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Dendritic cell-based immunotherapy of prostate and ovarian tumors

Imunoterapie na báze dendritických buněk pro léčbu nádoru prostaty a ovárií

Bachelor´s thesis

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Declaration/Vyhlásenie:

I hereby declare that I wrote this thesis on my own and listed all used sources of information and bibliography. Neither this work nor its significant part was previously used to obtain any other academic degree.

Vyhlasujem, že som záverečnú prácu spracovala samostatne a uviedla všetky použité informačné zdroje a literatúru. Táto práca ani jej podstatná časť nebola predložená na získanie iného, prípadne rovnakého akademického titulu.

V Prahe, 22.06.2024 Miroslava Kolníková

Abstract:

Urogenital cancers place a great burden on global health. Despite many forms of treatment available, many patients with prostate or ovarian cancer experience remissions or progressive disease. Active cell-based immunotherapy utilizes immune cells acquired from the patient or a healthy donor to directly target and destroy tumour cells. Several therapies of this type have been approved for clinical use. However, their effectiveness is still far from being satisfactory. Dendritic cells (DCs) play an important role in initiating and modulating adaptive immune responses, making them a powerful tool for cancer therapies. In this work, current development and future perspectives on using DCs to treat prostate and ovarian cancer are discussed.

Keywords: dendritic cells, dendritic cell vaccines, immunotherapy, prostate cancer, ovarian cancer

Abstrakt:

Nádory urogenitálneho traktu patria k najviac závažným formám rakoviny. Napriek dostupnosti viacerých možností liečby u mnohých pacientov s rakovinou prostaty alebo ovárií dochádza k remisiám či pokročeniu choroby do vyšších štádií. Aktívna bunková imunoterapia využíva bunky imunitného systému získané od pacienta alebo zdravého darcu na cielené ničenie nádorových buniek. Terapie tohto typu schválené pre použitie v klinickej praxi však stále vykazujú neuspokojivú účinnosť. Dendritické bunky (DC) hrajú dôležitú rolu v iniciácii a modulácii špecifickej imunitnej odpovede a majú tak veľký potenciál na využitie v liečbe rakoviny. Cieľom tejto práce je popísať súčasný pokrok a načrtnúť budúce perspektívy využitia DC v liečbe rakoviny prostaty a ovárií.

Kľúčové slová: dendritické bunky, protinádorové vakcíny, imunoterapia, rakovina prostaty, rakovina ovárií

Contents

List of abbreviations

Ab	antibody
AE	adverse events
Ag	antigen
APC	antigen-presenting cell
CAR	chimeric antigen receptor
cDC	conventional/classical dendritic cell
CTC	circulating tumour cell
CTL	cytotoxic ($CD8^+$) T-cell
DC	dendritic cell
DTH	delayed-type hypersensitivity
Flt3	Fms-like tyrosine kinase 3
Flt3L	Flt3 ligand
FRα	folate receptor α
GM-CSF	granulocyte/macrophage colony stimulating factor
HLA	human leukocyte antigen
HPV	human papillomavirus
HPSC	hematopoietic stem/progenitor cell
HT	hormonal therapy
hTERT	human telomerase reverse transcriptase
ICI	immune checkpoint inhibitors
i.d.	intradermal [administration]
IFN	interferon
IL	interleukin
i.n.	intranodal [administration]
i.v.	intravenous [administration]
LN	lymph node
mAb	monoclonal antibody
MAGE	melanoma-associated antigen
MHC	major histocompatibility complex

mo-DC	monocyte-derived dendritic cells
NK	natural killer [cell]
OCa	ovarian cancer
OS	overall survival
PAP	prostatic acid phosphatase
PBMC	peripheral blood mononuclear cell
PCa	prostate cancer
pDC	plasmacytoid dendritic cell
PFS	progression-free survival
poly(I:C)	polyinosinic:polycytidylic acid
PRR	pattern recognition receptor
PSA	prostate-specific antigen
PSADT	PSA doubling time
STAT3	Signal Transducer and Activator of Transcription proteins 3
STING	STimulator of INterferon Genes
TAA	tumour-associated antigen
TAP	transporter associated with antigen processing
TARP	TCR alternate reading frame protein
TCR	T-cell receptor
Th	helper T-cell
TLR	Toll-like receptor
TNF	tumour necrosis factor
Treg	regulatory T-cell

Introduction

Cancer has a considerable impact on the global mortality rate. According to the WHO, respiratory cancers alone were the 6th most prevalent cause of death in 2019, with all types of cancer combined accounting for an estimated 9 297 000 deaths worldwide [1]. Although there are differences between the sexes, the mortality rankings are generally dominated by lung, liver and colorectal cancers along with the respective genital cancers. While non-solid cancer types are no less dangerous, they are usually less prevalent than the cancer types mentioned above. Many solid cancers are preventable by maintaining a healthy lifestyle and avoiding exposure to carcinogens [2]. Infections by some pathogens may increase the risk of several cancer types, such as gastric cancer (associated with *Helicobacter pylori*) [3]. Naturally, there have been many efforts to develop vaccines against cancer-linked infectious agents. Some are in early development (ex. SH02, a *H. pylori* vaccine), others are being tested in clinical trials (ex. an Epstein-Barr virus nanoparticle vaccine) and several are already approved and widely used (e.g. Hepatitis B vaccines, with global immunization estimate at 84 %) [4–6].

Urogenital cancers, including cervical, prostate, and ovarian cancers, also pose a significant burden on global health, affecting individuals and healthcare systems. Cervical cancer in particular is the leading cancer type responsible for premature deaths in countries with lower Human Development Index (HDI) [7]. Vaccination against the human papillomavirus (HPV) plays a crucial role in urogenital cancer prevention [8]. Despite the availability of vaccines, the global vaccination rate remains very low [6]. Moreover, the mortality rates of cancers not directly associated with HPV infection, such as ovarian and prostate cancer (OCa and PCa, respectively), are still quite high. Therefore, a new treatment strategy is needed.

There are several treatment modalities for urogenital cancers. The primary approach in most cases is a cytoreductive surgery [9,10]. However, a complete resection of a tumour is not always possible, especially in the late stage of the disease, where the process of metastasis has already begun. Thus, surgical procedure is often coupled with chemotherapy or hormonal therapy (HT). Radiation therapy is also used with a high success rate, however it poses a risk of secondary malignancies in proximate organs [11,12]. HT, not applicable in every case, has varying response rates depending on the form of therapy and cancer type [13,14]. Moreover, the long-term effectiveness may be diminished by the tumour cells exploiting various mechanisms, such as alternative activation of hormone receptors, leading to the development of hormone-resistant tumours [15]. (In the case of PCa patients, these are referred to as hormone-refractory tumours.) Likewise, chemotherapy is rather effective, though any progress is often blocked by either intrinsic or acquired drug resistance [16]. Patients also report a high frequency of side effects, negatively impacting their quality of life [17]. As a result, there is a demand for effective therapies that would decrease the burden on the patient's body and increase the chances of survival and long-term remission.

One of the options is to use targeted therapies. Based either on small organic compounds or monoclonal antibodies (mAbs), the drugs interfere with signalling pathways overexpressed only in the cancer cells [18]. Thus, other dividing cell populations are spared from their cytotoxic effects. Another way to approach this is to make use of the body's natural defence line – the immune system. By specifically targeting cancer cells, immunotherapies are able to reduce collateral damage.

Immunotherapies are classified as passive or active based on how they employ the host immune system [19]. Components of passive immunotherapies do not require additional activation to enact a response, making them a good choice for patients with weakened immune system. Adoptive cell transfer (ACT) therapy involves collecting the patient's lymphocytes and returning them after *ex vivo* enhancement – in the form of activation, expansion, or receptor modification [20–22]. Oncolytic viruses have also been considered as a potential vector for cancer treatment, either for their natural capacity to kill cancer cells or in a genetically modified form [23,24]. In a phenomenon known as the abscopal effect, lysis of tumour cells resulting from localized treatment with an oncolytic virus can lead to immune system activation and systemic anti-tumour immune responses [24]. So far, few oncolytic virus therapies have been approved for clinical use [25–27]. Monoclonal antibody-based therapies are usually included in this category as well [19]. Some drugs of this type harm the cancer cells by linking tumour-targeted mAbs with cytotoxic reagents [28].

On the other hand, active immunotherapies do not act on the tumour directly. Instead, they work by triggering or modifying the patient's own anti-tumour response. In practice, this can be achieved by administration of cancer vaccines, immune checkpoint inhibitors or cytokines. Cytokines commonly used for cancer therapies include (but are not limited to) IL-2, IL-12 and interferon (IFN)-α [29–31]. Immune checkpoint inhibitor (ICI) therapies target proteins involved in inhibitory signalling pathways (immune checkpoint molecules), such as CTLA-4, PD-1 and its ligands, or LAG3 [32,33]. They are mostly expressed on T-cell or cancer cell surface [34]. The interaction of an immune checkpoint molecule (receptor) and its ligand leads to T-cell suppression. ICIs are mAbs which prevent loss of T-cell function by blocking the interaction. Cancer vaccines aim to trigger the anti-tumour response by employing tumour-associated antigens (TAAs) in the form of peptides or DNA constructs (which ensure ectopic expression of TAAs) [19]. An alternative approach uses *ex-vivo* generated dendritic cells (DCs).

Success of the therapy depends on multiple factors, such as health conditions, age, lifestyle and immune activity within the tumour microenvironment. Tumour infiltration by lymphocytes plays a critical role; tumours with robust immune cell infiltration are considered "hot tumours" and are often more responsive to immunotherapies such as ICIs [35]. Tumour-infiltrating lymphocytes (TILs) are frequently used in ACT therapies [21]. Notable cells used for ACT are chimeric antigen receptor (CAR) T-cells, approved for clinical use in patients with haematological cancers [36]. However, recently, there have been concerns about the risk of secondary malignancies [37].

Taking note of leukocytes responsible for innate immunity, natural killer (NK) cells and macrophages respond well to certain ICIs [38,39], though there were efforts to develop CAR-NK and CAR-macrophages as well [40,41]. So far, neutrophils have limited use in cancer immunotherapy apart from being a response indicator [42]. However, the same does not hold true for dendritic cells (DCs), professional antigen (Ag) presenting cells. The appeal of using DCs lies in their ability to mount an immune response to multiple tumour Ags. This allows the immune system to counter cancer resistance based on loss of antigens (for example, by reversible dedifferentiation of the cancer cells [43]). What's more, they provide a variety of signals needed for T-cell activation, from co-stimulatory molecules to cytokines.

The aim of this work is to explore recent developments and future possibilities of using DCs in prostate and ovarian cancer immunotherapy.

Dendritic cell biology

DCs generally arise from Fms-like tyrosine kinase 3 (Flt3)$^+$ hematopoietic progenitor cells and are present in both lymphoid and non-lymphoid tissue [44].

Non-lymphoid tissue (also known as migratory) DCs fulfill a surveillance role, constantly sampling their environment by macropinocytosis and capturing Ags from their surroundings [45,46]. To detect them, they employ a variety of pattern recognition receptors (PRRs), mostly Toll-like receptors (TLRs) or C-type lectin receptors (CLRs). PRR expression varies between DC subsets [47].

Upon recognition and internalization of an Ag, DCs initiate the maturation process. This involves upregulating CCR7, which is necessary for lymph node (LN) entry, and major histocompatibility complex (MHC) II [48,54]. The expression of co-stimulatory molecules (such as CD40, CD80 and CD86) required for T-cell activation also increases [49,50]. As this is happening, the cells migrate through lymphatic vessels and enter lymph nodes. Here, they present the captured Ag to thousands of naïve T-lymphocytes inhabiting the organ. By extending long membrane protrusions in all directions, DCs are able to engage several cells at once. It was estimated that, on average, one DC can "scan" up to five hundred T-cells per hour [51]. The T-lymphocyte that recognizes the presented Ag undergoes activation and becomes an effector cell. To successfully activate a naïve T-cell, additional signals are required – ligation of co-stimulatory receptors and cytokines - both of which can be provided by DCs.

T-cells forming contacts with DCs can be divided into two types based on their expressed co-receptors. CD8$^+$ T-cells gain cytotoxic capabilities once activated and are then referred to as cytotoxic lymphocytes (CTLs). CD4$^+$ T-cells develop into helper T-cells (Ths), which are responsible for directing the immune response as needed. Although direct activation of CD8$^+$ T-cells is possible [52], usually, assistance of CD4$^+$ T-cells is required [53]. This is referred to as DC licensing. Among its effects is prolongation of DC survival. Overall, DCs do not live long after entering a LN - only three to seven days (in rare cases, they can last up to two weeks) [54]. Interaction of CD40 (present on the DC surface) and CD40 ligand carried by helper T-cells lowers susceptibility to MHC II-mediated apoptosis and promotes full maturation of the DC [55]. Earlier studies indicated that CD40 ligation is crucial for launching an effective anti-tumour response [56]. Additionally, cytokines secreted by the DC can skew T-cell differentiation towards a particular subset.

Lymphoid tissue-resident DCs perform various duties. Thymic DCs help establish central tolerance by thymocyte deletion [57]. Ags captured by migratory DCs can be transferred to resident DCs, relaying on them the task of activating naïve T-cells [58]. They do not have to rely on other cells to acquire Ags, though – DCs residing within lymphatic sinuses take up material from the lymph, providing an additional line of defence against pathogens [59].

DCs are considered to be the most potent antigen-presenting cells (APCs), essential for initiating and regulating immune responses. DC phagolysosome tends to be rather alkaline, with a low level of lysosomal proteases [60]. This allows for longer preservation of the engulfed Ags and prevents the destruction of peptide epitopes recognized by T-cells. Presentation to T-cells is facilitated by MHC molecules depending on the antigen origin – MHC I is used for endogenous and MHC II for exogenous substances.

Extracellular Ag uptake can be either non-specific or specific – mediated through PRRs [47]. DCs can also take up immune complexes via Fc receptors [61]. Following endocytosis (or macropinocytosis), the Ag-containing endosome or phagosome usually merges with lysosomal vesicles containing processing enzymes [62]. The captured Ags are digested into short peptides and bound to MHC II. Here, the assistance of human leukocyte antigen (HLA)-DM is required, from quality control to unblocking the grooves of newly made MHC II molecules (which are occupied by a short peptide called CLIP) [63]. The loaded peptide:MHC II complexes are then displayed on the surface of the cell, ready to interact with a $CD4^+$ T-cell receptor (TCR). In immature DCs, empty MHC II along with HLA-DM are also present on the plasmatic membrane; it was suggested that they can directly capture and present exogenous Ags [63].

MHC I is typically loaded in the endoplasmic reticulum, assisted by a protein complex containing a transporter associated with antigen processing (TAP) – a protein that imports peptide fragments from the cytosol into the lumen. After, it is transported through the Golgi apparatus to the cell membrane. This "classical" pathway is present in virtually all cells and is used to display self-peptides or peptides derived from intracellular pathogens [64]. However, MHC I can also be used to present peptides obtained from the external environment of the cell. Given that $CD8^+$ T-cell receptors recognize MHC I only, this process, named cross-presentation, is the key to launching a CTL response. There is evidence of cross-presentation in various phagocytic cell types, including osteoclasts and endothelial cells, although its significance remains to be clarified [65].

