Dendritic cell engineering for selective targeting of female reproductive tract cancers

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Female reproductive tract cancers (FRCs) are considered as one of the most frequently occurring malignancies and a foremost cause of death among women. The late-stage diagnosis and limited clinical effectiveness of currently available mainstay therapies, primarily due to the developed drug resistance properties of tumour cells, further increase disease severity. In the past decade, dendritic cell (DC)-based immunotherapy has shown remarkable success and appeared as a feasible therapeutic alternative to treat several malignancies, including FRCs. Importantly, the clinical efficacy of this therapy is shown to be restricted by the established immunosuppressive tumour microenvironment. However, combining nanoengineered approaches can significantly assist DCs to overcome this tumour-induced immune tolerance. The prolonged release of nanoencapsulated tumour antigens helps improve the ability of DC-based therapeutics to selectively target and remove residual tumour cells. Incorporation of surface ligands and co-adjuvants may further aid DC targeting (in vivo) to overcome the issues associated with the short DC lifespan, immunosuppression and imprecise uptake. We herein briefly discuss the necessity and progress of DC-based therapeutics in FRCs. The review also sheds lights on the future challenges to design and develop clinically effective nanoparticles-DC combinations that can induce efficient anti-tumour immune responses and prolong patients’ survival.

Key words Cancer therapy - dendritic cells - nanomedicine - translational oncology

Introduction

Cancer accounted for approximately 9.6 million deaths and 18.1 million new cases globally in 2018¹. Among all female reproductive tract cancers (FRCs), the most common are the cancers of the cervix (cervical cancer), ovary and endometrium, which contribute to more than three-fourth of the total reported cases. Cervical cancer is the third most common malignancy in women which resulted in almost 300,000 deaths worldwide in 2018 (about 7.5% of all deaths from cancer in females)². The overall mortality to incidence ratio of cervical cancer is almost 50 per cent due to which it
is a major problem in developing and underdeveloped countries\textsuperscript{1,2}. Other frequently occurring FRCs also display a similar scenario, as cancers of endometrium and ovary, respectively, stand sixth and seventh most frequently occurring malignancies in women worldwide. Approximately, 382,069 and 295,414 new cases of endometrial and ovarian cancers, respectively, were reported worldwide in 2018. The occurrence rate of endometrial cancer in developing countries is more than three times to cervical cancer\textsuperscript{1-4}. Despite several efforts, stage-dependent therapeutic response is considered as a major reason for the higher mortality rate observed in these cancers. This creates a pressing need to develop novel approaches for the prevention and treatment of FRCs.

**Standard therapeutics and their limitations**

The late detection and limited therapeutic options available for FRCs significantly contribute to the global rise in mortality due to these malignancies. Similar to other malignancies, the primary therapy of FRCs is the surgical tumour removal which may be used alone or with other therapies (radio- or chemotherapy). Occasionally, hormone therapy such as progestin (a synthetic drug similar to the hormone progesterone) is also administered. In general, the treatment option mainly relies on the tumour type, occurrence site and disease phase\textsuperscript{5,6}. For instance, in endometrial cancers, radiation therapy may be given in combination to surgery if the disease is malignant and spreading to the nearby cervix, ovary or lymph nodes, which results in a better prognosis. Chemotherapeutic drugs such as carboplatin, cisplatin, doxorubicin and paclitaxel remain the choice of treatment if the disease is recurring or spreading beyond the uterus and cervix. These standard therapies have been shown to reduce the tumour size and control its spread; however, recurrence-induced toxicity and side effects remain severe issues\textsuperscript{7,8}. Furthermore, a large number of female reproductive tract tumours metastasize before disease identification which significantly reduces the effectiveness of these traditional procedures\textsuperscript{9}. For example, in cervical cancer, the disease starts as the growth of pre-cancerous lesions in the cervix region (stage I) and extends within pelvic cavity, side wall or lower vagina (stages II and III) and other body parts (stage IV)\textsuperscript{10}. The prognosis after treatment gets even worse with increasing stages for advanced cancer; the cure rate (5 year survival) drops from about 45 per cent for earlier stages to as low as 15 per cent in advanced stages\textsuperscript{11,12}. Due to various reasons including unawareness and fear, >80 per cent of affected population seeks help at later stages, thus worsening the disease outcomes. Moreover, the restricted response to high-dose standard therapies observed among different gynaecological malignancies increases the probability of disease recurrence as resistant disease. The unavailability of systemic therapies causes major harm to other normally growing cells of different vital systems including immune system, which may lead to an immunocompromised state, thereby increasing the probability of patients to develop various opportunistic infections\textsuperscript{13}.

