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REVIEW

The subtle interplay between gamma delta T lymphocytes and dendritic cells: is there a role for a therapeutic cancer vaccine in the era of combinatorial strategies?

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Abstract

Human gamma delta (γδ) T cells represent heterogeneous subsets of unconventional lymphocytes with an HLA-unrestricted target cell recognition. γδ T cells display adaptive clonally restricted specificities coupled to a powerful cytotoxic function against transformed/injured cells. Dendritic cells (DCs) are documented to be the most potent professional antigen-presenting cells (APCs) able to induce adaptive immunity and support the innate immune response independently from T cells. Several data show that the cross-talk of γδ T lymphocytes with DCs can play a crucial role in the orchestration of immune response by bridging innate to adaptive immunity. In the last decade, DCs, as well as γδ T cells, have been of increasing clinical interest, especially as monotherapy for cancer immunotherapy, even though with unpredictable results mainly due to immune suppression and/or tumor-immune escape. For these reasons, new vaccine strategies have to be explored to reach cancer immunotherapy's full potential. The effect of DC-based vaccines on γδ T cell is less extensively investigated, and a combinatorial approach using DC-based vaccines with γδ T cells might promote a strong synergy for long-term tumor control and protection against escaping tumor clones. Here, we discuss the therapeutic potential of the interaction between DCs and γδ T cells to improve cancer vaccination. In particular, we describe the most relevant and updated evidence of such combinatorial approaches, including the use of Zoledronate, Interleukin-15, and protamine RNA, also looking towards future strategies such as CAR therapies.

Keywords Dendritic cells · γδ T cells · DC-based vaccines · Cancer immunotherapies · Combinatorial strategies

Abbreviations

AML	Acute myeloid leukemia
αβ	Alfa beta
ADCC	Antibody-dependent cellular cytotoxicity
APC	Antigen-presenting cells
BrHPP	Bromohydrin pyrophosphate
CAR	Chimeric antigen receptor
CRC	Colorectal cancer
cDCs	Conventional dendritic cells
CXCL10	C–X–C motif chemokine 10
DCs	Dendritic cells
DNAM-1	DNAX accessory molecule-1
γδ	Gamma delta
Fcγ	Receptors Fc portion of G immunoglobulins
GVHD	Graft Versus Host Disease
HCC	Hepatocellular carcinoma
IFN-γ	Interferon-γ
IL	Interleukin
iNKT	Invariant natural killer T cells
iDCs	Immature dendritic cells
IPP	Isopentenyl pyrophosphate
mDCs	Mature dendritic cells
MIC	MHC class I chain related
MDSC	Myeloid-derived suppressor cells
NK	Natural killer cells
NKRs	Natural killer receptors

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NKG2D	Natural killer group 2 member D
NSCLC	Non-small cell lung cancer
OK-432	Picibanil
PBMC	Peripheral blood mononuclear cells
pAgs	Phosphoantigens
pDCs	Plasmacytoid dendritic cells
pRNA	Protamine-RNA
RCC	Renal cell carcinoma
TCR	T cell receptors
TEM	T Effector memory
TRAIL	TNF-related apoptosis-inducing ligand
TLR	Toll-like receptors
TAA	Tumor-associated antigen
TILs	Tumor-infiltrating lymphocytes
TME	Tumor microenvironment
TNF-α	Tumor Necrosis Factor-α
ULBP	UL16-binding protein
VEGF	Vascular Endothelial Growth Factor
WT1	Wilms' tumor 1
ZOL	Zoledronate

Introduction

Human gamma delta (γδ) cells represent a small subset of unconventional T lymphocytes (1–10% of the blood circulating T cells) expressing T cell receptors (TCR). γδ T cells functionally bridge innate and adaptive immunity, playing a protective and unique role in immune surveillance [1, 2]. Notably, unlike alfa beta (αβ) T cells, γδ T lymphocytes recognize pathogens and cancer cells in an HLA-unrestricted manner and display an extraordinary cytotoxic capacity to kill transformed cells [1, 2]. Based on TCR arrangement and cellular functions, these cells are broadly classified into two main subgroups, namely Vδ2-positive and Vδ2-negative T cells, according to their Vδ chain expression.

The Vδ2-positive, paired to the Vγ9 chain (Vγ9Vδ2 T cells), subset represents a significant fraction of γδ T cells circulating in the lymph nodes and peripheral blood (1–5% in healthy adults). Targets of Vγ9Vδ2 T cells are non-protein pyrophosphate metabolites, named phosphoantigens (pAgs), arising either from the host mevalonate (MVA) pathway (isopentenyl pyrophosphate, IPP) or the microbial non-mevalonate Rohmer pathway ((E)-4-Hydroxy-3-methylbut-2-enyl pyrophosphate, HMB-PP) [3]. An essential prerequisite for pAgs recognition is the surface expression by target cells of butyrophilin (BTN) BTN3A1 [4]. The binding of pAgs to the cytosolic BTN3A1 B30.2 domain induces a structural change of BTN3A1 intracellular and extracellular domains essential for γδ TCR-mediated responses [5, 6]. However, several studies have demonstrated that BTN3A1 is necessary but not sufficient for pAgs sensing. In this context, the ligand BTN2A1 has been identified as a critical factor in pAgs recognition by γδ T cells [7]. In particular, Vγ9Vδ2 T cells recognize pAgs according to the contextual cell surface expression of butyrophilin 2A1 (BTN2A1) and BTN3A [3, 7]. Mainly, BTN2A1 directly binds the Vγ9 regions of the T cell receptor (TCR), while BTN3A1 binds the Vδ2 regions of the same TCR [7].

