Chapter

Pluripotent Stem Cell Derived Macrophages: Current Applications and Future Perspectives

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Abstract

The ability to derive macrophages from human-induced pluripotent stem cells (iPSCs) provides an unlimited source of genotype-specific cells with the potential to play a role in advancing our understanding of macrophage biology in both homeostasis and disease. While sharing many of the functional characteristics of monocyte-derived macrophages, iPSC-derived macrophages have also been shown to have phenotypical and functional features associated with tissue resident macrophages. These features present new opportunities to develop models of human disease and to understand the role of developmental or tissue context in innate immune cell function. iPSCs-derived macrophages have also been identified as a highly attractive source for cell and gene therapy in the treatment of diverse degenerative diseases based on their anti-inflammatory activity, their ability to clear scarred cells by phagocytosis, and providing extracellular matrices. We review and present a concise discussion on macrophage differentiation from stem cells highlighting their advantages over classical monocyte-derived macrophages in modelling organ specific macrophages. We summarize the various disease models utilizing iPSCs-derived macrophages including hereditary syndromes and host-pathogen interactions in tissue repair and the strategies used to mimic pathological phenotypes. Finally, we describe the preclinical studies that have addressed the application of iPSCs-derived macrophages as a therapeutic intervention.

Keywords: iPSCs, macrophages, polarization, inflammation, regeneration, cell therapy

1. Introduction

Macrophages were historically considered as specialized immune cells that are resident in every tissue. They are professional phagocytic cells and are considered to be one of the most evolutionary conserved components of the innate immune system [1]. However, studies of the past two decades identified several additional functions of macrophages particularly those involved in maintaining tissue homeostasis such as wound healing and regeneration [2]. Macrophage populations within the tissue were originally assumed to be continuously replaced by the differentiation of monocytes derived from peripheral blood [3, 4]. However recent studies using methods such as lineage tracing and single cell transcriptomics have established that several

macrophage populations resident in organs including brain, lung, intestine and liver, originate from yolk-sac (YS) myeloid precursor cells that were seeded within the tissues during early embryonic hematopoiesis [5]. These myeloid precursors differentiate into macrophages (microglia in case of brain) within their resident tissue site, and are self-maintained throughout the life course of the organism [5]. Tissue resident macrophages (TRMs) are functionally distinct from macrophages derived from the more accessible circulating blood monocytes but their yolk sac origin makes them difficult to study [5]. In order to successfully study these cells *in vitro*, researchers have develop methods that permit functional TRMs to be isolated [5]. *In vitro* strategies to replicate the tissue resident differentiation of macrophages in a way that mimics their tissue specific developmental and differentiation pathways have been developed using a variety of growth factors and cytokines [6]. However, cells produced using these methods do not completely recapitulate the properties of TRMs and it would be challenging to scale up and produce large numbers of cells.

The ground-breaking discovery that human somatic cells could be reprogramed into induced pluripotent stem cells (iPSCs) that are capable of differentiating into any cell type has revolutionized many areas of medical research including macrophage biology [7]. Several studies have shown that human iPSCs can be differentiated into macrophage populations that are phenotypically and functionally comparable to human macrophages. The major advantage of the iPSCs-derived macrophages is that they share some phenotypic and functional profiles with both tissue resident macrophages and monocyte derived macrophages (MDMs) [8]. Here, we review the potential of iPSCs derived macrophages in both classical immune function as well as their tissue repair and regeneration properties. We summarize the various protocols that have been used for macrophage production from iPSCs, discuss their disease modeling potential including hereditary and pathogen associated diseases and describe some of the pre-clinical trials lay the foundations for their use in cell therapies.