**Immunotherapeutics: An alternate strategy**

Successful treatment of FRCs remains a major challenge and requires urgent attention for developing novel therapeutic strategies. In the past decade, significant efforts have been made to utilize immune cells and mechanisms to remove unwanted tumour cells. Normally, different components of immune system comprehensively check and remove unnecessary cells and other entities through a tightly regulated mechanism known as immune surveillance. This involves innate and adaptive immune responses which work under strict coordination to generate anti-tumour responses. The key for the generation of an immunotherapeutic strategy lies within these responses as these can be induced or modulated through a number of immunological agents. These strategies mainly depend on three basic strategies, *i.e.* increasing processing and presentation of tumour antigens (Ag), modulating T-cell responses and overcoming cancer cell-induced immunosuppression (Fig. 1). These strategies have been widely utilized to develop an effective anti-cancer therapy for gynaecological malignancies\textsuperscript{14,15}. Among the various analyzed immune-based strategies, utilizing the ability of dendritic cells (DCs) to initiate immune responses makes these cells as appropriate therapeutic alternate for FRCs. Antigen presenting cells (APCs) such as DCs are one of the important constituents of immune mechanisms and possess the potential of being utilized for anti-cancer therapeutics. The superior capability of DCs to uptake, process and present foreign antigens holds optimum promise for utilizing these cells for developing a successful supportive care for gynaecological malignancies.

**Dendritic cell immunotherapeutics for female reproductive tract cancers**

DC, initially identified in 1973 by the pioneer work of R.M. Steinman and Z.A. Cohn, are the professional
APCs of our body. These cells possess a significant potential to initiate primary immunological responses. DCs identify and interact with foreign molecules through their pattern-recognition receptors to release different immunological mediators and trigger effective host immune responses\(^\text{16}\). This centralized role of antigen processing and presentation through specialized surface receptors makes DCs a key player in initiating and regulating responses against tumour cells. This vital role of DCs to regulate anti-tumour responses is being broadly utilized towards developing personalized cancer immunotherapeutics (Fig. 2). DCs take up foreign antigen, process through specialized major histocompatibility (MHC) complexes and present these processed antigen to T-cells for the generation of efficient immunological response. However, this also requires the appropriate presentation of different co-stimulatory and signalling molecules. Normally, after uptake, antigens are processed either via endogenous pathway in which intracellular antigens are processed through class-I MHC or by exogenous pathway whereas extracellular antigens are presented with MHC-II\(^\text{17}\). In endogenous pathway, the antigens are directed to proteasomal degradation through ubiquitination which helps them to fit in the peptide-binding region of MHC class-I. These peptides bind with a protein known as transport-associated protein-1 and 2 heterodimer, which aids their transfer to rough endoplasmic reticulum (ER)\(^\text{18}\). These peptides are co-presented with MHC-I on the DCs membrane. However, in exogenous pathway, the endocytosed antigens are degraded by endosomal proteases which then fuses with MHC class-II in the rough ER with the help of HLA-DM and the stable peptide-MHC composite is then presented on DCs\(^\text{18}\). The DCs also possess capability to cross-present exogenous antigens with class-I MHC to persuade a Th1-mediated response. In this pathway, the cells initiate antigen uptake in exogenous mode but later switches to the endogenous pathway. It involves the retrotranslocation of endosomal compartments and proteasomal complex to load exogenous antigens on class-I MHC\(^\text{19}\). This ability of DCs is important and has been widely assessed to develop a competent DC-based immunotherapy against FRCs.