Unlike Vδ2-positive, Vδ2-negative subtypes, such as Vδ1 and Vδ3, are more represented in tissues than in peripheral blood [2, 8]. Most of the Vδ1 cells encompass intraepithelial lymphocytes of the mucosal epithelia. They respond to stress signals by secreting chemo-cytokines promoting mononuclear cell infiltration into damaged, infected, or malignant epithelium. The $V\delta3^+$ subsets are enriched in the liver and gut and are implicated in response to herpes virus infections such as cytomegalovirus and Epstein–Barr virus [8]. The acknowledged complexity of γδ T cells is evolving about the different subsets discovered and new functional subtypes. While some γδ subsets can carry out robust anti-tumor immune response, some other subtypes are unexpected drivers of tumor development and progression since their functions are often impaired by immunosuppressive signals originating from the tumor microenvironment (TME). In particular, TME may polarize γδ T cell to produce Interleukin (IL)-17, which is associated with a series of tumor-promoting functions such as several roles, including stimulation of tumor cell proliferation, induction of angiogenesis, and mobilization of pro-inflammatory or immunosuppressive myeloid cells [9, 10]. In this context, γδ T cells producing IL-17 are related to poor outcome in patients affected by gallbladder and colon cancers, while frequencies of either total γδ T cells or specially γδ T cells expressing IFN-γ are associated with better patient survival in colon cancers and cutaneous squamous cell carcinoma [11, 12].

Dendritic cells (DCs) are recognized to be the most potent professional antigen-presenting cells (APCs). In humans, blood circulating DCs consist of $CD11C^+$ conventional DCs (cDCs), containing cDC2 $CD1C^+$, cDC1 $CD141^+$ cells, and $CD11C^-$ plasmacytoid DCs (pDCs), which include $CD123^+$ and $CD303^+$ cells [13, 14]. There is growing evidence that either quantitative reduction or dysfunction of DCs are features shared by different diseases, including neoplasms [15–18]. In this regard, mature DCs (mDCs) play a crucial role in the host immune response against cancer by sensing the effector cells of adaptive immunity. Likewise, DCs can orchestrate adaptive anti-cancer immune responses by the cross-presentation of tumor-associated antigen (TAA) to T lymphocytes [19–21]. The tumor microenvironment can negatively affect DC maturation and activity, inducing a tolerogenic functional condition leading to an impaired anti-tumor immune response. Therefore, a successful DC-based cancer vaccine needs an effective TAA cross-presentation accomplished by co-stimulatory molecules to overcome the immunosuppressive tumor milieu [19–21]. In particular, a

cancer vaccination must exploit the DCs' ability to infiltrate tumor lesions and eliminate malignant cells by activating T lymphocytes and natural killer (NK) cells [19–21]. In the last decade, DCs have emerged as a promising tool for cancer immunotherapy, which has favored the development of increasingly effective clinical protocols [22]. Nevertheless, the effect of DC-based vaccines on γδ T cell functions has been less extensively investigated.

The reciprocal interplay between γδ T cells and DCs

Several pieces of evidence support that the cross-talk of γδ T lymphocytes with DCs and other effectors can play a crucial role in the orchestration of host protection by bridging innate to adaptive immunity [23].

Specifically, γδ T cells, like the innate immune cells, can identify infected and/or transformed cells through a set of ligands different from Toll-like receptors (TLR) [24]; upon activation, they elicit a cytolytic activity along with a release of a large number of cytokines [25, 26]. In this context, γδ T cells also induce DCs maturation by the combination of cytokines, including Interferon-gamma (IFN-γ), Tumor Necrosis Factor-alfa (TNFα), and cell contact-dependent signals such as Fas-FasL, and CD40-CD40L [27]. Therefore, γδ T cells, either alone or in synergy with pathogen products, can prompt DCs maturation leading to an immunogenic APCs expressing co-stimulatory molecules that can produce cytokines and stimulate T cells [25, 26]. As a consequence, mature DCs, in turn, activate γδ T cells by inducing their proliferation and enhancing their cytotoxic and immune-regulatory functions [28–30]. Specifically, DCs produce cytokines such as Interleukin (IL)-1β, IL-12, IL-18, TNF-α, and type I IFNs acknowledged to stimulate γδ T cells [28, 31–33]. Moreover, DCs can trigger contact-dependent activation of γδ T cells through CD86–CD28 interactions and by the expression of γδ T cell-activating ligands, such as Lipid A and pAgs [34, 35]. As a result of these complex cross-interactions, γδ T cells regulate maturation of DCs that, in turn, increase innate responses and stimulate adaptive immunity.

Overall, these observations suggest that the interplay between DCs and γδ T lymphocytes represents a necessary critical process to generate an integrated immune response towards cancer cells. Consequently, DC–γδ T interaction (Fig. 1) may represent a therapeutically actionable interface to counteract tumor-induced immune escape. Hereunder, we discuss the therapeutic potential of targeting DCs and γδ T cells interaction pathways to improve DC-based cancer vaccination strategies.