The cross-presentation pathways can be divided into vacuolar or cytosolic [62]. Depending on the MHC I loading location, two variants of the cytosolic pathway can be distinguished - the phagosome-to-cytosol (P2C) and phagosome-to-cytosol-to-phagosome (P2C2P) variant. Both processes involve the release of exogenous Ags into cytosol, where they are processed by proteasomes and subsequently taken up by TAP. The peptides bind to MHC I after re-entering a phagosome containing loading complexes or enter the endoplasmic reticulum and continue along the classical pathway. In the vacuolar pathway, Ags are retained and processed in the phagosome. This is usually facilitated by cathepsins, particularly cathepsin S [62,64]. The recruitment of MHC I to Ag-containing phagosomes is mediated by TLR signalling [66]. Besides employing newly synthesized molecules from the endoplasmic reticulum, MHC I is also sourced from the endosomal recycling compartment. In the case of TAP dysfunction (due to viral blockade, for example), MHC I is recruited from the endoplasmic reticulum-Golgi intermediate compartment (ERGIC) instead [64].
It has been proposed that the pathway used depends on the form of Ag and the DC subset [60]. Engagement of certain receptors can direct captured Ags to a cross-presentation pathway instead of MHC II presentation. An example is C-type lectin Clec9a, which detects F-actin-myosin complex found on cell remains after necrosis [67].
Cross-dressing is an interesting ability that may play an important role in tumour Ag presentation [68]. It was discovered that APCs can acquire parts of cancer cell plasma membrane containing loaded MHC molecules and display them on their own cell membrane. The peptide:MHC complexes are likely obtained by the uptake of tumour exosomes or phagocytosis (trogocytosis has also been suggested) and subsequently recognized by the MHC I-recycling pathway. It was hypothesized that cross-dressing helps facilitate cross-presentation of tumour-associated Ags in the absence of necrotic cell death.

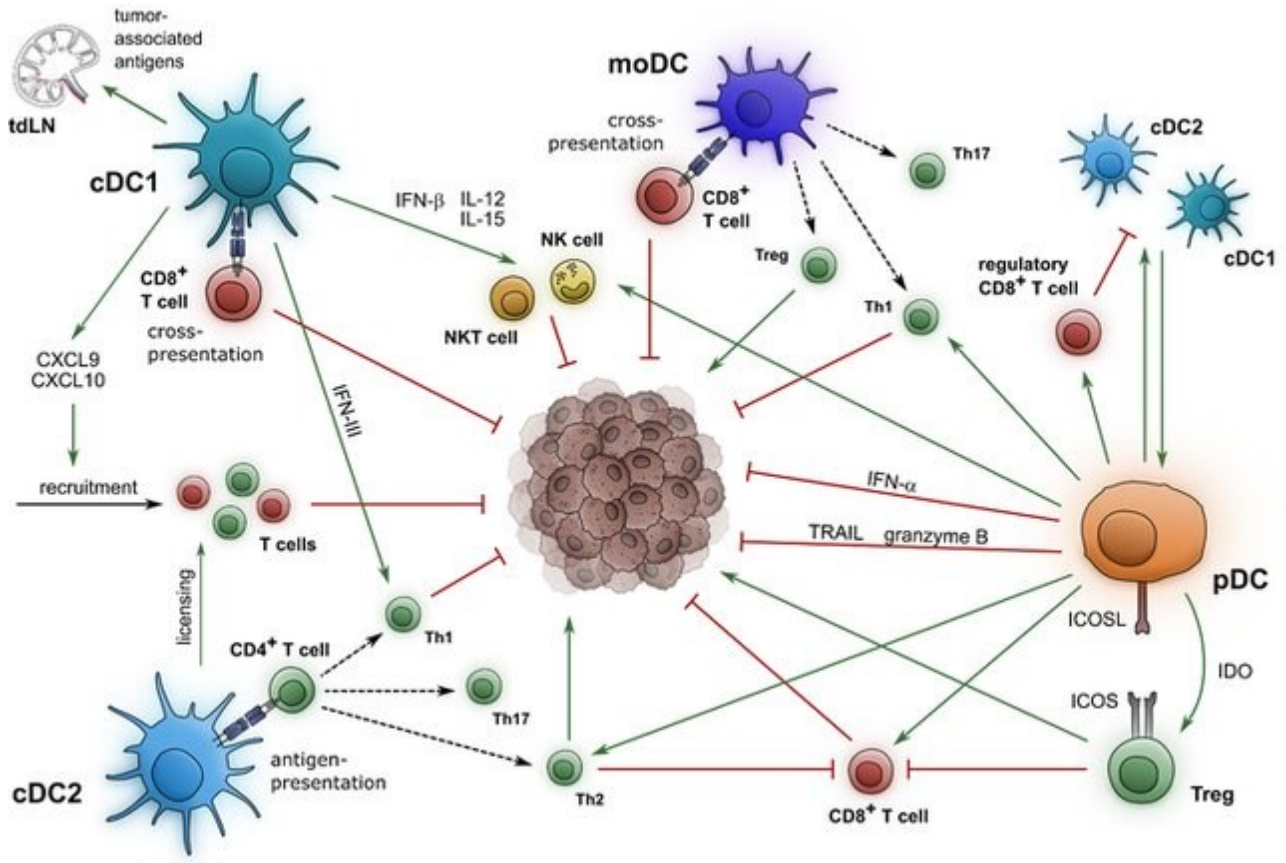

Figure 1. Main dendritic cell subsets and their roles in anti-tumour response. Adopted from Plesca et al, 2022[69].

Although the classification of DC subsets has seen many changes as new information emerges, several major types of DCs were identified (see Figure 1.) [70].

Conventional or classical DCs (cDCs) prime $CD8^+$ and $CD4^+$ T cell responses [71]. They form two subsets differing in developmental pathways, cytokine expression and behaviour, termed cDC1 and cDC2. cDC1 are found mostly in the spleen and the T cell areas of lymph nodes; also, to a lesser extent, in blood, bone marrow and peripheral tissues [72]. In humans, they are defined by expression of CD141/BDCA3 [73]. The presence of cDC1 in tumour microenvironment is often associated with a good prognosis, as their specialty is type III IFN secretion [74]. Furthermore, they possess a high ability to cross-present necrotic material [75], playing an important part in antiviral immunity. cDC1 express high levels of Clec9A, chemokine receptor XCR1 and TLR3, making them excellent inducers of CTL responses [76].

In comparison, cDC2 seems to prefer areas close to B-cell follicles and is much more frequent in non-lymphoid tissues than cDC1 (in some areas, the cDC1/cDC2 ratios reach 2:1) [72]. Moreover, the expression of CCR7 was observed more frequently in cDC2, suggesting a higher migratory ability. The human surface marker for cDC2 is CD1c/BDCA1 [73]. One of their functions is priming follicular helper T-cells (Tfh), essential in inducing B-cell responses [77]. Among human dendritic cells, cDC2 is the main producer of interleukin (IL)-23, allowing them to induce Th1 and Th17 response in $CD4^+$ memory T-cells[73]. Intestinal cDC2 express high levels of integrin αvβ8, which induces FOXP3 in $CD4^+$ T-cells, making them effective in promoting Treg differentiation [78].

Plasmacytoid DCs (pDCs) are specialized in type I IFN production [79]. The majority of these cells have a well-developed endoplasmic reticulum befitting their function; a minor subpopulation is adapted to $CD4^+$ T-cell

activation instead [80]. In contrast, their ability to induce $CD8^+$ proliferation is inferior to that of cDCs, as they express low levels of MHC I and CD86 [71,81]. Examples of human pDC markers are CD123 and BDCA2 [80]. Notably, in mice, $Flt3^+$ lymphoid progenitors are capable of developing into DCs, being more efficient at generating pDCs than cDCs [82].

Additionally, there is a distinct subset that does not require Flt3L to develop, for it differentiates from monocytes - known as monocyte-derived DCs (mo-DCs) [83]. Many of their abilities are shared with cDC2, from $CD8^+$ T-cell activation to Th17 induction [81,84]. In murine models, Th1, Th2, and Tfh induction by mo-DC was observed as well [85–87]. Compared to their common DC progenitor-derived counterparts, mo-DCs have a higher lysosomal degradation capacity and retain a part of monocytes' high phagocytic activity until maturation [60,88]. This has been suggested to help them process Ags of less soluble, even non-proteinaceous nature (typically of microbial origin). Initially, it was thought that monocytes differentiate into DC only under inflammatory conditions. However, there are steady-state DC populations in humans and mice that are suspected to have a monocytic origin [89,90].

Recently, a subset named DC3 was identified, sharing characteristics with both cDC2 and monocytes [91]; they possess a high number of co-stimulatory molecules necessary for the induction of tissue-resident memory T-cells [91,92]. Indeed, the variety present in DC activities is unprecedented – under certain circumstances, some subsets even display cytotoxic capabilities [23,93].

Dendritic cell-based immunotherapy

Anti-cancer strategies using DCs aim to maximize their immunostimulatory capacity by increasing their number, promoting their activation by specific Ags or enhancing Ag-presentation capacity.

In vivo expansion of DCs can be achieved by delivering Flt3L or granulocyte/macrophage colony stimulating factor (GM-CSF), preferably a combination of both [94]. Flt3L administration increases the DC (and overall leukocyte) number in peripheral blood, though that alone is not enough to induce an anti-tumour response [95]. As most DCs recruited by Flt3L are immature, it is mostly used to supplement DC-activating therapies [96]. In contrast, direct administration of GM-CSF showed some anti-tumour activity in cancer patients with recurrent disease [97]. In recent years, besides being a component of other immunotherapies, it is also applied in the form of genetically modified GM-CSF-expressing cancer cells (GVAX) [98].

Administration of adjuvants helps to stimulate immune cell activity and cytokine secretion. To support DC response during an ongoing therapy, adjuvants target the corresponding signalling pathways. For example, due to CD40's role in DC maturation, anti-CD40 antibodies are used to activate the receptor [99]. TLR agonists can reinforce activation signals by mimicking pathogen-associated molecular patterns. Polyinosinic:polycytidylic acid (poly-(I:C)) is a double-stranded RNA molecule often used to trigger TLR3 – and, subsequently, IFN production along with a $CD8^+$ response [100]. Since early studies found its activity is often hindered by cellular RNAses, a derivate stabilized with poly-L-lysine and carboxymethylcellulose, poly-ICLC, was developed [101]. Synthetic oligonucleotides can also be used to activate TLR9, usually consisting of deoxynucleotides and a CpG cap [102]. Besides eliciting CTL and Th response, TLR9 ligands have the added

benefit of simultaneously activating B cells. Likewise, TLR7 and TLR8 ligands such as resiquimod (R848) and imiquimod (applied topically) are commonly employed to stimulate APCs [102,103].
Pro-inflammatory cytokines may also be utilized as immunoadjuvants; some, e.g. IFN-γ, can be combined with other adjuvants for a synergistic effect [103].

Conversely, some approaches seek to treat cancer by inhibiting DCs' immunosuppressory functions. Indoleamine 2,3-dioxygenase (IDO) is one of the major signalling molecules responsible for tolerogenic activities in pDCs [104]. Its inhibitors have recently shown promising results (41% objective response rate) in children with recurrent brain tumours or newly diagnosed diffuse intrinsic pontine glioma, though to overcome resistance mechanisms, it might be necessary to inhibit additional targets [105]. An earlier terminated study indicated that tumour cells could escape this type of treatment by utilizing an analogous enzyme [106]. Signal Transducer and Activator of Transcription proteins 3 (STAT3) relays the signal from multiple cytokines including IL-6, IL-10, and its relatives or IFN-γ [100,107]. It plays an important role in mucosal tolerance, suppressing excessive pro-inflammatory activity in DCs and controlling their proliferation in conjunction with Flt3. Nonetheless, these functions can be detrimental to anti-tumour immunity, for example by inhibiting cDC1 interferon response [100]. The most common form of STAT3 inhibition is danvatirsen, an antisense oligonucleotide targeting STAT3 RNA, tested in combination with other cancer treatments [108].

Targeted delivery of TAAs/TSAs (tumour-specific Ags) to DCs has long been a hot topic in anti-cancer therapy research. Although vaccinations with synthetic peptides have been tested in clinical trials, the resulting Ag cross-presentation may be suboptimal due to the low frequency of DCs in target tissues [109]. To ensure that the molecules reach their intended destination, the delivery systems aim at DC- or APC-specific receptors. Antibodies serve this purpose well, as it is possible to engineer them to recognize virtually any protein. Typical targets are DEC-205 (CD205), an endocytosis-mediating receptor, and DC-SIGN (intercellular adhesion molecule 3– grabbing nonintegrin), both from the C-type lectin family [110,111]. In humans, the former is found on multiple leukocyte types, while the latter is expressed only on professional APCs (earlier studies indicated it may be restricted to $CD14^+$ cells and mo-DCs [112]). Additional targets include mannose receptor, XCR1 or Clec9A [76,109,110].
A study in mice showed that for a long-lasting response to injected Ags, it is ideal to deliver them along with maturation stimuli [99]. This can be streamlined by delivering the cargo in nanoparticles, liposomes or virus-like particles [102,113,114]. A system using Ag-loaded flagellate bacteria successfully induced antitumour immunity in mice [115]. By increasing the area of Ag distribution, the bacteria were able to more extensively activate intradermal DCs. Given that the composition of skin tissue limits the diffusion of injected Ags, this could strengthen immune responses to this type of vaccine. Instead of delivering whole peptides, immunization can be achieved by transfecting DCs with TAA- or adjuvant-encoding genetic information. For example, engineered lentiviral vectors increased pre-existing immune responses in patients with various cancer types [116]. Plasmid DNA vaccines have also been tested, though Rastogi *et al.* and McNeel *et al.* recently suggested that the primary APCs in these cases are actually B-cells [117]. Rather than actively presenting, DCs play a supportive but necessary role, licensing the B-cells to activate $CD8^+$ lymphocytes.

Ex vivo prepared DCs have shown promising results in both murine models and humans. The first FDA-approved DC vaccine, PROVENGE® (sipuleucel-T), is commercially available for the treatment of

castration-resistant prostate cancer – and many others are currently tested in clinical trials [118]. While the response rates vary, further optimization could develop them into a standard therapy for many cancer types.

Preparation of dendritic cell vaccines

The first step in preparing DC-based vaccines is acquiring the cells, preferably from the patient themselves. In several studies, vaccines based on DCs from allogeneic healthy donors were tested as well [119,120]. Given that DCs are distributed mostly in the tissues, obtaining them would ordinarily require a biopsy. Methods for isolation of human DCs from skin grafts have been developed, for example, though their efficiency is limited by the difficulty of purification [121]. The relative rarity of DCs is a further complication. Therefore, using leukocytes extracted from blood is preferred.
Leukapheresis allows for obtaining large amounts of cells while being less invasive and labour intensive than a biopsy. However, the frequency of DCs in the blood is rather low [122]. Hence, DCs used in cancer vaccines are usually mo-DCs induced from the blood-circulating $CD14^+$ monocytes or $CD34^+$ hematopoietic stem/progenitor cell (HPSC)-derived DCs [123,124]. cDCs are particularly rare, though it is possible to generate them from $CD34^+$ HPSCs [125–127]. Recently, it was shown that cDC1 may be obtained by reprogramming human embryonic fibroblasts [128]. Plasmacytoid DCs may be derived from $CD34^+$ HPSCs as well [129].

Monocytes are enriched from peripheral blood mononuclear cell (PBMC) fraction of isolated leukocytes. A common method for purifying PBMCs is density gradient centrifugation [122]. Monocytes are then obtained by adhesion to plastic, immunomagnetic separation using Ab-coated microbeads, elutriation, or cold-aggregation (not recommended due to low yields) [130,131,123]. Next, the differentiation into mo-DCs is induced by adding GM-CSF and IL-4 to the cell culture medium [132]. A successful DC generation is marked by the loss of CD14 expression [133]. Substituting IL-4 with IL-15 produces Langerhans cell-like phenotype [134].

