

COMMENTARY

Immunotherapy with iPSC-derived dendritic cells brings a new perspective to an old debate: autologous versus allogeneic?

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The advent of induced pluripotency has raised the prospect of personalized therapies based on the derivation of induced pluripotent stem cells (iPSC) derived from a patient's own somatic cells. Such bespoke cell products may successfully circumvent issues of rejection by the recipient's immune system but raise questions of affordability, the costs of generating patient-specific cell lines and their subsequent differentiation under cGMP conditions, proving a challenging business model. However, principles that have guided the decision between autologous and allogeneic cell products in the past may prove less reliable when considering the therapeutic use of dendritic cells (DC) differentiated from iPSC, whose role in the immune system would be adversely compromised in a fully allogeneic setting. Here, we review the immunological concepts that inform the debate between autologous and allogeneic cell therapies and discuss whether recent breakthroughs might provide a novel solution to this long-standing issue, paving the way for the widespread adoption of DC-based immunotherapy and increasing its reach from immune oncology (IO) to the induction of immunological tolerance.

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INTRODUCTION

The field of regenerative medicine has begun to mature over recent years, fueled by advances in reprogramming technologies, optimization of protocols for the directed differentiation of iPSC and the establishment of significant manufacturing capacity. These advances have resulted in on-going clinical trials for disease states as distinct as age-related macular degeneration, diabetes, Parkinson's disease, myocardial infarction and spinal cord injury [1,2]. Furthermore, the refinement of differentiation protocols for the production of more-specialized cell types continues to offer new avenues for subtle intervention in rare conditions that constitute unmet medical needs. Given that iPSC may be generated from the somatic cells of any patient, the opportunity to develop personalized therapies, tailored to the needs of the individual, remains an alluring prospect but one responsible for rekindling the debate as to whether autologous or allogeneic stem cells should ultimately be pursued for clinical applications.

This debate has traditionally revolved around two issues that are naturally in tension with one another. The production of an allogeneic cell line that serves as an off-the-shelf product for the treatment of numerous patients is clearly attractive but risks immunological rejection of the very cells required to restore the function of affected tissues [3,4]. So-called 'alloreactivity' that underlies allograft rejection, is precipitated by the recognition of products of the major histocompatibility complex (MHC), a series of highly polymorphic proteins that define an individual's immunological identity

(Box 1): by resembling a molecular barcode, MHC molecules mark tissues as belonging to an individual while simultaneously identifying those from a donor as foreign to the body with no legitimacy to remain. The successful use of an allogeneic source of iPSC is, therefore, dependent on the judicious use of immune suppression, the long-term risks of which may paradoxically outweigh those of the very disease state being treated, making such a strategy ethically contentious. Under such circumstances, the production of autologous iPSC as a source of cells that would be accepted indefinitely without recourse to immune suppression would clearly be preferable, were it not for the inevitable time lag involved in creating appropriate cell lines and the current costs of manufacture which threaten to undermine the economic viability of such an approach. In most cases, companies producing cell therapy products have opted for an allogeneic source in the unproven anticipation that the transient application of immune suppression may secure long-term survival of replacement tissues. While the veracity of this assumption has yet to be fully determined for the variety of cell types and tissues currently in use, the arguments on which such decisions are based are eclipsed by issues of efficacy when considering DC differentiated from iPSC for immunotherapeutic purposes.

HARNESSING THE POTENTIAL OF iPSC-DERIVED DC

DC are attractive vehicles for immunotherapy since they are responsible for setting the underlying tone of the

immune system, either establishing and maintaining a state of self-tolerance or breaking the *status quo* to initiate protective immune responses. These diametrically-opposed outcomes are equally dependent on the presentation of antigenic peptides via products of the MHC (Box 1), the outcome of antigen recognition by responding T cells being determined by the balance of auxiliary signals supplied by the DC in the form of cell surface receptors and secreted cytokines (Figure 1). Provision of peptide-MHC complexes in combination with the co-stimulatory molecules CD40, CD80 and CD86 and the pro-inflammatory cytokine IL-12, provokes a potentially destructive immune response. In contrast, circumstances that encourage expression of inhibitory receptors by DC, such as PD-L1/2 and ILT3/4, together with their secretion of the anti-inflammatory cytokine IL-10, favor tolerance

through the polarization of responding T cells towards a regulatory phenotype (Figure 1). While the use of DC to re-establish a tolerant state to self-proteins implicated in autoimmunity or to induce tolerance *de novo* to therapeutic proteins remains largely in its infancy [5], more than 200 clinical trials to date have exploited the properties of DC for vaccination to defined tumor associated antigens (TAA) for the treatment of melanoma, glioblastoma, prostate cancer and renal cell carcinoma [6].