γδ T cells and cancer

Effector functions of γδ T cells

γδ T cells boost the anti-cancer immune response against a broad range of solid tumors and hematological neoplasms. In particular, tumor cells recognition by γδ T cells relies on the

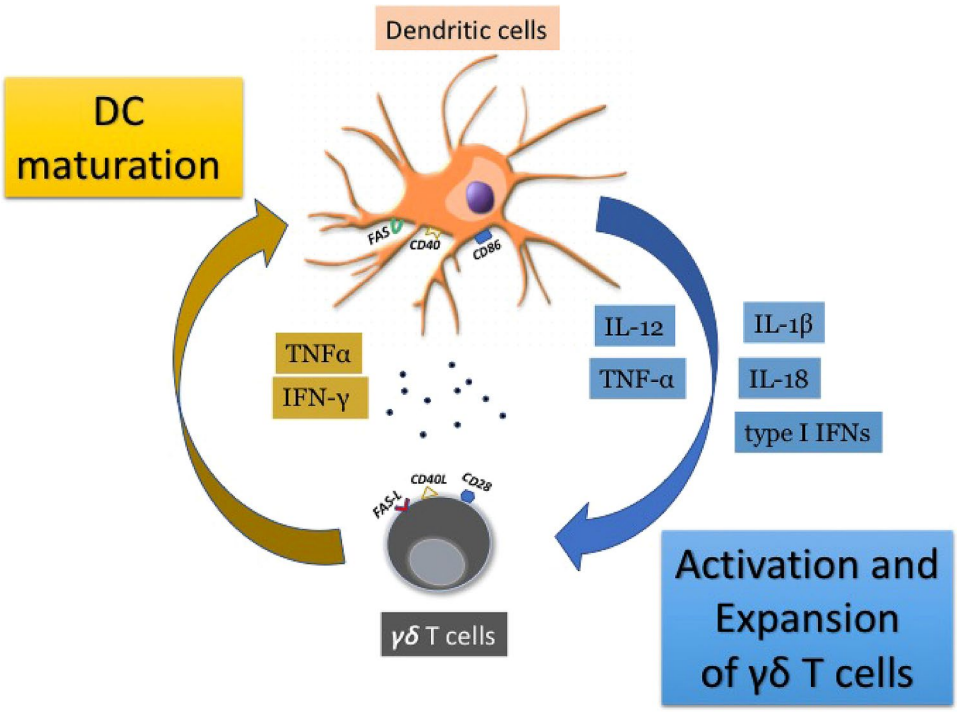

Fig. 1 The interplay between dendritic cells and γδ T cells. Schematic representation of the reciprocal interactions between DCs and gamma delta T cell with related opportunities for improvement of DC-based cancer vaccinations' anti-tumor efficacy. *CCR5* C–C chemokine receptor type 5, *IFN* Interferon, *IL* Interleukins, *TNF* Tumor Necrosis Factor, *TAA* tumor antigens

engagement of their TCR and/or natural killer cell receptors (NKRs) [36]. The main targets of γδ TCR comprise pAgs that are mostly metabolic intermediates of the isoprenoid biosynthesis, including IPP, endo- and exogenous lipids, presented by CD1-receptors, and stress-induced molecules displayed upon DNA damage, viral infection products, or transformation-related molecules [37]. On the other hand, the main targets of both Vδ1$^+$ and Vδ2$^+$ T cells, expressing natural-killer group 2 member D (NKG2D) receptors, are tumor cell ligands such as the MHC class I chain-related (MIC-A/B) [38, 39] or the UL16-binding protein (ULBP) 1–6 [40–43]. In this regard, increased frequencies of infiltrating Vδ1$^+$ T cells can mediate cytotoxicity in MICA or MICB positive lung, breast, kidney, ovary, and prostate human carcinoma cells [38, 39]. Likewise, DNAX accessory molecule-1 (DNAM-1) and NKG2D expressed by Vδ2$^+$ T cells are critical for recognition and lysis of hepatocellular carcinoma (HCC) and acute and chronic myeloid leukemia cells [44–46]. γδ2 T cell-mediated killing of tumor cells involves death receptors such as Fas/FasL, TNF/TNF-R, and TNF-related apoptosis-inducing ligand (TRAIL)-TRAIL-R or the perforin/granzyme pathways [47, 48]. In addition, γδ T cells can exploit antibody-dependent cellular cytotoxicity (ADCC) to kill cancer cells through the expression of CD16, which is a receptor that binds to the Fc portion of G immunoglobulins (Fcγ receptors) on the target cells [49, 50].

A further strategy adopted by Vγ9Vδ2 T cells is to indirectly enhance anti-tumor activities through the secretion of pro-inflammatory cytokines such as IFN-γ and TNF-α and to inhibit cancer angiogenesis [12]. In this respect, DCs can strengthen the IFN-γ and TNF-α secretion from human γδ T cells by releasing IL-12 or IL-18 [51]. Interestingly, investigation on different neoplasms has unveiled that γδ T cells are part of tumor-infiltrating lymphocytes (TILs) pool. Their presence represents a robust prognostic biomarker of favorable survival [52]. In particular, an analysis of 585 patients with colorectal cancer (CRC) has demonstrated that a high number of γδ T cells, with reduced production of IFN-γ, within TILs is associated with the probability of 5-year disease-free survival [12].