To obtain $CD34^+$ HPSCs, it is necessary to stimulate their mobilization from the bone marrow. Afterwards, they are easily accessible from the blood stream. The agent used for this purpose is usually granulocyte colony-stimulating factor (G-CSF), which was found to be more effective than GM-CSF or previously used chemotherapy regimens [135]. Human embryonic stem cells can be differentiated into HPSCs as well [136]. Density gradient centrifugation is used for HPSC purification, along with other methods such as magnetic-activated cell sorting or $CD34^+$ microbeads [137,138]. It is possible to expand the acquired cell culture by incubation with thrombopoietin, Flt3-ligand (Flt3L) and stem-cell factor, in addition to other cytokines [139]. For the generation of DCs, the combination of GM-CSF and tumour necrosis factor (TNF)-α is a common choice [133]. TNF-α may be substituted by other cytokines, e.g. IL-3 or IL-13 [124,140]. DC differentiation using GM-CSF (also known as colony-stimulating factor 2, CSF2) and IL-4 in the presence of Flt3L was also described [139].

Comparing the cells generated from both precursors, the $CD14^+$-derived DCs were found to express higher levels of CD86 and HLA-DR, though there was no difference in function between the two populations [133].
Alternative strategies aim to induce DC differentiation by genetically reprogramming their precursors. As an example, lentiviral vectors were used to successfully produce mo-DCs from $CD14^+$ monocytes *in vitro* [141].

In order to induce an anti-tumour response, the newly generated DCs need to be pulsed (loaded) with the targeted tumour Ags and matured. For the process, two factors are necessary: the Ag and an adjuvant to promote the maturation of the cells. It is vital to ensure that DCs to be used in the vaccine have matured properly, since immature DCs have the ability to induce tolerance [142].
This function is usually attained by cytokines such as IFN-γ; from the TNF family, TNF-α and CD40L in a soluble form may be used [143]. Curiously, despite being a key factor in DC differentiation, IL-4 was found to impair their functions during a TNF-α-induced maturation [144]. Occasionally, PRR ligands are also used – e.g. lipopolysaccharide [145]. While a single adjuvant is technically sufficient to induce DC maturation, most studies now favour using a mixture of cytokines, a so-called "cocktail". The classic combination developed by Jonuleit *et al.* includes TNF-α, IL-6, IL-1β and prostaglandin E2 [146]. (The study also sets the standard for DC maturation in serum-free conditions.)
Various specialized cocktails have been developed since, such as the "α-type-1-polarizing" mix consisting of IL-1β, TNF-α, IFN-γ, IFN-α, and poly-(I:C) [147]. DCs stimulated with this cocktail were found to have higher expression levels of Th1-associated chemokines along with IL-12, though their migratory capacity was lower than that of DCs matured with the "classic" cocktail [148]. Thus, the functional characteristics of the resulting mature DCs may be adjusted by choosing a particular combination of cocktail "ingredients". The choice can also depend on the DC subset needed.

Antigen loading is provided by incubating the DCs with a cell lysate prepared from a tumour tissue harvested from the patient or using established cancer cell lines.. Although many opt for the freeze-thaw method, it was shown that using this method may interfere with DC functions [149]. This can be partially avoided by stressing the tumour cells using heat or radiation before lysis [149,150]. Alternative methods of lysate preparation including oxidation with hypochlorous acid (HOCl) or sonication have been tested [151,152]. Utilizing electroporation to boost the effectiveness of Ag uptake results in more potent DCs [153].
Co-culturing DCs with killed cancer cells can also lead to efficient T cell stimulation [154]. It was suggested that live injured cells, rather than dead cells, promote certain anti-cancer responses and can be used as an effective adjuvant [155].

Tumour cells also help DCs acquire Ags by secreting extracellular vesicles [156]. These can be obtained by centrifugation of the cancer cell supernatant. Exosomes (30-150 nm) induced $CD8^+$ and $CD4^+$ anti-tumour responses in mice, but tolerogenic effects were also observed [157]. The reason is likely the influence of stress – exosomes produced after γ-irradiation had stronger immunogenic properties compared to exosomes derived from non-irradiated cells, which induced a semi-mature DC phenotype. Microparticles are larger (100-1000 nm) and are more easily taken up by DCs than apoptotic cells [156]. Besides successfully stimulating DCs, they were shown to upregulate co-stimulatory molecule and cytokine expression [156].

Methods used for Ag-loading *in vivo*, such as Ab-linked TAAs, nanoparticles or liposomes, may also be used *in vitro*, though simply utilizing dead tumour cells is less technologically and financially demanding. Still, nucleic acid-based delivery of TAAs is intensively studied. In clinical trials, electroporation is commonly used to deliver TAA mRNA into the cells [158,159]. Using this technique, both $CD8^+$ and $CD4^+$ T cell responses can be generated. It is possible to further promote $CD4^+$ response by attaching an MHC-II compartment sorting signal to the peptide sequence [160]. Other delivery methods have been explored, such as nanoparticles carrying self-amplifying RNA, nanochannel electroinjection or sonoporation with Ag-loaded

microbubbles [161–163]. Lipofection was also used but proved to be less effective than electroporation [164].
TriMix-DCs are DCs pulsed with a combination of constitutively active TLR4, CD40L, and CD70 mRNA in addition to TAA mRNA [165]. Adding the mixture greatly improves their immunostimulatory capacity.
A TriMix-DC-based vaccine was able to induce long-lasting immune responses (> 24 months) in melanoma patients, with an objective response rate of 27%.
Viral vectors (usually lentivirus or adenovirus-derived) carrying Ag-encoding genetic constructs were successfully tested as well [166,167]. Alternatively, DCs transfected with RNA obtained from tumour cells were able to stimulate CTL activity, however autoimmune response was also observed [168]. Plasmid DNA was used in experiments in mice, though DCs activated this way performed poorly compared to peptide-pulsed DCs or direct DNA injection [169].
A potential disadvantage of TAA-based methods is the lack of nucleic acid fragments that provide additional danger signals.

A rare but interesting phenomenon occurring in cancer cells is their spontaneous fusion with other cancer cells [170]. It is not limited to one cell type - fusions with other tumour-associated cells, including leukocytes, are also possible [171]. The resulting hybrid cells tend to have properties of both "parent" cells. While this can be very troublesome in cases where tumour cells acquire migratory ability, it can also be exploited for therapeutic purposes. Fusing a dendritic cell with a cancer cell gives the DC access to the full repertoire of tumour Ags, including those not yet identified [172]. Naturally, the possibility of generating tumorigenic cells raises safety concerns. In a clinical trial in melanoma patients, a DC/cancer cell hybrid vaccine showed no severe adverse effects and its effectiveness was comparable to vaccines utilizing lysates or dead tumour cells for DC pulsation [173]. However, analysis of murine DC-melanoma hybrids showed changes in cytokine secretion – a downregulation of IL-12 and IL-15 in favor of IL-4, along with decreased expression of chemokines and co-stimulatory molecules [172]. To counter this effect, additional administration of signalling modulators is necessary. The typical approaches to induce cell fusion are incubation with polyethylene glycol or electroporation [174].
Okeyo *et al.* have developed a single-cell surgery technique that allows for the generation of tumour nuclei-free DC/cancer cell hybrids [175]. Despite being a great safety improvement, it is not applicable for large-scale hybrid cell production yet.

Finally, the administration route also has an impact on the following immune response [176]. There is evidence that DC location influences the expression of homing receptors on CD8+ T cells they activate; whether this holds true for *ex vivo*-activated DCs is unknown, but possible [177,178]. The only currently FDA-approved vaccine, sipuleucel-T, is administered intravenously (i.v.) [118]. Intradermal (i.d.) and intralymphatic routes were also tested in clinical trials [179]. In comparison to i.v. application, Th1 response was observed more frequently, but lower titers of TAA-specific Abs were produced [176]. DC vaccines may also be administered intratumorally, though this is less common [180].

After the application, DCs often need to be additionally stimulated in order to effectively migrate. Failure to enter the lymph nodes might be a result of unfavourable conditions in the environment [181]. The tumour can also inhibit DC migration by inhibiting CCR7 expression [182]. Previously, this was solved by pre-treating the injection site with pro-inflammatory cytokines [181]. Reducing the number of injected DCs may lead to an improved migration rate. There is no consensus on the ideal number of DCs to use in a vaccine;

the PROVENGE® vaccine uses 50 million at minimum [118]. Notably, in a clinical trial led by Bedrosian *et al.*, patients who received 5 mil. DCs had a slightly higher frequency of adverse effects, but of a lower grade than patients who received 50 mil. DCs [183].

A way to circumvent migration problems is by administering DCs directly into the lymph nodes. In a comparative study, the intranodal (i.n.) DC injection induced a weaker response than i.d. injected DCs [184]. Possible reasons include damage to the LN during the administration or a higher proportion of immature DCs present in the LN (as DCs immigrating from other tissues usually mature en route) [185]. It was also suggested that injected DCs are carried off to more distant LNs by the lymph flow. In contrast, an earlier study reported superior ability of i.n. injected DCs to induce delayed-type sensitivity response and prime reactivity towards new peptides [183].

Ultimately, there is no administration route that is clearly superior to the others, and the tumour characteristics in each case should be considered when choosing the routes.

Dendritic cell vaccines in prostate cancer therapy

The greatest success in DC cancer vaccine research so far is the development of sipuleucel-T, commercially available under the name of PROVENGE®. Approved for the treatment of asymptomatic or minimally symptomatic metastatic castration-resistant PCa, it remains the only DC-based cancer vaccine endorsed by the FDA [186]. Besides DCs, each PROVENGE® dose contains other PBMCs obtained from the patient; the level of immune cell activation is assessed by measuring CD54 expression [118]. Activation is achieved by incubation with prostatic acid phosphatase (PAP):GM-CSF fusion protein. The vaccine is administered intravenously.

The safety and efficacy were tested in four placebo-controlled clinical trials (NCT00005947, NCT01133704, NCT00065442, NCT00779402) followed by a single-arm, long-term study (PROCEED, NCT01306890) [187]. In general, longer overall survival (OS) time was observed in patients with lower prostate-specific antigen (PSA) levels. Other factors impacting OS included, for instance, ethnicity (this is particularly notable as most clinical trials, enlisting predominantly white patients, do not take the factor into account), age, and prior administration of androgen-targeting therapy or chemotherapy. Patients treated with sipuleucel-T had a median OS improvement of 4.1 months [187].

The first clinical trials testing DC vaccines in PCa patients date back to 1999. Alongside NCT00005947, the pilot study for sipuleucel-T [188], Duke University's NCT00004211 was launched that year - one of the first trials to be completed [189]. Mo-DCs were activated by co-incubation with PSA-encoding RNA; no additional maturation agents were used. The vaccination regimen consisted of a low, medium, or high DC dose administered intravenously and a low dose administered intradermally. The results were encouraging – of the nine patients analyzed, all had a measurable increase in the frequency of PSA-reactive IFN-γ-producing cells [190]. Moreover, no adverse events (AE) above grade 1 were observed in any of the (thirteen) patients participating. Although the the follow-up showed the vaccine effects to be only temporary, the study demonstrated that DC vaccines are safe and have potential for further development.

A study especially notable for its large scale is VIABLE (NCT02111577). Evaluating 1182 patients from across Europe and the United States, the trial sought to assess the efficacy and safety of DCVAC, a DC

vaccine developed by SOTIO Biotech company, in combination with chemotherapy. No statistically significant differences between the experimental and control group treated were observed, whether in OS, radiological progression-free survival (PFS) or time to PSA progression. The vaccine was administered subcutaneously. Patients previously treated with abiraterone or enzalutamide had shorter median OS, but not significantly. Similarly, there was a positive trend in median OS as the number of doses increased; however, there was little difference between the groups.

Previously, multiple studies were conducted at the University Hospital Motol in order to evaluate DCVAC safety. The outcome of an immunotherapy-only trial (EudraCT 2009-017259-91) was favorable – after vaccination, PSA doubling time (PSADT) exceeded 15 months in 17 out of 25 participants [191]. A shorter increase was observed in 5 patients, and 3 had stable or decreased PSADT. The difference in median PSADT was significant, as it increased from 5.67 months pre-vaccination to 18.85 months post-vaccination. Twelve patients who underwent a second vaccination cycle maintained stable PSADT during both cycles. Three received a subsequent third cycle.

Immune response against PSA, melanoma-associated Ag (MAGE)-1 and MAGE-3 was evaluated. There was a significant increase in PSA-specific T-cells compared to healthy donors. The frequency also increased between the vaccination cycles and at all tested time points. In comparison to healthy donors, MAGE-1- and MAGE-3-specific T-cells were also increased, though an increase during the study was observed only in MAGE-1-specific T-cells, limited to the first time point. Patients receiving additional vaccination cycles had stable frequencies of monitored cell types. A significant decrease in regulatory T-cell (Treg) frequency was observed after the second cycle.

No treatment-related AE higher than grade 2 were recorded.

Another trial tested the safety of DCVAC combined with chemotherapy (EudraCT 2009-017295-24) [192]. Docetaxel therapy was initiated following the first two DC doses. The vaccination resumed after intolerance or toxicity was observed. DCs were pulsed with UV-irradiated LNCaP prostate cancer cell line and matured with poly-(I:C), a TLR-3 ligand. The administration route was subcutaneous, with imiquimod applied at the injection site pre- and post-vaccination. Although serious AE were observed, none of them were related to the vaccination. Other related AE were of grades 1 and 2, suggesting acceptable tolerability. Initially, PSA response (50 ≤ % decrease maintained for a minimum of 6 weeks) was observed in 39.1 % of patients. Six months after initiation of the treatment, 34.8 % demonstrated a complete and 21.7 % a partial (25-50 % decrease) PSA response. Compared to healthy donors, 47.8 % of the patients had a significant increase in PSA-specific, 26.1 % in MAGE-1-specific, and 13.0 % in MAGE-3-specific T cells. In comparison to baseline levels, a statistically significant increase was observed only in PSA-specific T cells. Overall, there was an increase in the activated CD3+/HLA-DR+ cell and CD8+ T cell frequency. On the contrary, a significant decrease in Treg frequency was observed.

The relationship of multiple biological parameters with the clinical outcome was also assessed. Haemoglobin was found to be correlated with a good prognosis in contrast to C-reactive protein, associated with a poor prognosis. The median OS was 19 months, significantly higher than predicted.

The most recent clinical trial testing DCVAC in PCa patients combined the vaccine with an oncolytic adenovirus [193]. However, the trial was terminated, and results were not published.

Kongsted *et al.* also investigated the capability of a DC vaccine to induce an immune response in patients treated with docetaxel [194]. Mo-DCs were transfected by mRNA encoding PSA, PAP, survivin and human telomerase reverse transcriptase (hTERT). The vaccine was administered intradermally.
Between the experimental (DCs + chemotherapy) and control (chemotherapy only) group, the difference in PSA response rates and median PFS was not statistically significant. In patients receiving DC vaccine, a significant decrease in myeloid-derived suppressor cells was observed. Of the 11 patients with measurable disease, 4 achieved a partial response (confirmed for 1 patient in each group), 6 had stable disease, and 1 patient had progressive disease. TAA-specific T-cell responses were monitored *in vitro* and were observed in 9 of 18 evaluated patients, though only a minority had lasting responses.

