CELL & GENE THERAPY INSIGHTS

CELLULAR IMMUNO-ONCOLOGY: OVERCOMING MANUFACTURING & DEVELOPMENT OBSTACLES TO COMMERCIAL SUCCESS

SPOTLIGHT

COMMENTARY/OPINION

Towards the rational design of a next-generation dendritic cell vaccine for cancer immunotherapy

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As professional antigen presenting cells (APCs) capable of eliciting primary immune responses among naïve T cells, dendritic cells (DCs) offer an attractive target for immune intervention. While some strategies for vaccination have sought to deliver antigens direct to DCs *in vivo*, others have pulsed DCs with target antigens *ex vivo* prior to administration. Indeed, numerous clinical studies of cancer immunotherapy have been conducted over the past two decades based on this approach, most of them benefitting from the ease with which DCs may be differentiated *in vitro* from the peripheral blood monocytes of individual patients. Nevertheless, while therapies exploiting monocyte-derived DCs (moDCs) have been shown to be safe, clinical outcomes have been disappointing, efficacy having been limited by factors including the type of DCs used and the source of tumor antigens. Here we review recent developments in identifying DC subsets with more favorable properties for use in cancer vaccination, with particular emphasis on CD141^{+} DCs capable of antigen cross-presentation and discuss alternative sources, such as induced pluripotent stem cells (iPSCs), amenable to manufacture at scale. Furthermore, we assess how different sources of tumor antigens may complement this approach for the design of next generation DC vaccines.

Cell & Gene Therapy Insights 2021; 7(5), 637–650

DOI: 10.18609/cgti.2021.086

INTRODUCTION

Dendritic cells (DCs) are the most efficient antigen-presenting cells (APCs) for the activation of naïve T cells and play a critical role in initiating and regulating both innate and adaptive immune responses. Commonly referred to as 'nature's adjuvant', DCs have been considered attractive candidates for immunotherapy and have been used extensively for the treatment of a range of cancers [1], indeed, DC vaccines have been deployed against various malignancies in over 200 clinical trials, the four most targeted cancer types being melanoma (>1000 patients), prostate cancer (>750 patients), glioblastoma (GBM; >500 patients), and renal cell carcinoma (RCC; >250 patients) [2]. The extensive body of evidence obtained from these trials has shown that DC-based immunotherapy is safe and can induce anti-tumor immunity, both in patients with minimal residual disease following tumor resection and those at advanced stages of disease progression. Nevertheless, clinical responses have been disappointing, with objective response rates (ORRs) rarely exceeding 15% [3]. Furthermore, on the basis of a 4.1 month survival advantage and despite less than 5% of patients achieving an objective response, Sipuleucel-T (Provenge®) was approved by the US Food and Drug Administration in 2010 but was subsequently withdrawn from the market [4].

As other emerging immunotherapies such as immune checkpoint inhibitors and chimeric antigen receptor (CAR)-T cells have started delivering encouraging results, the interest in DC therapies has waned in recent years. At present, there is only a small number of Phase 3 trials underway in patients with advanced melanoma, glioma, and renal cell carcinoma which use overall survival as the primary endpoint [3]. Nevertheless, new clinical data and a reappraisal of existing evidence, have begun to shed new insights that are putting DC vaccines back in the spotlight.

THE RE-EMERGENCE OF DC VACCINES FOR CANCER TREATMENT

Anguille and colleagues have proposed that the assessment criteria typically used as the primary endpoint in most early trials of DC vaccines are suboptimal [3]. Typically, the primary endpoint used has been the classic response assessment criteria, such as the Response Evaluation Criteria in Solid Tumors (RECIST), which are based on a measure of tumor burden. However, Anguille *et al.* demonstrated that an increasing number of trials that had secondary endpoints for survival confirmed that DC therapy could confer a survival benefit. Specifically, an increase in median overall survival (OS) of at least 20% has been documented in most studies that had a secondary survival endpoint. Although many of these trials were early phase and not designed primarily to measure survival, the results obtained are nevertheless promising given that the bar for establishment of a clinically-meaningful improvement in median OS is generally set at 20% [3]. Interestingly, evidence is also accumulating that Sipuleucel-T may have had more efficacy in earlier stages of prostate cancer than previously appreciated [5]. Given that, in spite of the varying degree of success of chemotherapy, checkpoint inhibitors and cell-based therapies, a large fraction of patients remain unresponsive to intervention or are prone to relapse, there is renewed interest in exploring DC vaccination either alone or in combination with other forms of immune intervention, such as immune checkpoint inhibition [6].

