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Review article

Advances in therapeutic cancer vaccines: Harnessing immune adjuvants for enhanced efficacy and future perspectives

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ABSTRACT

Preventive cancer vaccines are highly effective in preventing viral infection-induced cancer, but advances in therapeutic cancer vaccines with a focus on eliminating cancer cells through immunotherapy are limited. To develop therapeutic cancer vaccines, the integration of optimal adjuvants is a potential strategy to enhance or complement existing therapeutic approaches. However, conventional adjuvants do not satisfy the criteria of clinical trials for therapeutic cancer vaccines. To improve the effects of adjuvants in therapeutic cancer vaccines, effective vaccination strategies must be formulated and novel adjuvants must be identified. This review offers an overview of the current advancements in therapeutic cancer vaccines and highlights in situ vaccination approaches that can be synergistically combined with other immunotherapies by harnessing the adjuvant effects. Additionally, the refinement of adjuvant systems using cutting-edge technologies and the elucidation of molecular mechanisms underlying immunogenic cell death to facilitate the development of innovative adjuvants have been discussed.

1. Introduction

Vaccination is one of the most successful and cost-effective preventive therapeutic strategies. Adjuvants are as critical as antigens for vaccine development, and they can enhance the efficacy of vaccines by augmenting the immune response [1]. Since 1930, relevant aluminum-based adjuvants have been optimized for use in tetanus, pertussis, and COVID-19 vaccines (Fig. 1). Therapeutic cancer vaccines, which are emerging as a new option for personalized tumor immunotherapy, activate the immune system to clear tumor cells [1,2]. Currently used preventive vaccines target cancers caused by viral infections [3], including hepatitis B virus (HBV) vaccine and human papillomavirus (HPV) vaccine [4,5]. In contrast to preventive cancer vaccines that target diseases associated with the cancer process, therapeutic cancer vaccines aim to induce tumor regression, eradicate minimal residual disease, establish durable anti-tumor memory, and mitigate unspecific or adverse reactions [6]. Based on the antigens used by the vaccines that are recognized by the immune system, these cancer vaccines are categorized as mRNA vaccines, DNA vaccines (based on antigen sequences encoded by plasmid DNA), peptide vaccines (comprising short peptides conjugated to specific major histocompatibility complex (MHC) class I molecules), and vector-based vaccines [7]. An ideal adjuvant in therapeutic cancer vaccine activates T cell activation signal I by modulating the persistence, concentration, and presentation efficiency of antigen-presenting cells (APCs), as well as enhances the immune response by expressing co-stimulatory molecules induced by APCs and cytokines [8]. However, tumor antigens vary widely among tumor types and patients, and latent antigens are not effectively recognized by the immune system even with the addition of adjuvants, limiting the development of effective therapeutic cancer vaccines. Therefore, the adjuvants to be used in cancer vaccines must be extensively studied to ensure that tumor antigens generate durable and specific immune responses [9,10].

The immunogenicity of cancer vaccines is determined by adjuvants and vaccination strategy [11]. To improve the efficacy of cancer vaccines, factors, such as the selection of adjuvants for cancer vaccines, in vivo vaccine delivery modalities, and screening of tumor-specific antigens must be considered [12]. Clinical trials have demonstrated that

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Montanide ISA-51, a mixture of mineral oil and a mannide monooleate surfactant, enhances the systemic immune response by generating tertiary lymphoid-like structures in the vaccine site microenvironment (VSME) with repeated vaccination at one site [13]. Furthermore, endogenous adjuvants synergize with natural antibodies, such as those against interleukin (IL)−4 that can activate specific CD8 + T cells [14]. Compared with traditional types of adjuvants, endogenous adjuvants can be generated by inducing cell death processes, such as immunogenic death of tumor cells that release large amounts of tumor-associated antigens. These adjuvants activate dendritic cells (DCs) and consequently elicit enhanced adaptive responses in combination with cancer vaccines [15]. The inducers of immunogenic tumor cell death function as immune adjuvants in vaccines, inducing immunogenic cell death (ICD), a potential adjuvant-like signal for cancer vaccines [16]. Additionally, ICD results in the spontaneous release of large amounts of antigens and adjuvants to activate DCs, providing an excellent opportunity to establish effective in situ vaccination (ISV) strategies [17].

To understand the stimulation of the innate immune system by adjuvants through vaccination strategies, this review aimed to discuss the mechanisms of adjuvants in various cancer vaccines and their clinical applications, including an appropriate vaccination approach and effective adjuvants for tumor antigens in ICD. Additionally, the molecular mechanisms involved in the immune death of adjuvants and tumor cells were also discussed to design therapeutic tumor vaccines with enhanced efficacy that can induce ICD. This study lies in providing crucial insights into the molecular mechanisms and clinical applications for designing more effective therapeutic cancer vaccines, thereby facilitating the development and application of tumor vaccines that promote immunogenic cell death.

2. Adjuvants in cancer vaccines and mechanisms

Adjuvants are vaccine components that can enhance the strength, spectrum, and durability of immune responses and elicit adaptive immune responses by activating the pattern recognition receptor (PRR)-regulated immune responses through various mechanisms, such as the induction of tissue damage and cell death [18,19]. Endogenous molecular adjuvants have a broad application in therapeutic cancer vaccines. For example, nucleic acid vaccines (directly deliver antigens) and DC vaccines (load antigens onto DC cells) are types of vaccines with endogenous molecular adjuvants. These vaccines are in contrast to the first Food and Drug Administration (FDA)-approved protein/peptide therapeutic cancer vaccine sipuleucel-T, which contains the adjuvant granulocyte-macrophage colony-stimulating factor (GM-CSF) [20,21]. Adjuvant delivery platforms can ensure that the therapeutic cancer vaccine components are delivered to the tumor-proximal lymph nodes and tumor microenvironment (TME) [6].