Regulatory functions of γδ T cells

Recent studies unexpectedly report that TIL γδ cells might also elicit a pro-tumor role, fostering non-cytotoxic inflammation and regulatory functions that subvert anti-tumor immunity and promote tumorigenesis [53]. In this regard, the presence of TIL γδ T cells in breast cancer positively correlates with advanced tumor stages and is inversely associated with patients' survival [53]. New reports have identified a subset of γδ T cells capable of secreting IL-17, a cytokine correlated with metastatic dissemination and poor prognosis in different neoplasms. In line with these data, high numbers of TIL γδ Th17$^+$ cells have also been associated with advanced tumor stages in cancer patients [11, 54, 55]. Notably, an experimental study based on a murine model of HCC has established that γδ Th17$^+$ cells enhance myeloid-derived suppressor cells (MDSC) infiltration to tumor sites, favoring the immune suppression of cytotoxic anti-cancer response [56]. Similar mechanisms are observed in human CRC, where the Vδ1$^+$ T cells are the primary microenvironmental source of IL-17, contributing alongside MDSC to CRC's development and progression [54]. Accordingly, data obtained on breast cancer murine models have shown that γδ Th17$^+$ can induce the expansion and polarization of neutrophils capable of suppressing cytotoxic CD8$^+$ T function, favoring cancer cells spread [57]. Finally, IL-17 in TME can promote tumor development, stimulating angiogenesis through the production of Vascular Endothelial Growth Factor (VEGF), IL-6, and IL-8 [58].

γδ T cells can also play a regulatory role (γδ Treg) in the harmful modulation of anti-tumor immune responses. As an example, it has been shown that the tumor-derived C-X-C motif chemokine 10 (CXCL10) (also known as IP-10) as secreted by human breast cancer cells increases the expansion of a dominant Vδ1 cell population among TIL. Vδ1 can suppress naive and effector T cells and inhibit DCs maturation and function, resulting in an immunosuppressive TME [59, 60]. Furthermore, recent data have demonstrated that γδ Treg derived from breast cancer tissues can induce immunesenescence in both effector T cell and DCs, turning them into suppressive cells with reduced functions and altered phenotypes [61].

Considering these findings, strategies aimed to boost the anti-tumor activities of γδ T cells, instead of their suppressive properties, need to be developed.

Supplementary Figure shows the effector and regulatory functions of γδ T cells, describing the cross-talk between cancer microenvironment and immune cells.

γδ T cells in cancer immunotherapy

Several clinical trials have tested the use of γδ T cells for cancer immunotherapy, given their relatively high frequency in peripheral blood and their capacity to be selectively activated by the use of specific agents. γδ T cell-based cancer immunotherapy can be classified into two categories according to in vivo and ex vivo approaches [9].

First, in vivo strategies rely on γδ T cells activation through systemic administration of pAgs or bisphosphonates. Other utilized compounds include *N*-bis (zoledronate, pamidronate) and synthetic pAgs (bromohydrin pyrophosphate BrHPP), especially in association with IL-2. Several clinical studies have employed this approach in solid tumors and hematological malignancies [62]. In this regard, a phase

I clinical trial, performed in 18 patients with metastatic hormone-refractory prostate cancer, has evaluated the zoledronate's anti-tumor effects (ZOL) as a single agent vs. the combined administration of ZOL plus low-dose IL-2. Such a combinatorial approach has induced a significant and protracted expansion of activated γδ T effector memory (TEM) cells that can secrete IFN-gamma and perforin.

Moreover, the numbers of TEM γδ cells show a significant correlation with declining prostate-specific antigen levels and objective clinical outcomes [63]. BrHPP (IPH 1101) with low-dose IL-2, employed in two phase I clinical studies in patients with solid tumors, has proved that the combinatorial approach is safe, well-tolerated, inducing a significant expansion of γδ T lymphocytes without obtaining an objective clinical response [64, 65]. Successively, the anti-tumor efficacy and safety of zoledronic acid in combination with IL-2 has been evaluated in adults with advanced malignancies, refractory, or not eligible for standard therapeutic regimens. The clinical study has evidenced positive immune-modulatory effects without an objective clinical response in patients with solid tumors. Differently, two patients with acute myeloid leukemia (AML) have achieved a partial remission [66]. A small phase I clinical trial has recently demonstrated a significant increase in circulating γδ T cells, associated with a moderate expansion of NK cells, in four patients with refractory neuroblastoma treated with a combinatorial approach Zol plus subcutaneous IL-2 [67]. Wilhelm et al. have used in vivo-activated haploidentical γδ T cells for adoptive infusion in four patients with refractory hematological malignancies. Results showed a significant expansion of donor γδ T cells with no adverse effects, including Graft Versus Host Disease (GVHD). Interestingly, three patients obtained a complete remission, lasting even for 8 months [68].

The second strategy for γδ T cell-based cancer immunotherapy is the adoptive transfer of ex vivo expanded autologous γδ T cells through pAgs or *N*-bis. A pilot study has described the safety and feasibility of the adoptive transfer of γδ T cells combined with a low dose of recombinant IL-2 in advanced renal cell carcinoma (RCC) patients [69]. Further, a phase I/II trial, performed in 11 RCC patients with lung metastasis, has shown that the ex vivo-activated γδ T cells plus IL-2, and Zol enhances the frequency of circulating γδ T cells 3–5 days after the infusion, resulting in a potent cytotoxic activity in vitro and objective clinical responses in all patients [70]. Accordingly, the same combinatorial approach used in subjects with non-small cell lung cancer (NSCLC), refractory or intolerant to conventional treatments, has demonstrated that increasing administrations are safe and feasible, gradually inducing an expansion of circulating γδ T cells [71, 72]. Interestingly, data obtained in patients with advanced solid tumors have reported that radiolabeled γδ T cells trafficked predominately to the lungs, liver, and metastatic tumors sites [73]. Overall results from these clinical studies are summarized in Table 1.