DC used in clinical trials are conventionally derived from the patient's own peripheral blood monocytes (moDC) for ease of access, however, this preferred source may help explain the disappointingly low objective response rates reported so far: by lacking appreciable capacity for the cross-presentation of TAA to CD8⁺ cytotoxic T cells (CTL), the ability of this population of DC to effect tumor regression is inevitably limited.

Box 1 The Major Histocompatibility Complex

The Major Histocompatibility Complex (MHC) represents a large genetic locus on chromosome 6 in humans containing genes encoding so-called MHC molecules. In man, these molecules are referred to as human leukocyte associated antigens (HLA) and are of two types known as class I and class II. Although class I and II molecules differ in their structure, they share a peptide binding groove which confers on them the capacity to bind epitopes derived from foreign antigen and present them to the T-cell repertoire: indeed, the T-cell receptor (TCR) is inherently MHC-restricted, preferentially recognizing peptides bound to self-MHC molecules. MHC class I determinants are responsible for the presentation of epitopes to CD8⁺ cytotoxic T cells and are expressed by all nucleated somatic cells. By contrast, epitopes bound to MHC class II molecules are recognised by CD4⁺ Th cells and Treg and are far more restricted in their pattern of expression to dedicated antigen presenting cells, of which DC are uniquely capable of eliciting a primary immune response. In man, there are three loci encoding MHC class I molecules, HLA-A, HLA-B and HLA-C, and likewise three class II loci known as HLA-DR, HLA-DP and HLA-DQ. Each of these loci is highly polymorphic, existing in thousands of different allelic forms within the human population: given that each individual co-dominantly expresses two alleles at each locus, up to 12 different MHC molecules may be expressed by an individual, defining their unique MHC 'haplotype'. While diversity within the MHC is critical for establishing herd immunity to emerging pathogens, it creates a significant barrier to the success of tissue and organ transplantation, allogeneic MHC molecules marking tissues as foreign to the body. Indeed, a high precursor frequency of T cells is capable of recognising allogeneic MHC molecules, irrespective of the peptides bound, eliciting polyclonal T-cell responses that prove highly damaging to transplanted tissues. It is the balance between the roles played by MHC molecules in allograft rejection and the physiological function of DC that must be held in tension when seeking to develop a DC product for downstream clinical applications.

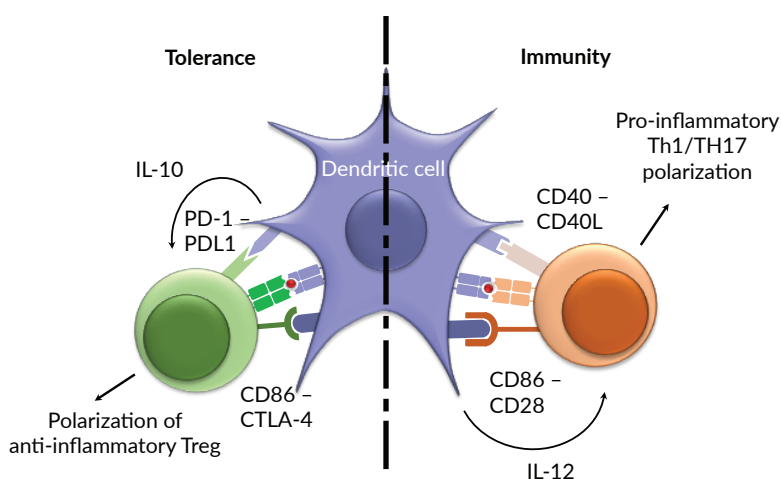
Under these circumstances iPSC offer a credible alternative source of DC that circumvents many of the difficulties encountered previously [7]. For instance, an appropriate iPSC line provides a permanent and scalable resource conducive to genome editing and the provision of an unlimited supply of DC proven to be safe and efficacious in animal models of IO [8,9]. More importantly, however, iPSC provide access to rare yet desirable subsets of DC previously beyond the reach of clinicians, including plasmacytoid DC that facilitate anti-viral responses [10,11] and the elusive CD141⁺ subset whose unrivalled capacity for antigen cross-presentation is essential for anti-tumor immunity [12]. Furthermore, by subtly altering the conditions for their differentiation, DC committed to tolerance induction may be readily obtained. In the mouse, these so-called regulatory DC (DCreg) carry

a tolerogenic signature defined by constitutive expression of inhibitory receptors and IL-10 secretion which elicits potent Treg responses *in vivo* [13,14]. Human iPSC, cultured under similar conditions, have likewise been shown to spawn DC that share with DCreg from peripheral blood [15], a CD141⁺ phenotype and capacity for copious IL-10 synthesis [16]. While the access afforded by induced pluripotency to functionally-distinct populations of DC offers unparalleled opportunities for their use in immunotherapy, it raises, once again, the question of whether an autologous or allogeneic source would be preferable.

THE PROS & CONS OF AN ALLOGENEIC DC PRODUCT

► FIGURE 1

Dendritic cells determine the outcome of antigen recognition by T cells.