2.1. Adjuvants in nucleic acid-based cancer vaccines

Nucleic acid vaccines can elicit humoral and cellular anti-tumor immune responses by delivering multiple tumor antigens in a single

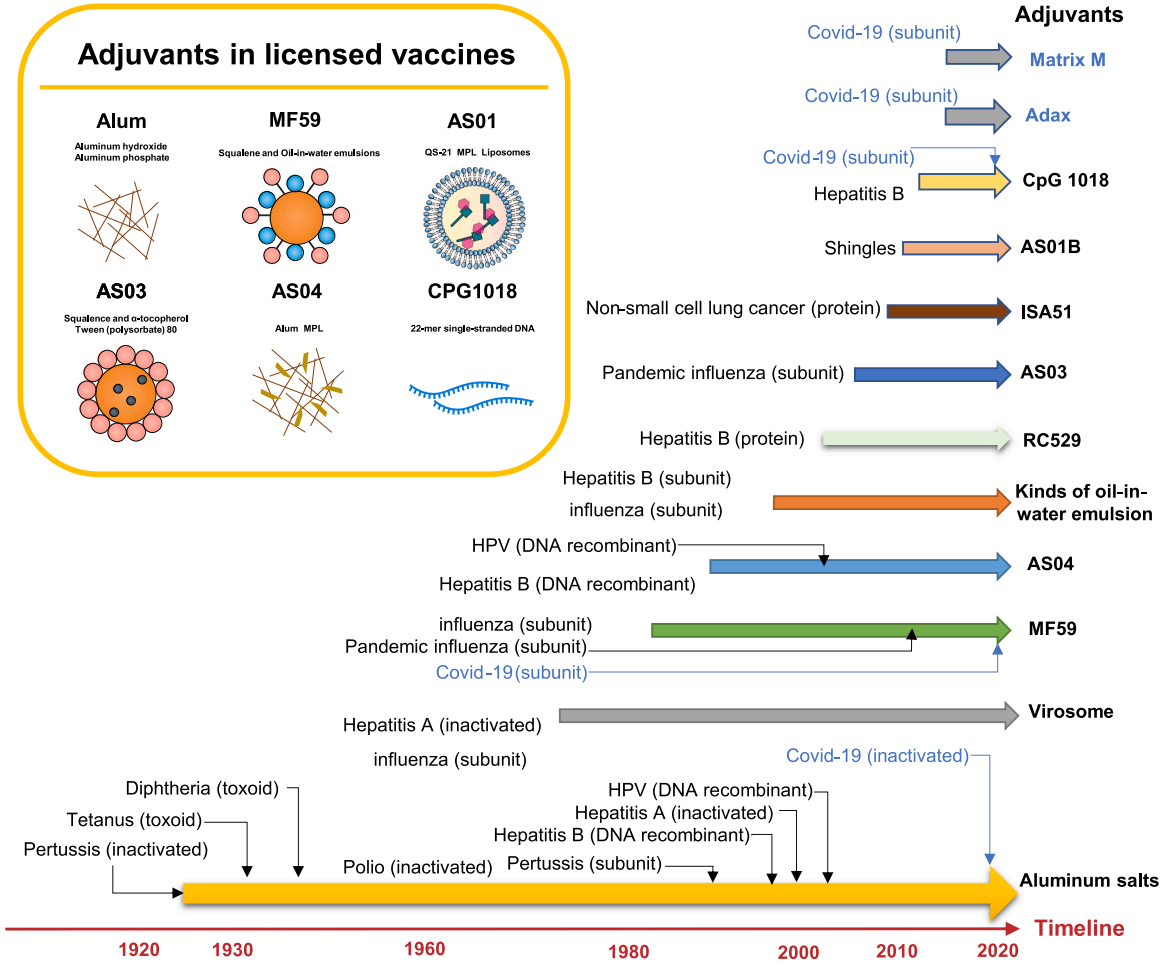

Fig. 1. The development and application of adjuvants in licensed vaccines. In the process of developing vaccines for influenza virus, hepatitis virus, cervical cancer, and other diseases in this century, new ingredient ratios have been determined and innovative ingredients have been added, which will contribute to the development of new adjuvants.

immunization [8]. APCs expressing mRNA or DNA vaccines elicit durable anti-tumor T cell responses. Tumor antigens used in nucleic acid cancer vaccines are combined with endogenous adjuvant molecules to elicit robust anti-tumor responses [2,10].

DNA vaccines are antigen-encoding genes inserted into bacterial plasmids, which are efficient gene delivery tools [22]. DNA cancer vaccination introduces a potentially effective tumor antigen into the host and activates the immune response of the host against tumor cells [10]. This process requires adjuvants to optimally modulate the immune system to recognize the tumor antigen [23]. However, in contrast to cell-based cancer vaccines, DNA vaccines require immune adjuvants to adequately stimulate the immune response [24]. Adjuvants, such as TLR3 agonist or TLR9 activating unmethylated cytosine phosphate guanine (CpG) oligodeoxynucleotides (ODNs) and conventional adjuvants (including aluminum salts), are used as an integral part of DNA vaccines [25,26]. In a Phase II clinical trial, immune adjuvants containing unmethylated CpG-ODN and interferon (IFN)-alpha were co-injected with autologous tumor cell lysate, which resulted in immunogenicity and induced anti-tumor responses in patients with metastatic renal cell carcinoma (RCC) [27]. Adenosine triphosphate (ATP)-modified calcium phosphate (ACP) nanoparticles, which serve as an immune adjuvant, upregulate the levels of antigen-specific antibody and potently inhibit tumor in mice immunized with the ACP-DNA vaccine [28].

mRNA vaccines are potential therapeutics for cancer immunotherapy owing to several advantages, including the ease of production, which is comparable to the best available traditional vaccine manufacturing technology [29]. Although mRNAs can stimulate innate immunity by functioning as an adjuvant to activate downstream IFN-related pathways, their inherent immunogenicity can reduce antigenic expression. This can limit the application of mRNAs in cancer vaccine development [2]. However, the results of mRNA vaccines for COVID-19 support their potential as a platform for cancer immunotherapy [30]. Lipid nanoparticles (LNPs) act as carriers to assist the mRNAs to enter the target cells and express sufficient amounts of proteins for the mRNA vaccines to exert therapeutic effects. Thus, the development of effective delivery systems is critical for the efficacy of mRNA cancer vaccines. Exogenous mRNAs bind to endogenous molecules, such as retinoic acid-inducible gene 1 (RIG-1) and melanoma differentiation-associated protein 5 (MDA5), as well as to the Toll-like receptors during translation, activating multiple signaling pathways to release pro-inflammatory cytokines [31]. For example, cytokine-encoding mRNAs, such as those encoding GM-CSF, IL-12, and IL-2, contribute to immune enhancement by vaccines that are loaded with whole tumor mRNA preparations or synthetic mRNAs encoding tumor-associated antigens [32]. Furthermore, the formulation integrates antigen-encoding mRNA and immunostimulatory adjuvants without compromising the expression of antigenic proteins. The formulations containing optimally structured double-stranded RNA (dsRNA)-conjugated mRNAs effectively activated DCs in both mice and humans, inducing the secretion of broad-spectrum pro-inflammatory cytokines without increasing the secretion of anti-inflammatory cytokines [33]. In a Phase I/II clinical trial involving 30 patients with stage IV RCC, intradermal administration of naked mRNA encoding mucin 1 (MUC1) in combination with GM-CSF resulted in good performance with 15 patients experiencing stable disease and one patient experiencing partial remission [34]. mRNA vaccines have strong intrinsic adjuvant characteristics. mRNA-encoded cytokines are another type of adjuvant molecule that is used to promote the maturation of DCs and the induction of cytotoxic T cells [2,9]. Compared with tumor antigen-binding adjuvants, nucleic acid vaccines are advantageous as the nucleic acid molecules bind to integrated adjuvant molecules during transcription and translation, yielding a robust immune response [8].

