", which stems from the biological overlap between CSCs and normal tissue stem cells. Research indicates that approximately 73% of surface markers currently used to identify CSCs are also expressed on normal embryonic or adult stem cells [37]. This is not a minor issue; it is the primary source of risk for significant "on-target, off-tumour" toxicity, where a vaccine successfully targets the intended antigen on a CSC but also leads to the immune-mediated destruction of healthy, regenerative tissues like hematopoietic progenitors or intestinal crypts. This risk is exemplified by markers like ALDH1, which is also expressed by normal mammary stem cells, and LGR5, a marker for colorectal CSCs that is also critical for the maintenance of intestinal stem cells [39]. Furthermore, the concept of CSC identification markers must be clearly distinguished from that of therapeutic vaccine antigens. An identification marker is useful in a research context if it can enrich for a CSC population, even a rare one. A therapeutic antigen, however, must be stably and highly expressed on the surface of the vast majority of cancer cells, including CSCs, while being absent from essential normal tissues. CSC-based therapies are being explored to eradicate cancer. The current trend is to target multiple markers in combination as reliable means of characterisation and, hence, therapeutic approaches [40]. Various markers and functional assays effectively capture a broader spectrum of the CSC population, increasing their identification's specificity and mitigating individual methods' limitations. This allows for the validation and reproducibility of findings [41].

Treatment against CSCs

Conventional cancer therapies often fail to eliminate CSCs, which can cause treatment resistance and relapse. Therefore, it is crucial to develop efficient methods that mainly target CSCs. Anticancer drug research has focused on the regulatory network of CSCs, which includes microRNAs and signalling pathways, including the Wnt/β-catenin, Notch, and Hedgehog pathways, which regulate CSC properties [42]. Several existing drugs have demonstrated efficacy in targeting CSCs. Metformin, a drug routinely used to treat diabetes, has been shown to reduce CSC characteristics in a variety of malignancies [43]. Other repurposed drugs include thioridazine (an antipsychotic), chloroquine (an antimalarial), and disulfiram (used for alcoholism), all of which have shown anti-CSC activity in preclinical tests. These medications could operate by altering the milieu that promotes CSCs or by triggering death in these robust cell types [44]. Research has also resulted in the development of new small compounds explicitly designed for CSCs. For example, substituted chloroacetamides have been synthesised and tested for their capacity to suppress CSC self-renewal in a variety of cancer types, including breast and prostate cancer. These drugs have shown promise in preclinical studies, effectively lowering CSC viability while protecting normal cells.

There are now more options for targeting CSCs due to recent developments in immunotherapy. Immunotherapy is a therapeutic approach that utilises the endogenous immune system's capacity, engaging innate immune mechanisms and adaptive immune responses to combat pathological conditions. One example is the use of dendritic cells (DCs) loaded with CSC antigens, such as CSC lysates or NANOG peptides, which can elicit a particular anti-tumour T-cell response and encourage the development of immunological memory [45, 46]. In animal models, these tactics have shown significant preventive effects, such as longer lifespans and less tumour growth. The approaches towards CSC treatments have been summarised in **Figure 1**.

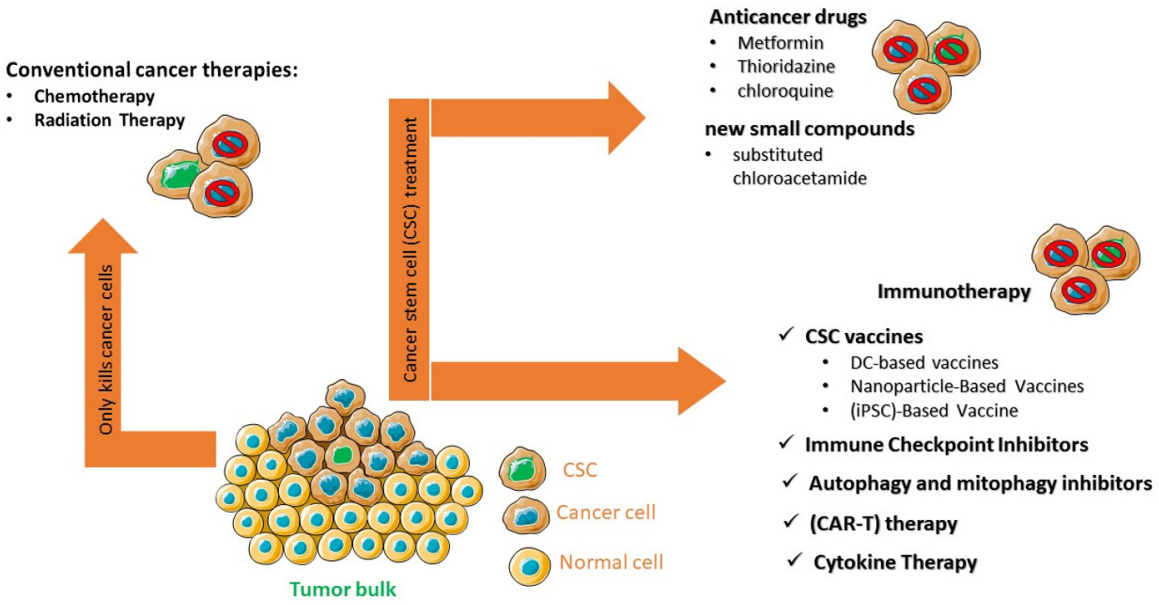


Figure 1. Different approaches of cancer stem cell therapy.

Significantly, novel high-throughput methodologies are expediting the identification of CSC markers. Single-cell RNA sequencing has elucidated the transcriptional variability of cancer stem cell types and found novel combinations of markers. Similarly, genome-scale CRISPR or RNAi screenings have begun identifying genes critical for CSC self-renewal, resulting in more potential antigens [47, 48]. Integrating these RNA-based and functional genomic techniques will enhance the discovery of reliable CSC markers for vaccine targeting.