2. Human induced pluripotent stem cells for macrophage production *in vitro*

The generation of iPSCs from adult somatic cells by the introduction of four genes encoding the "Yamanaka" transcription factors, octamer-binding transcription factor 3/4 (OCT3/4), sex determining region Y-box 2 (SOX2), Kruppel-like factor 4 (KLF4), and myelocytomatosis viral oncogene homolog (MYC), was first reported in 2006 [9]. Resultant iPSCs were shown to be comparable to embryonic stem cells (ESCs), thus providing an ethical strategy for creating clonal PSC lines that did not involve the destruction of human embryos [10]. Initially reprogramming of somatic cells to iPSCs was successfully performed using genome integrating retroviral or lentiviral vectors such as retroviruses and lentiviruses [7]. Though viral integration was efficient, it was associated with risks of random mutational insertions into the genome. Alternate non-viral integration methods such as plasmids, synthetic mRNA, minicircle DNA molecules and small chemical molecules to generate iPSCs reduced random mutations but the efficiency of generating iPSCs was low [7, 8]. Sendai virus is now commonly used for iPSC generation as its replication is limited within the cytoplasm and it does not integrate into the genome. Initially skin fibroblasts were used for reprogramming iPSCs, but other easily accessible cells such as peripheral blood monocytes and renal epithelial cells from urine have been successfully used as the starting cells [11, 12]. iPSCs are able to be differentiated into the three embryonic germ layers namely

ectoderm, endoderm and mesoderm and can differentiate into somatic cells associated with all cell lineages [13].

Following the generation of stable iPSCs, their pluripotent properties are maintained using specific growth factors [9]. Removal of these maintenance factors results in spontaneous differentiation and, under appropriate conditions the formation of 3-dimensional (3D) embryoid bodies (EBs) that are considered to mimic early embryogenesis [14]. Differentiation factors such as cytokines and small molecules have been used to stimulate specific differentiation pathways resulting in the production of cells displaying phenotypes and gene expression patterns of almost any cell type including cells of the blood and immune system [15].

Doetschman and colleagues were the first to report that hematopoietic cell types could be produced from mouse embryonic stem cells (ESCs) by demonstrating structures resembling blood islands in cystic embryoid bodies (EBs) that were comparable to the primitive wave of hematopoiesis in the yolk sac [16]. Differentiation protocols were subsequently developed and refined to include the use of feeder cells, extracellular matrices and specific growth factors [17–20]. Many of these protocols failed to generate the long-term reconstituting hematopoietic stem cells (HSCs) associated with the definitive wave of hematopoietic development and a significant amount of research has gone into addressing this problem in both the mouse and human systems [21–24]. The production of macrophages was reported even in the first, rather crude differentiation protocols with the ability to harvest on regular basis for several weeks represented a significant advance in the field [18, 25]. The fact that these differentiation protocols most likely mimic the primitive wave of hematopoiesis it is not surprising that resultant cells have some features that are comparable to TRMs.

The production of macrophages from human iPSCs is now well established and they are considered to have features associated with both YS-derived TRMs as well as MDMs [5]. As iPSCs can been maintained indefinitely in culture and can be readily genetically manipulated, they can therefore provide an inexhaustible source of macrophages carrying any desired genetic alteration. The first protocols that were developed involved the co-culture of iPSCs with OP9 mouse stromal cell monolayers to induce hematopoietic differentiation, followed by expansion of myeloid progenitors and selective differentiation into macrophages by using growth factors to differentiate dendritic cells and macrophages [26, 27]. These protocols were further modified to establish embryoid body (EB)-based protocols for IPSC-derived macrophage differentiation. We have used a modified serum-free protocol in which EB-based hematopoiesis is used to generate monocyte-like cells in suspension that can then be differentiated into mature macrophages [28]. Briefly, differentiation from human iPSCs is initiated by the removal of pluripotency factors and the addition of stem cell factor (SCF), bone morphogenetic protein (BMP)-4 and vascular endothelial growth factor (VEGF) to induce EB formation. The addition of interleukin (IL)-3 and macrophage colony stimulating factor (M-CSF) to EBs that are then plated down onto the culture plates results in the production of monocyte precursors that are released into suspension. Monocyte-like cells are then plated down and differentiated into mature macrophages by the addition of M-CSF [29]. iPSCs derived macrophages express macrophage-specific markers including cluster of differentiation (CD)11b, CD163, and CD169 [30]. Macrophages generated from iPSCs that carried the Zeiss Green reporter gene integrated into the adeno-associated virus integration site 1 (*AAVS1*) locus showed that neither expression of the reporter nor the targeting of the *AAVS1* locus affected of macrophage phenotype confirming the idea that genetically manipulated macrophages can be generated using this strategy [30].