The use of established cancer cell lines for DC loading was tested in multiple Rockefeller University studies, two of which (NCT00289341 and NCT00345293) were conducted in PCa patients [195]. In the initial placebo-controlled trial, the LNCaP cancer cell line was used for pulsation. The vaccines were administered subcutaneously. Delayed-type hypersensitivity (DTH) reaction to LNCaP Ags was observed in 67 % of the patients [196]. There was also a positive correlation between a DTH response, *in vitro* T-cell proliferation in response to apoptotic LNCaP cells and a statistically significant decrease in the PSA slope. Unexpectedly, there was an *in vitro* T-cell response to apoptotic cells of the PC3 tumour cell line as well, suggesting a high immunogenicity of the cell line. This led to subsequent testing of a vaccine based on PC3-loaded DCs, however, the T-cell proliferation responses were strikingly lower than expected, and data on clinical outcome was not collected [195,197].

A trial in the Radboud University Medical Center compared clinical outcomes of cDC, pDC, and combined (cDC2 + pDC) vaccines in chemotherapy-naïve patients [198]. DCs were isolated from apheresis products, expanded, and loaded with TAA peptides (NY-ESO-1, MAGE-C2, MUC1). Additional stimulation was achieved by co-culturing with protamine and mRNA. The vaccine was administered intranodally in a tumour-free LN.
Overall, no significant differences between the groups were found. Keyhole limpet hemocyanin (KLH) was used as a control Ag (hapten) in vaccines containing cDCs. A significant T-cell response was observed in both groups, though more notably in the cDC group (5/7 patients), suggesting that the cDC-only vaccine may be more effective than a mixed one. After 6 months, a partial response was observed in 1 patient (5 %), stable disease in 12 (57%), and disease progression in 8 patients (38 %). Only 2 of 21 patients showed a decline in the PSA level. The median radiological PFS for all participants was 9.5 months. Longer radiological PFS correlated with the presence of TAA-specific T-cells, being significantly higher in patients with both IFN-γ-producing and skin-test infiltrating T-cells. Interestingly, the presence of TAA-specific T-cells was detected more often in skin biopsies than in peripheral blood. In 5 patients (24 %), specific T-cells against all three TAAs were found.

More recently, Wood *et al.* reported on a vaccine trial targeting TCR alternate reading frame protein (TARP), an Ag often found on PCa and breast cancer cells [199]. One cohort received an emulsion of TARP peptides (a wild type plus an epitope-enhanced one) and GM-CSF, while the other received mo-DCs pulsed individually with each peptide, along with KLH for additional stimulation. DCs were administered intradermally and the peptides subcutaneously. The response was evaluated by calculating changes in Slope Log PSA, a decrease in the value reflecting lengthened PSA doubling time. At week 36, 26 of 40

patients received a booster dose of the respective vaccine, which showed no impact on the effect of the treatment. No statistically significant differences between the cohorts in IFN-γ reactivity or post-vaccination changes in Slope Log PSA were found.
Compared to baseline slopes, a significant slope decrease in the DC vaccine group only was observed. When pooled together, a decrease was achieved in 71.8% of the patients evaluated at week 24 and 74.2% of those evaluated at week 48, with a significant response observed at week 72 as well.
In 15 % of the patients, there was a decline in absolute PSA value at week 24. Notably, there was a significant decrease in estimated PSA growth rate, as the post-treatment median (*g* = 0,0042/day) was 50 % lower than pre-treatment (*g* = 0.0021/day). Furthermore, there was an increase of specific T cells recognizing not only both peptides used in the vaccine, but an additional wild type TARP peptide as well.
A study testing an improved version of the vaccine on the same participants (NCT02362464) has since finished, and data analysis is in progress [200].

Investigators at Haukeland University Hospital tested a unique approach combining DC immunotherapy with tumour cryoablation [201]. After the disruption of tumour tissue by freezing, immature mo-DCs were administered intratumorally, exposing them to the whole range of TAAs. Three dose levels were tested in the first part of the study, the highest reaching 2 x 10^8 DCs. Additionally, the patients were given cyclophosphamide to limit Treg activity. In the second part of the study, the tolerability of adding ICIs to the regimen was evaluated. The combination proved to be safe, with no evident correlation between the occurrences or severity of AE and ICI addition. Most of the reported AE were grade 1 or 2, suggesting only moderate toxicity. The sequencing of TCRs in blood samples detected a median of 35.5 novel and (> fivefold) expanded clonotypes two weeks after the treatment. Four weeks later, the median number rose to 70.5. Notably, the expanded TCR clonotypes demonstrated higher longevity than the novel ones. In all patients with circulating tumour cells (CTCs), a transient decrease or complete disappearance was observed. The complete disappearance of CTCs also correlated with longer PFS. OS was higher in CTC-free patients and patients with lower tissue ratios of $CD4^+/CD3^+$ cells.
The median OS of all participants was 40.7 months and median PFS was 10.5 months. Long-term clinical benefit (stabilization of the disease, partial or complete response observed 46 weeks after treatment) was achieved in 33 % of patients.

An Oslo University clinical trial (NCT02326805) is currently marked as active, despite preliminary results being published [202] (It is likely that the follow-up is still ongoing, as the vaccination phase is limited to three years.). Autologous tumour mRNA, as well as survivin and hTERT mRNA, was used to load the mo-DCs. There was a significant correlation between baseline and vaccination immune responses. The autologous immune response was mostly directed towards PAP, PSMA1 and STEAP1; high CD8+ responses were often aimed at hTERT. Eleven of twenty patients achieved biochemical remission, while nine developed biochemical relapse. Of the latter, all achieved stable disease. Notably, intraductal carcinoma was significantly associated with negative outcomes.
The study was preceded by a phase I/II trial using DCs pulsed with mRNA from several PCa cell lines (DU145, LNCaP, PC3) [203]. Six out of twenty patients demonstrated a partial PSA response, while eleven maintained a stable disease. A significant increase in the mean frequency of Ag-specific T cells was observed.

After promising results from initial studies, the safety of a second-generation APC vaccine administered along with AP1903, an activating agent, was evaluated [204]. For pulsation, prostate-specific membrane antigen and iMyD88/CD40-enconding adenoviral vector were used. The results were not made public, and there is no indication of further development.

Lastly, there are multiple clinical trials recruiting patients at the time of this writing. A novel vaccine by Shanghai Humantech Biotechnology is to be tested in patients with chemotherapy and androgen deprivation therapy failure [205]. Other studies explore variants of sipuleucel-T immunotherapy, whether in combination with hormonal agents or an extended course of treatment [206,207].

Dendritic cell vaccines in ovarian cancer therapy

Although studies testing DC immunotherapy in OCa patients are less abundant than those conducted in PCa patients, a fair number has been completed and reported on thus far.

One of the earliest trials compared peptide and DC vaccines in patients with p53-overexpressing tumours. No significant difference in median OS nor PFS was observed between the groups [208]. Wild type p53:264-272 peptide was administered either subcutaneously mixed with GM-CSF or used to pulse DCs administered i.v.. Overall, there was a significant increase in activated CD4+ T cells, including Tregs. This was associated with the administration of IL-2, which was given to all patients. Notably, 85 % of AE occurred during the IL-2 cycles. At the end of follow-up, 2 out of 13 patients (15.4 %) in the peptide group and 2 out of 7 patients (28.6 %) in the DC group had no evidence of disease; the remaining patients had disease recurrence.

The safety and efficacy of using a DC vaccine as a maintenance therapy for patients with a low tumour burden were tested in an international trial [209]. During the preparation of the vaccine, developed by Prima BioMed, a mucin-1:glutathione-S-transferase fusion protein coupled to oxidized mannan was used to activate the cells. Several grade 3 and 4 AE were observed, some possibly related to the vaccination. There was no statistically significant difference in OS or PFS between the experimental and the control group. However, when comparing patients with first and second clinical remission, a difference in median PFS was found between vaccinated and unvaccinated participants from the latter subgroup (13 and 5 months, respectively).

A HER2-targeting vaccine using an adenoviral vector for DC pulsation was tested in patients with various cancer types, including OCa [210]. Five patients with ovarian cancer enrolled in the first (dose escalation) part of the study. Progressive disease was observed in three of them, given low or medium DC dose. Of the OCa patients given a high dose of the vaccine (20×10^6 DCs), one had stable disease for 48 weeks and one had a complete response. A maximum of 24.8 % regression of target lesions was observed in the patient with stable disease, though the response was halted by the progression of a non-target lesion. Likewise, although all the target lesions in the patient with a complete response regressed fully and polyclonal Abs against HER2 were observed, the cancer recurred with $HER2^-$ tumours.

Several of the SOTIO Biotech company trials tested a combination of carboplatin and DCVAC with additional chemotherapies. The OCa-targeting versions of the vaccine consist of DCs loaded with epithelial

OCa cell lines and are administered subcutaneously [211,212]. In NCT02107950, thirty-two patients received the vaccine along with carboplatin and gemcitabine; the control group received chemotherapy only [212]. Similar frequencies of treatment-related AE were reported for both groups. Six patients in the experimental group experienced AE related to the vaccine. During initial analysis, no significant difference in median OS, PFS, or biological progression-free interval was observed. However, later analyses showed a significant increase in median OS in the experimental group versus the control group (35.5 and 22.1 months, respectively).

In order to determine whether the therapy schedule has an impact on its effectiveness, a three-arm study was conducted [211]. All patients received carboplatin and paclitaxel chemotherapy. While the first (parallel) experimental group was given DCVAC concomitantly with chemotherapy, the second (sequential) started vaccination after chemotherapy was completed. Treatment-related AE of grade 3 or higher were observed in over 55% of the patients, and two patients in each experimental group experienced AE related to the vaccine. One patient had to discontinue immunotherapy due to drug hypersensitivity. The data for the final survival analysis are still being collected, though preliminary results indicate significant prolongation of PFS associated with sequential vaccination. Likewise, OS estimations suggest a positive effect of DC immunotherapy, more notably for the sequential group. As a result, the benefits of an extended vaccination regimen are being assessed in the second part of the study.

To target APCs directly in the patients' bodies, Tan *et al.* engineered an adenoviral vector encoding a mucin-1:CD40L fusion protein [213]. The virus-based vaccine was tested on multiple adenocarcinoma types, including the ovarian. A significant increase in MUC1-specific IFN-γ response was noted, as well as a general increase in IFN-γ and granzyme B-expressing CTLs, along with $CD14^+$ monocytes. No response was observed in any of the 17 evaluable patients; of the 7 patients with OCa, 2 had stable and 4 had progressive disease.

A recent study at the Denvax Clinic in India showed a notable increase in survival compared to predictions (estimated based on blood CA125 levels) [214]. After vaccination with DCs loaded with autologous tumour lysate, the 95 % confidence interval for OS was 22.6 to 33.6 months. The 95 % confidence interval for expected survival was 7.23 to 8.39 months. No AE related to the treatment were observed. Complete or partial response was achieved in 54 % of the patients, 28 % had stable disease and 16 % showed disease progression. Six patients did not finish the trial.

To reduce the number of circulating Tregs and induce an IFN-γ anti-tumour response, investigators at Loyola University employed α-DC-1 cells – DCs matured with an α-type 1-polarizing cocktail, known to produce higher amounts of IL-12p70 [147,215]. IFN-γ secretion was indeed present in 25 % of the patients. A Treg decrease equal to or higher than 20 % was observed in 57 % of the evaluable patients.

Survivin and hTERT are common targets for DC vaccines, including Procure – tested in a trial in Austria and Hungary [216]. A double-loading method was used to activate the DCs, utilizing both mRNA and peptides. The investigators reported high frequency of strong immune responses and a positive trend in PFS.

Corr *et al.* tested a vaccine using DCs loaded with autologous cancer stem cell lysate [217]. Though the study ended prematurely during the SARS-CoV-2 pandemic, results were published: there was no

difference either in PFS or in OS between the experimental and the control group. A significant increase in IFN-γ response was observed after DC vaccination, though.

The goal of NCT01132014 was to determine the optimal immunotherapy combination [218]. A DC vaccine was administered either alone, alongside bevacizumab (a humanized antibody targeting vascular endothelial growth factor A, VEGF-A), or combined with both bevacizumab and cyclophosphamide. The final combination seems to be the most effective, as there was a significant increase in OS rate compared to patients not treated with cyclophosphamide. Between the bevacizumab-treated cohorts, T cell response to tumour Ags was more frequently observed in patients who received all three types of therapy. One patient from the vaccine-only cohort achieved a five-year cancer remission, while partial response was seen in two patients, each from one of the the remaining cohorts. Overall, 13 of 25 patients achieved stable disease and 10 patients were non-responders.

Multiple trials are listed as active at the time of this writing. A Mayo Clinic study saw a significant increase in folate receptor α (FRα)-specific Abs and IFN-γ+ T cells [219]. Five FRα epitopes were used to activate Th17-inducing mo-DCs, as previous studies indicated that IL-17 response might reduce Treg-mediated suppression of anti-tumour immunity. In most patients, a decrease in Treg levels was indeed observed, though not high enough to be statistically significant. However, patients with progressive disease had a significant Treg decline in recurrent tumours. Disease recurrence was associated with higher levels of Ab-dependent cellular cytotoxicity. The frequency of responses against the individual FRα epitopes varied: Ab responses ranged between 39 to 94 % of the patients, while IFN-γ+ T-cell response rates reached 89-100%. Notably, serum IgG specific for other TAAs such as hTERT and p53 were also increased post-vaccination. The median relapse-free survival was 12.1 months; the median OS has not been reached yet.

In 2022, the Lithuania-based biotechnology company Froceth started a trial assessing the safety of a DC vaccine combined with carboplatin and paclitaxel chemotherapy (EudraCT 2020-003166-39) [220]. No reports have been published so far.

An unique study in the Beth Israel Deaconess Medical Center (NCT00799110) evaluates the efficacy and safety of a DC/autologous tumour cell hybrid vaccine [221]. The product is administered subcutaneously along with GM-CSF injections at the vaccination site; in one of the two experimental groups, imiquimod will be applied topically as well. The trial is estimated to end in July 2024.

The currently recruiting trials offer a variety of approaches. The NEODOC study (NCT05773859) aims to test a cDC1-based vaccine [222]. Autologous tumour lysate and KLH are used to load the cells administered concomitantly with standard-of-care therapy. In the ALISON trial (NCT04739527), the ability of an allogeneic DC vaccine to prime a T cell response against multiple TAAs will be evaluated [119]. Patients with recurrent OCa are eligible for a Mayo Clinic trial evaluating the safety of pembrolizumab in combination with multi-epitope folate receptor α-loaded DCs (NCT05920798) [223]. Investigators at the University Hospital Antwerp plan to prime DCs to induce a response against a tumour Ag known as Wilms' Tumor-1, enhanced by IL-15 trans-presentation [224].

A clinical trial testing personalized DC vaccines (NCT05714306) is currently in planning [225]. Both arms of the study will be given DCs loaded with patient-specific peptides, identified either at screening or

later during vaccination. As it would seem from the number of currently ongoing studies, the development of anti-OCa DC vaccines is only just beginning.

Dendritic cells in combined immunotherapy

Given that the results of the treatment largely depend on function of effector cells such as lymphocytes, combining DC-based therapy with other immunotherapy types could significantly raise the success rates.

ICI have gained attention in recent years as a way to enhance immune cell function. It was suggested that blocking of PD-L1 on DCs may improve T cell reinvigoration, as the molecule is able to interact with CD80 (B7.1) on the same cell and therefore compete with CD28 [226]. Xenograft OCa models showed that CD28 co-stimulation and the presence of activated APCs are key to overcoming TIL exhaustion and resistance to PD-1 blockade [227]. Furthermore, it could be beneficial to block multiple targets simultaneously. Indeed, results from some *in vivo* studies indicate that using bispecific Abs or a combination of inhibitors can improve the effect of immune checkpoint blockade [228,229].