Cancer stem cell (CSC) vaccines

Another promising immunotherapy strategy that has gained attention in recent years is the development of cancer stem cell (CSC) vaccines. CSC vaccines represent a promising approach to cancer treatment by specifically targeting cancer stem cells, often resistant to conventional therapies. These vaccines present tumour-associated antigens to the immune system, leading to antibody generation and activation of T cells against CSCs. By training the immune system to recognise and attack these resilient cancer cells, CSC vaccines offer a potentially powerful method to achieve long-term cancer control. Unlike preventive vaccines that protect against cancer-causing viruses (such as HPV vaccines), CSC vaccines are designed to treat existing cancer by stimulating the immune system to recognise and attack cancer cells with greater specificity and potency than conventional chemotherapy [49]. The immune response induced by CSC vaccines includes the activation of cytotoxic T lymphocytes (CTLs) and the production of antibodies that specifically target and lyse CSCs. These vaccines can modulate the tumour microenvironment by increasing the infiltration of immune cells like dendritic and natural killer cells while reducing immunosuppressive cells such as regulatory T cells and myeloid-derived suppressor cells [50]. This enhanced immune response addresses the limitations of standard treatments that often fail to eliminate resilient CSCs responsible for tumour recurrence. Several innovative approaches are being explored in CSC vaccine development: (1) CSC-enriched populations have shown effectiveness in inducing strong immune responses, with vaccines causing the production of IgG antibodies that attach to CSCs and trigger complement-mediated lysis, as well as CTLs capable of killing CSCs in vitro [51]. (2) Vaccine nanodiscs specifically target $ALDH^{high}$ CSCs associated with tumour metastasis and recurrence. When combined with anti-PD-L1 treatment, these nanodiscs enhance antigen trafficking to lymph nodes and produce robust T-cell responses specific to ALDH, demonstrating significant antitumor efficacy in mouse models [52]. (3) Adjuvant CSC-DC vaccinations

Table 2. Pros and cons of each vaccine type

Vaccine Type	Advantages	Limitations	References
Dendritic Cell-Based Vaccines	● Targets elusive CSCs. ● Stimulates antitumor immune response.	● Immunosuppressive tumour microenvironment can hinder effectiveness. ● Identifying the most immunogenic tumour antigens (TAAs) is ongoing. ● Optimising DC maturation and delivery requires further research.	[61, 63, 65]
Nanoparticle-Based Vaccines	● Targets and eliminates CSCs. ● Potential to prevent cancer recurrence and promote long-term remission. ● Various strategies for targeting and delivering antigens. ● Enhances immune system activation. ● Immunogenicity of cancer vaccines.	● Challenges in overcoming the similarity between cancer cells and normal cells	[66, 67, 71, 83]
iPSC (Induced Pluripotent Stem Cell) Based Vaccine	● Targets shared antigens between iPSCs and CSCs. ● Creates human T cells with tumour targets. ● Efficiently targets CSCs and alters tumour microenvironment. ● Cutting-edge approach for simulating actual disease and creating new treatments.	● Technical difficulties in producing iPSCs. ● Concerns about immunogenicity and tumorigenicity.	[79, 84]

have shown efficacy in reducing local tumour recurrence and spontaneous metastases while extending host survival [45].

The development of effective CSC vaccines continues to advance with promising results in preclinical investigations, showing potential for long-term tumour control and prevention of recurrence. Specific examples of these innovative vaccine approaches and their clinical applications are described below and compared in **Table 2**. Nonetheless, CSC-targeted vaccinations have further challenges beyond those associated with bulk tumour vaccines. CSCs demonstrate phenotypic plasticity; for example, non-stem cancer cells can dedifferentiate into CSC-like states [53] and they can actively dodge immune responses by downregulating antigen presentation and upregulating inhibitory molecules, such as N-glycosylated PD-L1 [54]. No universal antigen identifies all cancer stem cells, as marker expression differs among tumour types. These characteristics necessitate that CSC vaccines frequently target numerous antigens or employ combination techniques. A recent study found that a nanoparticle vaccination targeting $ALDH^{high}$ CSCs elicited robust T-cell responses only in conjunction with anti-PD-L1 treatment [52].

We concentrate on dendritic cell-based, nanoparticle, and induced pluripotent stem cell-based cancer stem cell vaccines because to their capacity to present a diverse range of cancer stem cell antigens. Dendritic cell vaccines infused with whole cancer stem cell lysates can activate T lymphocytes against several stem cell antigens concurrently [45]. Nanoparticle vaccines can simultaneously deliver CSC peptides and adjuvants to dendritic cells, therefore augmenting antigen presentation and T-cell activation [52]. iPSC vaccines use the significant antigenic similarity between pluripotent stem cells and cancer stem cells, showcasing several oncofoetal antigens common to tumours [48]. Conversely, peptide-only or oncolytic viral strategies generally address fewer targets or encounter further limitations (e.g., restricted epitopes or pre-existing immunity), which is the rationale for this review's focus on the aforementioned platforms.

Dendritic cell-based vaccines

Dendritic cell (DC)-based vaccines represent a cutting-edge approach in the fight against cancer, particularly those targeting elusive cancer stem cells (CSCs) that are often responsible for tumour relapse and resistance to conventional therapies. Having remarkable antigen-presenting abilities, DCs are a special kind of immune cell that makes them excellent choices for cancer immunotherapy. Generally, they play a crucial role in initiating and controlling immunological responses, acting as a link between the innate and adaptive immune systems [55-57]. **Figure 2** illustrates an immunotherapy strategy targeting cancer stem cells (CSCs) using dendritic cells (DCs). The process begins with isolating immature DCs from the patient and tumour tissue containing CSCs and cancer cells. CSCs are lysed to generate a CSC lysate rich in tumour-specific antigens. Immature DCs are then matured ex vivo and loaded with CSC antigens, enabling them to present these antigens to T cells. Upon reintroduction into the patient, antigen-loaded mature DCs activate T cells, which recognise and destroy CSCs, addressing a key source of tumour recurrence and resistance. This approach leverages DCs' role as antigen-presenting cells to enhance antitumor immunity [58]. In a mouse model of malignant melanoma, research has shown that DCs pulsed with CSC lysates might produce strong antitumor immune responses, indicating that CSCs are potential targets for DC-based immunotherapy [45]. An essential aspect of using DCs in cancer vaccines is their capacity to deliver tumour antigens to T cells [55]. DC vaccines have shown promise in early clinical studies; some patients have experienced tumour regression and activation of antitumor immune responses [59]. However, because these reactions are not always intense, studies to improve the effectiveness of DC vaccinations are still being conducted [59]. Enhancing antigen presentation, stimulating T cells, and overcoming the immunosuppressive nature of the tumour microenvironment are all part of the DC vaccine optimisation process [56, 57].