Antigen specificity of the immune response is conferred through recognition by naïve T cells of peptide epitopes from foreign antigen bound to MHC molecules. T-cell receptor (TCR) engagement leads to T-cell activation when accompanied by ligation of the co-stimulatory receptors CD28 and CD40L and provision of the pro-inflammatory cytokine IL-12. In contrast, antigen recognition accompanied by ligation of inhibitory receptors in an environment replete with IL-10 polarises responding T cells towards a regulatory phenotype involved in the establishment and maintenance of tolerance.

There is little doubt that economic considerations would favor an allogeneic source of DC, the generation of an off-the-shelf product available to a broad spectrum of recipients, justifying the significant financial investment required for the derivation of an iPSC line under cGMP conditions. However, unlike any other cell type whose efficacy *in vivo* is unrelated to its MHC haplotype, MHC molecules play an essential role in antigen presentation by DC and are inextricably linked to their physiological function, making the cost–benefit analysis rather more nuanced. Most importantly, a fully allogeneic source of DC would have no capacity to interact productively with recipient T cells in an antigen-specific manner: the debate between autologous and allogeneic sources therefore strikes at the very heart of efficacy of the DC product itself.

Given that fully allogeneic DC are physiologically impotent, such a cell therapy product would fail to fulfil the very function for which it was intended. Consequently, as a minimum requirement, the source of DC would need to be semi-allogeneic, sharing with the recipient one or more MHC class I loci through which TAA could be productively presented to the CD8⁺ T cell repertoire. Given that some MHC class I loci, such as HLA-A*0201, are particularly prevalent, being expressed by approximately 27% of the US Caucasian population [17], a source of iPSC derived from an HLA-A*0201⁺ donor would be compatible with a significant proportion of the population. Indeed, this reasoning has already led to the development of a plasmacytoid DC product based on a leukemic cell line derived from

an HLA-A*0201⁺ patient [18]. Ensuring provision for the remainder of the population expressing alleles other than HLA-A*0201 would, however, require the generation of iPSC lines relevant to progressively smaller cohorts of potential patients, rapidly invoking the law of diminishing returns. Importantly, patients with rare MHC haplotypes poorly represented within the population would be unlikely to ever have access to treatment, raising ethical issues of equitability. But although a semi-allogeneic source may potentially fulfil the economic advantages of a fully allogeneic product, the downstream technological risks are far from insignificant. While presentation of TAA may occur through the shared MHC class I molecules, the allogeneic MHC determinants would inevitably provoke the polyclonal activation of antigen non-specific alloreactive T cells. Given that the phenotype of DC renders them uniquely immunogenic, such alloresponses are especially dramatic, engaging an estimated 7% of the entire T-cell repertoire [19], and are, therefore, responsible for the ultimate demise of the administered cells. Consequently, while semi-allogeneic DC may theoretically succeed in provoking a TAA-specific response, they inevitably set in motion a race against time to vaccinate the recipient before they themselves are actively targeted for destruction.

While the ultimate demise of semi-allogeneic DC is inescapable, it has been argued that the alloresponse elicited against them may, paradoxically, contribute to the concurrent activation of TAA-specific CTL by mimicking the activity of an adjuvant [20]: indeed,

DC have often been described as 'nature's adjuvant' to reflect their inherent capacity to provoke potent inflammatory responses. It is feasible, for instance, that the polyclonal activation of CD4⁺ helper T cells (Th cells) through recognition of allogeneic MHC class II molecules, may provide bystander help in the form of secreted IL-2 and IFN- γ to CTL engaged in the cognate recognition of TAA (Figure 2). That such a pathway may operate *in vivo* is evidenced by the induction of alloantibody responses to vascularized organ allografts which has been shown to be wholly dependent on DC carried over within the graft eliciting CD4⁺ T cell activation as a potent source of B-cell help [20]. More direct evidence in support of this notion comes from studies in mice of DC differentiated from ES cells [8]. Administration of DC loaded with a nominal TAA to semi-allogeneic recipients induced antigen-specific responses that restricted tumor progression *in vivo*, despite the simultaneous induction of significant alloreactivity [8]. That the secretion of pro-inflammatory cytokines was fundamental to this outcome was suggested by the findings of Martín-Fontecha and colleagues who injected recombinant TNF- α subcutaneously to mice followed by administration of TAA-laden DC at the same location. Prior exposure to TNF- α induced the up-regulation of the chemokine CCL21 by local lymphatic endothelium resulting in a 40-fold increase in the numbers of DC reaching the draining lymph nodes [21], a highly-relevant finding given that less than 5% of injected cells are normally expected to reach the site of T-cell activation [22].