Adoptive cell transfer strategies could complement DC therapy well, as seen in a recent case of a patient with stage IV metastatic OCa. A significant reduction in cancer lesions was seen after administration of a combined treatment consisting of a DC vaccine, highly activated NK cells, and nivolumab [230].

Furthermore, utilizing vaccines based on immune system stimulators might have additional benefits. For example, using a DNA vaccine as a booster shot after peptide-loaded DC vaccination in mice led to a significant increase in central memory CD8+ T cells, prolonging the immune response [169].

Analysis of gene signatures in peripheral blood of patients treated with DCVAC indicated that PCa patients with higher expression of immunostimulatory (mostly NK and T cell-associated) genes have improved responses to DC immunotherapy [231]. In OCa patients, the benefit from vaccination was associated with low levels of immunosuppressive gene signature instead. However, OCa patients generally showed increased expression of immunosuppressive genes such as *FOXP3* and decreased levels of immunosuppressive genes compared to PCa patients. While these observations may not hold true for the overall population, they suggest that PCa patients may benefit from adjuvant therapy that focuses on stimulating the immune system; unlike OCa patients, for whom a therapy intended to reduce immunosuppression may be advantageous.

Since patients with cold tumours respond less avidly to immunotherapy, it would be desirable to induce a shift to the hot tumour phenotype. STAT3 is a transcription factor that plays a major role in cold-to-hot tumour conversion, as tumour cells exploit STAT3 signalling in the TME to suppress the process [232]. It was found that inhibiting the Janus kinase 2/STAT3 pathway in DCs has a significant positive impact on their activation and maturation [233]. Moreover, the results suggested this approach may improve DC differentiation in the presence of tumour-derived inhibiting factors. As STAT3 inhibition was shown to boost cDC1 function, it is a good candidate for adjunctive therapy.

However, investigators of the NCT02107937 trial noted that patients with lower tumour mutational burden and T-cell infiltration responded better to the vaccine. It was suggested that cold tumours could be more susceptible to DC immunotherapy as their immunosuppressive activity would be lower due to the low

presence of lymphocytes in the TME [234]. Furthermore, in hot tumours, the CTL-driven selection pressure may lead to higher heterogeneity and thus resistance to Ag-specific therapies [235]. In such cases, additional vaccinations aimed at different, perhaps even newly emergent Ags could lower the chances of a recurrence.

Stimulator of interferon genes (STING) is a part of a pro-inflammatory signalling pathway, activated by presence of DNA in the cytosol [236]. Its stimulation leads to production of type I IFNs and could be exploited to help overcome immunosuppressive environment. Murine PCa models showed that treatment with a STING agonist increased median survival [236]. The effect was even more pronounced in combination with modified IL-15. In OCa murine models and xenografts, administration of a STING agonist increased the number of MHC I^+ DCs in the tumour and promoted DC activation [237].

It must be noted that results from preclinical studies may be influenced by the choice of model organism, as DC phenotypes can vary slightly between different mice strains [169].

Many therapies focus either on immune or cancer cells while the rest of TME is left out of the equation. Since cell types such as tumour-associated macrophages or fibroblasts play an important role in cancer immunosuppression, mitigating their influence could have a positive impact on the treatment results.

Discussion and conclusion

Despite promising results of the sipuleucel-T development, trials of alternative vaccines in patients with prostate or ovarian cancer were less successful. Response rates rarely reached 50 %; the responses were often transient and usually led to stabilization of the disease rather than remission.

As DC vaccines rely on the patient's immune system, response in patients with weakened immunity would be decreased. It should be noted that in the vast majority of the trials, the age median of participants was 60 or higher. Although it was observed that elderly mo-DCs perform similarly to mo-DCs generated from young donors, the function of the remaining DC subsets and cell types is often impaired [50,238]. Other negative effects associated with aging include decrease in HPSC regeneration ability, haematopoiesis disruptions or decline in lymphocyte repertoire [238].
Moreover, the immunotherapy was often administered to patients after other forms of treatment had failed. Despite their benefits, it was observed that both chemotherapy and HT may also have immunosuppressive effects [239,240]. It is not clear how a DC vaccine would perform when administered as the first line of therapy.

In a recent study, mature mo-DCs generated from PBMC of PCa patients showed reduced expression of MHC II, CD80 and CD86 compared to mature mo-DCs generated from healthy donors [241]. Moreover, in the blood samples from PCa patients, less monocytes were present. This likely has a significant impact on the effectiveness of the therapy. The exact mechanism behind the effect remains to be uncovered. A possible solution to this problem would be to use monocytes from healthy allogeneic donors.

Another factor behind the mixed results was likely the heterogeneity of patients' genetic backgrounds. Particularly, HLA polymorphism would have an impact on the success of Ag presentation, as the MHC-binding affinity of a specific epitope can vary between alleles [242].

The administration route and dosage varied largely between the studies; neither seems to influence the effectiveness of the tested therapies. The impact of administration route on possible AE should be considered, though. A study in melanoma patents reported an increase in pro-inflammatory cytokine levels after i.v. administration of a DC vaccine [165]. There was also a low rate of cerebrovascular events reported after sipuleucel-T administration [118].

The effectiveness may be impacted by the Ag chosen as the therapeutic target. Often, the molecules overexpressed by tumour cells are widespread in other tissues. Such Ags are unfit for immunotherapy purposes as the immune system is already tolerized against them. Likewise, highly immunogenic Ags can be subject to selective pressure and may be lost in a case of immune escape. Hence, Tourdot *et al.* suggested the use of low affinity epitopes - peptides with decreased MHC-binding affinity or stability of peptide:MHC complexes [243]. While this results in reduced presentation (and therefore lower immunogenicity) of these peptides *in vivo,* it is possible to counteract this by modifying the amino acid sequence. The study showed that CTLs specific against one such epitope were able to recognize both modified and native variants of the peptide.

It was also suggested that overly purifying DCs might be detrimental to the vaccine efficacy; instead, mixing in other cell types could be beneficial. Some evidence in favour of this approach comes from the Rockefeller University clinical trials. The studies found that DCs isolated using PBMC adherence to plastic induced significantly higher lymphocyte proliferation responses than DCs selected by Ab-conjugated beads [195]. The cause of this was the presence of lymphocytes in the "Adherence" vaccine, some of them even activated. The authors proposed that injecting a mixed vaccine into a tumour might induce local inflammation, attracting additional lymphocytes and reducing the need for DC migration to LNs. Furthermore, interactions with T cells may lead to prolonged DC survival in vivo.

Ex vivo manipulation of cells bears the risk of contamination or damage during delivery. In some trials (not mentioned), factors stimulating DC proliferation, such as GM-CSF or Flt3L were administered along with TAAs [244]. However, no methods of directly inducing DC differentiation in the patients have been tested. It would perhaps be possible to utilize viral vectors or nanoparticles to directly target DC precursors and differentiate them into the desired DC subset. However, markers used to isolate these cells are found on many other cell types. Ensuring proper maturation and targeting Ag delivery would pose additional challenges, making this approach arguably less efficient.
Utilizing DCs in a novel way, a vaccine consisting of polymer nanoparticles coated with cell membrane obtained from tumour lysate-pulsed DCs was developed [245]. The so-called "mini DCs" showed remarkable anti-tumour effects in both *in vitro* and *in vivo* OCa models. The authors argue that mini DCs have multiple advantages over living cells, including longer shelf life and insusceptibility to an immunosuppressive environment.

In summary, DC-based immunotherapies have shown promising results in the past years. Nevertheless, there is a lot of potential for further development and improvements. Moving forward, it

seems that a move into personalized therapies that take the patient's genetic background and tumour characteristics into account will be necessary. If a suitable combination of treatments can be determined, the goal of achieving a complete biochemical remission would not be impossible.

References

1. World Health Organization. Global Health Estimates 2019: Deaths by Cause, Age, Sex, by Country and by Region, 2000-2019. (2020).
2. M, S. & E, G. Preventable Incidence and Mortality of Carcinoma Associated With Lifestyle Factors Among White Adults in the United States. *JAMA oncology* **2**, (2016).
3. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis - The Lancet Global Health. https://www.thelancet.com/journals/langlo/article/PIIS2214-109X(19)30488-7/fulltext.
4. Zhang, X. *et al.* Oral Administration of a Shigella 2aT32-Based Vaccine Expressing UreB-HspA Fusion Antigen With and Without Parenteral rUreB-HspA Boost Confers Protection Against Helicobacter pylori in Mice Model. *Front. Immunol.* **13**, (2022).
5. National Institute of Allergy and Infectious Diseases (NIAID). *Phase 1 Study of the Safety and Immunogenicity of an Epstein-Barr Virus (EBV)Gp350- Ferritin Nanoparticle Vaccine in Healthy Adults With or Without EBV Infection*. https://clinicaltrials.gov/study/NCT04645147 (2024).
6. Immunization coverage. *World Health Organization* https://www.who.int/news-room/fact-sheets/detail/immunization-coverage.
7. Frick, C. *et al.* Quantitative estimates of preventable and treatable deaths from 36 cancers worldwide: a population-based study. *The Lancet Global Health* **11**, e1700–e1712 (2023).
8. Human papillomavirus vaccines: WHO position paper, December 2022. https://www.who.int/publications-detail-redirect/who-wer9750-645-672.
9. Ledermann, J. A. *et al.* ESGO–ESMO–ESP consensus conference recommendations on ovarian cancer: pathology and molecular biology and early, advanced and recurrent disease. *Annals of Oncology* **35**, 248–266 (2024). **Review.**
10. Horwich, A. *et al.* Prostate cancer: ESMO Consensus Conference Guidelines 2012. *Annals of Oncology* **24**, 1141–1162 (2013). **Review.**
11. Bárcena, P. G. Q., Aprikian, A. G. & Dragomir, A. Secondary bladder and colorectal cancer after treatments for prostate cancer: A population based study. *Cancer Med* (2024) doi:10.1002/cam4.6922.
12. Huynh, M. J. *et al.* Incidence and survival of secondary malignancies after external beam radiotherapy for prostate cancer in the Surveillance, Epidemiology, and End Results database. *Can Urol Assoc J* (2023) doi:10.5489/cuaj.8508.
13. Mitra, S. *et al.* Hormonal Therapy for Gynecological Cancers: How Far Has Science Progressed toward Clinical Applications? *Cancers* **14**, 759 (2022). **Review.**
14. Iversen, P. *et al.* Bicalutamide monotherapy compared with castration in patients with nonmetastatic locally advanced prostate cancer: 6.3 years of followup. *J Urol* **164**, 1579–1582 (2000).
15. Yuan, J. *et al.* The role of the tumor microenvironment in endocrine therapy resistance in hormone receptor-positive breast cancer. *Front Endocrinol (Lausanne)* **14**, 1261283 (2023). **Review.**
16. Longley, D. & Johnston, P. Molecular mechanisms of drug resistance. *The Journal of Pathology* **205**, 275–292 (2005). **Review.**
17. Katta, B., Vijayakumar, C., Dutta, S., Dubashi, B. & Nelamangala Ramakrishnaiah, V. P. The Incidence and Severity of Patient-Reported Side Effects of Chemotherapy in Routine Clinical Care: A Prospective Observational Study. *Cureus* **15**, e38301.

18. Joo, W. D., Visintin, I. & Mor, G. Targeted cancer therapy – Are the days of systemic chemotherapy numbered? *Maturitas* **76**, 308–314 (2013). **Review.**
19. Galluzzi, L. *et al.* Classification of current anticancer immunotherapies. *Oncotarget* **5**, 12472–12508 (2014). **Review.**
20. Rosenberg Steven A. *et al.* Observations on the Systemic Administration of Autologous Lymphokine-Activated Killer Cells and Recombinant Interleukin-2 to Patients with Metastatic Cancer. *New England Journal of Medicine* **313**, 1485–1492 (1985).
21. Rosenberg, S. A. *et al.* Durable Complete Responses in Heavily Pretreated Patients with Metastatic Melanoma Using T-Cell Transfer Immunotherapy. *Clinical Cancer Research* **17**, 4550–4557 (2011).
22. Robbins, P. F. *et al.* Single and Dual Amino Acid Substitutions in TCR CDRs Can Enhance Antigen-Specific T Cell Functions. *J Immunol* **180**, 6116–6131 (2008).
23. Achard, C. *et al.* Oncolytic measles virus induces tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated cytotoxicity by human myeloid and plasmacytoid dendritic cells. *OncoImmunology* **6**, e1261240 (2017).
24. Havunen, R. *et al.* Abscopal Effect in Non-injected Tumors Achieved with Cytokine-Armed Oncolytic Adenovirus. *Mol Ther Oncolytics* **11**, 109–121 (2018).
25. Liang, M. Oncorine, the World First Oncolytic Virus Medicine and its Update in China. *Current Cancer Drug Targets* **18**, 171–176. **Review.**
26. IMLYGIC. *FDA* https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/imlygic (2023).
27. Otani, Y. *et al.* Implications of immune cells in oncolytic herpes simplex virotherapy for glioma. *Brain Tumor Pathol* **39**, 57–64 (2022). **Review.**
28. Leal, M. *et al.* Antibody-drug conjugates: an emerging modality for the treatment of cancer. *Ann N Y Acad Sci* **1321**, 41–54 (2014).
29. Klein, C. *et al.* Cergutuzumab amunaleukin (CEA-IL2v), a CEA-targeted IL-2 variant-based immunocytokine for combination cancer immunotherapy: Overcoming limitations of aldesleukin and conventional IL-2-based immunocytokines. *Oncoimmunology* **6**, e1277306 (2017).
30. Liu, J.-Q. *et al.* Intratumoral delivery of IL-12 and IL-27 mRNA using lipid nanoparticles for cancer immunotherapy. *J Control Release* **345**, 306–313 (2022).
31. Latagliata, R. *et al.* Discontinuation of alpha-interferon treatment in patients with chronic myeloid leukemia in long-lasting complete molecular response. *Leukemia & Lymphoma* **57**, 99–102 (2016).
32. Hodi F. Stephen *et al.* Improved Survival with Ipilimumab in Patients with Metastatic Melanoma. *New England Journal of Medicine* **363**, 711–723 (2010).
33. Tawbi, H. A. *et al.* Relatlimab and Nivolumab versus Nivolumab in Untreated Advanced Melanoma. *N Engl J Med* **386**, 24–34 (2022).
34. P, P., Jc, V. & J, R. Immune checkpoint blockade in hematological malignancies: current state and future potential. *Frontiers in oncology* **14**, (2024). **Review.**
35. Wang, L. *et al.* Hot and cold tumors: Immunological features and the therapeutic strategies. *MedComm (2020)* **4**, e343 (2023). **Review.**
36. Sun, D. *et al.* CAR-T cell therapy: A breakthrough in traditional cancer treatment strategies (Review). *Molecular Medicine Reports* **29**, 1–9 (2024). **Review.**
37. Verdun, N. & Marks, P. Secondary Cancers after Chimeric Antigen Receptor T-Cell Therapy. *New England Journal of Medicine* (2024) doi:10.1056/NEJMp2400209. **Review.**