Many studies have been performed on modifying DCs to increase their immunogenicity. Genetically engineered DCs expressing tumour antigens or immunostimulatory molecules have shown promise in preclinical and clinical contexts. The goal of these changes is to improve the capacity of DCs to stimulate cytotoxic T lymphocytes (CTLs), which can attack and destroy cancer cells, including CSCs [60]. A key immu-

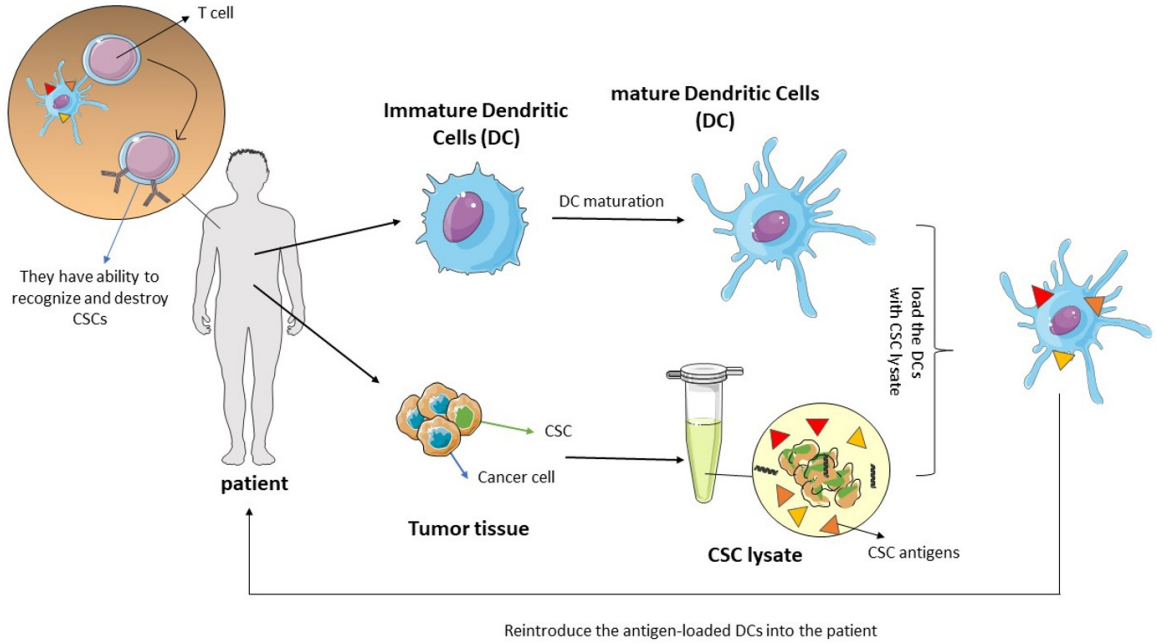


Figure 2. The procedure for developing a dendritic cell-based cancer stem cell therapy vaccine.

nological advantage of DC-based vaccines is the ability of DCs to perform cross-presentation. This is the process whereby DCs take up exogenous antigens, such as those from a CSC lysate, and present the resulting peptides on MHC class I molecules. This is critical for priming and activating cytotoxic $CD8^+$ T cells, the primary immune effectors capable of directly killing tumour cells. Following antigen loading and maturation [61] ex vivo, the DCs are infused back into the patient, where they migrate to draining lymph nodes to orchestrate this potent T-cell response [62]. Vaccines based on DC-tumour fusion cells provide an additional novel approach. A wide range of tumour-associated antigens (TAAs) may be generated by fusing DCs with entire tumour cells, activating $CD4^+$ and $CD8^+$ T cells. One benefit of this approach is that it produces endogenous TAAs with major histocompatibility complex (MHC) molecules, which are essential for successful T-cell activation [63]. DC-based vaccines show promise, but some hurdles need to be solved. The immunosuppressive nature of the tumour microenvironment may prevent T-cell activation and DC function [56]. The maturation and activation of DCs can sometimes trigger an overactive immune response, causing damage to normal tissues [64]. While DC activation involves the necessary release of pro-inflammatory cytokines, the risk of severe Cytokine Release Syndrome (CRS), or "cytokine storm", is low with DC vaccines. This specific adverse event is more commonly and severely associated with therapies that induce massive and rapid T-cell proliferation in vivo, such as CAR-T (Chimeric Antigen Receptor T-cell) therapy [65]. In some cases, DC vaccines may induce immune tolerance rather than an active immune response, particularly if the DCs present antigens in a non-inflammatory context [64]. This can lead to the suppression of the immune response against cancer cells. Furthermore, research is still being conducted to determine which TAAs are the most immunogenic and to optimise DC maturation and delivery [61].

Nanoparticle-based vaccines

With a focus on the elusive CSCs linked to tumorigenesis, metastasis, and resistance to conventional therapies, nanoparticle-based vaccines represent a state-of-the-art approach in the battle against cancer. By training the immune system to identify and eliminate CSCs, these vaccines hope to prevent cancer from returning and encourage long-term remission. Using mannose-modified nanoparticles coated in cancer cell membranes to improve their absorption by antigen-presenting cells such as

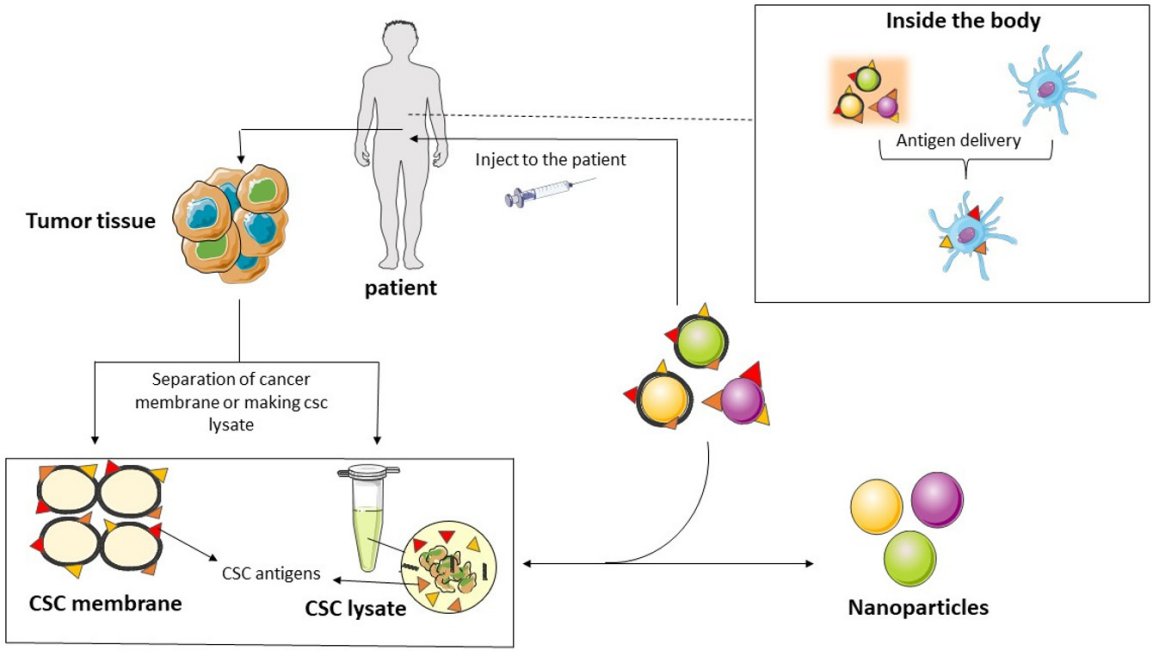


Figure 3. The procedure of developing nanoparticle-based vaccines for cancer stem cell therapy.