**Clinical efficiency and impediments**

DC-based therapeutic vaccines for FRCs has undergone a series of *in vitro* examinations and now reached the stage of preclinical and clinical trials. A set of clinical considerations, including host- and tumour-related factors such as age, immunosuppression, stage of disease, HLA and co-infection have been recognized by the Cancer Vaccine Clinical Trial...
Working Group to develop a successful DC vaccine. It is also vital to have a standardized protocol for optimum DC culture and vaccination. Several ongoing and completed clinical studies have successfully established the clinical efficiency of DC-based vaccines in FRCs (Table I). A series of investigations conducted with the peptide-pulsed DCs has shown the generation of significant anti-tumour responses without any significant toxicity. In one such phase II investigation among patients of ovarian cancer with elevated recurrence risk, it was reported that p53 peptide-pulsed DC vaccines were safe and efficient in generating specific responses against p53 peptide. In another phase I trial, therapeutic

![Diagram of developing dendritic cell vaccines](image)

**Fig. 2.** A diagrammatic representation of developing dendritic cell vaccines for female reproductive tract cancers. Dendritic cells are isolated from peripheral blood and cultured with growth cytokines. These cultured dendritic cells are then pulsed with appropriate tumour antigens and administered to the patients. IL, interleukin; GM-CSF, granulocyte macrophage-colony stimulating factor; Ag, antigen.

| Cancer           | Strategy used                                                                 | Reference |
|------------------|-------------------------------------------------------------------------------|-----------|
| Ovarian          | DCs loaded with peptides of MUC1/HER-2/NEU                                   | 21        |
| Ovarian and uterine | DC pulsed with KLH and autologous tumour antigens                          | 22        |
| Ovarian          | DCs pulsed with p53 peptide along with IL2                                  | 23        |
| Ovarian          | Chemotherapy followed by doses of TL-KLH co-loaded pulsed DCs followed by IL2 dosage as an immune adjuvant | 24        |
| Cervical         | HPV protein loaded DC                                                         | 25        |
| Ovarian          | DCs loaded with HER-2/NEU, hTERT, and PADRE peptides, with or without low-dose intravenous cyclophosphamide | 26        |
| Ovarian          | TL co-incubated DC                                                           | 27        |
| Uterine          | DCs loaded with WT1-mRNA                                                     | 28        |
| Ovarian          | DCs loaded with oxidized TL along with bevacizumab and cyclophosphamide     | 29        |
| Ovarian and cervical | Autologous DC formulation                                                   | 30        |
| Cervical         | TL primed DC followed by cisplatin                                           | 31        |

MUC1, mucin 1; HER-2, hereceptin-2; KLH, keyhole limpet haemocyanin; DC, dendritic cells; TL, tumour lysate; HPV, human papilloma virus; IL, interleukin; h-TERT, telomerase reverse transcriptase; PADRE, pan HLA DR-binding epitope; WT1, wilms tumour 1
administration of DCs pulsed with the recombinant HER-2-granulocyte macrophage-colony stimulating factor (GMCSF) peptide blend to a group of patients comprising metastatic ovarian cancer was observed to generate a strong anti-HER-2 response. Studies have also shown keyhole limpet haemocyanin (KLH) act as a vital antigen among different gynaecological malignancies. A phase I study conducted on patients suffering from uterine and ovarian cancers suggested that administration of KLH and whole T-lymphocytes (TLs) (autologous) activated DCs was safe and immunologically active. Similarly, subcutaneous (s.c.) vaccination of KLH-loaded DCs to the patients resulted in significant induction of protein-specific cellular immune responses and delayed-type hypersensitivity reactions, which reaffirmed the safety and effectiveness of these vaccines.