As of April 2019, there were 20 ongoing clinical trials evaluating personalized DC-based vaccines, 11 of which used tumor lysates as a source of antigen [1]. Among these, there are several promising Phase 3 trials including one testing an autologous monocyte-derived DC (moDC) vaccine loaded with autologous tumor lysate (DCVaxL) in patients with newly diagnosed glioblastoma [7], another study evaluating the efficacy of adjuvant vaccination

DOI: 10.18609/cgti.2021.086

using an autologous moDC vaccine loaded with autologous tumor RNA in patients with uveal melanoma [8] and a trial evaluating active immunization in adjuvant therapy of patients with stage 3 melanoma using natural CD141$^+$ DCs pulsed with appropriate peptides [9]. Although most of the current trials are based on autologous DCs differentiated *ex vivo* from peripheral blood monocytes and loaded with tumor cell lysate as a source of antigen, these Phase 3 trials highlight the breadth of 'design' modifications that are being explored to overcome the current limitations of standard moDC vaccines.

CONSIDERATIONS FOR VACCINE DESIGN: DC SOURCE

The two design elements that have most impact on the potential efficacy of a DC-based cancer vaccine are the source of DCs and the approach used to 'arm' the vaccine with an appropriate tumor-associated antigen (TAA). The reduced success of clinical trials has been variously attributed to the limited ability of administered DCs to directly prime T cells *in vivo* where they serve not only as APCs but as a source of antigen for processing and presentation by endogenous DCs [10–12]. Other confounding factors may include the late stage of disease progression of the patients recruited [13] and the suppressive tumor microenvironment [14]. Nevertheless, it has become evident over recent years that there are also significant limitations inherent in moDCs which have inspired efforts to identify alternative sources of DCs with properties more amenable to the induction of potent cell-mediated immunity.

The need for alternatives to moDCs

In order to achieve tumor eradication, cancer vaccines must elicit potent CD8$^+$ cytotoxic T lymphocyte (CTL) responses as well as the activation of CD4$^+$ Th1 cells required for optimal priming of CTLs and expansion of memory T cells [15]. Although all DCs function as efficient APCs, specific subsets are tasked with activating either CD8$^+$ and/or CD4$^+$ T cells [14]. Conventional DCs (cDCs) are broadly divided into two subsets, namely CD141$^+$ DCs (the so-called cDC1 subset) and CD1c$^+$ DCs, referred to as cDC2. The CD1c$^+$ population consists of highly-migratory cells which primarily stimulate CD4$^+$ T cells as a prelude to eliciting humoral immunity. In contrast, CD141$^+$ DCs are resident predominantly in secondary lymphoid tissues and have enhanced capacity to cross-present antigen to CD8$^+$ T cells [14,16,17] while the equivalent population in the mouse has also been demonstrated to stimulate the necessary CD4$^+$ T cell help to achieve optimal CTL priming [18]. Although the specific deletion of the cDC1 subset in mice has been shown to abrogate anti-tumor immunity, highlighting the importance of antigen cross-presentation [13,19], *in vitro* studies with human DC subsets have been rather more controversial. However, on the question of the ability of moDCs to induce antigen-specific CTL responses, a comprehensive study has been conducted by DanDrit Biotech, who undertook several clinical trials with their discontinued moDC-based vaccine, MelCancerVac. Attempts to generate TAA-specific T cell clones resulted primarily in CD4$^+$ clones, suggesting that T cell responses mounted against lysate-loaded moDCs were directed predominantly towards MHC class II-restricted epitopes consistent with the limited ability of these cells to cross-present exogenous antigen. Consequently, although it is relatively straightforward to differentiate sufficient numbers of moDCs from the peripheral blood monocytes of patients for subsequent vaccination, these cells fail to emulate the efficient cross-presenting capacity of CD141$^+$ DCs, highlighting the need to identify alternative sources with more appropriate credentials (Table 1).

Human blood dendritic cells

Accumulating evidence suggests that DC-based vaccines, consisting of naturally-

TABLE 1

Comparison of the advantages and disadvantages of different sources of DCs for cancer immunotherapy.