2.2. Adjuvants in DC-based cancer vaccines

DCs are vital for the induction and regulation of innate and adaptive immunity in the TME [35,36]. Therefore, targeted vaccines that deliver antigens and adjuvants to DCs in vivo are emerging as a viable approach to improve the efficacy of cancer immunotherapy [36]. Sipuleucel-T, a recombinant autologous DC and GM-CSF fusion protein vaccine, was the first FDA-approved cancer vaccine for metastatic prostate cancer that achieved enhanced efficacy [37,38]. In a lymphoma prevention model, Man-EG7-CpG, a novel vaccine comprising mannose-modified CpG-ODN and natural tumor cell vectors, enhanced the targeting ability of DCs and efficiently suppressed tumor formation [39]. However, autologous DC cancer vaccines, which have been extensively studied in clinical trials, do not provide a strong clinical benefit to patients. This can be attributed to three reasons. Firstly, matured type 1 conventional DCs in the TME are less reactive, resulting in less effective presentation of tumor antigens [40]. Secondly, activated DCs present antigen to naive antigen-specific CD4 + T helper cells residing in T cell-enriched regions of lymphoid organs, and the magnitude and nature of the induced T helper cell response are highly dependent on the nature of the adjuvant and the induced PRRs [41,42]. Thirdly, the systemic delivery of mRNA to DCs promotes transgene expression activity and maturation in DCs [43]. DCs transfected with melanoma antigen recognized by T cells exhibit enhanced ex vivo migratory and immunostimulatory capacity due to the co-delivery of IL-12-encoding mRNA, suggesting the significant upregulation of the proliferation, effector function, and memory potential of specific CD8 + T cells [44]. Therefore, DC-based targeted cancer vaccines must comprise appropriate endogenous adjuvants or adjuvant delivery systems.

2.3. Delivery function of adjuvants in cancer vaccines

Adjuvants are multi-component systems, which include adjuvant systems and adjuvant delivery platforms, complement the efficacy of licensed vaccines. The adjuvant system, which is a combination of classical adjuvant molecules and immunostimulatory molecules, exerts a strong adjuvant effect [45]. AS04 has been used in HBV and HPV vaccines to enhance the production of antibodies against recombinant antigens [46,47], but analysis of the summary of clinical trials related to therapeutic cancer adjuvants in clinicaltrails.gov (Table 1) revealed that AS01, AS03, and AS04 have not yielded promising results in clinical trials of cancer vaccines. Thus, conventional adjuvant-based delivery systems are not a suitable option for therapeutic cancer vaccines.

Adjuvant delivery platforms, which play a specific role in antigen delivery in cancer immunotherapy, including various materials, such as emulsions, lipid particles, and microparticles [48]. If these molecules fail to deliver the antigen precisely to the intended site, non-specific immune mechanisms can be activated, adversely affecting vaccine efficacy [49]. Improvements in the composition of traditional adjuvants have resulted in the development of effective adjuvants with enhanced safety. Montanide ISA-51 (a modified formulation of incomplete Freund's adjuvant (IFA)) has a stable toxicity profile when compared with IFA (used in water-in-oil emulsions for veterinary vaccines). In Cuba, Montanide ISA-51 has been approved for use in a lung cancer vaccine [50]. Additionally, a combination of cancer peptide vaccine and Montanide ISA-51 emulsion promoted the infiltration of CD8 + T cells and CD4 + T cells in VSME [51,52]. The novel combined adjuvant delivery platform in cancer vaccines improves the efficiency of tumor antigen presentation through the structure-guided design of molecular agonists for immune cell receptors [53]. New nanomaterials, which provide a multifunctional platform for immune activation and antigen delivery to promote the rapid development of cancer vaccines, can induce antigen-specific anti-tumor immune responses and simplify vaccine preparation [54]. Immune-stimulating complex and CpG-ODN prepared as nanoparticles can enhance the anti-tumor immune response by effectively delivering antigens to tumor sites or APCs [55]. Currently, nanomaterials that can be used as immune adjuvants include inorganic materials (such as metal nanoparticles and spiked titanium dioxide particles) and organic materials (such as polymers and LNPs)

Table 1
Adjuvant in completed cancer therapeutic vaccine clinical trials selected from ClinicalTrails.gov.