To validate the clinical efficacy of this adjuvant therapy, the vaccines have been systematically administered and assessed in different combinatorial modes. In a randomized phase I trial, loaded DCs along with a chemotherapy drug cisplatin were administered to cervical cancer patients. Absolute clinical response with no recurrence for five years was reported in one of three individuals with the metastatic disease which suggested that the strategy might act as appropriate adjuvant therapy for advanced cervical cancers. Similarly, a trial conducted on patients with recurrent ovarian cancers further reaffirmed the clinical efficiency of these vaccines. The study utilized DCs co-incubated with oxidized TL together with a chemotherapy drug, bevacizumab and observed strong anti-tumour response among the treated patients which later strongly correlated with the disease stability. In one randomized trial vaccination of HER-2 + telomerase reverse transcriptase (h-TERT) + pan HLA DR-binding epitope (PADRE) peptides, pulsed DCs in combination with cyclophosphamide to the advanced ovarian cancer patients resulted in elicited tumour cell-specific responses with promising survival ability. In a different strategy, treatment of WT1 mRNA electroporated DCs to ovarian cancer patients showed higher anti-tumour immunological responses which also correlated with the extended patient survival. In the past decade, these clinical trials have shown promising results and suggested that the establishment of a successful DC-based approach requires utilization of synergistic therapy combinations and incorporation of novel modalities which can generate profound anti-tumour responses and prolong patients’ survival in FRCs.

**Immunosuppressive microenvironment**

The efficiency of DC-based vaccines against FRCs has been assessed in several pre-clinical and clinical studies; however, the key problem in the clinical triumph of DC immunotherapy is the well-established immunosuppressive surroundings. These tolerogenic surroundings limit the immune activation ability of DCs and result in a limited therapeutic response. Different mechanisms have been described which help tumour cells to manipulate host immune system and escape surveillance for progression. These mechanisms primarily aim to downregulate the expression of tumour antigens and MHC molecules, facilitating cancer cells to get away from infiltrating T-cells. The immunosuppressive tumour cell surroundings primarily comprise numerous suppressive molecules and cells which jointly work to create a tolerogenic microenvironment. These molecules suppress the secretion of inflammatory molecules and assist in recruiting Treg populations to downregulate the activity of T-cell subsets, natural killer cells and DCs. Although exact molecular pathways and their inter-linking mechanisms are still a matter of investigation, evidence signifies the vital function of tumour growth factor (TGF)-β in tumour development and its metastatic progression. Interleukin (IL)-10 has also been observed to significantly limit the appearance of different co-stimulatory molecules and inhibit the vital process of antigen processing and presentation. T-cells interacting with IL-10 expressing DCs are shown to be functionally anergic. Molecules such as vascular endothelial growth factor-A (VEGF-A) and prostaglandin E2 (PGE2) interfere with nuclear factor-κB, G protein-coupled receptor and other transcription factors to negatively influence DC maturation and function. Moreover, PGE2 is also shown to influence the levels of an immunoregulatory enzyme, indoleamine-2,3-dioxygenase (IDO) which later suppresses the antitumour responses. Tumours also release soluble Wnt ligands to actively tolerize DC populations within the tumour microenvironment. It has also been shown that the involvement of Wnt-β-catenin pathways have a vital role in suppression of pro-inflammatory cytokines and elevated expression of the immunosuppressive cytokines. Different studies have shown that this tumour-associated immunosuppression is significantly linked to the aggressiveness and metastasis of different cancers of the female reproductive tract. Patients...
with higher TGF expression are more closely related to the worse prognosis of post-surgical therapeutic interventions. Similarly, differential expression of cell surface molecules such as intercellular adhesion molecule and MHC on tumour cells supports their escape from DC vaccine-generated specific immune responses. Therefore, to develop clinically effective DC-based cancer therapeutics, it is important to design strategies which can potentially counter this immunosuppressive microenvironment.