dendritic cells is one creative tactic [66]. This method uses poly(d,l-lactide-co-glycolide) nanoparticles coated with cancer cell membranes that contain antigens unique to tumours and loaded with imiquimod, a Toll-like receptor 7 agonist. Mannose modification enhances the targeting of dendritic cells, promoting their maturity and initiating anticancer immune responses. This nano vaccine, particularly when combined with checkpoint blockade therapy, has shown promising results in delaying tumour development and treating established tumours [66]. Numerous reviews have examined the application of biodegradable nanoparticles in cancer vaccines, emphasising their ability to deliver adjuvants and tumour-associated antigens to dendritic cells. In this way, the immune system is effectively activated to combat tumour cells. Poly(lactic-co-glycolic acid) (PLGA) and poly-ε-caprolactone (PCL) are two examples of biodegradable aliphatic polyesters that show great promise since they may modify the size, surface charge, and surface functionalisation of nanoparticles that are absorbed by dendritic cells [67]. **Figure 3** illustrates a nanotechnology-based immunotherapy strategy for targeting cancer stem cells (CSCs). The process begins with isolating tumour tissue containing cancer cells and CSCs. The CSC membrane is separated or lysed to create a CSC lysate rich in tumour-specific antigens. These antigens, which may include proteins like ALDH and Nanog, are then loaded onto nanoparticles that serve as targeted delivery vehicles. The antigen-loaded nanoparticles are administered to the patient, facilitating antigen presentation to immune cells [52, 68, 69]. This approach aims to activate the immune system against CSCs, addressing a key source of tumour recurrence and therapy resistance. The strategy leverages nanotechnology to improve antigen stability, targeting, and immune activation against these critical cancer-initiating cells, as demonstrated in preclinical studies showing reduced CSC frequency and enhanced survival outcomes.

Additionally, technologies for delivering drugs via nanoparticles are being developed to enhance the effectiveness of immunotherapies and the immunogenicity of cancer vaccines. These systems can potentially improve the precise delivery of medicines and tumour antigens, boost immune activation, and increase the effectiveness of adoptive cell treatments [70]. The evaluation of existing approaches highlights the potential of nanoparticles to enhance cancer immunotherapies. Dendritic cells are the target of chitosan nanoparticles loaded with tumour cell lysate, which improves

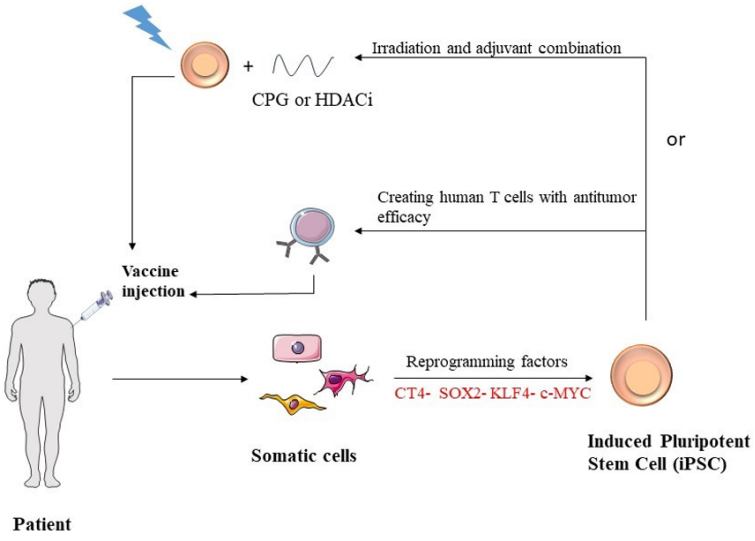


Figure 4. Production of induced pluripotent stem cell (iPSC)-based vaccine.

antigen presentation. When used as a vaccine, these nanoparticles have demonstrated the capacity to stimulate dendritic cell maturation, increase serum IFN-γ (interferon-gamma) and IL-4 (interleukin) levels, and halt tumour growth in vivo, suggesting their potential as potent cancer vaccines [71]. One of the most critical aspects of creating potent anticancer vaccines is the modification of antigen-presenting cells utilising nanoparticle technology. The ability of nanoparticles to trigger the correct immunological reactions is a problem caused by the similarity between cancer cells and normal cells [72]. It has been shown that blocking the TGF-β signalling system increases the ability of nanoparticles to penetrate tumours and aids in the delivery of CSC treatment. With this method, tumour growth is suppressed, and the fraction of CSCs is decreased in vivo by improving the intertumoral distribution of nanoparticles and their internalisation by CSCs [73].

Induced pluripotent stem cell (iPSC)-based vaccine

An innovative technique that has the potential to revolutionise cancer immunotherapy is the use of induced pluripotent stem cells (iPSCs). The iPSCs are adult somatic cells reprogrammed into a pluripotent state, enabling them to differentiate into any cell type. This remarkable quality of iPSCs has been used to create novel cancer therapy strategies, most notably in creating cancer vaccines targeting cancer stem cells (CSCs) [74]. Because iPSCs and CSCs share several proteins, researchers are developing vaccines based on iPSCs that specifically target these shared antigens [75]. iPSCs also express tumour-associated antigens that can be used to trigger antitumor immune responses. Therefore, their use as a source for cancer vaccines is logical [48]. iPSCs are a viable option for cancer immunotherapy [76, 77] because they can elicit an immune response against antigens that cancer cells share. Preclinical research has demonstrated the promise of autologous iPSC-based vaccinations. In several cancer models, vaccination with irradiated iPSCs has been shown to stop tumour growth and decrease the number of metastatic tumours. This impact is linked to a T-cell response against tumours, indicating that iPSC vaccination can enhance immunity against particular antigens [48]. Furthermore, it has been demonstrated that iPSC vaccines efficiently target CSCs, changing the tumour microenvironment and increasing survival rates [75]. **Figure 4** illustrates the process of developing an induced pluripotent stem cell (iPSC)-based vaccine. iPSCs are chosen due to their expression of tumour-associated antigens (TAAs), also present in cancer stem cells (CSCs) [48]. These shared antigens are crucial for eliciting a strong immune response. Somatic cells are first harvested from either a patient (autologous approach) or a donor (allogeneic approach). These cells are reprogrammed using transcription factors (Oct4, SOX2, KLF4, and c-MYC) and undergo modifications such as irradiation to prevent teratoma formation and are processed into a vaccine formulation [48]. The vaccine aims to stimulate cytotoxic $CD8^+$ T cells and memory $CD4^+$ T cells, reduce immunosuppressive cells, and induce a cancer-specific humoral immune system [48, 78].