NCT Number	Application of Adjuvants	Conditions	Phases
NCT00952692	AS15	Metastatic breast cancer	Phase 1/2
NCT00299728	CpG 7909	Tumors	Phase 1
NCT00199836	CpG 7909/ Montanide ISA 51	Cancer/Neoplasm	Phase 1
NCT00665002	GM-CSF	Leukemia	Not Applicable
NCT00293423	GP96	Brain and central nervous system tumors/leukemia/ myelodysplastic syndrome	Phase 1/2
NCT00923910	IL-4	Leukemia/myelodysplastic syndrome/chronic myelogenous/non-Hodgkin's lymphoma	Phase 1/2
NCT00091273	Incomplete Freund's adjuvant	Ovarian cancer	Phase 1
NCT00398138	Incomplete Freund's adjuvant	Leukemia/lung cancer/ primary peritoneal cavity cancer/malignant mesothelioma	Phase 1
NCT00433745	Incomplete Freund's adjuvant	Myelodysplastic syndrome/ acute myeloid leukemia/ chronic myeloid leukemia	Phase 2
NCT02151448	Interferon Alfa-2b	Malignant neoplasm	Phase 1/2
NCT00199901	ISCOMATRIX® adjuvant	Melanoma	Phase 2
NCT00518206	ISCOMATRIX® adjuvant	Melanoma	Phase 2
NCT03199872	Montanide ISA 51	Prostate cancer	Phase 1/2
NCT01390064	Montanide ISA 51	Stage IV breast cancer	Phase 1
NCT00304096	Montanide ISA 51	Breast cancer	Phase 1
NCT00923195	Montanide ISA 51	Melanoma/skin cancer	Phase 2
NCT00857545	OBI-821	Stage IA fallopian tube cancer Stage IB ovarian cancer Primary peritoneal cancer Stage IA ovarian cancer	Phase 2
NCT00773097	Poly-ICLC	Risk for colorectal cancer	Phase 2
NCT00616941	Poly-ICLC	Epithelial ovarian cancer Primary peritoneal cancer	Phase 1
NCT03391232	Poly-ICLC	Colorectal cancer	Phase 1/2
NCT02129075	Poly-ICLC	Cutaneous melanoma/ melanoma	Phase 2

The immunotherapy strategies for the therapeutic cancer vaccines in the table were obtained from https://clinicaltrials.gov/, which collected clinical trial staging as well as cancer type and trial number, and the adjuvants used in them were obtained by mining the ingredients. GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; ICLC, polyinosinic-polycytidylic acid stabilized with poly-l-lysine.

[56]. The design of programmable nanoparticles can automatically change according to specific environments, meeting the antigen requirements for delivery to different types of cells [57]. A multifunctional adjuvant platform based on the two-dimensional nanomaterial black phosphorus nanosheets significantly enhanced antigen uptake by DCs in vitro and in vivo. A combination of this adjuvant platform and anti-PD-L1 antibodies exerted potent therapeutic effects in a B16-OVA melanoma mouse model [58]. Nanotechnology must be used to develop novel adjuvant delivery platforms as adjuvants for therapeutic cancer vaccines, such as nanoparticles of immune agonists coupled with tumor-specific immune responses stimulated with tumor-associated antigens to facilitate the immune activation process. In particular, nanoparticles loaded with cytotoxic drugs and immune agonists can be used as in situ vaccines against tumors [59].

Thus, adjuvants in nucleic acid vaccines and DC vaccines have been widely used in therapeutic cancer vaccines, including the adjuvant delivery systems that play a key role in mRNA vaccines, and adjuvants also contribute to enhancing the immune response in different vaccine types (Table 2).

Table 2
The role of adjuvants and type of vaccine application.

Name	Vaccine category	Function	Reference
CpG-ODN/IFN-alpha	DNA vaccine	Induces anti-tumor responses and improves immunogenicity	[27]
ATP-modified calcium phosphate nanoparticles	DNA vaccine	Upregulates antigen-specific antibody production and potently inhibits tumor growth	[28]
IL-2	mRNA vaccine	Contributes to vaccine-mediated immune enhancement	[65]
RIG1/MDA5	mRNA vaccine	Activates multiple signaling pathways to release pro-inflammatory cytokines	[31]
GM-CSF	mRNA vaccine	Assists intradermal administration of naked mRNA	[34]
Lipid nanoparticles	mRNA vaccine	Functions as carriers to assist the mRNAs to enter the target cells and to express sufficient amounts of proteins	[30]
CpG-ODN	DC vaccine	Enhances the targeting ability of DCs and efficiently suppresses tumor formation	[39]
IL-12	DC vaccine	Enhances the ex vivo migratory and immunostimulatory capacity	[44]

CpG, unmethylated cytosine phosphate guanine; ODN, oligodeoxynucleotide; IFN, interferon; IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; DC, dendritic cell.

3. ISV as a platform for immunoadjuvant

Conventional vaccines comprise antigens and an immune adjuvant. In contrast, ISV uses antigens from the tumor, and the immune adjuvant is injected directly into the site of tumorigenesis during treatment [60]. A comparative analysis of the conventional vaccination method and the ISV method is shown in Fig. 2. Current immunotherapies often fail to eradicate cancer due to tumor-mediated local immune suppression [61]. Effective ISV changes the TME from an immunosuppressive state to an immunostimulatory state, stimulating APCs to present tumor antigens to cytotoxic T cells and eliciting systemic anti-tumor immunity by promoting antigen-specific effector T cells to attack treated and untreated metastatic tumors [11].

3.1. Cytokines for ISV

Cytokines secreted by various immune cells and their receptors play a critical role in innate and adaptive immune activation [32]. Various cytokines can bind to tumor-associated antigens in VSME to activate the immune system (Table 3), such as IFN-γ secreted by lymphocytes, which can upregulate MHC class I and MHC class II molecules [62]. However, cytokines are not suitable as adjuvants to be combined synergistically with tumor antigens using traditional vaccination methods. Cytokines are released in small amounts at specific sites and have a short half-life, which limits the stimulation of anti-tumor immunity through the systemic administration of cytokines [63]. Alternatively, the locally restricted cytokines in combination with ISV can potentially promote immune cell-mediated tumor elimination [64].

IL-2, IL-12, and GM-CSF are the most commonly used cytokine adjuvants in cancer vaccines. IL-2 is a key homeostatic factor for establishing and maintaining natural and peripheral T regulatory (Treg) cells [65]. In a Phase I clinical trial, ISV of IL-2 with a tumor-specific antibody at the tumor site in patients with metastatic melanoma undergoing radiotherapy increased the diversity of peripheral CD8 + T cell effectors [66]. IL-12 limits tumor progression in cancer therapy by secreting IFN-γ and inhibiting angiogenesis [67]. In ISV, adenovirus-based IL-12 delivery plasmids, which were engineered to express IL-12 in tumors through intertumoral electroporation, have been used in the treatment of advanced melanoma, glioblastoma, and breast cancer [44]. GM-CSF