Source	Advantages	Disadvantages
Peripheral blood monocytes	▶ Autologous ▶ Readily accessible ▶ Well characterized ▶ Good safety profile	▶ Donor-to-donor variation ▶ Adversely affected by chemotherapy ▶ Poor capacity for antigen ▶ Cross-presentation ▶ Genome editing difficult
Circulating DCs	▶ Autologous ▶ Readily accessible ▶ Provides access to distinct DC subsets	▶ Cell numbers limited ▶ Adversely affected by chemotherapy ▶ Genome editing difficult
$CD34^+$ HSCs	▶ Good cellular yield ▶ Amenable to scale-up ▶ Provides access to distinct DC subsets	▶ Access is compromised ▶ Protracted timescale for differentiation ▶ Genome editing difficult
iPSCs	▶ Autologous or allogeneic sources available ▶ Amenable to scale-up ▶ Provides access to rare DC subsets ▶ Tractable for genome editing ▶ Refractory to chemotherapy	▶ Protracted timescale for differentiation ▶ Risks of tumorigenesis

occurring blood-borne DCs loaded with TAA-derived peptides, display promising efficacy in melanoma patients [2]. Tel and colleagues reported on 15 patients with metastatic melanoma that received intranodal injections of plasmacytoid dendritic cells (pDC) loaded *ex vivo* with TAA peptides. *In vivo* imaging showed that administered pDCs were capable of migrating to multiple lymph nodes. Several patients mounted anti-vaccine $CD4^+$ and $CD8^+$ T-cell responses indicating that vaccination with naturally-occurring pDC is not only feasible with minimal toxicity but induces favorable immune responses in patients with metastatic melanoma [20]. Promising results using naturally-circulating DCs have subsequently been reported in Phase 1 trials of prostrate carcinoma [21] as well as acute leukemia [22].

Nevertheless, although peripheral blood DCs may provide an obvious alternative to moDC, this approach must overcome multiple hurdles. Circulating DCs constitute less than 1% of leukocytes in peripheral blood which may be further reduced by the impact of chemotherapy. In a study by Almand and colleagues, the number of DCs in the peripheral blood of cancer patients was dramatically reduced but was accompanied by the accumulation of cells lacking markers of mature hematopoietic cells, the appearance of which closely correlated with the stage and duration of the disease [23]. Consequently, isolating sufficient DCs may be challenging, especially given that multiple vaccinations may be required [1]. Another major limitation is that several studies have shown that DCs isolated from peripheral blood and lymph nodes of cancer patients are functionally compromised, displaying decreased expression of MHC class II and co-stimulatory molecules, and impaired T cell stimulatory capacity. Three studies, including one of breast cancer patients, have correlated DC phenotype and function with the stage of cancer, reporting that both functionality and expression of maturation markers decreases with advancing stages of cancer [24]. Furthermore,

DOI: 10.18609/cgti.2021.086

Almand and colleagues investigated 93 patients with breast, head and neck, or lung cancer and observed that the function of peripheral blood and tumor-draining lymph node DCs was equally impaired, consistent with a systemic rather than a local effect on DC function [23].

Given these limitations, methods for expanding DC subsets *in vivo* are of significant interest. One such approach uses Flt3L, a key cytokine involved in commitment of progenitors to the DC lineage, to expand DC numbers *in vivo*, even in patients with advanced cancer [25]. This approach may facilitate the isolation of different DC subsets in sufficient quantities to enable multiple rounds of vaccination. Balan and colleagues have reported trials of Flt3L administration in combination with poly-I:C:LC in melanoma and B cell lymphoma demonstrating safety and immunogenicity [13]. Furthermore, a recent study by the same group has demonstrated the capacity of Flt3L to augment all subsets of DCs when administered to high-risk melanoma patients, leading to responses to the TAA NY-ESO-1 when administered as a fusion protein with anti-Dec-205 monoclonal antibodies as a way of targeting the antigen to the DC compartment [26]. Nevertheless, there have so far been no vaccine trials using peripheral blood DCs expanded *in vivo* through administration of Flt3L which might serve as a source for purification and antigen loading *ex vivo* prior to reinfusion.