38. Zhang, Q. *et al.* Blockade of the checkpoint receptor TIGIT prevents NK cell exhaustion and elicits potent anti-tumor immunity. *Nat Immunol* **19**, 723–732 (2018).
39. Shi, Y. *et al.* Trastuzumab triggers phagocytic killing of high HER2 cancer cells in vitro and in vivo by interaction with Fcγ receptors on macrophages. *J Immunol* **194**, 4379–4386 (2015).
40. Rezvani, K., Rouce, R., Liu, E. & Shpall, E. Engineering Natural Killer Cells for Cancer Immunotherapy. *Mol Ther* **25**, 1769–1781 (2017). **Review.**
41. Klichinsky, M. *et al.* Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol* **38**, 947–953 (2020).
42. Benguigui, M. *et al.* Interferon-stimulated neutrophils as a predictor of immunotherapy response. *Cancer Cell* **42**, 253-265.e12 (2024).
43. Landsberg, J. *et al.* Melanomas resist T-cell therapy through inflammation-induced reversible dedifferentiation. *Nature* **490**, 412–416 (2012).
44. Karsunky, H., Merad, M., Cozzio, A., Weissman, I. L. & Manz, M. G. Flt3 Ligand Regulates Dendritic Cell Development from Flt3+ Lymphoid and Myeloid-committed Progenitors to Flt3+ Dendritic Cells In Vivo. *J Exp Med* **198**, 305–313 (2003).
45. Norbury, C. C., Chambers, B. J., Prescott, A. R., Ljunggren, H.-G. & Watts, C. Constitutive macropinocytosis allows TAP-dependent major histocompatibility compex class I presentation of exogenous soluble antigen by bone marrow-derived dendritic cells. *European Journal of Immunology* **27**, 280–288 (1997).
46. Dendritic cells use macropinocytosis and the mannose receptor to concentrate macromolecules in the major histocompatibility complex class II compartment: downregulation by cytokines and bacterial products. *J Exp Med* **182**, 389–400 (1995).
47. Lundberg, K., Rydnert, F., Greiff, L. & Lindstedt, M. Human blood dendritic cell subsets exhibit discriminative pattern recognition receptor profiles. *Immunology* **142**, 279–288 (2014).
48. Ohl, L. *et al.* CCR7 Governs Skin Dendritic Cell Migration under Inflammatory and Steady-State Conditions. *Immunity* **21**, 279–288 (2004).
49. O'Doherty, U. *et al.* Dendritic cells freshly isolated from human blood express CD4 and mature into typical immunostimulatory dendritic cells after culture in monocyte-conditioned medium. *Journal of Experimental Medicine* **178**, 1067–1076 (1993).
50. Gardner, J. K. *et al.* Elderly dendritic cells respond to LPS/IFN-γ and CD40L stimulation despite incomplete maturation. *PLoS One* **13**, e0195313 (2018).
51. Bousso, P. & Robey, E. Dynamics of CD8+ T cell priming by dendritic cells in intact lymph nodes. *Nat Immunol* **4**, 579–585 (2003).
52. Ruedl, C., Kopf, M. & Bachmann, M. F. CD8+ T Cells Mediate CD40-independent Maturation of Dendritic Cells In Vivo. *Journal of Experimental Medicine* **189**, 1875–1884 (1999).
53. Bennett, S. R. M., Carbone, F. R., Karamalis, F., Miller, J. F. A. P. & Heath, W. R. Induction of a CD8+ Cytotoxic T Lymphocyte Response by Cross-priming Requires Cognate CD4+ T Cell Help. *J Exp Med* **186**, 65–70 (1997).
54. Garg, S. *et al.* Genetic tagging shows increased frequency and longevity of antigen-presenting, skin-derived dendritic cells in vivo. *Nat Immunol* **4**, 907–912 (2003).
55. McLellan, A. *et al.* MHC class II and CD40 play opposing roles in dendritic cell survival. *European Journal of Immunology* **30**, 2612–2619 (2000).

56. Mackey, M. F. *et al.* Dendritic cells require maturation via CD40 to generate protective antitumor immunity. *J Immunol* **161**, 2094–2098 (1998).
57. Herbin, O. *et al.* Medullary Thymic Epithelial Cells and CD8α+ Dendritic Cells coordinately regulate central tolerance but CD8α+ cells are Dispensable for Thymic Regulatory T cell production. *J Autoimmun* **75**, 141–149 (2016).
58. Ruhland, M. K. *et al.* Visualizing Synaptic Transfer of Tumor Antigens among Dendritic Cells. *Cancer Cell* **37**, 786-799.e5 (2020).
59. Gerner, M. Y., Torabi-Parizi, P. & Germain, R. N. Strategically Localized Dendritic Cells Promote Rapid T Cell Responses to Lymph-Borne Particulate Antigens. *Immunity* **42**, 172–185 (2015).
60. McCurley, N. & Mellman, I. Monocyte-Derived Dendritic Cells Exhibit Increased Levels of Lysosomal Proteolysis as Compared to Other Human Dendritic Cell Populations. *PLOS ONE* **5**, e11949 (2010).
61. Boross, P. *et al.* FcRγ-Chain ITAM Signaling Is Critically Required for Cross-Presentation of Soluble Antibody–Antigen Complexes by Dendritic Cells. *The Journal of Immunology* **193**, 5506–5514 (2014).
62. Cruz, F. M., Chan, A. & Rock, K. L. Pathways of MHC I cross-presentation of exogenous antigens. *Semin Immunol* **66**, 101729 (2023). **Review.**
63. Arndt, S. O. *et al.* Functional HLA-DM on the surface of B cells and immature dendritic cells. *EMBO J* **19**, 1241–1251 (2000). **Review.**
64. Barbet, G. *et al.* TAP dysfunction in dendritic cells enables non-canonical cross-presentation for T cell priming. *Nat Immunol* **22**, 497–509 (2021).
65. Cruz, F. M., Colbert, J. D., Merino, E., Kriegsman, B. A. & Rock, K. L. The biology and underlying mechanisms of cross-presentation of exogenous antigens on MHC I molecules. *Annu Rev Immunol* **35**, 149–176 (2017). **Review.**
66. Nair-Gupta, P. *et al.* TLR Signals Induce Phagosomal MHC-I Delivery from the Endosomal Recycling Compartment to Allow Cross-Presentation. *Cell* **158**, 506–521 (2014).
67. Canton, J. *et al.* The receptor DNGR-1 signals for phagosomal rupture to promote cross-presentation of dead cell-associated antigens. *Nat Immunol* **22**, 140–153 (2021).
68. MacNabb, B. W. *et al.* Dendritic cells can prime anti-tumor CD8+ T cell responses through major histocompatibility complex cross-dressing. *Immunity* **55**, 982-997.e8 (2022).
69. Plesca, I. *et al.* Tumor-associated human dendritic cell subsets: Phenotype, functional orientation, and clinical relevance. *European Journal of Immunology* **52**, 1750–1758 (2022). **Review.**
70. Guilliams, M. *et al.* Dendritic cells, monocytes and macrophages: a unified nomenclature based on ontogeny. *Nat Rev Immunol* **14**, 571–578 (2014).
71. Nizzoli, G. *et al.* Human CD1c+ dendritic cells secrete high levels of IL-12 and potently prime cytotoxic T-cell responses. *Blood* **122**, 932–942 (2013).
72. Granot, T. *et al.* Dendritic Cells Display Subset and Tissue-Specific Maturation Dynamics over Human Life. *Immunity* **46**, 504–515 (2017).
73. Leal Rojas, I. M. *et al.* Human Blood CD1c+ Dendritic Cells Promote Th1 and Th17 Effector Function in Memory CD4+ T Cells. *Front. Immunol.* **8**, (2017).
74. Hubert, M. *et al.* IFN-III is selectively produced by cDC1 and predicts good clinical outcome in breast cancer. *Science Immunology* **5**, eaav3942 (2020).
75. Jongbloed, S. L. *et al.* Human CD141+ (BDCA-3)+ dendritic cells (DCs) represent a unique myeloid DC subset that cross-presents necrotic cell antigens. *Journal of Experimental Medicine* **207**, 1247–1260 (2010).

76. Tullett, K. M. *et al.* Targeting CLEC9A delivers antigen to human CD141+ DC for CD4+ and CD8+T cell recognition. *JCI Insight* **1**, e87102.
77. Krishnaswamy, J. K. *et al.* Migratory CD11b+ conventional dendritic cells induce T follicular helper cell–dependent antibody responses. *Science Immunology* **2**, eaam9169 (2017).
78. Fenton, T. M. *et al.* Inflammatory cues enhance TGFβ activation by distinct subsets of human intestinal dendritic cells via integrin αvβ8. *Mucosal Immunol* **10**, 624–634 (2017).
79. Cella, M. *et al.* Plasmacytoid monocytes migrate to inflamed lymph nodes and produce large amounts of type I interferon. *Nat Med* **5**, 919–923 (1999).
80. Alculumbre, S. G. *et al.* Diversification of human plasmacytoid predendritic cells in response to a single stimulus. *Nat Immunol* **19**, 63–75 (2018).
81. Tang-Huau, T.-L. *et al.* Human in vivo-generated monocyte-derived dendritic cells and macrophages cross-present antigens through a vacuolar pathway. *Nat Commun* **9**, 2570 (2018).
82. Yang, G.-X. *et al.* Plasmacytoid Dendritic Cells of Different Origins Have Distinct Characteristics and Function: Studies of Lymphoid Progenitors versus Myeloid Progenitors. *The Journal of Immunology* **175**, 7281–7287 (2005).
83. Randolph, G. J., Inaba, K., Robbiani, D. F., Steinman, R. M. & Muller, W. A. Differentiation of Phagocytic Monocytes into Lymph Node Dendritic Cells In Vivo. *Immunity* **11**, 753–761 (1999).
84. Segura, E. *et al.* Human Inflammatory Dendritic Cells Induce Th17 Cell Differentiation. *Immunity* **38**, 336–348 (2013).
85. León, B., López-Bravo, M. & Ardavín, C. Monocyte-Derived Dendritic Cells Formed at the Infection Site Control the Induction of Protective T Helper 1 Responses against *Leishmania*. *Immunity* **26**, 519–531 (2007).
86. Plantinga, M. *et al.* Conventional and monocyte-derived CD11b(+) dendritic cells initiate and maintain T helper 2 cell-mediated immunity to house dust mite allergen. *Immunity* **38**, 322–335 (2013).
87. Chakarov, S. & Fazilleau, N. Monocyte-derived dendritic cells promote T follicular helper cell differentiation. *EMBO Mol Med* **6**, 590–603 (2014).
88. Kiama, S. G., Cochand, L., Karlsson, L., Nicod, L. P. & Gehr, P. Evaluation of Phagocytic Activity in Human Monocyte-Derived Dendritic Cells. *Journal of Aerosol Medicine* **14**, 289–299 (2001).
89. Liao, C.-T. *et al.* Peritoneal macrophage heterogeneity is associated with different peritoneal dialysis outcomes. *Kidney International* **91**, (2017).
90. Langlet, C. *et al.* CD64 Expression Distinguishes Monocyte-Derived and Conventional Dendritic Cells and Reveals Their Distinct Role during Intramuscular Immunization. *The Journal of Immunology* **188**, 1751–1760 (2012).
91. Bourdely, P. *et al.* Transcriptional and Functional Analysis of CD1c+ Human Dendritic Cells Identifies a CD163+ Subset Priming CD8+CD103+ T Cells. *Immunity* **53**, 335-352.e8 (2020).
92. Girard, M., Law, J. C., Edilova, M. I. & Watts, T. H. Type I interferons drive the maturation of human DC3s with a distinct costimulatory profile characterized by high GITRL. *Science Immunology* **5**, eabe0347 (2020).
93. Koya, T., Yanagisawa, R., Higuchi, Y., Sano, K. & Shimodaira, S. Interferon-α-inducible Dendritic Cells Matured with OK-432 Exhibit TRAIL and Fas Ligand Pathway-mediated Killer Activity. *Sci Rep* **7**, 42145 (2017).
94. Peretz, Y., Zhou, Z. F., Halwani, F. & Prud'homme, G. J. In vivo generation of dendritic cells by intramuscular codelivery of FLT3 ligand and GM-CSF plasmids. *Mol Ther* **6**, 407–414 (2002).

95. Freedman, R. S. *et al.* Pilot Study of Flt3 Ligand Comparing Intraperitoneal with Subcutaneous Routes on Hematologic and Immunologic Responses in Patients with Peritoneal Carcinomatosis and Mesotheliomas. *Clinical Cancer Research* **9**, 5228–5237 (2003).
96. Svensson-Arvelund, J. *et al.* Expanding cross-presenting dendritic cells enhances oncolytic virotherapy and is critical for long-term anti-tumor immunity. *Nat Commun* **13**, 7149 (2022).
97. Kurbacher, C. M. *et al.* Continuous low-dose GM-CSF as salvage therapy in refractory recurrent breast or female genital tract carcinoma. *Oncology (Williston Park)* **19**, 23–26 (2005).
98. Higano, C. S. *et al.* Phase 1/2 dose-escalation study of a GM-CSF-secreting, allogeneic, cellular immunotherapy for metastatic hormone-refractory prostate cancer. *Cancer* **113**, 975–984 (2008).
99. Bonifaz, L. C. *et al.* In Vivo Targeting of Antigens to Maturing Dendritic Cells via the DEC-205 Receptor Improves T Cell Vaccination. *J Exp Med* **199**, 815–824 (2004).
100. Chrisikos, T. T. *et al.* STAT3 Inhibits Autocrine Interferon Signaling in Type I Conventional Dendritic Cells. *J Immunol* **209**, 1286–1299 (2022).
101. Levy, H. B. *et al.* A Modified Polyriboinosinic-Polyribocytidylic Acid Complex That Induces Interferon in Primates. *The Journal of Infectious Diseases* **132**, 434–439 (1975).
102. Goldinger, S. M. *et al.* Nano-particle vaccination combined with TLR-7 and -9 ligands triggers memory and effector CD8+ T-cell responses in melanoma patients. *Eur J Immunol* **42**, 3049–3061 (2012).
103. Bian, Y., Walter, D. L. & Zhang, C. Efficiency of Interferon-γ in Activating Dendritic Cells and Its Potential Synergy with Toll-like Receptor Agonists. *Viruses* **15**, 1198 (2023).
104. Pallotta, M. T. *et al.* Indoleamine 2,3-dioxygenase is a signaling protein in long-term tolerance by dendritic cells. *Nat Immunol* **12**, 870–878 (2011).
105. Johnson, T. S. *et al.* Indoximod-based chemo-immunotherapy for pediatric brain tumors: A first-in-children phase I trial. *Neuro Oncol* **26**, 348–361 (2023).
106. Kotecki, N. *et al.* A Phase I Study of an IDO-1 Inhibitor (LY3381916) as Monotherapy and in Combination With an Anti-PD-L1 Antibody (LY3300054) in Patients With Advanced Cancer. *Journal of Immunotherapy* **44**, 264 (2021).
107. Melillo, J. A. *et al.* Dendritic Cell (DC)-Specific Targeting Reveals Stat3 as a Negative Regulator of DC Function. *J Immunol* **184**, 2638–2645 (2010).
108. Nishina, T. *et al.* Safety, tolerability, pharmacokinetics and preliminary antitumour activity of an antisense oligonucleotide targeting STAT3 (danvatirsen) as monotherapy and in combination with durvalumab in Japanese patients with advanced solid malignancies: a phase 1 study. *BMJ Open* **12**, e055718 (2022).
109. Botelho, N. K. *et al.* Combination of Synthetic Long Peptides and XCL1 Fusion Proteins Results in Superior Tumor Control. *Front Immunol* **10**, 294 (2019).
110. Tsuji, T. *et al.* Antibody-Targeted NY-ESO-1 to Mannose Receptor or DEC-205 In Vitro Elicits Dual Human CD8+ and CD4+ T Cell Responses with Broad Antigen Specificity. *The Journal of Immunology* **186**, 1218–1227 (2011).
111. Tacken, P. J. *et al.* Effective induction of naive and recall T-cell responses by targeting antigen to human dendritic cells via a humanized anti–DC-SIGN antibody. *Blood* **106**, 1278–1285 (2005).
112. Turville, S. G. *et al.* Diversity of receptors binding HIV on dendritic cell subsets. *Nat Immunol* **3**, 975–983 (2002).
113. Fang, R. H. *et al.* Cancer Cell Membrane-Coated Nanoparticles for Anticancer Vaccination and Drug Delivery. *Nano Lett* **14**, 2181–2188 (2014).