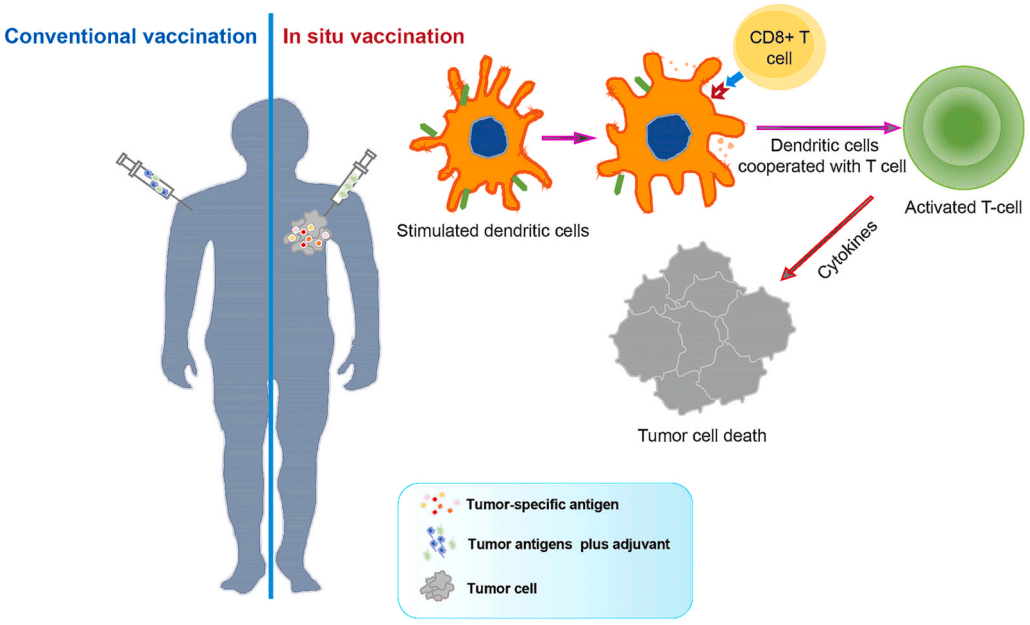

Fig. 2. Schematic diagram of in situ vaccination (ISV) and the mechanism of dendritic cell (DC) activation. Compared with the traditional inoculation strategy, the ISV strategy allows the adjuvant to directly activate DCs in the tumor microenvironment, facilitating the clearance of tumor cells.

recruits antigen-presenting DCs and promotes their maturation at tumor sites, transporting tumor antigens to lymph nodes and presenting these antigens to naive T cells in an immunostimulatory manner [68]. Clinical trials on GM-CSF injection into tumor sites are ongoing with patients with breast cancer, prostate cancer, and lymphoma [68,69]. The injection of low doses of CpG into tumors as an adjuvant induces OX40 expression on the CD4 + T cell surface in the TME to trigger a systemic immune response. The combination of TLR ligands and anti-OX40 antibodies exerts potent therapeutic effects on multiple cancers and prevents spontaneous gene mutation-driven cancers [70]. The intra-tumoral injection of low-dose anti-CTLA-4 monoclonal antibody and IL-2 can be used to treat advanced melanoma and lymphoma [71,72]. Neoadjuvant tumor-specific anti-GD2 immune cytokine generates immune memory despite limited efficacy in large primary melanomas, prevents distant metastasis of tumor cells, and mitigates the toxicity of local radiotherapy in the treatment regimen [73].

3.2. *Immune agonists for ISV*

Immune agonists, such as TLR and stimulator of IFN gene (STING) agonists are used as adjuvants to recruit and stimulate DCs and modulate the transition of the TME from an immune suppression state to an immune activation state [74,75]. Additionally, immune agonists have been used in some in situ cancer vaccines [12].

CpG, a TLR9 receptor agonist, activates adaptive and innate immunity upon recognition by TLR9 expressed on the APC and B cell surfaces [75]. TLR9 agonists improved the prognosis of melanoma in two clinical trials involving ISV [76]. A new "cancer vaccine" with the combination of Flt3L and a TLR3 agonist, which recruited and activated intra-tumoral, cross-presenting antigen-loaded DCs, has been developed for application in ISV and may improve the treatment outcomes of patients with indolent non-Hodgkin's lymphoma [74]. This vaccination strategy, which incorporates a specific antigen, is delivered directly to the same tumor site, activating DCs, inducing cytotoxic T cells to attack tumor cells, and significantly decreasing the number of somatic tumors in mice [70]. Additionally, this therapy increases the success rate of anti-PD-1 antibodies and prolongs the survival of cancer-bearing mice [70,77]. ISV is reported to exhibit a broad therapeutic spectrum when combined with other immunotherapies, such as immune checkpoint inhibitors. The strategy is being tested in patients with breast, head, and neck cancers [78]. In a clinical trial of 3M-052, a novel TLR7/TLR8 agonist-based adjuvant, that induced T cell inflammation through the activation of DCs and the production of type I IFN and other pro-inflammatory cytokines, promoted tumor cell antigen presentation and reduced the metastatic spread of tumor cells to the lung [79,80]. Flagellin functions as a TLR5 agonist to support the therapeutic effects of tumor-specific peptide vaccination, and flagellin-mediated TLR5 activation provides an additional signal that can mediate innate immune responses and exert potent growth-inhibitory effects against breast cancer cells [81,82].

The process of generating tumor-specific T cell responses involves STING [83]. STING agonists regulate the immune response of other vaccines in therapy, including the nucleotide second messenger c-di-GMP and cyclic GMP-AMP (cGAMP), a diffusible cyclic dinucleotide that activates the antiviral response through the adaptor protein STING (Table 4). The intra-tumoral administration of STING induced anti-tumor responses, leading to tumor regression, inhibition of tumor metastasis, and prolonged immune memory in a murine tumor-bearing model [84]. Furthermore, the intra-tumoral administration of STING agonists dose-dependently upregulated the frequency of cytokine-positive monocytes and amplified cell-type responses in tumor cells at the injection site [85]. The anti-tumor efficacy of a combination of TLR7/8 and STING agonist adjuvants was higher than that of single agonist vaccines in the B16F10 melanoma and MB49 bladder tumor models, indicating the potential of the combination to activate innate and adaptive immune responses [74,86].

Thus, ISV is an excellent choice for therapeutic cancer vaccines as it utilizes tumor-associated antigens and maximizes the ability of the adjuvant to effectively initiate APCs and stimulate cytotoxic T cells. In the ISV approach, cytokines and immune agonists yielded promising results in eliciting tumor-specific immune responses with tumor-specific antigen stimulation.