DCs differentiated from CD34$^+$ hematopoietic stem cells

Early studies of DC vaccination included several clinical trials in which DCs were differentiated from CD34$^+$ hematopoietic stem cells (HSCs). For example, Mackensen and colleagues reported promising results from a Phase 1 trial in melanoma patients of a vaccine consisting of peptide-pulsed DCs generated *in vitro* from CD34$^+$ HSCs [27]. Furthermore, Banchereau *et al.* reported immune and clinical responses in patients with metastatic melanoma who received a HSC-derived DC vaccine, also known to contain Langerhans cells (LCs) [28]. Syme and colleagues subsequently performed the first and only study in which a direct comparison was made between moDCs and DCs derived from CD34$^+$ HSCs in a group of cancer patients [29]. They concluded that DCs differentiated from HSCs may prove a more attractive source for clinical vaccination protocols, since cellular yield was superior and differences in patterns of costimulatory molecule expression did not appear to create a functional impediment. Based on these early studies, there has been renewed interest in this source of DCs and several groups are currently developing platforms exploiting CD34$^+$ HSCs for the large scale production of specific DC subsets, such as CD141$^+$ DCs, pDCs, LCs and CD1d$^+$ DCs [30]. Nevertheless, given that CD34$^+$ HSCs are found in trace numbers in peripheral blood making access difficult, and the timescale for their differentiation *in vitro* is protracted, moDCs have prevailed as the most common source of DCs currently employed in clinical trials [31].

DC vaccines based on iPSC-derived CD141$^+$ DCs

A recent development has been to exploit the potential of induced pluripotent stem cells (iPSCs) whose unlimited self-renewal capacity and inherent pluripotency may give rise to specific cell types that would otherwise prove inaccessible in patients. Indeed, an unlimited number of DCs with little variability could be derived from iPSCs, reprogrammed from cells such as dermal fibroblasts that are least affected by long-term chemotherapy, an advantage for cancer patients displaying functional defects among moDCs [32]. Several groups have successfully derived DCs from iPSCs: Senju and colleagues first reported the generation of DCs from human iPSCs that exhibited the morphology of typical DCs and the capacity for efficient antigen

presentation and activation of naïve T-cells [33]. However, Silk *et al.* subsequently developed protocols for the directed differentiation of CD141$^+$ DCs from patient-specific iPSCs, displaying the additional capacity for cross-presentation of TAAs to CD8$^+$ T cells [34]. Given the proliferative capacity of iPSCs, this process therefore has the potential for mass production of otherwise inaccessible subsets of DCs required for vaccination purposes.

Turnis and Rooney have suggested that for optimal induction of tumor-specific T cells, an ideal DC vaccine should exhibit three essential qualities: the ability to migrate to lymph nodes where T cell activation first occurs; maintenance of a mature phenotype over time to activate and expand tumor-specific T cells; and the capacity to cross-present TAAs as a prelude to the activation of CTLs [35]. In addition, the DC vaccine should be amenable to scale-up of manufacturing to ensure the availability of cells at a scale necessary for repeated vaccination. It is in these four areas that iPSC-derived CD141$^+$ DCs show advantages compared to other sources of DCs since they share many characteristics of the rare lymph node-resident human cDC1 subset. Unlike moDCs, this novel population co-expresses the chemokine receptors CCR7 and XCR1 which guide migration towards secondary lymphoid tissues and CD8$^+$ T cells, respectively [36]. Indeed, XCR1 has been found to be selectively expressed among cDC1 cells and to confer on them the unique ability to migrate in response to its ligand XCL1 [37]. Accordingly, the selective expression of XCR1 by this novel source of DCs may promote their recruitment to sites of CTL activation in the lymph nodes [38] and to peripheral sites of inflammation where natural killer (NK) cells and CTLs may actively secrete XCL1 [39].

Primary cDC1 were initially identified as a unique subset based on their propensity for antigen cross-presentation when tested *in vitro* with soluble or cell-associated antigen [37,40–42]. In common with their *in vivo* counterparts, Silk and colleagues demonstrated that iPSC-derived CD141$^+$ DCs cross-present exogenous TAA directly to MHC class I restricted CTL clones as well as naïve primary T cells [34], properties which permit target antigens to be introduced either as recombinant proteins or whole tumor cell lysates from which appropriate MHC class I and class II-restricted epitopes may be selected during antigen processing.

Finally, the central role played by iPSCs in this source of DCs provides opportunities to apply genome engineering to the rational design of DC vaccines displaying additional functionality. Coupled with opportunities for the mass production of large numbers of high-quality cells, iPSC-derived CD141$^+$ DCs have multiple advantages that make them attractive candidates for the next generation of DC vaccines.