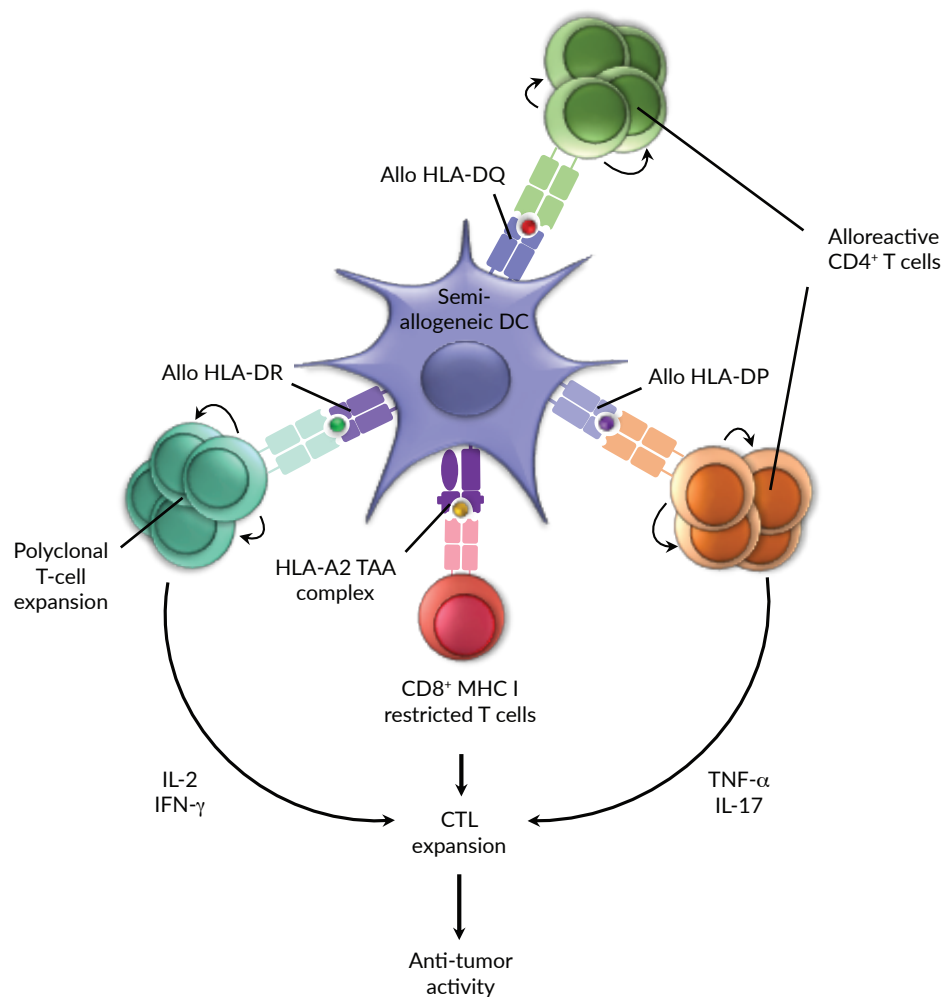
These encouraging findings have recently gained further traction

from studies of cancer immunotherapy in man which likewise suggest that on-going inflammation at the site of DC administration may substantially increase the efficacy of cancer vaccination. By using tetanus/diphtheria toxoid (Td) as a potent recall antigen, Mitchell *et al.* induced local inflammatory responses in patients with glioblastoma multiforme at the same site to which they subsequently administered DC pulsed with the TAA, pp65. This regimen showed significantly enhanced accumulation of DC in the draining lymph nodes, as assessed by Indium-111 labelling of the administered cells, which correlated with enhanced progression-free and overall survival of patients [23]. The role played by CD4⁺ T cells as the principal source of pro-inflammatory cytokines was subsequently confirmed in mice, their depletion abrogating any beneficial impact of prior conditioning with Td [23].

Although these findings support the notion that alloresponses by CD4⁺ T cells elicited by semi-allogeneic DC may, paradoxically, enhance concurrent activation of TAA-specific CTL (Figure 2), enthusiasm for this strategy must be tempered by two important considerations. Firstly, the polyclonal activation of CD4⁺ alloreactive T cells inevitably leads to a broad repertoire of memory T cells capable of evoking far more dramatic responses upon subsequent exposure to the same inoculum. Indeed, careful experiments evaluating the survival of TAA-pulsed DC in mice revealed their greatly accelerated clearance following prior immunization with the same source of DC [24]. Consequently, the desired effects of DC vaccination in a semi-allogeneic setting would need to be achieved

► FIGURE 2

Alloreactivity among CD4⁺ T cells may serve to bolster anti-tumour immunity.