4. Application of adjuvants released after cell death in cancer vaccine

Regular cell death is not recognized by the immune system. Only the death of a few cells infected by pathogens can trigger a strong antigen-

Table 3
The sources and functions of adjuvant-related cytokines in the innate immune mechanism.

Family	Reference (PMID)	Members	Secretion	Function
IL-1 family	29656546 [117]	IL-1α (IL-1F1)	Macrophages	Induces pro-inflammatory response;
		IL-1Ra (IL-1F3)	B cells	Promotes Th17 cell differentiation;
			Dendritic cells	Functions as an alarmin to induce Th2 cell response
		IL-1β (IL-1F2)	Endothelial cells	Induces IFN-γ in the presence of IL-12;
		IL-33 (IL-1F11)	Macrophages	Inhibits dendritic cell function
		IL-18 (IL-1F4)		
		IL-37 (IL-1F7)		
		IL-36α (IL-1F6)	Smooth muscle cells	Antagonizes IL-36-α, IL-36-β, and IL-36γ;
		IL-36β (IL-1F8)	Intestinal fibroblasts	Exhibits anti-inflammatory activity in the brain by upregulating IL-4 expression in glial cells
		IL-36γ (IL-1F9)		
IL-6 family	30254251 [118]	IL-6	Myofibroblasts	Promotes the synthesis of acute-phase proteins in the liver; Induces chemokine secretion;
			Epithelial cells	
			T helper cells	Induces neutrophil apoptosis, B cell differentiation, and production of IgG, IgM, and IgA
			Macrophages	
		IL-11	Fibroblasts	Serves as a growth factor for myeloid erythroid and megakaryocyte progenitors;
			BM stromal cells	Promotes bone remodeling; Protects epithelial cells and connective tissue
IL-12 family	33418929 [119]	IL-12	T cells	Increases cytotoxic activity of NK cells and CD8 + T cells and production of IFN-γ
			Dendritic cells	
	22814351 [120]	IL-23	Macrophages	Promotes Th17 cell expansion; maintains the activation and secretion of IL-17A, IL-22, and GM-CSF
		IL-27	B cells	Induces Th1 response and suppresses the CD4 + T cell-mediated production of IL-2; Inhibits Th2 and Th17 cells
Interferon	12133794[63]	IFN-α	Leukocytes	Upregulates class I MHC molecules; Activates NK cells;
		IFN-β		Upregulates class I MHC molecules; Activates NK cells
		IFN-γ	T cells	Promotes cytotoxic activity; Upregulates class I and class II MHC molecules
Tumor necrosis factor	36358688 [121]	TNF-α	Macrophages Monocytes	Promotes phagocyte cell activation and endotoxic shock and exerts tumor cytotoxic effects
	12133794[63]	TNF-β	T cells	Promotes chemotactic phagocytosis; Induces other cytokines
Colony-stimulating factor	12133794[63]	G-CSF	Fibroblasts	Promotes granulocyte production
			Endothelium	
		GM-CSF	T cells	Promotes granulocyte, monocyte, and eosinophil production
			Macrophages Fibroblasts	
		M-CSF	Fibroblasts Endothelium	Promotes monocyte production and activation
		Erythropoietin	Endothelium	Promotes red blood cell production
IL-2 family	21889323 [122]	IL-4	T cells	Mediates T helper 2 cell (Th2) development and function
			NKT cells	
			Mast cells	
		IL-7	Fibroblasts	Promotes T cell development
			Epithelial cells	
		IL-15	Dendritic cells	Promotes NK cell development and CD8 + T cell homeostasis
			Epithelial cells	
		IL-21	NKT CD4 + T cells	Promotes the terminal differentiation of B cells to plasma cells

IL, interleukin; Th, T helper; IFN, interferon; Ig, immunoglobulin; NK, natural killer; GM-CSF, granulocyte-macrophage colony-stimulating factor; MHC, major histocompatibility complex; TNF, tumor necrosis factor; G-CSF, granulocyte colony-stimulating factor; NKT cells, natural killer T cells.

specific immune response, leading to the clearance of invading pathogens and the establishment of long-term immune memory [87]. The immunogenicity of cell death depends on a combination of antigenicity provided by neoepitopes and adjuvant properties conferred by specific microbe-associated molecular patterns or damage-associated molecular patterns (DAMPs). Similar to microorganisms, DAMPs produced by dying cells can function as adjuvants to communicate a danger signal. Dead cells generated by diverse cell death mechanisms function as antigens and adjuvants in DC-mediated cross-presentation. A study examining the adjuvanticity of MF59 demonstrated that MF59 can mediate the function of necrosis receptor-interacting protein kinase 3, which co-operates with NF-κB-dependent inflammation to sequentially promote CD8 + T cell responses [88].

ICD is a specific type of cell death that produces tumor-specific antigens. These antigens trigger an immune response mediated by IFN-γ, eliminating any remaining tumor cells [89,90]. Thus, patients can derive long-term clinical benefits from cytotoxic chemotherapy and physical induction of ICD [15,16]. ICD results in the spontaneous release of large amounts of antigens and adjuvants to activate DCs, providing an excellent opportunity to establish effective ISV strategies [17,91]. Adjuvant-like signals generated by DAMPs promote the recruitment, activation, differentiation, and maturation of APCs, facilitating the recruitment of cytotoxic T cells and further activating immunity [92]. Dying cells produce new epitopes and release DAMPs, including calreticulin, high-mobility group box protein B1 (HMGB1), type I IFN, and annexin 1 [15,92]. The released DAMPs bind to APCs, especially DCs, and recognize dead cell antigens and present them to T cells to activate adaptive immune responses (Fig. 3). The mechanisms of DAMP production during ICD include the cell death program signal-mediated activation of transcription factors, leading to the release of inducible DAMPs, such as receptor-interacting protein kinase 1, which induces both cell death and NF-κB activity. Additionally, the cell death program simultaneously activates endogenous DAMPs through post-translational modification of proteins, such as the activation of inflammatory vesicles, cystatin-dependent focal hypoplasia, and the induction of pro-IL-1β maturation [45,93–95].

ICD is a key process in transforming cancer cells into tumor vaccines and mediating the immune clearance of cancer cells through the interface interaction between DAMPs and DCs. ICD is a unique and highly beneficial target for cancer therapy [16,41,90]. The inducers of ICD are new targets for adjuvants of therapeutic cancer vaccines. In chemotherapy, adriamycin and anthracyclines are used to induce ICD in acute

Table 4
STING agonist-regulated Immune responses and application in therapy.