2.1 iPSCs-derived macrophages share features of MDM

Yeung and colleagues demonstrated that the iPSCs-derived macrophages exhibited gene expression profiles and responsiveness to external stimuli that were comparable to MDMs. Their data demonstrated that untreated iPSCs-derived macrophages and MDMs expressed 12,599 human genes overlapping and a further 93% of these genes were expressed to a similar level [31]. This pattern of gene expression iPSCs-derived macrophages and MDMs remained consistent even after stimulation, as upon *Chlamydia trachomatis* infection, the two cell types had more than 2000 differentially expressed genes in common [31].

iPSCs-derived macrophages secrete comparable levels of cytokines as MDMs upon stimulation with pattern recognition receptors such as toll-like receptor (TLR) agonist [32]. They are able to phagocytose live *Salmonella typhi* or fungal particles like zymosan [30, 33]. Most importantly, iPSCs-derived macrophages retain plasticity which is one of the key characteristic of macrophages [34]. Naive macrophages express an M0-like steady state phenotype, which can be switch to either an inflammatory (M1) or immunosuppressive (M2) function based on their microenvironment. It is proposed that due to the genetic and functional similarities between iPSCs-derived macrophages and primary macrophages, the former could be used as a tool to model macrophage polarization in inflammatory diseases and genetic diseases such as autosomal recessive disorders. A study from Matsuo et al modeling a disease *fibro dysplasia ossificans progressiva* using iPSCs derived macrophages showed the potential of 2D and 3D based differentiation of macrophages having a differential role in their polarization potential [35]. While the 2D iPSCs-derived macrophages could be polarized to either M1 or M2, the 3D differentiated macrophages showed a mixed M1 and M2 like functional features, highlighting the complexity of macrophage plasticity.

2.2 iPSCs derived macrophages model tissue resident macrophages

Although iPSCs-derived macrophages have demonstrated similar phenotypic, functional, and transcriptomic characteristics to MDMs as discussed above, they are also reported to have comparable characteristics to tissue resident macrophages (TRMs). This TRM like phenotype gives iPSCs-derived macrophages an advantage over other models such as MDMs or monocytic cell lines such as THP-1. It has been recognized that the standard hematopoietic differentiation protocols of iPSCs resemble the primitive rather than definitive wave of hematopoiesis *in vivo* [36]. To confirm this theory, Vanhee and colleagues used a reporter PSC line with myeloblastosis proto-oncogene with a green fluorescent protein (*MYB-eGFP*), a marker for definitive hematopoietic stem cell (HSC)-dependent hematopoiesis, and as expected the *in vitro* generation of EB-based iPSCs derived macrophages lacked *MYB*+ HSCs [37]. The results were further validated by demonstrating that clustered regularly interspaced short palindromic repeats and its associated protein 9 (CRISPR/Cas9) knockout of *MYB* in human iPSCs, did not impact macrophage differentiation [38]. The theory was further verified in experiments where two important transcription regulators of YS hematopoiesis transcription factor PU.1 encoding gene *SPI1* and Runt-related transcription factor 1 gene *RUNX1* were knocked out in iPSCs, they were unable to produce mature macrophages [37, 38]. Several studies have indicated the TRM nature of iPSCs-derived macrophages is based on *in vitro* microglia modeling. Indeed, several cytokine mediated iPSCs to microglia-like cell differentiation protocols were published in recent years using coculture with iPSCs-derived neurons and astrocytes