DC semi-allogeneic to the recipient have the capacity to present epitopes from TAA to CTL via shared MHC class I molecules. The simultaneous recognition of allogeneic MHC class II molecules by alloreactive CD4⁺ T cells may enhance anti-tumor immunity by the provision of pro-inflammatory cytokines and bystander help for the activation and clonal expansion of TAA-specific CTL.

through the administration of a single inoculum, since subsequent doses would be rapidly destroyed by an anamnestic response. Importantly, such a regimen runs counter to current evidence suggesting that the efficacy of DC vaccination correlates positively with the number of doses given, successive inocula serving to boost immunity over time. For instance, Teramoto and colleagues demonstrated that 1-year survival of patients with refractory non-small cell lung cancer increased

from 25% in patients receiving 1–2 injections of autologous MUC-1-pulsed DC, to 39% in those receiving six or more vaccinations. Furthermore, the median survival time increased from 2.7 to 9.5 months, strongly supporting the expediency of progressively augmenting immunity over time [25]. Secondly, while the pro-inflammatory microenvironment elicited by semi-allogeneic DC may be compatible with vaccination protocols, it would doubtless prove profoundly antagonistic

to the induction of immunological tolerance, the release of inflammatory cytokines inhibiting the induction of Treg and most likely favoring aggressive Th17 responses instead. It is, therefore, challenging to envisage how semi-allogeneic DC could ever be re-purposed for tolerance induction, greatly limiting the reach of DC-based immunotherapy to IO. Such constraints naturally raise questions as to the feasibility of working towards an autologous cell therapy product instead.

THE PROS & CONS OF AN AUTOLOGOUS DC PRODUCT

The scientific mandate for an autologous DC product is beyond dispute. In the absence of confounding alloreactivity, the use of DC to establish or reinforce immunological tolerance becomes a far more realistic prospect [5]. Such a strategy would pave the way for the potential use of DC to establish tolerance to defined protein antigens serving as biological therapeutics, such as the recombinant enzymes required for the treatment of lysosomal storage diseases or clotting factors such as Factor VIII for the treatment of hemophilia A. The recent demonstration of pre-existing immunity to the bacterial enzyme Cas9 [26,27] may threaten the very future of *in vivo* gene editing, suggesting that new targets continue to emerge for which the establishment of immunological tolerance is necessary [28]. Furthermore, the role played by DC in autoimmunity and allograft rejection suggests that these indications may also serve as potential, albeit ambitious targets for the future establishment of tolerance [29].

In the context of IO, the availability of autologous DC would likewise prove a significant advantage since DC sharing all MHC class I loci with the recipient would be able to present a broad spectrum of epitopes generated from an appropriate TAA, provoking a polyclonal yet antigen-specific CD8⁺ T-cell response against an established tumor. In contrast, the token expression of a single MHC class I allele in common between semi-allogeneic DC and recipient, would necessarily restrict the response to the small number of epitopes presented by the relevant MHC molecule. Given that the number and diversity of tumor infiltrating lymphocytes (TIL) serves as a biomarker of favorable prognosis [30], diversity in the immune response is an important goal with significant implications for efficacy. Furthermore, the absence of foreign MHC molecules that would provoke potent non-specific alloreactivity paves the way for the delivery of multiple doses of an autologous DC product over an extended period of time, a strategy that might establish and progressively augment immunity to the desired TAA. The administration of multiple small doses of DC is also preferable since it is less likely to provoke adverse reactions, such as cytokine release syndrome, than the delivery of a single large inoculum that a semi-allogeneic product would necessitate. Such considerations are clearly important, since, to date, the vast majority of clinical trials have made use of autologous moDC, on the basis of which, this form of immune intervention has been deemed safe and well-tolerated by patients [6]. The use of a semi-allogeneic source would, however, involve stepping into the unknown, the safety and efficacy data that have been acquired

over the past decade bearing limited relevance to this new scenario.

While the scientific credentials of an autologous DC product are indisputable, the economic argument is undoubtedly rather less persuasive since the more bespoke a treatment, the greater the cost of manufacture is likely to be: a fully autologous product is clearly at one end of the spectrum taking little advantage of the economies of scale [31]. Nevertheless, there is little doubt that the costs of manufacture of a cGMP-compliant product are likely to fall substantially in the future, fueled by the increased success and consequent uptake of cell therapies, the introduction of competition into market forces and the streamlining of regulatory pathways. Furthermore, in the context of tolerance induction,