114. Moon, J. J. *et al.* Interbilayer-Crosslinked Multilamellar Vesicles as Synthetic Vaccines for Potent Humoral and Cellular Immune Responses. *Nat Mater* **10**, 243–251 (2011).

115. Tao, F. *et al.* Antigen-loaded flagellate bacteria for enhanced adaptive immune response by intradermal injection. *Journal of Controlled Release* **364**, 562–575 (2023).

116. Somaiah, N. *et al.* First-in-Class, First-in-Human Study Evaluating LV305, a Dendritic-Cell Tropic Lentiviral Vector, in Sarcoma and Other Solid Tumors Expressing NY-ESO-1. *Clinical Cancer Research* **25**, 5808–5817 (2019).

117. Rastogi, I. & McNeel, D. G. B cells require licensing by dendritic cells to serve as primary antigen-presenting cells for plasmid DNA. *Oncoimmunology* **12**, 2212550.

118. PROVENGE Full Prescribing Information. (2017).

119. Fröbom, R. *et al.* Phase I trial evaluating safety and efficacy of intratumorally administered inflammatory allogeneic dendritic cells (ilixadencel) in advanced gastrointestinal stromal tumors. *Cancer Immunol Immunother* **69**, 2393–2401 (2020).

120. Van Zeeburg, H. *et al.* Vaccination Using an Allogeneic Leukemia-Derived Dendritic Cell Vaccine, Maintains and Improves Frequencies of Circulating Antigen Presenting Dendritic Cells Correlating with Relapse Free and Overall Survival in AML Patients. *Blood* **142**, 2957 (2023).

121. Bond, E. *et al.* Techniques for time-efficient isolation of human skin dendritic cell subsets and assessment of their antigen uptake capacity. *Journal of Immunological Methods* **348**, 42–56 (2009).

122. Lubin, R., Gvili, R., Hazan, I. & Yona, S. Human Dendritic Cell Enrichment and Their Activation of T Cells. *Current Protocols* **3**, e873 (2023).

123. Chometon, T. Q. *et al.* A protocol for rapid monocyte isolation and generation of singular human monocyte-derived dendritic cells. *PLOS ONE* **15**, e0231132 (2020).

124. Taborska, P., Bartunkova, J. & Smrz, D. Simultaneous *in vitro* generation of human CD34+-derived dendritic cells and mast cells from non-mobilized peripheral blood mononuclear cells. *Journal of Immunological Methods* **458**, 63–73 (2018).

125. Balan, S. *et al.* Large-Scale Human Dendritic Cell Differentiation Revealing Notch-Dependent Lineage Bifurcation and Heterogeneity. *Cell Reports* **24**, 1902-1915.e6 (2018).

126. Kirkling, M. E. *et al.* Notch Signaling Facilitates *In Vitro* Generation of Cross-Presenting Classical Dendritic Cells. *Cell Reports* **23**, 3658-3672.e6 (2018).

127. Swartz, A. M. & Nair, S. K. The In Vitro Differentiation of Human CD141+CLEC9A+ Dendritic Cells from Mobilized Peripheral Blood CD34+ Hematopoietic Stem Cells. *Current Protocols* **2**, e410 (2022).

128. Rosa, F. F. *et al.* Single-cell transcriptional profiling informs efficient reprogramming of human somatic cells to cross-presenting dendritic cells. *Science Immunology* **7**, eabg5539 (2022).

129. Charles, J. *et al.* An innovative plasmacytoid dendritic cell line-based cancer vaccine primes and expands antitumor T-cells in melanoma patients in a first-in-human trial. *OncoImmunology* **9**, 1738812 (2020).

130. Nair, S., Archer, G. E. & Tedder, T. F. Isolation and Generation of Human Dendritic Cells. *Current Protocols in Immunology* **99**, 7.32.1-7.32.23 (2012).

131. Faradji, A. *et al.* Apheresis-Elutriation Program for Adoptive Immunotherapy with Autologous Activated Monocytes in Cancer Patients. *Int J Artif Organs* **14**, 304–312 (1991).

132. Sallusto, F. & Lanzavecchia, A. Efficient presentation of soluble antigen by cultured human dendritic cells is maintained by granulocyte/macrophage colony-stimulating factor plus interleukin 4 and downregulated by tumor necrosis factor alpha. *Journal of Experimental Medicine* **179**, 1109–1118 (1994).

133. Syme, R., Bajwa, R., Robertson, L., Stewart, D. & Glück, S. Comparison of CD34 and Monocyte-Derived Dendritic Cells from Mobilized Peripheral Blood from Cancer Patients. *Stem Cells* **23**, 74–81 (2005).

134. Mohamadzadeh, M. *et al.* Interleukin 15 Skews Monocyte Differentiation into Dendritic Cells with Features of Langerhans Cells. *Journal of Experimental Medicine* **194**, 1013–1020 (2001).

135. Nowrousian, M. R. *et al.* Impact of chemotherapy regimen and hematopoietic growth factor on mobilization and collection of peripheral blood stem cells in cancer patients. *Annals of Oncology* **14**, i29–i36 (2003).

136. Mohib, K. & Wang, L. Differentiation and Characterization of Dendritic Cells from Human Embryonic Stem Cells. *Current Protocols in Immunology* **98**, 22F.11.1-22F.11.22 (2012).

137. Proietto, A. I., Mittag, D., Roberts, A. W., Sprigg, N. & Wu, L. The equivalents of human blood and spleen dendritic cell subtypes can be generated in vitro from human CD34+ stem cells in the presence of fms-like tyrosine kinase 3 ligand and thrombopoietin. *Cell Mol Immunol* **9**, 446–454 (2012).

138. van Eck van der Sluijs, J. *et al.* Clinically applicable CD34+-derived blood dendritic cell subsets exhibit key subset-specific features and potently boost anti-tumor T and NK cell responses. *Cancer Immunol Immunother* **70**, 3167–3181 (2021).

139. Fransen, L. F. H. & Leonard, M. O. CD34+ derived macrophage and dendritic cells display differential responses to paraquat. *Toxicology in Vitro* **75**, 105198 (2021).

140. Lopez, M. *et al.* IL-13 induces CD34+ cells isolated from G-CSF mobilized blood to differentiate in vitro into potent antigen presenting cells. *Journal of Immunological Methods* **208**, 117–129 (1997).

141. Sundarasetty, B. S. *et al.* Lentivirus-induced 'Smart' dendritic cells: Pharmacodynamics and GMP-compliant production for immunotherapy against TRP2-positive melanoma. *Gene Ther* **22**, 707–720 (2015). **Review.**

142. Steinman, R. M. & Nussenzweig, M. C. Avoiding horror autotoxicus: The importance of dendritic cells in peripheral T cell tolerance. *Proceedings of the National Academy of Sciences* **99**, 351–358 (2002).

143. Moschella, F. *et al.* Transcript profiling of human dendritic cells maturation-induced under defined culture conditions: comparison of the effects of tumour necrosis factor alpha, soluble CD40 ligand trimer and interferon gamma. *British Journal of Haematology* **114**, 444–457 (2001).

144. Chabot, V. *et al.* Unexpected impairment of TNF-α-induced maturation of human dendritic cells in vitro by IL-4. *Journal of Translational Medicine* **14**, 93 (2016).

145. Matsunaga, T. *et al.* Analysis of Gene Expression During Maturation of Immature Dendritic Cells Derived from Peripheral Blood Monocytes. *Scandinavian Journal of Immunology* **56**, 593–601 (2002).

146. Jonuleit, H. *et al.* Pro-inflammatory cytokines and prostaglandins induce maturation of potent immunostimulatory dendritic cells under fetal calf serum-free conditions. *European Journal of Immunology* **27**, 3135–3142 (1997).

147. Mailliard, R. B. *et al.* α-Type-1 Polarized Dendritic Cells: A Novel Immunization Tool with Optimized CTL-inducing Activity. *Cancer Research* **64**, 5934–5937 (2004).

148. Möller, I. *et al.* Dendritic Cell Maturation With Poly(I:C)-based Versus PGE2-based Cytokine Combinations Results in Differential Functional Characteristics Relevant to Clinical Application. *Journal of Immunotherapy* **31**, 506 (2008).

149. Hatfield, P. *et al.* Optimization of Dendritic Cell Loading With Tumor Cell Lysates for Cancer Immunotherapy. *J Immunother* **31**, 620–632 (2008).

150. Qiu, J. *et al.* Heat-shocked tumor cell lysate-pulsed dendritic cells induce effective anti-tumor immune response in vivo. *World J Gastroenterol* **12**, 473–478 (2006).

151. Chiang, C. L.-L., Ledermann, J. A., Rad, A. N., Katz, D. R. & Chain, B. M. Hypochlorous acid enhances immunogenicity and uptake of allogeneic ovarian tumor cells by dendritic cells to cross-prime tumor-specific T cells. *Cancer Immunol Immunother* **55**, 1384–1395 (2006).

152. Dombroski, J. A. *et al.* Tumor nano-lysate activates dendritic cells to evoke a preventative immune response. *Journal of Immunological Methods* **524**, 113601 (2024).

153. Wolfraim, L. A. *et al.* Clinical scale electroloading of mature dendritic cells with melanoma whole tumor cell lysate is superior to conventional lysate co-incubation in triggering robust in vitro expansion of functional antigen-specific CTL. *International Immunopharmacology* **15**, 488–497 (2013).

154. Fucikova, J. *et al.* Human Tumor Cells Killed by Anthracyclines Induce a Tumor-Specific Immune Response. *Cancer Research* **71**, 4821–4833 (2011).

155. Sriram, G. *et al.* The injury response to DNA damage in live tumor cells promotes antitumor immunity. *Sci Signal* **14**, eabc4764 (2021).

156. Zhang, H. *et al.* Cell-free Tumor Microparticle Vaccines Stimulate Dendritic Cells via cGAS/STING Signaling. *Cancer Immunology Research* **3**, 196–205 (2015).

157. Kim, W. S. *et al.* Comparison of Exosomes Derived from Non- and Gamma-Irradiated Melanoma Cancer Cells as a Potential Antigenic and Immunogenic Source for Dendritic Cell-Based Immunotherapeutic Vaccine. *Vaccines (Basel)* **8**, 699 (2020).

158. Wilgenhof, S. *et al.* Long-term clinical outcome of melanoma patients treated with messenger RNA-electroporated dendritic cell therapy following complete resection of metastases. *Cancer Immunol Immunother* **64**, 381–388 (2015).

159. Aarntzen, E. H. J. G. *et al.* Vaccination with mRNA-Electroporated Dendritic Cells Induces Robust Tumor Antigen-Specific CD4+ and CD8+ T Cells Responses in Stage III and IV Melanoma Patients. *Clinical Cancer Research* **18**, 5460–5470 (2012).

160. Bonehill, A. *et al.* Messenger RNA-electroporated dendritic cells presenting MAGE-A3 simultaneously in HLA class I and class II molecules. *J Immunol* **172**, 6649–6657 (2004).

161. McCullough, K. C. *et al.* Self-replicating Replicon-RNA Delivery to Dendritic Cells by Chitosan-nanoparticles for Translation In Vitro and In Vivo. *Mol Ther Nucleic Acids* **3**, e173 (2014).

162. Liu, J. *et al.* Nanochannel Electro-Injection as a Versatile Platform for Efficient RNA/DNA Programming on Dendritic Cells. *Small* **19**, 2303088 (2023).

163. Dewitte, H. *et al.* The potential of antigen and TriMix sonoporation using mRNA-loaded microbubbles for ultrasound-triggered cancer immunotherapy. *Journal of Controlled Release* **194**, 28–36 (2014).

164. Grünebach, F., Müller, M. R., Nencioni, A. & Brossart, P. Delivery of tumor-derived RNA for the induction of cytotoxic T-lymphocytes. *Gene Ther* **10**, 367–374 (2003).

165. Wilgenhof, S. *et al.* A phase IB study on intravenous synthetic mRNA electroporated dendritic cell immunotherapy in pretreated advanced melanoma patients. *Annals of Oncology* **24**, 2686–2693 (2013).

166. He, Y., Zhang, J., Mi, Z., Robbins, P. & Falo, L. D., Jr. Immunization with Lentiviral Vector-Transduced Dendritic Cells Induces Strong and Long-Lasting T Cell Responses and Therapeutic Immunity 1. *The Journal of Immunology* **174**, 3808–3817 (2005).

167. Butterfield, L. H. *et al.* Adenovirus MART-1–engineered Autologous Dendritic Cell Vaccine for Metastatic Melanoma. *J Immunother* **31**, 294–309 (2008).

168. Heiser, A. *et al.* Induction of polyclonal prostate cancer-specific CTL using dendritic cells transfected with amplified tumor RNA. *J Immunol* **166**, 2953–2960 (2001).

169. Li, J. *et al.* DNA is an efficient booster of dendritic cell-based vaccine. *Hum Vaccin Immunother* **11**, 1927–1935 (2015).

170. Hass, R., von der Ohe, J. & Dittmar, T. Cancer Cell Fusion and Post-Hybrid Selection Process (PHSP). *Cancers (Basel)* **13**, 4636 (2021).

171. Gast, C. E. *et al.* Cell fusion potentiates tumor heterogeneity and reveals circulating hybrid cells that correlate with stage and survival. *Sci Adv* **4**, eaat7828 (2018).

172. Dannull, J. *et al.* Gene Expression Profile of Dendritic Cell-Tumor Cell Hybrids Determined by Microarrays and Its Implications for Cancer Immunotherapy. *Journal of Immunology Research* **2015**, e789136 (2015).

173. Geskin, L. J. *et al.* Three antigen-loading methods in dendritic cell vaccines for metastatic melanoma. *Melanoma Research* **28**, 211 (2018).

174. Scott-Taylor, T. H. *et al.* Human tumour and dendritic cell hybrids generated by electrofusion: potential for cancer vaccines. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease* **1500**, 265–279 (2000).

175. Okeyo, K. O., Hiyaji, R. & Oana, H. A single-cell surgery microfluidic device for transplanting tumor cytoplasm into dendritic cells without nuclei mixing. *Biotechnology Journal* **18**, 2200135 (2023).

176. Fong, L., Brockstedt, D., Benike, C., Wu, L. & Engleman, E. G. Dendritic Cells Injected Via Different Routes Induce Immunity in Cancer Patients1. *The Journal of Immunology* **166**, 4254–4259 (2001).

177. Sandoval, F. *et al.* Mucosal Imprinting of Vaccine-Induced CD8+ T Cells Is Crucial to Inhibit the Growth of Mucosal Tumors. *Science Translational Medicine* **5**, 172ra20-172ra20 (2013).

178. Dudda, J. C. *et al.* Dendritic cells govern induction and reprogramming of polarized tissue-selective homing receptor patterns of T cells: important roles for soluble factors and tissue microenvironments. *European Journal of Immunology* **35**, 1056–1065 (2005).

179. Radomski, M. *et al.* Prolonged intralymphatic delivery of dendritic cells through implantable lymphatic ports in patients with advanced cancer. *J Immunother Cancer* **4**, 24 (2016).