There are two sides to iPSC's immunogenicity. As discussed earlier, it is helpful for cancer vaccinations but has safety issues for regenerative applications. Since cells can be

altered to reduce these dangers, the tumorigenic qualities of iPSCs, which are a concern for transplantation, are less troublesome in the context of vaccines [76]. The use of iPSCs as a source of cancer vaccines is supported by their expression of oncofoetal antigens and ability to generate antigen-specific T-cell responses. However, the potential of iPSC-based immunotherapy as a pan-cancer treatment strategy is significant and warrants further investigation [79, 80]. iPSCs can potentially form teratomas or other types of tumours if not fully differentiated and purified before use. Even though iPSCs are derived from the patient's cells, immune rejection is still risky. This can occur if the iPSCs express antigens that are recognised as foreign by the immune system, leading to an adverse immune response. The use of iPSCs can sometimes trigger autoimmune reactions, particularly if the immune system begins to recognise normal tissues as targets. This can lead to chronic inflammation and damage to healthy tissues [64]. Notwithstanding its potential, the clinical implementation of iPSC-derived vaccine is confronted with noteworthy obstacles. These include difficulties in producing iPSCs technically, concerns about their immunogenic and tumorigenic qualities, and the need for more research to determine their therapeutic efficacy [76, 81]. While the antigenic overlap between iPSCs and CSCs is the foundational rationale for this vaccine strategy, it also presents unique and formidable challenges. A primary safety concern is the inherent ability of pluripotent stem cells to form teratomas in vivo. Although this risk is mitigated by lethally irradiating the iPSC vaccine product, a more fundamental biological hurdle remains: the potential loss of relevant antigens upon differentiation [74]. The very process that makes iPSCs safe (differentiation or irradiation-induced death) may eliminate the shared oncofoetal antigens that are the intended targets, questioning the long-term efficacy of the induced immune response against a plastic CSC population. Moreover, the clinical translation of iPSC-based therapies is constrained by immense manufacturing complexities, including the difficulty of achieving stable, controlled, and scalable cellular differentiation under cGMP (current good manufacturing practice) conditions [82]. However, the use of iPSCs is a cutting-edge approach for simulating real diseases and developing new treatments [74].

Emerging targets and next-generation platforms

Other vaccine strategies involve combining more than one strategy with potential as great as the vaccines discussed above. These other vaccine strategies lead to combination therapies and drug immunotherapies targeting potential underappreciated receptors or protein targets. These overlooked therapies can be used together for better outcomes. These strategies have been summarised in **Table 3**.

Targeting the xCT cystine-glutamate antiporter: System xc-, commonly referred to as the xCT cystine-glutamate antiporter, is a membrane transport protein that facilitates the exchange of extracellular cysteine for internal glutamate, which is essential for preserving cellular redox equilibrium. Due to its role in the synthesis of glutathione, a significant cellular antioxidant that shields cancer cells from oxidative stress and chemotherapeutic drugs, this antiporter is particularly important for cancer treatment. Targeting xCT as a therapeutic approach to treating cancer has been the focus of recent research, especially regarding taking advantage of its expression in cancer stem cells (CSCs), which are linked to tumour recurrence and metastasis because of their resistance to traditional therapies. The role of xCT in modulating the tumour microenvironment and impacting the cancer stem cell niche further underscores its potential as a target for cancer therapy [85]. The mechanistic link between xCT and cancer therapy is rooted in cellular redox balance. CSCs frequently overexpress the xCT antiporter (encoded by the SLC7A11 gene) to increase the import of extracellular cystine. Intracellularly, cystine is reduced to cysteine, which is the rate-limiting substrate for the synthesis of the master antioxidant glutathione (GSH). This elevated pool of GSH creates a powerful buffer that protects CSCs from the high levels of reactive oxygen species (ROS) induced by chemotherapy and radiotherapy, directly contributing to therapy resistance [86]. An anti-xCT vaccine, therefore, aims to disrupt this protective mechanism. By inducing polyclonal antibodies against xCT, the vaccine can flag CSCs for destruction by immune cells like Natural Killer (NK) cells via Antibody-Dependent Cell-mediated Cytotoxicity (ADCC), providing a killing mechanism that is independent

Table 3. Comparison of other vaccine strategies against CSCs

Strategy	Mechanism of Action	Target Molecule/Cell	Advantages	Disadvantages	Cancer Models	References
Targeting xCT cystine-glutamate antiporter	• Inhibits xCT antiporter, reducing cystine uptake and glutathione synthesis. • Increases susceptibility of CSCs to chemotherapy.	xCT antiporter on CSC membrane	• Targets CSCs which are often resistant to traditional therapies. • Enhances the effectiveness of chemotherapy.	• May require high drug doses to overcome compensatory mechanisms (e.g., increased expression of other transporters. • Potential for off-target effects on healthy cells expressing xCT.	Small-cell lung cancer (SCLC) & Breast cancer	[89, 90, 102]
xCT-Targeted Immunotherapy	• Stimulates immune response against CSCs expressing xCT. • Utilises various approaches (VLPs, vaccines).	• VLPs or vaccines targeting xCT on CSC surface. • T lymphocytes, Antibody-dependent cell cytotoxicity (ADCC)	• Specifically targets CSCs. • Can enhance the effectiveness of chemotherapy. • May offer a more durable antitumor response compared to drugs.	• Requires further development and clinical evaluation for efficacy and safety. • Potential for immune resistance mechanisms to develop.	Breast cancer	[89, 90]
GM-CSF Surface-Modified CSC Vaccine	• Modifies CSC surface with GM-CSF to attract and activate immune cells. • Stimulates T-cell response against CSCs.	GM-CSF on the surface of modified CSCs, Dendritic cells (DCs), T cells	• Targets CSCs directly. • Can be combined with other therapies. • May induce a broader immune response against various tumour antigens.	• Manufacturing challenges associated with generating and expanding patient-derived CSCs. • Potential for immune suppression in the tumour microenvironment to hinder vaccine effectiveness.	Prostate cancer; Bladder cancer & Various cancers (preventive) like Acute myeloid leukaemia (AML) & Melanoma	[95, 100, 101]
Combination Therapy	Combines xCT targeting or GM-CSF CSC vaccines with other therapies.	xCT targeting or GM-CSF CSC vaccines + Chemotherapy, PD-1 inhibitors, etc.	• May achieve a synergistic effect and overcome resistance mechanisms. • Targets multiple aspects of cancer progression (e.g., CSCs, tumour bulk, immune suppression).	• Requires careful design and optimisation to avoid adverse effects and drug interactions. • Potential for increased treatment complexity and cost.	Various cancers (depending on specific combination)	[90, 95]

of MHC-I presentation and can thus circumvent a common T-cell evasion strategy [39, 87]. Given the increase in xCT in gliomas and its involvement in neurodegenerative illnesses, research has focused on developing high-throughput screening assays to identify novel inhibitors of the xCT antiporter. These experiments have shown that in glioma cells, cystine uptake occurs more quickly than glutamate release, which sheds light on the role of xCT in cancer cells and helps guide drug discovery efforts [88].