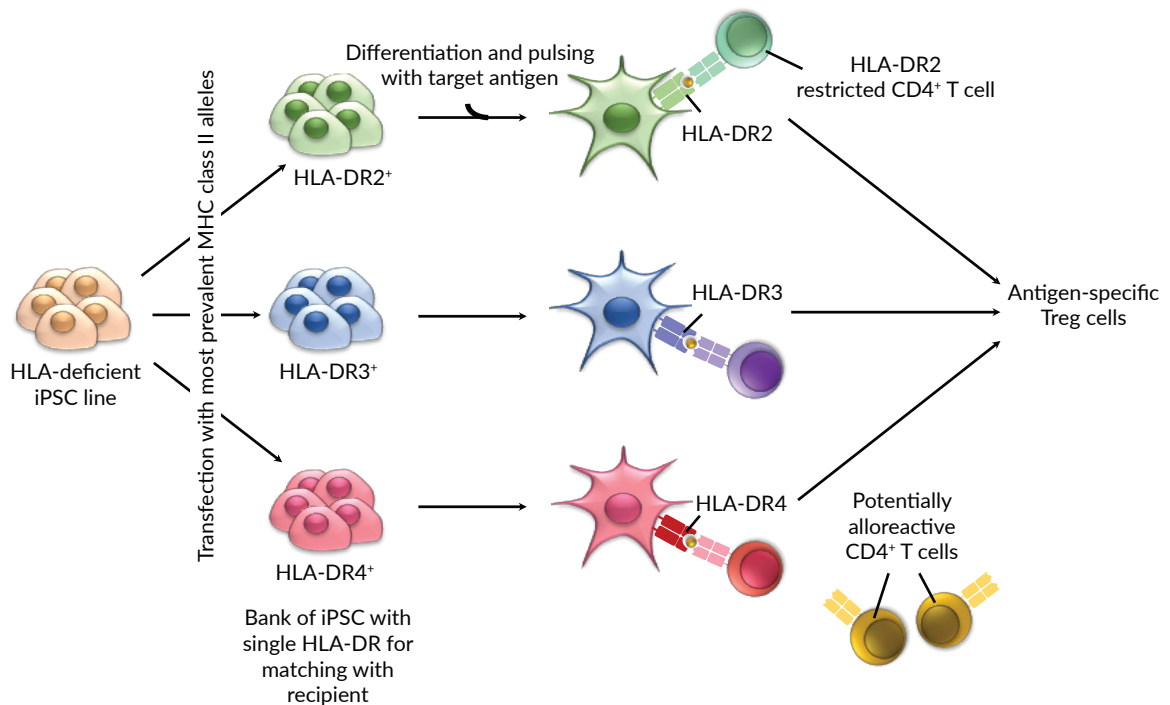
many indications may be considered orphan diseases with few alternative treatment options, greatly increasing the likelihood of reimbursement and altering the cost-benefit analysis [31]. Given that predictions of decreasing costs of manufacture have yet to be realized, however, it is doubtless pertinent to ask whether recent developments in the iPSC field might suggest solutions to the issue of alloreactivity that are compatible with the development of an off-the-shelf product.

TOWARDS A UNIVERSAL DC PRODUCT

Arguably the greatest impediment to the use of a semi-allogeneic DC product is the restriction that

► **FIGURE 3**

Design of an off-the-shelf product for tolerance induction.



MHC-deficient iPSC may be transfected with the most commonly expressed MHC class II alleles to create a bank of lines, each expressing a single MHC class II allele. Administration of antigen-pulsed DC differentiated from such lines to appropriately-matched recipients may permit the expansion of antigen-specific Treg cells in the absence of confounding alloreactivity.

alloreactivity imposes on the number of doses that can be administered. One approach to circumventing the anamnestic response might be to derive multiple iPSC lines from different donors for each MHC haplotype, each of which could be administered in turn. For the most prevalent haplotype based on HLA-A*0201, for instance, six unrelated iPSC lines might be derived, each expressing the HLA-A*0201 allele but differing at all other loci. Repeated exposure to HLA-A*0201-restricted epitopes derived from an appropriate TAA would, therefore, be expected to establish robust anti-tumor immunity over time without provoking memory responses to allogeneic MHC molecules which would elicit only primary T-cell responses on each occasion. Such a strategy would build on the demonstrated success of using multiple doses of a DC vaccine [25] while also preserving the potentially beneficial adjuvant effect of a semi-allogeneic product [20]. The obvious disadvantage of such an approach is the associated costs of deriving multiple iPSC lines for each MHC haplotype, significantly weakening the economic arguments for such a semi-allogeneic product.

An alternative strategy might be to exploit recent efforts to generate so-called 'universal' iPSC lines, compatible with all patients, irrespective of their MHC haplotype. Various groups have succeeded in the genome editing of PSC lines to render the cells deficient in MHC class I. For instance, Gornalusse and colleagues targeted the β_2 -microglobulin gene, a structural component of all MHC class I molecules, but protected the differentiated products of the resulting cells from Natural Killer (NK) cell lysis by

the forced expression of minimally-polymorphic HLA-E molecules that actively engage inhibitory receptors expressed by NK cells [32]. A more refined approach has since been reported which targets HLA-A and HLA-B alleles while preserving expression of HLA-C. This serves the dual function of facilitating residual antigen presentation to MHC class I-restricted CTL while pacifying NK cells through the ligation of KIR receptors [33]. Given the lower levels of polymorphism at the HLA-C locus, Xu *et al.* have calculated that as few as 12 iPSC lines could be immunologically compatible with more than 90% of the global population [33].