or using conditioned media from those cell types to recapitulate organ-specific microenvironment [39]. Takata and colleagues further demonstrated by macrophages derived from iPSCs using a protocol that specifically resembles primitive hematopoiesis and yolk sac macrophages that they are very similar to TRMs [5]. They did this by engrafting iPSCs derived macrophages into the mouse brain which then underwent functional and morphological changes to become microglia, while the iPSCs derived macrophages engrafted to the lung of the Pulmonary Alveolar Proteinosis (PAP) mouse model matured into alveolar macrophages. This study showed that the iPSC-derived macrophages developed to microglia-like cells *in vivo* and showed genomic profile similar to that of both human adult and foetal microglia, while in the lung of the PAP mouse model they eliminated the surfactant protein that had accumulated as a result of the disease. Our lab recently used iPSCs-derived macrophages to model the erythroblastic Island (EBI) niche *in vitro* by genetic programming with the transcription factor, KLF1 [29].

2.3 iPSCs derived macrophages in disease modeling

Another important feature of human iPSCs derived macrophages is that iPSCs are amenable to genetic engineering and thus can be manipulated to be model genetic disease. Disease modeling can be achieved either through the production of iPSCs from patients carrying disease-causing mutations and/or specific genome-wide associations or by targeted gene edited using the (CRISPR)/Cas9 system. iPSCs-derived macrophages are increasingly being used to study genetic disease, including validation of known causative genes or identifying novel mutations associated with single nucleotide polymorphisms (SNPs) [40]. iPSCs-derived macrophages helped overcome the limitations of the poor availability of disease-specific primary macrophages in studying these rare genetic diseases. The ability to derive macrophages from iPSCs provided new opportunities to develop models relevant to human genetics, resulting in a progressive accumulation of studies describing macrophage functions in both tissue homeostasis and disease. For example, patient iPSCs-derived macrophages have been utilized to investigate several genetically inherited diseases including Blau Syndrome, Tangier disease and Gaucher disease. In additions there are studies where a diseased condition such as Dyskeratosis Congenita has been generated in iPSCs using genetic engineering technology and macrophages or myeloid cells derived from these used to understand the disease mechanism [41–44]. **Table 1** lists some of the genetic studies performed using patient iPSCs-derived macrophages, that would not have been possible using primary cells.

As well as generating valuable disease models these studies also described the novel approach of immortalizing iPSCs-derived myeloid cells using transducing lentiviral vectors that encoded genes *MYC*, polycomb complex protein gene *BMI1* and mouse double minute 2 homolog gene *MDM2,* creating a strategy to generate monocytic cell lines, with the diseased phenotype [53]. These iPSC-derived immortalized myeloid cell lines have the advantage that they can be stored and differentiated into terminally differentiated progenies and expanded from one experimental batch with reduced financial and labour costs, overcoming many of the hurdles associated with iPSCs.

2.4 iPSCs-derived macrophages in host-pathogen interactions

iPSCs-derived macrophages have also been used widely in studies relating to their classical role in infection biology. iPSCs-derived macrophages can polarized