**Tumour-initiating cells**

Tumour-initiating cells (TRICs) have a key function in developing therapeutic tolerance and progression of FRCs. This dedicated cell population functions as tumour stem cells and shows higher tendency for metastasis and increased therapeutic resistance. However, the exact mechanisms depicting the development of such resistive abilities of TRICs are largely unknown. It is assumed that the reciprocal crosstalk between the malignant cells and tumour-infiltrating leukocytes probably regulates the expansion of stem cell-like population and facilitates developing therapeutic resistance among these cells. TRICs express membrane efflux transporters which further support their chemoresistance characteristics. In addition, enhanced DNA repair and low mitotic index are other properties which contribute to the development of drug resistance. Further, these self-renewing malignant progenitors form a group of cells to regulate cellular differentiation. TRICs are the major cause of clinical relapse as they escape the therapeutics and re-grow upon their cessation and can subsequently regenerate entire malignant phenotype. The well-known immunosuppressive tumour microenvironment in patients and significant toxicity of traditional therapeutics further assist TRICs to escape the immunological surveillance. This represents a decisive interface between vaccine effectiveness and tumour progression, so it is vital to devise and include new clinically effective approaches enabling DCs to overcome tumour-induced immunological suppression along with eliciting specific immune responses for removal of TRICs, a major cause of clinical relapse in gynaecological malignancies.

**Dendritic cell nanoengineering: An approach to move forward**

Nanotherapeutics offers an efficient inclusion in traditional DC-based vaccine to surpass the existing obstacle of immune suppressive microenvironment. It has been shown that tumour antigens have limited ability to activate DCs; thus, co-encapsulating tumour antigens with different adjuvants such as micro-emulsions, lipopolysaccharides and bacterial CpG...
DNA may prove beneficial\(^{51}\) (Fig. 3). Encapsulation of tumour antigens using different nanomaterials protects them from damage of proteases and assists in a prolonged and proscribed antigenic release to DCs. This increases the antigenic acquaintance to DCs and helps them to counter the suppressive microenvironment\(^{34}\). Incorporation of different surface modifiers and ligands may specifically direct nanocarriers to target cells and further aid the clinical and immune activation potential of DC vaccines. Earlier reports have shown that encapsulation of tumour antigens enhances the MHC-I-mediated antigen cross-presentation\(^{34,50}\). However, for efficient immune stimulation, different factors such as size, shape, surface chemistry, mode of administration, diffusion and solubility play an important role\(^{52,53}\). Evidence suggests that reduction in the ionic interaction of matrix material with the encapsulated protein significantly declines the burst effect and reduces the release rate\(^{54}\). Therefore, copolymers such as ethylene oxide-propylene oxide can be incorporated to a non-toxic polymer with appropriate molecular weight and chemically defined monomers to improve the efficiency of nanocarriers\(^{54}\).

**Vital factors for dendritic cell nanoengineering**

The therapeutic efficiency of DC vaccines can be further modulated by the mode of uptake by DCs, which have a primary function in shaping the antigenic fate, i.e. either interact with MHC-I or with MHC-II. However, relying on the physical and chemical compositions of the encapsulated Ag the mode of uptake may be endocytosis, macropinocytosis and phagocytosis. A pre-requisite for successful DC-based therapeutic vaccines is their ability to generate a potent Th1-mediated cytotoxic response. It is suggested that larger nanoparticles (NPs) with size approximately 500 nm are preferably taken up through phagocytosis, a process which assists humoral immune responses\(^{45}\). The NPs with a smaller size (approximately 100 nm) are effectively taken up through endocytosis which generates a potent Th1 response\(^{56}\). Thus, the use of NPs with size less than 100 nm should be preferred to ensure the generation of an optimum cytotoxic immune response. The larger particles (500 nm) predominantly reside at the injection site while the smaller ones freely move to the lymph node area where these interact with resident DCs. Smaller particles also provide higher surface area and superior membrane permeability which support their efficient uptake\(^{57}\). A quick particle uptake and longer post-injection retention of smaller particles (<100 nm) have also been reported\(^{58}\). This suggests the suitability of these particles for designing future in vivo targeting strategies. In addition, maintaining the appropriate particle shape is also crucial as spherical particles are supposed to have less premature clearance, more circulatory halftime and good organ distribution\(^{58,59}\).