Targeting xCT with immunotherapy has become a viable approach for preventing the spread of metastatic cancer. In preclinical models of breast cancer, a virus-like particle (VLP) immunotherapy targeting xCT expressed on cancer stem cells has been demonstrated to elicit a robust antibody response, hinder breast cancer stem cell development and self-renewal, and inhibit metastatic progression [89]. The relationship between breast cancer stem cells and immune targeting by xCT has also been investigated. xCT-specific vaccination approaches stimulate a humoral immune response that hinders metastasis development and slows tumour growth. Furthermore, it has been demonstrated that xCT immunotargeting increases doxorubicin-sensitive CSCs, indicating a function for this technique as an adjuvant to chemotherapy [90]. In both preventive and therapeutic contexts, a novel anti-xCT viral vaccination based on the bovine herpesvirus 4 (BoHV-4) vector has proven effective in reducing lung metastases caused by breast CSCs. In addition to directly affecting the CSC phenotype and self-renewal [91], the vaccine stimulated T lymphocyte activation and anti-xCT antibodies that could mediate antibody-dependent cell cytotoxicity (ADCC). One method of treating breast cancer has been to combine immunotherapy against xCT with the medication APR-246, which restores the wild-type function of p53 in tumours harbouring its mutation. This combination treatment reduces the viability of breast cancer cells, triggers apoptosis, influences the self-renewal of cancer stem cells, and inhibits the formation of lung metastases in vivo. These findings highlight the potential for xCT immune targeting to complement APR-246 treatment and improve its therapeutic effect [92].

GM-CSF surface-modified CSC vaccine: Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a cytokine that plays a pivotal role in the immune response against cancer by stimulating the differentiation and proliferation of granulocytes and macrophages. GM-CSF surface-modified cancer stem cell (CSC) vaccines represent a novel approach in cancer immunotherapy, aiming to target and eliminate CSCs. Using a variety of cancer models, the therapeutic potential of GM-CSF-modified CSC vaccines has been investigated. For example, a study showed that combining whole-tumour cell vaccination modified on the surface of GM-CSF with low-dose paclitaxel improved antitumor activity in a mouse model of prostate cancer. It was demonstrated that vaccination was more successful than irradiation of tumour cells plus free GM-CSF at encouraging dendritic cells (DCs) to absorb tumour antigens. Increased $CD8^+$ T-cell infiltration in tumour tissue due to combination therapy suggests an immune response specific to the tumour has been triggered [93]. Similarly, exosomes produced from embryonic stem cells (ESCs) expressing GM-CSF were used as a vaccine to prevent cancer. Strong tumour-reactive $CD8^+$ T effector responses and Th1 cytokine responses were observed in mice that received the vaccine, and these exosomes dramatically reduced or prevented the development of cancer cells that were implanted in the mice [94].

In the context of bladder cancer, a GM-CSF surface-modified bladder CSC vaccine was developed using protein-anchored technology. To efficiently eradicate CSCs, vaccination elicits a specific type of immune response. Nonetheless, immune resistance is induced by PD-1(programmed cell death protein-1)/PD-L1 signalling in the tumour microenvironment. The addition of PD-1 inhibition to CSC vaccination therapy improved the activities of the vaccine's tumour-specific T cells, demonstrating a potent and targeted antitumor immune response [95]. Another study used GM-CSF surface-modified MB49 bladder cancer cells to create a therapeutic vaccine. The vaccine was effective against bladder cancer that had spread to other areas and produced specific antitumor immunity. It causes peripheral blood and tumour sites to have higher concentrations of $CD4^+$ and $CD8^+$ T cells, which are highly cytotoxic to MB49 cells [96]. Furthermore, by immobilising TNF-α

(tumour necrosis factor-alpha) and GM-CSF on the surface of cancer cells, a therapeutic vaccine against prostate cancer was developed. This vaccine was better than single GM-CSF or TNF-alpha surface-modified vaccines in extending survival in an orthotopic model of prostate cancer [97]. After autologous stem cell transplantation (ASCT), a GM-CSF-based tumour vaccine demonstrated viability in producing a vaccine from autologous leukaemia cells in acute myeloid leukaemia (AML). There were indications of local injection site reactions, and the vaccination was well tolerated. WT1 (Wilms' Tumour gene 1) RT-PCR monitoring of minimal residual disease (MRD) is a standard indicator of vaccine effectiveness [98]. Strong T-cell-mediated antitumor immunity was provided by vaccination with irradiated induced pluripotent stem cells (iPSCs) that were genetically modified to produce GM-CSF. In syngeneic mouse models, the vaccination strategy demonstrated efficacy in both prophylactic and therapeutic contexts, indicating its potential as a cancer immunotherapy modality [99, 100]. Finally, a novel membrane-bound GM-CSF vaccine for the treatment of cancer was generated using B16F10 melanoma cells. The vaccine induced strong systemic immunity that was more effective than vaccines containing only secreted GM-CSF, protecting against wild-type tumours and therapeutic effects against established tumours [101].