Despite the encouraging results obtained in the pre-clinical setting, the clinical performance of γδ T cells and their use in cancer therapy may have been limited by their susceptibility to T cell hypo-responsiveness and the activation-induced γδ T cell exhaustion [74, 75]. On the other hand, functional limitations correlated with the challenge of (in vitro or in vivo expanded γδ T cells to reach and infiltrate tumors and counteract the immune-suppressive TME [74, 75]. Different immunotherapy approaches, which mainly involved DC-based vaccinations, have been pursued to overcome these limitations.

DC-based cancer immunotherapy

DC-based cancer vaccines have been applied against several malignancies (> 200 clinical trials) [76], including melanoma, prostate cancer, glioblastoma, and renal cell carcinoma [77]. In this regard, DC-vaccines can be subdivided into three distinct "generations." First generation vaccines relied on ex vivo-generated DCs, mainly obtained from peripheral blood mononuclear cells (PBMCs), monocyte-derived DC (MoDC), or $CD34^+$ hematopoietic progenitors. The ex vivo preparations were subsequently loaded with TAAs or mRNA encoding for TAAs [78]. First-generation DC vaccination was regarded as a safe and feasible therapeutic approach capable of mounting protective anti-tumor immunity. At the same time, the clinical success was limited, resulting in a tumor regression rate of only 3.3% in patients with cancers [76, 79]. The second-generation of DC-vaccines was matured and activated in the presence of specifically designed cytokine cocktails and pathogen-derived agonists to enforce the DC immunogenicity. Tumor antigens employed were mostly peptides from melanoma-associated antigens (e.g., MAGE antigens, MLANA, and gp100), Wilms tumor 1 (WT1), and NY-ESO-1 [31, 32] or tumor cell lysates [76]. Several phase I/II clinical trials performed with second-generation of DC-vaccines promoted both acquired and innate anti-cancer immunity. An objective clinical response was reported in 5–15% of cancer patients treated, while a trend to survival benefit was described in most studies [80]. Phase III clinical trials using MoDC-based cancer vaccination are ongoing for patients affected by uveal melanoma (NCT01983748) and metastatic colorectal cancer (NCT02503150). At the same time, no results are yet available for the concluded trial on castration-resistant prostate cancer (NCT02111577). Lastly, a large trial (NCT00045968) adding autologous tumor lysate-loaded MoDC vaccination (DCVax-L) to standard treatment of glioblastoma has demonstrated that such approach is safe and may extend the survival of patients [81].

Table 1 Clinical studies of gamma delta T cell-based cancer immunotherapy

Malignancy	Reference	Treatment	N of patients	Clinical outcome
Prostate cancer	Dieli et al. [54]	Zoledronate	9	SD 1, PR 1, PD 1 pts death 6 pts
		Zoledronate + IL-2	9	SD 4, PR 2, PD 1 pts death 2 pts
RCC	Bennouna et al. [55]	BrHPP + IL-2	10	SD 6, PD 4 pts
RCC Colon cancer Esophagus cancer Gastric cancer Ovarian cancer Breast cancer	Bennouna et al. [56]	BrHPP + IL-2	28	SD 12 pts PD 16 pts
RCC Melanoma AML	Kunzmann et al. [57]	Zoledronate + IL-2	21	PR in 2 AML patients
Hematological malignancies	Wilhelm et al. [59]	Zoledronate + IL-2	4	CR 3 pts
Neuroblastoma	Pressey et al. [58]	Zoledronate + IL-2	4	SD 1 pts PD 3 pts
RCC	Kobayashi et al. [61]	gd T cells plus Zoledronate + IL-2	7	SD 5, PD 5, CR 1 pts
NSCLC	Sakamoto et al. [62]	gd T cells plus Zoledronate + IL-2	15	SD 6, PD 6 pts
NSCLC	Nakajima et al. [63]	gd T cells plus Zoledronate + IL-2	10	SD 3, PD 5 pts
RCC Colon cancer Esophagus cancer Gastric cancer Ovarian cancer Breast cancer	Nicol et al. [64]	gd T cells plus Zoledronate	18	SD 3, PR 2, PD 11, CR 1 pts NE 1 pts

An organized summary of clinical trials performed against tumors

IL-2 Interleukin-2, *SD* stable disease, *PR* partial response, *PD* progression disease, *RCC* renal cell carcinoma, *BrHPP* bromohydrin pyrophosphate, *AML* acute myeloid leukemia, *CR* complete remission, *NSCLC* non-small cell lung carcinoma, *NE* not evaluable

The next-generation of DC-based vaccination has employed the naturally circulating DCs, suggesting that blood-derived specific subset of DCs may be superior for therapeutic immunization as compared to in vitro-generated DCs. Accordingly, intranodal injections of human pDCs activated and loaded with tumor antigen-associated peptides in 15 patients with metastatic melanoma have induced specific T cell responses [82]. In this context, two trials performed in patients affected by prostate cancer and melanoma have shown the safety and feasibility of blood-derived cDC2s loaded ex vivo with TAA peptides [83, 84]. Moreover, data obtained from melanoma patients demonstrated cytotoxic T cell responses which correlated with improved progression-free survival [84]. New clinical trials (NCT02993315, NCT02692976, NCT02574377, NCT03747744, and NCT03707808) are exploring the usage of pDCs and/or cDC2s in various cancer settings.