CONSIDERATIONS FOR VACCINE DESIGN: ANTIGEN SELECTION

The second critical factor in vaccine design for cancer immunotherapy is the choice of antigen or antigen cocktail with which to load DCs prior to administration.

Tumor-associated & tumor-specific antigens

Tumor-associated antigens (TAAs) include gene products that are involved in tissue differentiation that are preferentially over-expressed by cancer cells but may also have a wider distribution, being expressed at lower levels by some normal tissues. While over-expressed tumor antigens include HER2, TERT and anti-apoptotic proteins, such as BIRC5, tissue differentiation antigens include mammaglobin-A, PSA, Melan-A and PMEL [43]. Cancer testis antigens (CTAs) are a specialized subset of TAAs that are thought to provide higher tumor specificity, as they are not expressed in normal adult tissues with the exception of germline and trophoblastic cells, but are, nevertheless, highly expressed

DOI: 10.18609/cgti.2021.086

by numerous cancers. More than 60 genes encoding CTAs have been identified, the best studied of which are the MAGE family, SAGE1 and CTAG1A [44].

It is important to note, however, that in addition to lacking complete specificity for the tumor, TAAs are self-components and are, therefore, subject to some degree of central and peripheral tolerance. Breaking such immunological tolerance inevitably carries the risk of autoimmunity directed against those tissues expressing the relevant genes at low levels. Furthermore, those peripheral T cells specific for TAAs may have escaped normal tolerance mechanisms due to their moderate or low affinity for antigen: accordingly, vaccination against such antigens may lead to weak T cell responses with poor anti-tumor activity [45].

Tumor-specific antigens (TSAs) include proteins derived from oncogenic viruses associated with cancers such as cervical cancer, induced by human papillomavirus (HPV), hepatocellular carcinoma, secondary to hepatitis B virus infection, and human herpesvirus 8-associated Kaposi sarcoma [46]. As *bona fide* foreign antigens, these proteins play no part in central tolerance. Furthermore, being expressed solely by cancer cells they are highly specific for the tumor, making them ideal for use in cancer vaccines [44].

Defined antigen vaccines targeting a single TAA or TSA may, however, be ineffective due to immune escape via downregulation or mutation, these so-called escape mutants losing expression of key epitopes. Using multiple defined antigens mitigates against this risk and may be a crucial design component for achieving clinical benefit [47]. Another approach to mitigate this risk is to select TAAs that are essential for cell function and cannot, therefore, be downregulated by the tumor. An example is carcinoembryonic antigen (CEA), an adhesion molecule without which colorectal cancers could not metastasize. Another issue which may explain the limited clinical efficacy of earlier vaccines is that selection of appropriate antigens was based on their reported expression pattern in the relevant type of tumor; nevertheless, expression of these antigens by the tumor tissue of individual patients was rarely verified [44]. Consequently, where tumor biopsies are available, treatment eligibility criteria should be established based on confirmation of expression of the TAAs to be targeted [48].

Neoantigen vaccines

Recent years have witnessed a growing interest in the use of so-called neoantigens that arise from tumor-specific mutations, indeed, the high mutational rate of some tumors results in the expression of neoantigens that are exquisitely tumor specific and highly immunogenic due to the lack of central tolerance [49]. Although tumor neoantigens have long been conceptualized as ideal antigenic targets, their routine identification and evaluation has only recently become feasible with the advent of next generation sequencing and bioinformatics tools for detection of all coding mutations within tumors and algorithms to reliably predict those mutations capable of generating epitopes with high-affinity for the patient's MHC molecules [50]. Although targeting of neoantigens is a recent development, some groups have published promising results [45]. For example, Carreno and colleagues reported that a DC vaccine loaded with neoantigenic peptides elicited neoantigen-specific T cell responses as a result of which some patients showed stabilized or non-recurrent disease [51]. Furthermore, the use of RNA-vaccines that deliver patient-specific neoantigenic epitopes directly to DCs *in vivo*, has recently facilitated a personalized approach to cancer immunotherapy, leading to objective responses in two of five patients with metastatic melanoma [52].