Challenges & future prospects

The development of vaccines against cancer stem cells (CSCs) has numerous complex challenges that arise from the inherent characteristics of CSCs. Cancer stem cells (CSCs) are defined by their self-renewal and differentiation capabilities, but they exhibit significant marker plasticity and functional heterogeneity within tumours. This plasticity complicates the identification of stable, specific antigens for vaccine targeting, as CSC markers can change in response to environmental cues or therapy, and often overlap with normal stem cell markers, increasing the risk of off-target effects and reducing vaccine specificity [103, 104]. The intricate nature of the intracellular signalling pathways that govern CSCs, such as the Wnt, NF-κB, Notch, Hedgehog, and PI3K/AKT/mTOR pathways, contributes to the challenge of efficiently targeting these cells [27]. CSCs hijack signalling pathways (e.g., Notch, Hedgehog, Wnt/β-catenin) used by normal stem cells, making it difficult to selectively target CSCs without affecting healthy tissue. This overlap necessitates the identification of antigens or pathways unique to CSCs, a challenge not as pronounced in typical cancer vaccine development, which often targets more differentiated tumour cells [103, 104]. The tumour microenvironment (TME) presents a complex interplay of challenges for immunotherapy, including insufficient inflammation/chemokine levels, immune suppression via PD-L1/TGF-β, T-cell exhaustion, and barriers created by cancer stem cells (CSCs). CSCs contribute to tumour heterogeneity and are adept at evading immune responses, partly due to their ability to alter antigen expression and their role in creating an immunosuppressive microenvironment. This makes it harder for vaccines to generate durable and effective immune responses against CSCs compared to bulk tumour cells [103, 105]. Additionally, hurdles in developing CSC-targeted vaccines - such as off-target effects on normal stem cells, antigen heterogeneity, immune evasion by CSCs, and translating preclinical findings to clinical success - remain as critical obstacles. Addressing these multifaceted challenges requires innovative strategies to modulate the TME, enhance T-cell resilience, refine CSC antigen specificity, and bridge translational gaps.

Tumour microenvironment (TME)

The tumour microenvironment (TME) poses substantial obstacles to successful immunotherapy, such as insufficient levels of inflammation and chemokines required for T-cell movement, as well as prevailing suppression through immune inhibitory mechanisms [106]. These obstacles can cause T-cell malfunction and energy loss, which are worsened by factors such as programmed death ligand-1 (PD-L1) and transforming growth factor-beta (TGF-β) [107]. To address these difficulties, novel treatment strategies are being created, guided by a better understanding of the tumour microenvironment (TME). Furthermore, the lack of T-cell inflammation in the tumour microenvironment (TME) is a major obstacle, and current research is dedicated to devising strategies that can enhance the effectiveness of immunotherapies in these patients [108]. Combination ther-

apies involving therapeutic antibodies and chemotherapy have demonstrated promise in overcoming resistance inside tumour microenvironments that provide protection. Combining epigenetic therapies with immune therapies has the potential to reverse pathways of immune resistance in cancer and activate the body's immunological response against tumours. Moreover, it is essential to focus on cancer stem cells (CSCs) in the tumour microenvironment (TME) to achieve total regression of tumours. Further research should prioritise two-way communication between cancer stem cells (CSCs) and the tumour microenvironment (TME). CSCs can mould the TME into a barrier that suppresses the immune system, which is a crucial element in resistance to chemoradiotherapy and the recurrence of the illness. Furthermore, there are ongoing efforts to create novel treatment approaches that specifically target cancer stem cells (CSCs) within the tumour microenvironment (TME). These approaches include the use of small molecule inhibitors, immunotherapy, and microRNA-mediated inhibitors [109]. Translating these discoveries into clinical trials is crucial for overcoming the medication resistance and metastasis linked with CSCs. It is crucial to understand the impact of the tumour microenvironment (TME) on the response to therapy. The development of medication combinations that can target both tumour cells and the milieu could be a solution to overcome resistance to treatment.

Immune system-related challenges

CSC vaccines may also inadvertently target non-cancerous stem cells, leading to unintended consequences. This could occur if the vaccine targets a specific antigen or epitope that is also expressed on non-cancerous stem cells. Vaccines against CSCs are made to specifically target markers and pathways associated with CSCs. For example, normal stem cells also produce aldehyde dehydrogenase (ALDH), a marker used to identify CSCs. Strong antitumor efficaciousness has been demonstrated by vaccination against $ALDH^{high}$ CSCs utilising nanodiscs; however, the possible off-target effects on normal stem cells that express ALDH must be carefully considered [52]. Similarly, the pathways regulating CSCs, such as Wnt, Notch, and Hedgehog, are also involved in the self-renewal and differentiation of normal stem cells [27]. Studies have proven that CSCs are capable of triggering an immune response, and populations enriched in CSCs are more successful in stimulating a protective immune response against tumours than unselected tumour cells. Nevertheless, the production of immune responses that are sufficiently powerful to specifically target CSCs while leaving normal stem cells unaffected is a substantial hurdle. Another challenge lies in identifying suitable CSC antigens that can be effectively targeted by vaccination. Although there have been considerable advancements in the identification of markers such as ALDH and NANOG, the presence of diverse CSCs in several types of tumours makes it challenging to develop a universally effective anti-CSC vaccine. In addition, the ability of CSCs to avoid immunological responses by expressing immune checkpoint molecules requires the combination of CSC-targeted vaccines with other immunotherapeutic approaches, such as checkpoint inhibitors, to improve the effectiveness of the vaccine [52, 110]. Finally, the transfer of preclinical discoveries to clinical environments poses a difficulty, as the actions of CSCs in animal models may not completely mirror their actions in humans. Therefore, it is imperative to meticulously plan clinical studies and devise innovative approaches to assess the efficacy of CSC vaccination in patients [111].

Future considerations

Future CSC vaccine strategies should incorporate methods to modulate the TME, such as using selective preparative regimens to eliminate suppressive cellular populations and employing more effective immunologic adjuvants. Personaliased neoantigen-based vaccines have shown robust tumour-specific immunogenicity and preliminary evidence of antitumor activity in clinical studies. Applying similar approaches to CSC vaccines could enhance their specificity and efficacy. This involves the identification of patient-specific CSC antigens and the development of tailored vaccines that target these unique markers. Empirical evidence indicates that the diversity of antigen targeting, and immune regulation influence the efficacy of CSC vaccines. Vaccinations that include several CSC antigens or whole-cell lysates generate more robust and sustained T-cell responses compared to limited, single-

Table 4. Current status of clinical trials of tumour stem cell vaccines in various cancers