A different path to improve DC-vaccines consists of selecting the immunizing antigens such as tumor-specific neoantigens that are peptides derived from somatic mutations, absent in non-malignant cells, capable of stimulating an expansion of high-affinity $CD8^+$ T lymphocytes specific for the patient's neoplasm [85]. Accordingly, a phase I trial performed in three patients with advanced melanoma has revealed that DCs pulsed with high affinity, patient-specific, tumor-derived mutant peptides can induce neo-antigen-specific T cell response with some patients showing stabilized or non-recurrent disease [86].

In vivo

More recently, a distinctive alternative approach for cancer immunotherapy has been to deliver an Ag to DCs directly in vivo by chimeric proteins composed by an antigen (Ag) coupled to antibodies (Abs) specific to DC receptors and mixed with adjuvants to activate the immune system [87]. Among the most studied DC-targeting antibodies are those specific for c-type lectins such as DEC205, CLEC9A, and CLEC12A [88].

The principal advantage of in vivo-targeting strategies is the development of an off-the-shelf product. Many studies have demonstrated that in vivo DCs vaccination induces specific T cell immune response sufficiently robust and durable to be clinically active [89]. However, further research is necessary before clinical trials can be started.

Several causes have hampered the development and success of DC-based vaccines resulting in limited clinical benefits. The most relevant are: (1) the immunosuppressive

mechanisms establishing in the TME, especially in the advanced stage of cancer; (2) a decreased ability of systemically injected DCs to reach the tumor-draining lymph nodes; and (3) low strength of interaction among specific T cells and TAA loaded.

Consequently, the maximal benefits of DCs vaccines may be accomplished in combination with alternative cancer immunotherapies to circumvent the immunosuppressive TME and optimize DCs function in support of efficient anti-tumor immunity.

Dendritic cell-based immunotherapy empowers γδ T cell anti-tumor immunity

DCs have been reported to be potent stimulators of γδ T cells besides their role in triggering the adaptive immune response [90]. Similarly, activated γδ T cells can induce DCs maturation and activation, promoting T cell-dependent immune responses [34]. The earliest clinical trials performed in solid tumors or hematological neoplasms took advantage of amino bisphosphonates for the amplification of γδ T cells in vitro. However, an effective stimulation was observed in only about one-half of cancer patients [64, 91]. Cumulative evidence has revealed that DCs, stimulated by the bisphosphonate zoledronate, can acquire a strong activating-capacity towards γδ T cells [92]. In these studies, ZOL enhanced the immune-stimulatory ability of immature DCs (iDCs) and mDCs to provoke the rapid expansion of central memory and effector memory γδ T cells and to improve their anti-tumor efficiency. In addition, ZOL-treated DCs increased T cell numbers expressing CD62L, as the homing receptor for secondary lymphoid organs, and co-stimulatory molecules, including B7 family members [93, 94]. Accordingly, previous data have shown that iDCs, pulsed with TAAs alone or plus ZOL, activate Vγ9δ2T cells through the up-regulation of CD40L expression [95] and the secretion of Th1-cytokines, such as IFN-γ, thus promoting the expansion of tumor-antigen-specific $CD8^+$ T cells [95]. Moreover, Osada et al. analyzed the function of iDCs pulsed with ZOL. They showed that they could elicit an antigen-specific immunity similar to Picibanil (OK-432)-pulsed DCs [96].

Further clinical trials have adopted new protocols based on DC pretreatment with amino bisphosphonate to more efficiently expand γδ T cells. Notably, a clinical study performed in 20 patients with HCC and 22 patients with CRC with hepatic metastases has shown that co-cultures of PBMC/ZOL-pretreated DCs had high IPP levels, a metabolite of the mevalonate metabolic pathway likely involved in the PBMC activation. Of note, ZOL-treated DCs could powerfully activate Vγ9δ2 T cells as measured by their IFN-γ production [97]. Similarly, a phase I/II clinical trial was performed in three AML elderly patients with an injection of DCs pulsed with HLA-A*2402-restricted Wilms' tumor 1 (WT1) peptide and ZOL. Cancer immunotherapy induced detectable TAA-specific immune responses in two out of three AML patients, demonstrating the clinical feasibility of targeting γδ T cells in DC-based vaccination [98]. Table 2 summarizes results from these clinical studies.

IL-15 is a cytokine playing a pivotal role in regulating homeostasis and the activation of innate and adaptive immunity [99]. In this regard, the comparison between IL-15 DCs and conventional IL-4 DCs has demonstrated that IL-15 DCs possess a strong immunotherapeutic potential for DC-vaccines, expressing higher levels of chemokines involved in the attraction of anti-tumor immune effector cells such as γδ T cells with efficient cytotoxic function [100]. Accordingly, a recent study has shown that IL-15-DC-based vaccine is the primary mechanism behind enhancing the cytotoxic capacity of γδ T cell in AML patients [101]. In particular, IL-15 DCs up-regulates cytotoxicity-associated and co-stimulatory molecules, favoring the proliferation and the IFN-γ production by γδ T cells in the presence of neoplastic cells and phosphoantigens. Consequently, the secretion of soluble IL-15 can harness the anti-tumor activity of γδ T cells, supporting the idea of fulfilling IL-15 expressing DCs immunotherapy into future clinical trials [101] (Table 2).