Type	Formulation	Application	Reference (PMID)
cGAMP	Liposome	Influenza	32820126[123]
c-di-GMP	Liposome	HIV	18640167[124]
	Hollow nanoparticle	MERS-CoV infection	28956394[125]
Chitosan plus LPS	Hydrogel	Melanoma	31248461[126]
Chitosan	Nanoparticle	Hepatitis B virus	30744102[127]

STING, stimulator of interferon genes; cGAMP, 2′3′-cyclic GMP-AMP; HIV, human immunodeficiency virus; MERS-CoV, Middle East respiratory syndrome coronavirus; LPS, lipopolysaccharide.

myeloid leukemia, breast cancer, gastric cancer, lymphoma, ovarian cancer, and small-cell lung cancer [96]. Other cancer treatments, such as photodynamic therapy, irradiation, and high hydrostatic pressure induce ICD in mouse cancer cells, suggesting the availability of several modalities for combination therapies with immunotherapy [97]. Recent studies have reported that several ICD inducers exert indirect long-term anti-tumor effects [98,99]. In particular, combinations of lysing viruses and chemotherapeutic agents enhance immunogenicity [100]. The classic and novel inducers of ICD are shown in Table 5, and previous studies have demonstrated that a combination of adenovirus and oxaliplatin induced ICD in colorectal cancer [101]. Furthermore, the inoculation of cells undergoing ICD exerted immunogenic effects on cancer vaccines. The inoculation of immunocompetent mice with necrotic DD_RIPK3 cells treated with a potent cyclin resulted in the production of tumor-specific antigens, the proliferation of cytotoxic T lymphocytes, the release of HMGB1, the production of cytokines (such as CXCL1 and IFN-γ), and the phenotypic maturation of bone marrow-derived DCs [102]. HeLa cell-derived exosomes (Exos) are a type of DC vaccine and function as ICD inducers [103]. Human neutrophil elastase and TLR3 agonist were loaded into alpha-lactalbumin to engineer breast cancer Exos. The exogenous induction of ICD in cancer cells, followed by adequate exposure to tumor antigens and hiltonol (poly I:C plus poly-L-lysine), activates type I tumor-reactive CD8 + T cell responses in conventional DCs in situ and promotes cross-priming, resulting in effective tumor suppression in both triple-negative breast cancer mouse model and patient-derived tumor organs with insufficient immunogenicity [77,103,104]. Therefore, in contrast to autologous DC vaccine preparation, targeting the delivery of ICD inducers and adjuvants into tumor cells to activate DCs in situ can be extended to other cancers.

The small molecule signal released by ICD-related DAMP signaling can potently maintain systemic immune and inflammatory homeostasis. Thus, the distinct antigenic and adjuvant properties of these small molecule signals render them a promising target for the development of endogenous adjuvants for therapeutic cancer vaccines [95,105]. Moreover, therapeutic cancer vaccines using ISV strategies can eliminate tumor cells through the intrinsic tumor-specific antigens generated via ICD and can be universally applied to patients, alleviating the impact of human leukocyte antigen differences in tumor antigens among patients. Additionally, the combination of this strategy and ICD inducer treatment enables the development of appropriate immune adjuvants to enhance the immunogenicity of tumor antigens.

5. Perspective

In the past century, adjuvants have enabled vaccines to promote the activation of the human immune system for both preventive and therapeutic purposes. Cancer vaccines have been designed to prevent the progression of major malignancies and are now applied to treat cancer (Table 6). For example, Sipuleucel-T used for the treatment of advanced prostate cancer was the first therapeutic oncology vaccine to be approved for marketing by the FDA in 2010 [37]. In 2021, the FDA approved the mRNA cancer vaccine BNT111, an investigational cancer immunotherapy for the treatment of advanced melanoma that uses a combination of mRNA-encoded tumor-associated antigens to elicit a powerful and precise immune response against cancer [106]. BNT151 is an mRNA encoding IL-2 that will not activate Treg cells and is delivered as a complement to checkpoint blockers. Additionally, BNT151 elicited an enhanced vaccine response in preclinical studies [30,107].

To improve the efficacy of therapeutic cancer vaccines, the weak immunogenicity of the tumor antigens selected for the vaccine must be addressed [108]. The renewed interest in improving traditional endogenous adjuvant molecules through pharmacokinetics based on mRNA vaccines has also opened up new avenues for adjuvant development [33,109,110]. Current studies have focused on the tumor antigens and adjuvants required for cancer vaccines. However, ISV strategies have been adopted for therapeutic cancer vaccines in combination with therapies, such as immune checkpoints, improving the effects of both delivered immune adjuvants and endogenous genetic adjuvants on the vaccine efficacy [70,111]. Precise dosing systems and continuous modification of adjuvants have improved vaccine efficacy and mitigated vaccine-induced side effects. Thus, studies on adjuvant research and development are involved in the development of multi-component and multi-technology combinations. Matrix M, an adjuvant for the COVID-19 vaccine, combines the properties of a nanoparticle adjuvant with the classical vaccine adjuvant saponin. This suggests that the search for new adjuvants in future vaccine adjuvant development can be accompanied by extensive testing of existing adjuvants along with their safety [112]. However, adjuvant molecules associated with viral

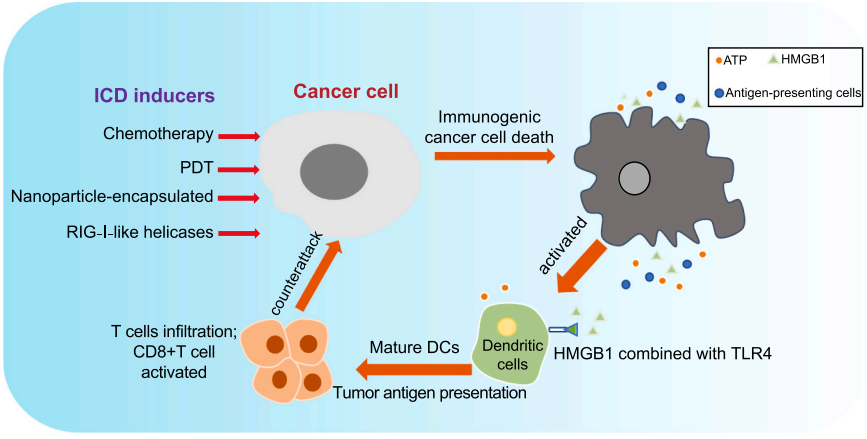

Fig. 3. Mechanisms of the induction of immunogenic cell death (ICD). After ICD induction, chronic exposure of damage-associated molecular patterns on the cancer cell surface attracts receptors and ligands on the dendritic cell (DC) surface and promotes the maturation of immature DCs.