Despite these successes, some tumors carry a higher mutational burden than others, creating a disparity between cancer types with respect to the likelihood of identifying appropriate neoantigens [51]. Furthermore, even in those so-called 'hot' tumors, which show enhanced responsiveness to treatments such as

immune checkpoint inhibitors, it is necessary to identify so-called 'trunk' mutations which, having contributed to the original transformation, are expressed ubiquitously throughout the tumor. Their identification must, however, be achieved against a background of high mutational burden creating numerous 'branch' mutations expressed as a patchwork throughout the tissue but representing inappropriate targets. This approach also requires the availability of fresh tumor material and is, therefore, applicable only to solid tumors that can be surgically resected. Consequently, by being inherently patient-specific, this approach may be limited by pragmatic issues of complexity, cost and challenging timelines between tumor resection and injection of the first vaccine, a delay of several months potentially proving a major challenge for uptake by patients.

Tumor lysates as a source of patient-specific antigens

For indications where surgery can be performed as part of treatment, a common approach to antigen loading has been the use of tumor lysates as a source of antigen [45]. Since these contain the full spectrum of relevant target antigens, both TAAs, TSAs and neoantigenic epitopes capable of activating both $CD4^+$ and $CD8^+$ tumor-specific T cells [53], their use may help reduce the chances of tumor escape. Accordingly, there have been several positive reports of the induction of a potent anti-tumor response using this approach. Notably, May and colleagues reported a significant OS advantage for renal cell carcinoma (RCC) patients treated with an autologous tumor lysate vaccine. Patients at an advanced tumor stage (pT3) revealed 5- and 10-year OS rates of 71.3% and 53.6%, respectively, among those treated compared to 65.4% and 36.2% in the control group. Significantly, patients in the vaccine group showed a significantly improved survival both across the whole treatment group and the subgroup with pT3 stage tumors [54]. Furthermore, a meta-analysis of approximately 1,800 patients showed that those who were immunized with whole tumor vaccines had a significantly higher ORR (8.1%) compared to patients vaccinated with defined tumor antigens (3.6%) [55], providing a strong rationale for using whole tumor cell lysate for cancer vaccination. Interestingly, these findings may be further enhanced in the future by the oxidation of tumor lysates which was found to augment the capacity of DCs to induce TSA-specific T cell responses both *in vitro* and *in vivo*. Indeed, of five patients with ovarian cancer treated with autologous DCs pulsed with oxidized tumor lysate, two experienced durable progression free survival of 24 months or more [56].

Although promising, a significant limitation to the use of autologous cancer tissue as a source of antigens is the requirement for sufficient patient material, making it applicable only to solid tumors that can be surgically resected [45]. An alternative approach that merits consideration is, however, the use of tumor lysates of allogeneic origin. Allogeneic vaccines based on a cocktail of human tumor cell lines might enable large-scale production and standardization of quality and composition [45]. Possibly the best example is TRIMEL, a cell lysate derived from three allogeneic melanoma cell lines established from metastatic lymph nodes and used in TAPCells, a DC vaccine tested in more than 120 stage 3 and 4 melanoma patients and 20 castration-resistant prostate cancer patients in a series of Phase 1 and 1/2 clinical trials. The TAPCells vaccine was shown to induce T cell-mediated memory that correlated with increased survival of melanoma patients while in patients with prostate cancer, is was shown to prolong prostate-specific antigen (PSA) doubling time [57]. TRIMEL was, therefore, shown to include all the necessary elements to induce a vigorous immune response, promote the recognition and destruction of tumors *in vitro* and the stabilization of the disease *in vivo* in a proportion of treated patients [58,59].