Table 2 Clinical studies testing dendritic cells in combination with gamma delta T cells for the treatment of cancer

Malignancy	References	Treatment	N of patients	Results
HCC	Cabillic et al. [74]	PBMC co-culture with ZOL-pretreated DC	20	γδT cells immune response in vitro
mCRC			22	
AML	Kitawaki et al. [75]	ZOL plus WT1 pulsed DC	3	SD 1, PD 2 pts
AML	van Acker et al. [78]	IL-15 DC	4	γδT cells immune response in vitro
Melanoma	van Beek et al. [80]	pDC and cDC2 vaccination	6	SD 3, CR 1, MR 2 pts

A schematic list of combinatorial studies performed

HCC hepatocellular carcinoma, *mCRC* metastatic colorectal cancer, *PBMC* peripheral blood mononuclear cell, *ZOL* zoledronate, *DC* dendritic cells, *γδ* gamma delta, *AML* acute myeloid leukemia, *SD* stable disease, *PD* progression disease, *IL-15* Interleukin-15, *pDC* plasmacytoid dendritic cells, *cDC* conventional DC, *CR* complete remission, *MR* mixed response

Finally, recent clinical trials have also reported that protamine-RNA (pRNA) complexes can induce a full maturation of both pDCs and CD1c$^+$ DCs, via agonists of the Toll-like receptors (TLR) 7 and 8, respectively. These findings suggest that pRNA can be used as a stimulus in a vaccine consisting of both DC subsets with an expected improved efficacy [102]. In addition, there is evidence in melanoma patients that pRNA activation of pDCs and cDC2s allows the secretion of a wide array of chemokines to recruit different types of immune effector cells, including γδ T cells. Therefore, a cancer vaccine consisting of pDCs and CD1c$^+$ DCs could take advantage of the strong Th1 type chemoattractive properties of pDCs and the excellent T cell priming properties of CD1c$^+$ DCs [103] (Table 2). Figure 2 illustrates the combinatorial approaches based on DC vaccines and γδ T cells.

Future strategies

Autologous T cells engineered to express T cell receptors (TCR) or chimeric antigen receptors (CAR) prompt a strong T cell immune response, with a specific killing of target cells, providing an alternative approach to treat different tumors [104–106]. In particular, CAR-T cell immunotherapy directed against the pan-B cell antigen CD19 have shown high remission rates in acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL) patients leading to accelerated FDA approvals [107, 108]. Despite high levels of response, growing data have revealed that a substantial number of patients do not respond to CAR-T cells or experience short-lasting remission due to low CAR T cell persistence and tumor escape deriving from antigen loss [109, 110].

Consequently, new alternative strategies have been pursued, some of them focusing on γδ T lymphocytes, due to their innate capacity for anti-tumor effects. The human application of CAR$^+$ γδ T cells could be a better approach to safely use allogeneic CARs and target small clones with lower antigen density, which may not be eradicated by the standard CAR T cells [37, 111].

To date, numerous pre-clinical studies have appraised CAR-transduced γδ T cells; in particular, one study has reported that GD2-CAR- and CD19-CAR-engineered γδ T cells efficiently recognize antigen-expressing tumor cells, displaying specific effector functions such as the IFN-γ secretion and MHC unrestricted tumor cell lysis [112]. More