While such developments hold promise for the generation of numerous cell types for the purpose of regenerative medicine, DC pose a greater challenge by virtue of their constitutive expression of MHC class II molecules as well as class I. To generate a universal DC product would, therefore, require the additional targeting of all class II loci, perhaps through disruption of the gene encoding the class II transactivator (CIITA) that controls all MHC class II expression. Notwithstanding the additional complexity of targeting CIITA in cell lines already devoid of MHC class I, such iPSC would provide a blank canvas in which to express individual MHC alleles prevalent within the population. TAA-pulsed DC differentiated from iPSC solely expressing HLA-A*0201 could be administered to all HLA-A*0201⁺ patients with impunity and as often as necessary to build up anti-tumor immunity over time without the confounding influence of alloreactivity. Furthermore, DC differentiated from iPSC lines uniquely expressing some of

the most prevalent MHC class II loci, such as HLA-DR2, could present target antigens to naïve T cells potentially polarizing them towards a Treg phenotype. Antigen-specific Treg cells might be expected to help establish and maintain a state of immunological tolerance that would otherwise be sabotaged by activation of alloreactive CD4⁺ T cells (Figure 3). Such an approach might, therefore, provide the potential for the development of the first off-the-shelf product fully compatible with tolerance induction.

TRANSLATION INSIGHT

For most cell therapies based on the differentiation of iPSC, the debate between autologous and allogeneic sources has already been determined primarily by economic drivers, the advantages of an autologous product being eclipsed by the high costs of manufacture. The arguments on which these decisions have been based are, however, rather less persuasive when considering a DC therapy for which the very gene products that normally provoke rejection of tissues are essential to the physiological function of the very cells themselves. The stakes are, therefore, uncomfortably high, the

development of a semi-allogeneic product potentially compromising efficacy and curtailing any further investment into an otherwise promising field. Under such circumstances, recent developments in the generation of broadly compatible iPSC lines through genome editing may provide a starting point for the rational design of off-the-shelf DC products suitable either for IO or indications requiring tolerance induction.

FINANCIAL & COMPETING INTERESTS DISCLOSURE

Paul J Fairchild, Timothy J Davies, Christopher Horton hold intellectual property relevant to the differentiation of DC from iPSC but otherwise have no relevant financial involvement with an organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in this manuscript. The other authors have no conflicts of interest to declare.

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REFERENCES

1. Trounson A, McDonald C. Stem cell therapies in clinical trials: Progress and challenges. *Cell Stem Cell* 2015; 17: 11–22.
2. Trounson A, DeWitt ND. Pluripotent stem cells progressing to the clinic. *Nat. Rev. Mol. Cell Biol.* 2016; 17: 194–200.
3. Fairchild PJ. The challenge of immunogenicity in the quest for induced pluripotency. *Nat. Rev. Immunol.* 2010; 10: 868–75.
4. Fairchild PJ, Horton C, Lahiri P, Shanmugarajah K, Davies TJ. Beneath the sword of Damocles: Regenerative medicine and the shadow of immunogenicity. *Regen. Med.* 2016; 11(8): 817–29
5. Horton C, Shanmugarajah K, Fairchild PJ. Harnessing the properties of dendritic cells in the pursuit of immunological tolerance. *Biomed. J.* 2017; 40: 80–93.
6. Anguille S, Smits EL, Lion E, van Tendeloo VF, Berneman ZN. Clinical