Following size and shape of the particle, another vital factor for developing effective nanoengineered DC vaccines is the mode of administration. NPs injected through intraperitoneal mode interact with the macrophages, while, upon intradermal (i.d.) administration, NPs are taken up by DCs. It has been reported that i.d. injected particles move preferentially to lymphatics with an average 50 per cent higher bioavailability as compared to intramuscular (i.m.) mode\(^{59}\). Moreover, the kinetics and magnitude of tumour cell-specific Th1 responses were reported to be strongly influenced by the mode of administration [i.d., i.m., intralymphatic (i.l.) and s.c.]. Administration of different NPs such as chitosan, liposomes and poly(lactic-co-glycolic acid) (PLGA) through i.l. route induces a strong Th1-type immunological response which is a determining factor for therapeutic use of nanoengineered DCs\(^{60}\).

**Nanocarriers for dendritic cell nanoengineering**

Different nanocarrier systems have been evaluated for the treatment of FRCs. These nanocarriers including lipid-based and/or polymer-based systems have been broadly used to deliver therapeutic drugs/tumour antigens and have also shown to possess the ability to target both tumour cells and microenvironment. The HPV16 E7 small interfering (si) RNA-loaded chitosan NPs were reported to successfully restrict the tumour cell proliferation and reverse the drug resistance of ovarian tumour cells, suggesting these nanocarriers as promising therapeutic mediators for treating gynaecological malignancies\(^{61}\). Similarly, cisplatin-loaded liposomal formulations effectively targeted cancer cells of the female reproductive tract. The observed growth inhibitory effects were better than that of free cisplatin\(^{62}\). The liposomal formulation comprising a new T7 peptide which exclusively attaches to the transferrin receptor, reported best in vivo growth inhibitory effect on ovarian carcinoma cells\(^{63}\). In addition, in comparison to the plain liposomal doxorubicin, peptide-conjugated liposomal doxorubicin was shown to be better in controlling tumours\(^{64}\).

Polymeric NPs such as PLGA are also effectively analyzed for the release of therapeutic molecules. The delivery of tumour-associated antigens through PLGA-NPs enables DCs to proficiently trigger tumour-specific responses. The encapsulation
angiogenesis inhibitors (TNP-470-NP-APRPG) through PLGAs showed promising results for utilizing the strategy for treating ovarian cancers\textsuperscript{65}. Administration of short hairpin (sh) RNA encapsulated with PLGA showed significantly higher anti-tumour effects against ovarian cancer cells\textsuperscript{66}. Therapeutic efficiency of novel folic acid decorated-PEG-PLGA NPs for targeting the drugs to cancer cells in endometrial cancers has also been shown\textsuperscript{67}. The dosage of PLGA-PRINT NPs loaded with chemotherapeutic drug docetaxel and chitosan NPs containing mEZH2 siRNA was reported to be associated with considerable anti-angiogenic and pro-apoptotic consequences in tumour cells\textsuperscript{68}. NPs carrying oligonucleotide duplexes dramatically raised the immune stimulatory activity of miR-155 in DCs related to ovarian cancers\textsuperscript{59}. It has been shown that surface-modified PEG-poly caprolactin-NPs strongly activate DCs for antigen cross-presentation and thus can be used as a potent immune adjuvant\textsuperscript{70}.