Table 5
The classic and novel inducers of immunogenic cell death.

ICD inducers	Induction pathway	Induction effects	Reference (PMID)
Hypericin photodynamic therapy	Classical secretory pathway and phosphoinositide 3-kinase in the plasma	Induces ICD; Inhibits tumor growth in non-immunized mice	26936504 [103] 22252132 [128]
Shikonin	Activates both receptors and mitochondria-mediated apoptotic pathway; upregulates the expression of DAMPs and tumor-associated antigens	Induces ICD; Promotes DC maturation; Mediates the priming of Th1/Th17 effector cells	22527248 [129]
Nanoparticle encapsulated oxaliplatin	Upregulates DAMPs	Induces; upregulates tumor-infiltrating activated cytotoxic T lymphocytes; exerts higher anti-tumor effects than the free ICD inducer	27343466 [97]
RIG-I-like helicases	Modulates the expression of pro-inflammatory cytokines type I IFN; Upregulates MHC-I molecules and CD95; Promotes CRT relocation to the cell surface	Induces ICD; promotes DC maturation; CD8 + T cells efficiently engulf apoptotic tumor cells; Induces efficient anti-tumor immunity in vivo	25012502 [130]
Oncolytic virus	Increases the number of HER-2-specific CD8 + TILs secreting IFN-γ; Increases the intra-tumoral infiltration of tumor antigen-specific CD8 + T cells	Induces ICD; upregulates immunogenicity of the tumor-associated antigens, disrupts immunological tolerance	29434366 [131] 24782988 [132]

ICD, immunogenic cell death; DAMPs, damage-associate molecular patterns; Th, T helper; MHC, major histocompatibility complex; CRT, calreticulin; TILs, tumor-infiltrating lymphocytes; IFN, interferon; DC, dendritic cell.

antigens generated by nonsynonymous somatic mutations and novel mutation sites cannot be ignored [113,114]. Finally, ICD induced by external stimuli mediates the anti-tumor immune response and is a key regulator of the immune response. To customize cancer vaccine adjuvant regimens, molecules that can be converted into suitable anti-tumor immune adjuvants as ICD inducers must be identified [77,115].

Endogenous genetic adjuvants or natural adjuvants have potential clinical applications and may aid in vaccine development. For example, ISG15, a potential therapeutic target in anticancer treatment, is a promising adjuvant that enhances immune responses against viral antigens and exhibits efficacy in cancer models [116]. In the era of precision medicine, customized therapeutic vaccines are designed for patients with cancer to specifically target and destroy malignant tumor cells by targeting different tumor-associated or tumor-specific antigens in each patient with cancer, as well as to promote the long-term clearance of malignant tumor cells through memory immunity. Therefore, the adjuvants used must be safe, stable, and enhance the immunogenicity of tumor antigens and the antigen presentation efficiency of APCs. Furthermore, the shortcomings of adjuvants for personalized vaccine therapies, such as easy proliferation, interference with immune system activation, and adverse reactions must be overcome by optimizing adjuvant systems, ICD inducers, and ISV through nanotechnology to gradually improve the efficacy of existing adjuvants and further enhance the immunogenicity of tumor antigens and the efficiency of APCs.

Table 6
Therapeutic cancer vaccines applied in clinical cancer treatment.

Vaccine	Adjuvant	Cancer	Date	Reference (PMID)
CIMAvax-EGF	Montanide ISA 51	Non-small-cell lung cancer	2008	28348561 [133]
Sipuleucel-T	GM-CSF	Prostate cancer	2010	21471425 [134]
BiovaxID®	GM-CSF	B cell lymphoma	2011	22085168 [135]
L-BLP25	Liposomes	Non-small-cell lung cancer	2014	24331154 [136]
MAGE-A3	AS15 or AS02B	Non-small-cell lung cancer	2013	23715562 [137]
mRNA-4157/V940	Unknown	Melanoma	2023	37062020 [138]

GM-CSF, granulocyte-macrophage colony-stimulating factor.

6. Conclusion

To develop therapeutic cancer vaccines, the available adjuvants and the delivery modalities must be optimized to rapidly activate immune cells in the VSME and the tumor immune microenvironment. The differences in receptor sites, relevant immune cell targets, and signaling pathways associated with adjuvants in diverse cancer types can interfere with the molecular mechanisms and pharmacokinetic properties of an adjuvant of cancer vaccines and consequently limit the application of heterogeneous cancer vaccine approaches. Hence, the recombination of proportional components in existing adjuvants and nanotechnology, which can enable adjuvants to further improve the efficiency of APCs and induce immunogenic death of tumor cells through novel ICD inducers to enhance the immunogenicity of tumor antigens, is a promising new strategy for adjuvant preparation. Therefore, ideal adjuvants, such as the combination of GM-CSF and IFA, can induce tumor antigen-specific immunity, enhancing the immunogenicity of tumor antigens, improving the efficacy of therapeutic cancer vaccines, and ensuring safety.

CRediT authorship contribution statement

Xueni Yang: Data curation, Formal analysis, Investigation, Methodology, Resources. **Shizheng Xiong:** Data curation, Methodology, Resources. **Xinmiao Zhao:** Data curation, Investigation. **Miaomiao Xu:** Data curation, Resources. **Jiaming Jin:** Data curation, Investigation, Methodology, Software. **Li Guo:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Supervision, Writing – original draft, Writing – review & editing. **Tingming Liang:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Resources, Supervision, Writing – review & editing. **Lixing Weng:** Conceptualization, Investigation, Resources, Supervision, Writing – review & editing, Funding acquisition. **Yujie Ren:** Data curation, Formal analysis, Methodology, Resources. **Dekang Ren:** Data curation, Investigation, Methodology, Resources, Writing – original draft.

Declaration of Competing Interest

The authors have no interests to declare.

Data availability

Data will be made available on request.

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