- use of dendritic cells for cancer therapy. *Lancet* 2014; 15: e257–e267.
7. Fairchild PJ, Leishman AJ, Sachamitr P, Telfer C, Hackett S, Davies TJ. Dendritic cells and pluripotency: unlikely allies in the pursuit of immunotherapy. *Regen. Med.* 2015; 10(3): 275–86.
 8. Fukuma D, Matsuyoshi H, Hirata S *et al.* Cancer prevention with semi-allogeneic ES cell-derived dendritic cells. *Biochem. Biophys. Res. Comm.* 2005; 335: 5–13.
 9. Kitadani J, Ojima T, Iwamoto H *et al.* Cancer vaccine therapy using carcinoma embryonic antigen-expressing dendritic cells generated from induced pluripotent stem cells. *Sci. Rep.* 2018; 8: 4569.
 10. Choi K-D, Vodyanik MA, Slukvin II. Generation of mature human myelomonocytic cells through expansion and differentiation of pluripotent stem cell-derived lin-CD34+CD43+CD45+ progenitors. *J. Clin. Invest.* 2009; 119(9): 2818–29.
 11. Sontag S, Förster M, Qin J *et al.* Modelling IRF8 deficient human hematopoiesis and dendritic cell development with engineered iPSC cells. *Stem Cells* 2017; 35: 898–908.
 12. Silk KM, Silk JD, Ichiryu N *et al.* Cross-presentation of tumor antigens by induced pluripotent stem cell-derived CD141+ XCR1+ dendritic cells. *Gene Ther.* 2012; 19(10): 1035–40.
 13. Zhang Q, Fujino M, Iwasaki S *et al.* Generation and characterization of regulatory dendritic cells derived from murine induced pluripotent stem cells. *Sci. Rep.* 2014; 4: 3979.
 14. Cai S, Hou J, Fujino M *et al.* iPSC-derived regulatory dendritic cells inhibit allograft rejection by generating alloantigen-specific regulatory T cells. *Stem Cell Rep.* 2017; 8: 1174–89.
 15. Comi M, Avancini D, Santoni de Sio F *et al.* Coexpression of CD163 and CD141 identifies human circulating IL-10-producing dendritic cells (DC-10). *Cell. Mol. Immunol.* 2019; doi.org/10.1038/s41423-019-0218-0 (Epub ahead of print).
 16. Sachamitr P, Leishman A, Davies T, Fairchild PJ. Directed differentiation of human induced pluripotent stem cells into dendritic cells displaying tolerogenic properties and resembling the CD141+ subset. *Frontiers Immunol.* 2018; 8: 1935.
 17. Middleton D, Menchaca L, Rood H, Komerofsky R. New allele frequency database: <http://www.allelefrequencies.net>. *Tissue Antigens* 2003; 61(5): 403–7.
 18. Aspod C, Charles J, Leccia M-T *et al.* A novel cancer vaccine strategy based on HLA-A*0201 matched allogeneic plasmacytoid dendritic cells. *PLoS One* 2010; 5(5): e10458.
 19. Suchin EJ, Langmuir PB, Palmer E *et al.* Quantifying the frequency of alloreactive T cells *in vivo*: New answers to an old questions. *J. Immunol.* 2001; 166: 973–81.
 20. Fabre JW. The allogeneic response and tumour immunity. *Nat. Med.* 2001; 7: 649–52.
 21. Martín-Fontecha A, Sebastiani S, Höpken UE *et al.* Regulation of dendritic cell migration to the draining lymph node: Impact on T lymphocyte traffic and priming. *J. Exp. Med.* 2003; 198: 615–21.
 22. Sabado RL, Bhardwaj N. Dendritic cell vaccines on the move. *Nature* 2015; 519: 300–1.
 23. Mitchell DA, Batich KA, Gunn MD *et al.* Tetanus toxoid and CCL3 improve dendritic cell vaccines in mice and glioblastoma patients. *Nature* 2015; 519: 366–9.
 24. Hermans IF, Ritchie DS, Yang J, Roberts JM, Ronchese F. CD8+ T cell-dependent elimination of dendritic cells *in vivo* limits the induction of antitumour immunity. *J. Immunol.* 2000; 164: 3095–101.
 25. Teramoto K, Ozaki Y, Hanaoka J *et al.* Predictive biomarkers and effectiveness of MUC1-targeted dendritic cell-based vaccine in patients with refractory non-small cell lung cancer. *Ther. Adv. Med. Oncol.* 2017; 9(3): 147–57.
 26. Simhadri VL, McGill J, McMahon S *et al.* Prevalence of pre-existing antibodies to CRISPR-associated nuclease Cas9 in the USA population. *Mol. Ther. Methods Clin. Develop.* 2018; 10: 105–12.
 27. Wagner DL, Amini L, Wendering DJ *et al.* High prevalence of Streptococcus pyogenes Cas9-reactive T cells within the adult human population. *Nat. Med.* 2018; 25: 242–8.
 28. Wignakumar T, Fairchild PJ. Evasion of pre-existing immunity to Cas9: A pre-requisite for successful genome editing *in vivo*? *Curr. Transpl. Rep.* 2019; doi: 10.1007/s40472-019-00237-2 (Epub ahead of print).
 29. Marín E, Cuturi MC, Moreau A. Tolerogenic dendritic cells in solid organ transplantation: Where do we stand? *Front. Immunol.* 2018; 9: 274.
 30. Vasaturo A, Halilovic A, Bol KF *et al.* T-cell landscape in a primary melanoma predicts the survival of patients with metastatic disease after their treatment with dendritic cell vaccines. *Cancer Res.* 2016; 76(12): 3496–506.

31. Cossu G, Birchall M, Brown T *et al.* Lancet commission: Stem cells and regenerative medicine. *Lancet* 2018; 391: 883–910.
32. Gornalusse GG, Hirata RK, Funk SE *et al.* HLA-E-expressing pluripotent stem cells escape allogeneic responses and lysis by NK cells. *Nat. Biotechnol.* 2017; 8: 765–72.
33. Xu H, Wang B, Ono M *et al.* Targeted disruption of HLA genes via CRISPR-Cas9 generates iPSCs with enhanced immune compatibility. *Cell Stem Cell* 2019; 24: 1–13.

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