Trial Number (NCT ID)	Cancer Type(s)	Phase	Status (as of last update)	Vaccine Type/Target	References
NCT03400917	Glioblastoma (GBM)	Phase 2	Completed (Results Published)	AV-GBM-1: Autologous DCs pulsed with autologous tumour-initiating cell (TIC) lysate	[112]
NCT03548571	Glioblastoma (GBM)	Phase 2/3	Recruiting	DEN-STEM: Autologous DCs transfected with mRNA for surviving, hTERT, and autologous tumour stem cell antigens	[113]
NCT01567202	Glioblastoma (GBM)	Phase 2	Unknown (Last verified 2020)	Autologous DCs loaded with autologous glioma stem-like cells (A2B5+)	[114]
NCT05163080	Glioblastoma (GBM)	Phase 2b	Active, not recruiting	SurVaxM: Peptide vaccine targeting surviving, a CSC-associated protein	[115]
NCT02074046	Pancreatic Cancer	Phase 1/2	Completed (Last update 2015)	Autologous DCs loaded with pancreatic CSC lysate	[116]
N/A (AMPLIFY-201)	Pancreatic & Colorectal Cancer	Phase 1/2	Phase 1 complete, Phase 2 open	ELI-002 2P: “Off-the-shelf” peptide vaccine targeting KRAS mutations (G12D, G12R)	[117]
NCT02176746	Colorectal Cancer (CRC)	Phase 1/2	Completed (Last update 2019)	Autologous DCs loaded with colorectal CSC (ALDH-high) lysate	[118]
NCT02089919	Hepatocellular Carcinoma (HCC)	Phase 1/2	Completed (Last update 2015)	Autologous DCs loaded with hepatocellular CSC lysate	[119]
NCT00022334	Hepatocellular Carcinoma (HCC)	Phase 1/2	Active, not recruiting	Autologous DCs pulsed with alpha-fetoprotein (AFP) peptides	[120]

epitope vaccinations. For example, dendritic cells pulsed with cancer stem cell lysate elicited strong cancer stem cell-specific immunity. A nanoparticle vaccination carrying two ALDH epitopes effectively controlled tumours only in conjunction with PD-L1 inhibition [52]. These findings suggest that combinatorial approaches, such as the use of checkpoint inhibitors, may be essential to counteract immune suppression mediated by cancer stem cells. Moving beyond preclinical models requires a rigorous analysis of ongoing clinical trials to understand which strategies are feasible and promising in humans. The current landscape provides key insights into the direction of the field (**Table 4**).

The tumour-specific T-cell repertoire is prone to immunologic exhaustion and senescence, which limits the effectiveness of vaccines. Future CSC vaccine strategies should include agents capable of antagonizing immune checkpoint pathways and rejuvenating exhausted T cells. This could involve the use of novel adjuvants or combination therapies that enhance T-cell responses and sustain their activity. To translate preclinical successes into clinical practice, well-designed clinical trials are essential. These studies should rigorously evaluate the safety, immunogenicity, and therapeutic efficacy of CSC vaccines in diverse patient populations. Additionally, they should explore the long-term memory potential of vaccine-induced T cells and their impact on tumour recurrence and metastasis. There is a need for more extensive studies evaluating the long-term efficacy and ability of these anti-CSC vaccines to induce durable immune memory. Current studies primarily focus on short-term safety and immunogenicity [121, 122]. Most studies have focused on specific cancer types, such as pancreatic and breast cancer. Research on the applicability of CSC-targeting vaccines across a broader range of cancers is needed. This includes understanding the commonalities and differences in CSC markers across different cancer types. More research is needed to understand the precise mechanisms by which these vaccines elicit immune responses against CSCs. This knowledge could help in optimising vaccine formulations and improving their effectiveness (**Table 5**).

Conclusion

Recent data highlights that tumours include a subset of cancer stem cells (CSCs) that facilitate metastasis and recurrence. Diverse vaccination approaches aimed against CSC-associated antigens have demonstrated potential in preclinical investigations. Systematic evaluations indicate that whole-CSC vaccinations routinely hinder tumour development and metastasis while eliciting robust anti-CSC immunity. Dendritic-cell vaccines infused with CSC lysates or peptides have demonstrated extended survival in animal models, while a MUC1-expressing colorectal CSC vaccine specifically diminished CSC markers ($CD133^+$ and $ALDH^+$ cells) and augmented NK-cell cytotoxicity, IFN-γ production, and anti-MUC1 antibody responses. These findings underscore the importance of CSC-targeted vaccination: by eliminating the tumour's regenerative core, such vaccinations may avert recurrence and provide lasting remissions. Nonetheless, targeting cancer stem cells with vaccines has distinct obstacles. CSC flexibility and heterogeneity enable tumours to renew stem-like cells, and CSCs frequently utilise immune evasion strategies, such as the upregulation of PD-1/PD-L1, to withstand treatment. The intrinsically poor immunogenicity of some CSC antigens, coupled with the immunosuppressive tumour microenvironment, further restricts vaccination effectiveness. Significantly, thus far, only early-phase clinical trials have evaluated CSC-targeting vaccines, concentrating on safety and immunological responses. Consequently, the clinical efficacy of CSC-targeted vaccinations in patients is unverified, necessitating further clinical research to confirm their therapeutic effects. Principal domains for forthcoming investigation encompasses: (A) Identifying CSC-restricted or neoantigen epitopes would facilitate the development of multi-epitope, personalised vaccination formulations tailored to each patient's tumour mutational profile; (B) Integrating CSC vaccines into combination therapies, such as with checkpoint inhibitors or traditional chemotherapy/radiotherapy, may mitigate immune suppression and augment efficacy, as demonstrated by preclinical models where CSC-DC vaccines paired with anti-PD-1/CTLA-4 antibodies or chemotherapy significantly enhanced tumour control; (C) Utilising sophisticated vaccination

Table 5. Factors a successful CSC vaccine must address

Factor	CSC Vaccine Requirement	Typical Cancer Vaccine Requirement	Citation
Antigen Selection	Must target stable, CSC-specific antigens with minimal overlap with normal stem cells	Can target overexpressed or mutated tumour antigens	[103, 104, 123]
Marker Plasticity	Must account for dynamic changes in CSC marker expression	Less concern, as bulk tumour antigens are more stable	[103, 123]
Tumour Heterogeneity	Must induce broad immune responses to cover diverse CSC subpopulations	Often targets dominant antigens in bulk tumour	[103, 105]
Immune Evasion Mechanisms	Must overcome CSC-driven immunosuppression and immune escape	Focuses on general tumour immune evasion	[103, 105, 124]
Risk of Off-Target Effects	High, due to shared markers with normal stem cells; requires high specificity	Lower, as targets are more tumour-restricted	[103, 104]

platforms (such as nanoparticle or viral-vector carriers, or iPSC-derived vaccines) might improve antigen delivery and adjuvant efficacy, possibly augmenting lymph node targeting and T-cell activation against cancer stem cells (CSCs); (D) Rigorous clinical trials are essential to assess the effectiveness of CSC vaccines and their long-term results, progressing beyond preliminary safety investigations. This entails identifying biomarkers for CSC vaccination response and delineating appropriate patient subpopulations.

In conclusion, CSC-targeting vaccines have considerable potential for enhancing cancer treatment by eliminating the source of tumour recurrence. Realising this objective necessitates progress in antigen selection (including neoantigens), the invention of personalised vaccinations, and the incorporation of CSC vaccines into multi-modal therapy regimens capable of counteracting CSC plasticity and immune evasion.

Disclosure of conflict of interest

None.

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