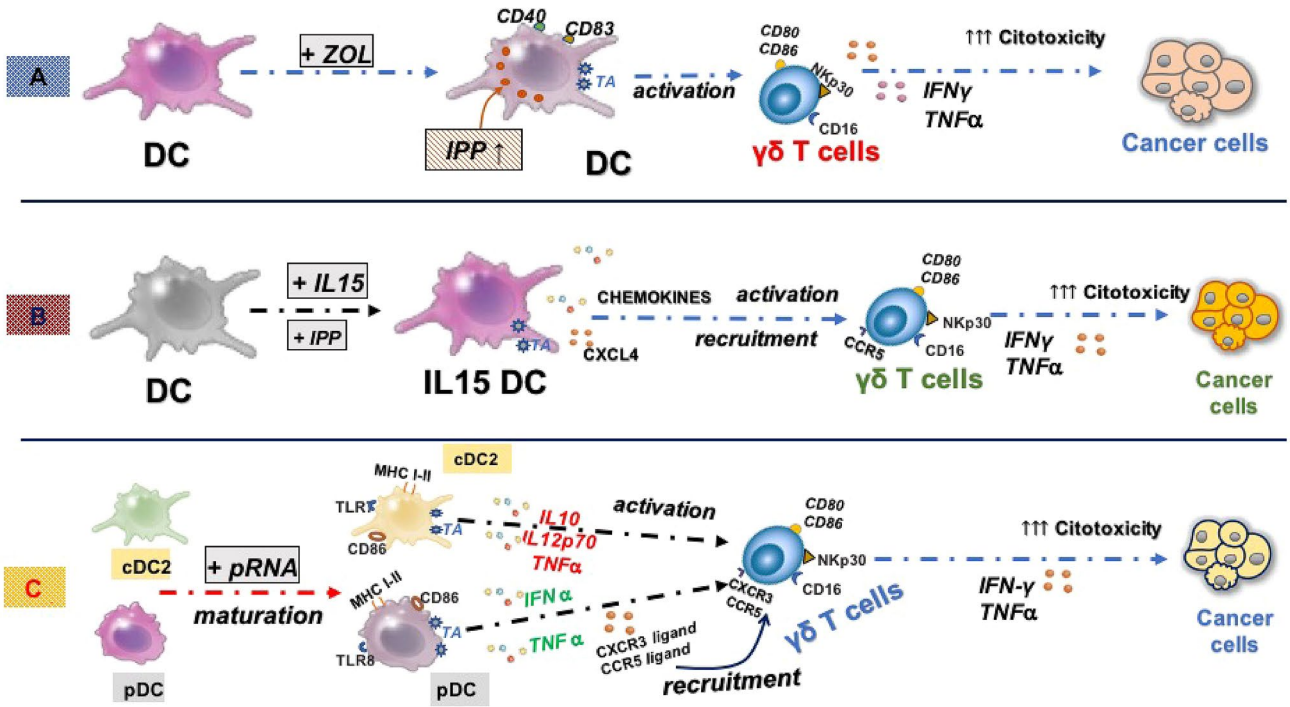

Fig. 2 Overview of combinatorial strategies based on DC-based vaccines and γδ T cells. The figure illustrates different combinatorial approaches to boost the immune effector response and counteract the immunosuppressive cancer microenvironment. Multiple agents such as zoledronate (**a**), Interleukin-15 (**b**), and protamine-RNA (**c**) can support an efficient anti-tumor immunity induced by γδ T cells. *CCR5* C–C chemokine receptor type 5, *CCXL4* C–X–C motif chemokine 4, *IFN* Interferon, *IL* Interleukins, *IPP* isopentenyl pyrophosphate, *MHC* major histocompatibility complex, *NKp30* natural cytotoxicity receptors, *pRNA* protamine-RNA, *TNF* Tumor Necrosis Factor, *TLR* Toll-like receptors, *TAA* tumor antigens

recently, aminobisphosphonate-pretreated Vγ9Vδ2 T cells could express CD19-CAR by the electroporation of a Sleeping Beauty transposon. Data obtained have shown that CAR$^+$ γδ T cells can secrete pro-inflammatory cytokines, resulting in anti-tumor cytotoxicity in both the in vitro and in vivo settings [112]. In this context, a recent study has employed a new approach for CAR$^+$ γδ T cells using GD2 antigen transduction. GD2 is a ganglioside widely expressed by neuroblastoma cells and several other cancer cells. Results demonstrate an increased cytotoxicity activity toward GD2-expressing cancer cell lines, suggesting a possible future therapeutic evaluation in solid tumors [113]. More recent data have shown CD19-CAR-engineered γδ T cells' efficacy against CD19 hematologic malignancies in vitro and in vivo. Notably, CD19-CAR γδ T cells keep the activity against leukemic clones lacking CD19 expression, avoiding the loss or cancer antigen modulation promoting relapse following CAR therapy [111].

An alternative and new strategy is based on transducing high-affinity Vγ9Vδ2 TCRs into αβ T cells of cancer patients, named termed T cells engineered with defined γδ TCRs (TEGs) [114, 115]. TEGs conjugate the ability to kill tumor cells by γδ TCR with high proliferation and memory features of conventional αβ T cells, overcoming the low persistence and impaired activation of γδ T cells in the tumor microenvironment. Notably, this strategy's attractive advantage is that selected Vγ9Vδ2 TCR are transduced in both CD8 effector and CD4 T helper cells, thereby enabling CD4 T cells to exert professional help by inducing a full maturation of DCs [114, 116]. The specific use of the new TEG could target a wide range of solid and hematological tumors. In this regard, TEGs have already entered in phase I clinical trials performed in patients with relapsed and refractory acute myeloid leukemia and multiple myeloma (NTR6541) [116].

Interestingly, a different approach has revealed that CAR transduction (pCCL-anti-CD33-4-1BB-CD3z-T2A-GFP) in DC precursors and peripheral T cells can enhance in vitro the anti-AML CAR T cytotoxicity [117].

These data may suggest that future strategies combining CAR-DC with CAR$^+$γδ T cells could further increase cancer immunotherapy's efficiency.

Conclusions

Several reports have described a new generation of DC-based vaccines with enhanced potency in the years. Most DC vaccination-related studies rely on the stimulation of antigen-specific immune cells, particularly CD8$^+$ T cells and Th1-polarized CD4$^+$ T cells [118]. Besides these prototypical immune effector cells, it is becoming well defined that DCs cross-talk with innate and innate-like immune cells may play a key role in cancer immune-therapy [27]. A fascinating immune-therapeutic strategy could fully exploit the anti-cancer potency of γδ T cells, considering that their activation is MHC unrestricted. Accordingly, ex vivo-matured DCs can induce in situ activation of NK, iNKT, and γδ T cells involved in cytotoxic activities against tumor cells [28–30].

Moreover, these effector cells can sustain immune system responses by prompting further activation of tissue DCs [34, 35]. Interestingly, innate immunity could play a vital role in the activation and polarization of adaptive immune responses, strongly supporting DC-based vaccines [119, 120]. The single cancer immunotherapy strategies have obtained unpredictable results, mainly due to immune suppression and cancer immune escape mechanisms [121]. For these reasons, new vaccine strategies warrant exploration to exploit the potential of cancer immune-therapy fully. The combinatorial approach using DC-based vaccines with γδ T cells might promote a more potent synergy for long-term tumor control and protection against escaping tumor variants [27]. However, even though pre-clinical data show that γδ T cells represent a therapeutic promise, the clinical performance of γδ T cells may be limited by the established plasticity of γδ T cells, particularly by their regulatory role upon interaction with TME, which could restrain the efficacy of immune-therapy [9, 122].

The future of cancer immune-therapy will likely rely on balancing these two critical aspects, namely empowering anti-tumor immune responses and overwhelming tumor-induced immune escape. A better comprehension of the complex interactions occurring in the TME, along with the modulation of γδ T cell function in combination with DC-based vaccines, will undoubtedly improve the design of really efficient and durable immunotherapeutic approaches for tumor disease control.

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Compliance with ethical standards

Conflict of interest All the other authors declare that they have no conflict of interest.

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