DOI: 10.18609/cgti.2021.086

iPSCs as source of TAAs

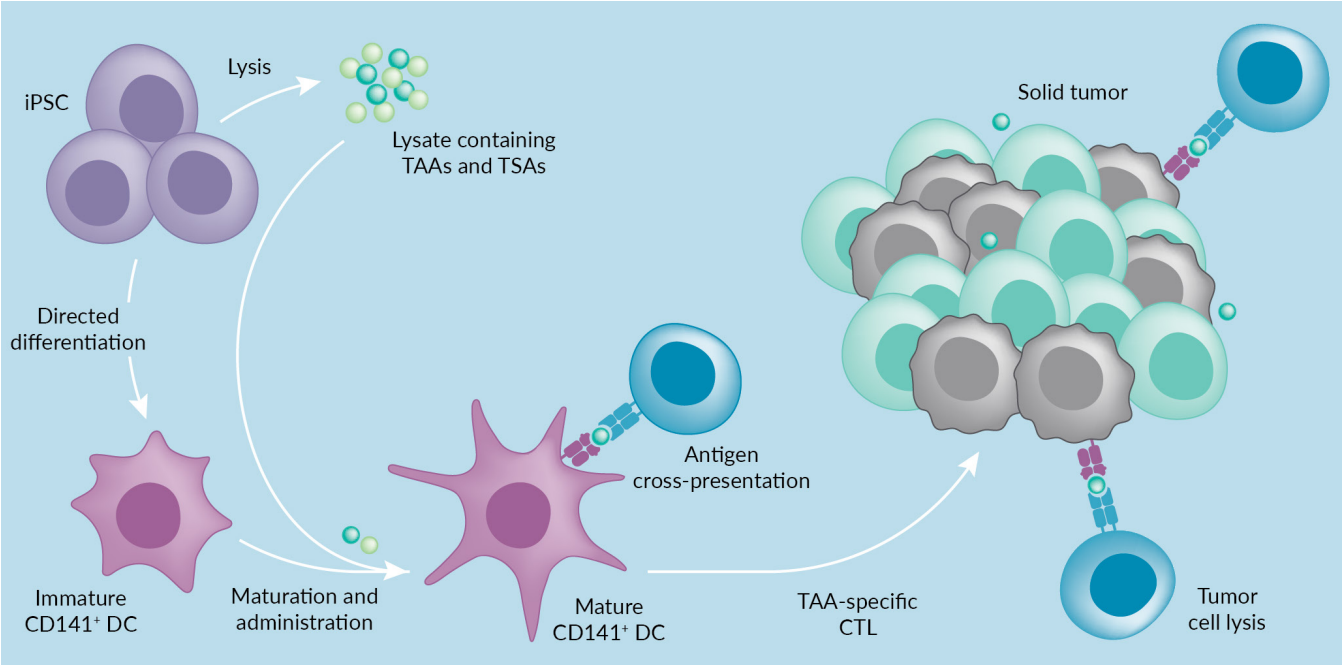
It has been known for over a century, that immunization with embryonic or fetal tissue could lead to the rejection of transplanted tumors in animal models [60]. More recently, studies identified antigens shared between tumors and embryonic cells which led to the hypothesis that embryonic stem cells (ESCs) might be used to induce anti-tumor immunity. Indeed, cancer cells and ESCs share many cellular and molecular features including a rapid proliferation rate, upregulation of telomerase, increased expression levels of oncogenes, and similar gene expression profiles, microRNA signature and epigenetic status. Similar to ESCs, iPSCs share genetic and transcriptomic signatures with cancer cells [61], as well as the ectopic expression of certain genes encoding 'developmental antigens'. These are strongly expressed in the pluripotent state but would normally be down-regulated early during ontogeny, being lost prior to development of the immune system and the induction of self-tolerance [62]. Upon reprograming somatic cells to pluripotency, these genes are strongly upregulated but may not be silenced upon subsequent differentiation *in vitro*, potentially prompting the rejection of tissues differentiated from them, even in syngeneic recipients [63]. Nevertheless, the same genes that are making the application of iPSCs challenging in regenerative medicine may be the key to their use as a source of antigen to drive anti-tumor responses as they are shared by many tumors. For example, CT46/HORMAD1 is a CTA which is strongly up-regulated by iPSCs but has also been shown to be expressed in 31% of carcinomas [64].

While it is undoubtedly early days in exploring the application of iPSCs as cancer vaccines, Li and colleagues evaluated the use of a human xenogeneic iPSC line as a cancer vaccine in a transplantable mouse model of colon cancer. They found that iPSCs were able to induce significant expansion of IFNγ- and IL-4-producing cells, although this did not result in tumor rejection [65]. More recently, however, Kooreman *et al.* reported proof of principle experiments using irradiated iPSCs as an autologous anti-tumor vaccine. Vaccination of mice was shown to protect against growth of tumors as distinct as mesothelioma, melanoma and breast cancer. Furthermore, adoptive transfer of T cells from vaccinated mice protected unvaccinated recipients from tumor growth, consistent with the induction of antigen-specific T cell responses. Interestingly, this study also used RNA sequencing to compare expression profiles between human iPSCs and cancer tissues and demonstrated the shared expression of numerous TAAs and TSAs [66]. Subsequent studies by the same group have further demonstrated how shared expression of cancer signature genes between iPSCs and pancreatic ductal adenocarcinomas (PDAC) enabled the generation of $CD8^+$ effector and memory T cells specific for tumor antigens in mice vaccinated with iPSCs, thereby preventing tumorigenesis in 75% of PDAC mice [67].