A number of other NPs have been used for directing immune cells to target and increase the eradication of gynaecological tract tumours. Recently, the combination of NPs with albumin-bound paclitaxel and nedaplatin was observed to be active and well tolerable for treating cervical cancer patients with late-stage, frequent or metastatic disease\textsuperscript{79}. Carbon NPs were shown to precisely envisage the pathological status of pelvic lymph nodes in early cervical tumours, suggesting their larger function towards generating DC immunotherapy against these cancers\textsuperscript{80}. Synergistic combination of micellar-based telodendrimer nanocarrier systems with chemotherapeutic drugs paclitaxel and cisplatin resulted in a competent and targeted delivery of drug to tumour cells along with reduced toxicity and potent immunological effect\textsuperscript{72}. The efficiency of this system has been re-validated with bortezomib and doxorubicin where these multifunctional telodendrimer nanocarriers were reported to restore therapeutic synergy with minimum cytotoxicity in ovarian cancers\textsuperscript{83}. PEGylated peptide dianimocyclohexyl-platinum conjugates are suggested to be new prospective drug release method with enhanced anti-tumour efficacy and clinical possibility in treatment of ovarian cancer malignancies\textsuperscript{74}. The superiority of cationic liposomes and Toll-like receptor (TLR)-3 agonist complexes towards improving the tumour-specific immune response has been attributed to the TLR3-interferon regulatory factor signalling within DCs\textsuperscript{75}.

Several NP formulations including polymeric formulations and lipid-based systems have been utilized for developing a successful nanocarrier system for efficient antigen delivery to DCs. Among the studied NPs, solid-lipid NPs (SLN) offer the most efficient delivery system. SLN systems initially prepared in the early 1990s belong to class of the standard colloidal systems which have a solid-lipid core making internal core minimally exposed to water, thereby possessing increased stability of the loaded material\textsuperscript{76}. Besides, the surface of these NPs can be simply attached with appropriate ligands for targeting, has superior payload and is minimal toxic\textsuperscript{52}. SLN is prepared by utilizing lipids such as triglycerides which have high melting points and their lipid core is stabilized by different emulsifiers. In addition, the ease of surface modification enables and ability to co-encapsulate adjuvants further improve their capability to activate DCs and provoke a strong anti-tumour response. These properties have been significantly supported by the reports which suggest SLN as an optimum nano-carrier system\textsuperscript{77,78}. Cationic SLN loaded with STAT3 decoy oligodeoxynucleotides was reported to efficiently transfect genes, inhibit invasion and stimulate apoptosis in ovarian cancer cells\textsuperscript{79}. Wang et al\textsuperscript{80} suggested that SLN surface modified with hyaluronic acid act as a potential carrier to target tumour cells and overcome multidrug resistance associated with in cervical cancers. These studies provide a platform to utilize nanocarrier systems for developing optimum DC-based vaccines. However, the choice of an optimum system is vital but confusing. In a study, a relative evaluation of lipophilic NPs was performed to recognize and develop an optimum NP-based strategy for DC-based targeting of TRICS in gynaecological cancers. The study reported that in comparison to other studied lipid-based nanocarriers, mannosylated-SLN was a feasible and optimum approach. Mannosylated SLN-pulsed DCs were observed to be efficiently taken up by DCs, induced minimal toxicity and were more potent inducers of tumour cell-specific immune responses\textsuperscript{80}.