180. Rodríguez-Ruiz, M. E. *et al.* Combined immunotherapy encompassing intratumoral poly-ICLC, dendritic-cell vaccination and radiotherapy in advanced cancer patients. *Ann Oncol* **29**, 1312–1319 (2018).

181. Aarntzen, E. H. *et al.* Reducing cell number improves the homing of dendritic cells to lymph nodes upon intradermal vaccination. *OncoImmunology* **2**, e24661 (2013).

182. Villablanca, E. J. *et al.* Tumor-mediated liver X receptor-α activation inhibits CC chemokine receptor-7 expression on dendritic cells and dampens antitumor responses. *Nat Med* **16**, 98–105 (2010).

183. Bedrosian, I. *et al.* Intranodal administration of peptide-pulsed mature dendritic cell vaccines results in superior CD8+ T-cell function in melanoma patients. *Journal of Clinical Oncology* **21**, 3826–3835 (2003).

184. Lesterhuis, W. J. *et al.* Route of Administration Modulates the Induction of Dendritic Cell Vaccine–Induced Antigen-Specific T Cells in Advanced Melanoma Patients. *Clinical Cancer Research* **17**, 5725–5735 (2011).

185. de Vries, I. J. M. *et al.* Effective Migration of Antigen-pulsed Dendritic Cells to Lymph Nodes in Melanoma Patients Is Determined by Their Maturation State12. *Cancer Research* **63**, 12–17 (2003).

186. Approved Cellular and Gene Therapy Products. *FDA* https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products (2024).

187. Higano, C. S. *et al.* Real-world outcomes of sipuleucel-T treatment in PROCEED, a prospective registry of men with metastatic castration-resistant prostate cancer. *Cancer* **125**, 4172–4180 (2019).

188. *Vaccine Therapy in Treating Patients With Metastatic Prostate Cancer That Has Not Responded to Hormone Therapy*. https://www.clinicaltrials.gov/study/NCT00005947.

189. Duke University. *A Safety and Feasibility Study of Active Immunotherapy in Patients With Metastatic Prostate Carcinoma Using Autologous Dendritic Cells Pulsed With RNA Encoding Prostate Specific Antigen, PSA*. https://clinicaltrials.gov/study/NCT00004211 (2013).

190. Heiser, A. *et al.* Autologous dendritic cells transfected with prostate-specific antigen RNA stimulate CTL responses against metastatic prostate tumors. *J Clin Invest* **109**, 409–417 (2002).

191. Fucikova, J. *et al.* Phase I/II trial of dendritic cell-based active cellular immunotherapy with DCVAC/PCa in patients with rising PSA after primary prostatectomy or salvage radiotherapy for the treatment of prostate cancer. *Cancer Immunol Immunother* **67**, 89–100 (2018).

192. Podrazil, M. *et al.* Phase I/II clinical trial of dendritic-cell based immunotherapy (DCVAC/PCa) combined with chemotherapy in patients with metastatic, castration-resistant prostate cancer. *Oncotarget* **6**, 18192–18205 (2015).

193. EudraCT Number 2015-004314-15 - Clinical trial results - EU Clinical Trials Register. https://www.clinicaltrialsregister.eu/ctr-search/trial/2015-004314-15/results.

194. Kongsted, P. *et al.* Dendritic cell vaccination in combination with docetaxel for patients with metastatic castration-resistant prostate cancer: A randomized phase II study. *Cytotherapy* **19**, 500–513 (2017).

195. Frank, M. O. *et al.* Dendritic cell vaccines containing lymphocytes produce improved immunogenicity in patients with cancer. *J Transl Med* **12**, 338 (2014).

196. Frank, M. O. *et al.* Harnessing Naturally Occurring Tumor Immunity: A Clinical Vaccine Trial in Prostate Cancer. *PLOS ONE* **5**, e12367 (2010).

197. Rockefeller University. *A Phase I/II Study of Autologous Dendritic Cells Pulsed With Apoptotic Tumor Cells (DC/PC3) Administered Subcutaneously to Prostate Cancer Patients.* https://clinicaltrials.gov/study/NCT00345293 (2016).

198. Westdorp, H. *et al.* Blood-derived dendritic cell vaccinations induce immune responses that correlate with clinical outcome in patients with chemo-naive castration-resistant prostate cancer. *J Immunother Cancer* **7**, 302 (2019).

199. Wood, L. V. *et al.* TARP vaccination is associated with slowing in PSA velocity and decreasing tumor growth rates in patients with Stage D0 prostate cancer. *OncoImmunology* **5**, e1197459 (2016).

200. Protocol Details: A Pilot Study of Long Term TARP Vaccination Using A Multi-Epitope TARP Peptide Autologous Dendritic Cell Vaccination in Previously Vaccinated Men on NCI 09-C-0139. *NIH Clinical Center* https://clinicalstudies.info.nih.gov/protocoldetails.aspx?id=15-C-0076.

201. Thomsen, L. C. V. *et al.* A phase I prospective, non-randomized trial of autologous dendritic cell-based cryoimmunotherapy in patients with metastatic castration-resistant prostate cancer. *Cancer Immunol Immunother* **72**, 2357–2373 (2023).

202. Tryggestad, A. *et al.* Long-term first-in-man Phase I/II study of an adjuvant dendritic cell vaccine in patients with high-risk prostate cancer after radical prostatectomy. *The Prostate* **82**, (2021).

203. Mu, L. J. *et al.* Immunotherapy with allotumour mRNA-transfected dendritic cells in androgen-resistant prostate cancer patients. *Br J Cancer* **93**, 749–756 (2005).

204. Sonpavde, G. *et al.* A phase I study of BPX-201 vaccine plus AP1903 for chemo-naive metastatic castrate-resistant prostate cancer (mCRPC). *JCO* **32**, TPS3132–TPS3132 (2014).

205. Shanghai Humantech Biotechnology Co. Ltd. *A Multicenter, Non-Randomized, Open-Label, and Dose-Escalation Phase I Study to Evaluate the Safety of Prodencel Treatment in Patients With Metastatic Castration-Resistant Prostate Cancer (mCRPC)*. https://clinicaltrials.gov/study/NCT05533203 (2023).

206. H. Lee Moffitt Cancer Center and Research Institute. *A Phase II Randomized Study of Sipuleucel-T With or Without Continuing New Hormonal Agents (NHA) in Metastatic Prostate Cancer With PSA Progression While on NHA and LHRH Analog*. https://clinicaltrials.gov/study/NCT05751941 (2024).

207. University of Oklahoma. *Pilot Trial to Investigate Immune Response to an Extended Course of Sipuleucel-T Immunotherapy in Patients With Metastatic Castration-Resistant Prostate Cancer (OU-SCC-EXCITE)*. https://clinicaltrials.gov/study/NCT05806814 (2024).

208. Rahma, O. E. *et al.* A gynecologic oncology group phase II trial of two p53 peptide vaccine approaches: subcutaneous injection and intravenous pulsed dendritic cells in high recurrence risk ovarian cancer patients. *Cancer Immunol Immunother* **61**, 373–384 (2012).

209. Gray, H. J. *et al.* Progression-free and overall survival in ovarian cancer patients treated with CVac, a mucin 1 dendritic cell therapy in a randomized phase 2 trial. *J Immunother Cancer* **4**, 34 (2016).

210. Maeng, H. M. *et al.* Phase I Clinical Trial of an Autologous Dendritic Cell Vaccine Against HER2 Shows Safety and Preliminary Clinical Efficacy. *Front Oncol* **11**, 789078 (2021).

211. Rob, L. *et al.* Safety and efficacy of dendritic cell-based immunotherapy DCVAC/OvCa added to first-line chemotherapy (carboplatin plus paclitaxel) for epithelial ovarian cancer: a phase 2, open-label, multicenter, randomized trial. *J Immunother Cancer* **10**, e003190 (2022).

212. Cibula, D. *et al.* Dendritic cell-based immunotherapy (DCVAC/OvCa) combined with second-line chemotherapy in platinum-sensitive ovarian cancer (SOV02): A randomized, open-label, phase 2 trial. *Gynecologic Oncology* **162**, 652–660 (2021).

213. Tan, T. J. *et al.* A phase I study of an adenoviral vector delivering a MUC1/CD40-ligand fusion protein in patients with advanced adenocarcinoma. *Nat Commun* **13**, 6453 (2022).

214. Khan, J. A., Yaqin, S. & Deswal, M. A Pilot Study to Analyse the Effectiveness of Dendritic Cell Immunotherapy in Ovarian Cancer. *J Oncol Res Rev Rep* 1–4 (2023) doi:10.47363/JONRR/2023(4)167.

215. Czerlanis, C. M. *et al.* A study of mature alpha-DC-1 vaccine to induce immune responses in ovarian cancer. *JCO* **33**, e14033–e14033 (2015).

216. Imhof, M. *et al.* Double-loaded mature dendritic cell (DC) therapy for non-HLA-restricted patients with advanced ovarian cancer: Final results of a clinical phase I study. *JCO* **31**, 3052–3052 (2013).

217. Corr, B. *et al.* Randomized phase 2 trial of personal dendritic cell (DC)-autologous tumor antigen (ATA) vaccines in newly diagnosed advanced ovary cancer. *JCO* **41**, 5560–5560 (2023).

218. Tanyi, J. L. *et al.* Personalized cancer vaccine effectively mobilizes antitumor T cell immunity in ovarian cancer. *Science Translational Medicine* **10**, eaao5931 (2018).

219. Block, M. S. *et al.* Th17-inducing autologous dendritic cell vaccination promotes antigen-specific cellular and humoral immunity in ovarian cancer patients. *Nat Commun* **11**, 5173 (2020).

220. EudraCT Number 2020-003166-39 - Clinical trial protocol - EU Clinical Trials Register. *Clinical Trials Register* https://www.clinicaltrialsregister.eu/ctr-search/trial/2020-003166-39/LT.

221. Avigan, D. *Vaccination of Patients With Ovarian Cancer With Dendritic Cell/Tumor Fusions With GM-CSF and Imiquimod*. https://clinicaltrials.gov/study/NCT00799110 (2023).

222. Radboud University Medical Center. *Induction of Neo-Antigen Specific Cytotoxic T Cells by Autologous Tumor Lysate-Loaded Specialized Cross-Presenting Dendritic Cells in Epithelial Ovarian Cancer Patients Treated With Neoadjuvant Chemotherapy, the NEODOC Study*. https://clinicaltrials.gov/study/NCT05773859 (2023).

223. Mayo Clinic. *FRαDCs Plus Pembrolizumab for Patients With Advanced Stage Ovarian Cancer*. https://clinicaltrials.gov/study/NCT05920798 (2023).

224. University Hospital, Antwerp. *First-in-Human Interleukin-15-Transpresenting Wilms' Tumor Protein 1-Targeting Autologous Dendritic Cell Vaccination in Cancer Patients*. https://clinicaltrials.gov/study/NCT05964361 (2024).

225. Sarivalasis, D. A. *Phase I/II Study to Test the Immunogenicity, Feasibility, and Safety of Autologous PEP-DC Vaccine vs. Autologous OC-DC Vaccine Followed by PEP-DC Vaccine, in Combination With Low-Dose Cyclophosphamide, in Patients With Advanced HGSOC*. https://clinicaltrials.gov/study/NCT05714306 (2023).

226. Mayoux, M. *et al.* Dendritic cells dictate responses to PD-L1 blockade cancer immunotherapy. *Science Translational Medicine* **12**, eaav7431 (2020).

227. Duraiswamy, J. *et al.* Myeloid Antigen-Presenting Cell Niches Sustain Antitumor T Cells and License PD-1 Blockade via CD28 Costimulation. *Cancer Cell* **39**, 1623-1642.e20 (2021).

228. Zahm, C. D., Moseman, J. E., Delmastro, L. E. & G. Mcneel, D. PD-1 and LAG-3 blockade improve anti-tumor vaccine efficacy. *Oncoimmunology* **10**, 1912892.

229. Wan, C. *et al.* Enhanced efficacy of simultaneous PD-1 and PD-L1 immune checkpoint blockade in high grade serous ovarian cancer. *Cancer Res* **81**, 158–173 (2021).

230. Nagai, H. & Karube, R. Late-Stage Ovarian Cancer With Systemic Multiple Metastases Shows Marked Shrinkage Using a Combination of Wilms' Tumor Antigen 1 (WT1) Dendritic Cell Vaccine, Natural Killer (NK) Cell Therapy, and Nivolumab. *Cureus* **16**, e56685 (2024).

231. Hensler, M. *et al.* Peripheral gene signatures reveal distinct cancer patient immunotypes with therapeutic implications for autologous DC-based vaccines. *OncoImmunology* **11**, 2101596 (2022). **Review.**

232. Hu, R., Han, Q. & Zhang, J. STAT3: A key signaling molecule for converting cold to hot tumors. *Cancer Letters* **489**, 29–40 (2020).

233. Nefedova, Y. *et al.* Activation of dendritic cells via inhibition of Jak2/STAT3 signaling. *J Immunol* **175**, 4338–4346 (2005).

234. Fucikova, J. *et al.* An Autologous Dendritic Cell Vaccine Promotes Anticancer Immunity in Patients with Ovarian Cancer with Low Mutational Burden and Cold Tumors. *Clinical Cancer Research* **28**, 3053–3065 (2022).

235. Vitale, I., Shema, E., Loi, S. & Galluzzi, L. Intratumoral heterogeneity in cancer progression and response to immunotherapy. *Nat Med* **27**, 212–224 (2021). **Review.**

236. Papaevangelou, E., Esteves, A. M., Dasgupta, P. & Galustian, C. Cyto-IL-15 synergizes with the STING agonist ADU-S100 to eliminate prostate tumors and confer durable immunity in mouse models. *Front Immunol* **14**, 1196829 (2023).

237. Ding, L. *et al.* STING agonism overcomes STAT3-mediated immunosuppression and adaptive resistance to PARP inhibition in ovarian cancer. *J Immunother Cancer* **11**, e005627 (2023).

238. Shevyrev, D., Tereshchenko, V., Berezina, T. N. & Rybtsov, S. Hematopoietic Stem Cells and the Immune System in Development and Aging. *Int J Mol Sci* **24**, 5862 (2023). **Review.**

239. Ren, Y. *et al.* Single-cell sequencing reveals effects of chemotherapy on the immune landscape and TCR/BCR clonal expansion in a relapsed ovarian cancer patient. *Front Immunol* **13**, 985187 (2022).

240. Xu, P. *et al.* Androgen receptor blockade resistance with enzalutamide in prostate cancer results in immunosuppressive alterations in the tumor immune microenvironment. *J Immunother Cancer* **11**, e006581 (2023).

241. Bakhshi, P. *et al.* Impaired monocyte-derived dendritic cell phenotype in prostate cancer patients: A phenotypic comparison with healthy donors. *Cancer Rep (Hoboken)* **7**, e1996 (2024).

242. Paul, S. *et al.* HLA class I alleles are associated with peptide binding repertoires of different size, affinity and immunogenicity. *J Immunol* **191**, 10.4049/jimmunol.1302101 (2013).

243. Tourdot, S. *et al.* A general strategy to enhance immunogenicity of low-affinity HLA-A2.1-associated peptides: implication in the identification of cryptic tumor epitopes. *European Journal of Immunology* **30**, 3411–3421 (2000).

244. McNeel, D. G. *et al.* Phase 2 trial of a DNA vaccine (pTVG-HP) and nivolumab in patients with castration-sensitive non-metastatic (M0) prostate cancer. *J Immunother Cancer* **11**, e008067 (2023).

245. Cheng, S. *et al.* Artificial Mini Dendritic Cells Boost T Cell–Based Immunotherapy for Ovarian Cancer. *Advanced Science* **7**, 1903301 (2020).