While these researchers have explored the use of iPSCs as whole-cell cancer vaccines, there is a significant opportunity to use iPSCs as the source of antigen in combination with a DC vaccine. This approach would ensure that tumor antigens are processed for presentation to CTLs, provided the DCs used in the vaccine have cross-presenting capacity. In this context, the recent optimization of protocols for the directed differentiation of the $CD141^+$ DCs from human iPSCs [34, 36] suggests a compelling scenario in which a signature iPSC line may not only provide a ready source of tumor antigens but an inexhaustible supply of cDC1 cells, capable of their cross-presentation to the patient's T cell repertoire (Figure 1). Although iPSCs could be produced in a patient-specific manner, benefit may also be derived from the use of a semi-allogeneic source, sharing with the patient one or more MHC class I loci to allow for cross-presentation [68]. A source of iPSCs derived under cGMP conditions from an HLA-A*0201^+ donor would, for example, be compatible with >20% of the US Caucasian population whilst providing an ongoing source of tumor antigens, an approach which would pave the way for the manufacture of

FIGURE 1

Scheme showing the potential use of iPSCs as a novel source of DC subsets, such as the CD141⁺ cDC1 subset capable of antigen cross-presentation to MHC class I-restricted CTLs.



The parent iPSC line may serve as a rich source of TAAs and TSAs with which to load the DCs prior to maturation and administration to recipients, thereby eliciting a TAA-specific CTL response capable of inducing tumor regression.

a readily available off-the-shelf product. The derivation of additional iPSC lines expressing the most prevalent MHC class I alleles could cater for a significant proportion of the population [68].

TRANSLATIONAL INSIGHT

Translation of novel sources of DCs to the clinic is likely to be challenging: for blood borne DCs and DCs differentiated *in vitro* from CD34⁺ HSCs, scale up and consistency of the cell therapy product poses significant issues, while the specter of tumorigenicity continues to cloud the use of iPSCs. Nevertheless, exploiting pluripotency as a means of accessing those rare subsets of DCs most suited to the induction of anti-tumor responses may avoid many of the anticipated issues likely to be encountered upon the use of iPSCs in the context of regenerative medicine. In particular, the success of immunotherapy does not depend on the long-term survival of administered DCs but rather the legacy they leave behind within the T cell repertoire: the eventual demise of the administered cells is not, therefore, an obstacle to be overcome, but rather a strategic advantage, ensuring the clearance of all material derived from iPSCs and greatly improving the safety profile of the cell therapy product. Consequently, although the promise of enlisting nature's adjuvant to elicit anti-tumor immunity has beguiled researchers for more than 20 years, recent developments that have diversified the sources of tumor antigens available while providing access to alternative populations of DCs, suggest that the field may now be ripe for a renaissance.

ACKNOWLEDGEMENTS

Research into dendritic cells in the authors' laboratory is supported by grants from the Edward Penley Abraham (EPA) Trust (Grant: RF278), the Guy Newton Translation Fund (Grant GN05 (10)), and the University Challenge Seed Fund (Grant: UCSF 443).

DOI: 10.18609/cgti.2021.086

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Contributions: *All named authors take responsibility for the integrity of the work as a whole, and have given their approval for this version to be published.*

Acknowledgements: *Research into dendritic cells in the authors' laboratory is supported by grants from the Edward Penley Abraham (EPA) Trust (Grant: RF278), the Guy Newton Translation Fund (Grant GN05 (10)), and the University Challenge Seed Fund (Grant: UCSF 443).*

Disclosure and potential conflicts of interest: *Marcelo Bravo, Timothy J Davies and Paul J Fairchild are founders of OxVax Ltd, a company focused on the development of an advanced next-generation dendritic cell vaccine platform for the treatment of solid tumors. Paul J Fairchild is an inventor on two patents that relate to the differentiation of dendritic cells from iPSC. Timothy J Davies is a contributor to a patent that relates to the differentiation of dendritic cells from iPSC*

Funding declaration: *The authors received no financial support for the research, authorship and/or publication of this article.*

ARTICLE & COPYRIGHT INFORMATION

Article source: *Invited; externally peer reviewed.*

Submitted for peer review: *Apr 27 2021;* ***Revised manuscript received:*** *May 25 2021;* ***Publication date:*** *Jun 8 2021.*

DOI: 10.18609/cgti.2021.086