\textbf{Nanocarriers for in vivo targeting}

The limited existence of the administered DCs restricts their activity and not many of these reach to the lymph nodes which is a major reason for the observed limited effectiveness of these vaccines\textsuperscript{49}. Therefore, focusing on the DCs within the body instead of culturing them \textit{ex vivo} may be an option. This can be done using NP-mediated DC-targeting approaches which consist of a blend of antigens and other vaccine constituents. Further, addition of immune activating adjuvants may help generate a surrounding environment which aid effective DC recruitment and activation to increase the vaccine efficiency\textsuperscript{80}. In addition, surface modification
of the NPs with ligands specific for DC membrane receptors enable the NPs to dynamically target DC with no or minimal non-specific uptake\textsuperscript{81-83}. Upon administration, the NPs interact with the \textit{in vivo} residing DCs, and due to the prolonged antigenic release, these cells come out of suppressive microenvironment and trigger tumour cell-specific immune responses\textsuperscript{84-86}. Earlier evidence also suggests that \textit{in vivo} DCs targeting is an efficient option with huge immunological prospective\textsuperscript{87-89}. In this regard, C-type lectin receptors (CLR) is one of the vital ligands analyzed for precise DC targeting. CLR holds the ability to bind with the antigenic carbohydrates residues. Broadly, CLR is divided into two categories, \textit{i.e.} type I and type II. Targeting DCs with DEC-205 and CD11c labelled liposomes showed significantly increased tumour-cell specific immune responses\textsuperscript{90}. Perhaps preparing the blend of MHC-I and T-helper cell epitopes with CLR-specific molecules on NPs might hold as a promising approach for inducing a competent tumour cell-specific T-cell responses\textsuperscript{56}. Similarly, liposomes surface modified with glycans exhibited an enhanced encounter with DCs\textsuperscript{91}. Incorporating LPS additionally augmented T-cell activation signifying the anti-tumour potential of the approach\textsuperscript{91}. Upon administration of the NPs (gold nanocarriers and liposomes) modified with Fc receptor, efficient interaction with DC receptor was observed which suggested their potential ability to target DCs and activating tumour cell-specific immune responses\textsuperscript{92}. Investigations have also shown the ability of superoxide iron NPs towards DC targeting and inducing efficient migration \textit{in vivo}, with superior bio-compatibility\textsuperscript{93,94}. This ability of NPs can be further improved by including imaging mediators which enable simultaneous monitoring of nanocarriers and assist in further designing the improved targeted approaches\textsuperscript{81}. Developing such DC targeting strategies will help towards overcoming immune suppressive tumour surroundings and designing efficient strategies.

| Table II. Nanoparticles for engineering dendritic cells to target tumour re-initiating cells in gynaecological malignancies |
|-----------------|-----------------|-----------------|
| Nanoparticle    | Properties                                      | References     |
| Liposomes       | Non-toxic, biocompatible, biodegradable NP       | 51, 95         |
| Solid lipid nanoparticles | Non-toxic, biocompatible and biodegradable NP | 32, 50, 96     |
| Poly-(lactic-co-glycolic acid) | Non-toxic, biocompatible, biodegradable NP | 97, 98         |
| Poly-ε-caprolactone | Non-toxic, biocompatible, biodegradable NP | 99, 100        |
| Poly (propylene sulphide) | Non-toxic, biocompatible and biodegradable NP | 101            |
| Poly (γ-glutamic acid) nanoparticles | Biocompatible and biodegradable NP | 102            |
| Chitosan        | Non-toxic, biocompatible, biodegradable NP       | 103, 104       |
| Gelatin         | Non-toxic, biocompatible, biodegradable NP       | 105            |

NP, nanoparticles; RES, reticular endothelial system; FDA, Food and Drug Administration
for cancer therapy in future. A brief detail of different NPs which can be used for generating nanoengineered DCs is provided in Table II.

Conclusion

DCs vaccines offer a potential therapeutic modality for FRCs. The promising results observed in pre-clinical and clinical studies encourage continuing efforts towards further optimizing these methodologies and validating combinatorial therapeutic approaches. A major effort must be dedicated towards designing approaches to overwhelm the well-identified issue of tumour-associated immunological suppression. In this regard, incorporation of nanoengineering approaches may prove vital as these possess the ability to enhance the immune activation property of DCs. These engineered DCs are suggested to be safe and hold significant potential to eradicate TRICs. Encapsulation helps DCs to overcome immune suppression which later initiates a cascade of tumour-specific immune responses. Designing multifunctional NPs may further open the opportunities of direct in vivo targeting and may be vital as it will help in overcoming some important concerns related to ex vivo tumour antigen loading, short life-span of loaded DCs and faulty migration. In addition, the presence of substantial amount of resident DCs in the lymph nodes will assist in the efficient antigen uptake, processing and its cross-presentation to the neighbouring T-cell population to generate specific immune responses. These nanotherapeutic approaches have profoundly worked under different in vitro and in vivo conditions, however; their successful clinical translation relies on the ability of these vaccines to overcome different limiting factors. The present article was an attempt to showcase the need and significance of developing nanoengineered approaches, likely to maximize the therapeutic success of DCs against different FRCs.

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