Studies utilizing iPSCs derived macrophages for disease modeling		
Disease	**Research findings**	**References**
Tangier Disease (TD)	iPSC-derived macrophages from TD patients recapitulate the clinical defect of failed cholesterol efflux resulting in reverse cholesterol transport.	[45]
	TD effect of reverse cholesterol transport in macrophages derived from CRISPR/Cas9 induced adenosine triphosphate binding cassette subfamily A member 1 gene (*ABCA1*) knockout iPSCs.	[43]
Gaucher disease (GD)	iPSCs-derived macrophages from GD patients exhibited delayed clearance of phagocytosed RBC which was reversed when treated with recombinant glucocerebrosidase enzyme.	[42]
	Reversal of GD phenotype in iPSCs-derived macrophages using small-molecule chaperone drug.	[46]
Chronic granulomatous disease	iPSCs-derived macrophages from dihydronicotinamide-adenine dinucleotide phosphate (NADPH) oxidase defective patient showed normal phagocytic properties unlike patient MDMs, however showed a lack in reactive oxygen species production, correlating with clinical diagnosis.	[47]
Blau syndrome	iPSCs-derived macrophages from nucleotide-binding oligomerization domain-containing protein 2 (*NOD2*) mutated patient showed ligand-independent pro-inflammatory cytokine production *in vitro* upon Interferon (IFN)-γ treatment. The cytokine production was terminated upon NOD2 mutation correction by CRISPR/Cas9.	[41]
Type 1 diabetes	iPSCs-derived macrophages from Diabetic patient showed potential for antigen presentation to proinsulin-specific T cell receptors from donor-matched islet-infiltrating T cells.	[48]
Familial Mediterranean fever	Patient iPSCs-derived macrophages exhibited the disease characteristics including enhanced IL-1β secretion and hyperactivation of the pyrin inflammasome.	[49]
Mendelian Susceptibility to Mycobacterial Disease (MSMD)	iPSCs-derived macrophages from MSMD patients with autosomal recessive complete- and partial IFN-γR2 deficiency, partial IFN-γR1 deficiency and complete STAT1 deficiency demonstrated varying phenotypes including cytokine secretion for the partial and complete deficiencies.	[50]
Idiopathic Parkinson's disease	Patient iPSCs-derived microglia to confirmed findings in patients brain tissue of having elevated *IL1B, IL10* and NLRP3 expression after *in vitro* LPS stimulation. iPSCs-derived microglia showed high phagocytic capacity under basal conditions that was exacerbated upon stimulation with LPS.	[51]
Chronic infantile neurologic cutaneous and articular syndrome	iPSCs-derived macrophages from NLRP3 mutated patient showed the disease relevant phenotype of abnormal IL-1β secretion which were inhibited by anti-inflammatory compounds.	[52]
	Immortalized iPSCs-derived myeloid cells from patient recapitulated the disease phenotypes *in vitro.*	[53]

Table 1.
Summary of studies that have used iPSCs-derived monocytes and macrophages for in vitro modeling of genetic diseases.

to a pro-inflammatory or anti-inflammatory phenotype by treating with lipopolysaccharide (LPS)/IFN-γ or IL-4/IL-10, respectively [30]. These features make them a powerful *in vitro* tool to study bacterial, viral, and parasitic infections and their resultant immune responses. Hale et al infected iPSC-derived macrophages with *Salmonella enterica serovar Typhimurium* (*S. Typhimurium*) and *S. Typhi* and reported comparable data to that observed using the commonly used human monocyte-like THP1 cell line thus opening the way for their application to other bacterial infections [33].

One such example was the study of the interaction between *Staphylococcus aureus* toxin leukocidins with macrophages, which are the initial targets during *S. aureus* lung infection [54]. iPSCs-derived macrophages were susceptible to the leukocidins and triggered NLPR3 inflammasome activation resulting in IL-1β secretion and eventual cell death. CRISPR/Cas9-mediated deletion of the leukocidin receptor, complement component 5a receptor 1 (C5aR1) also known as CD88 protected the macrophages from cytotoxicity [54]. Another bacterial study using the iPSCs-derived macrophages model was on *C. trachomatis,* which causes bacterial sexually transmitted infections and preventable blindness worldwide. Yeung and colleagues demonstrated that iPSC-derived macrophages supported the full infectious life cycle of *C. trachomatis in vitro* in a manner that resembled the infection of human blood *in vivo* [31]. Using transcriptomic and proteomic profiling of the macrophage they identified that the key players in response to chlamydial infection are type I interferon and interleukin 10. This was further confirmed by knocking-out IRF5 and IL-10RA in iPSCs, which resulted in limited chlamydial infection in genetically-deficient macrophages. Though the studies mentioned above showed that bacterial infection studies using iPSCs-derived macrophages are comparable with the data generated using MDMs, one recent study clearly highlights an additional advantage of using iPSCs-derived macrophages. Nenasheva et al reported that iPSCs-derived macrophages differed from MDMs by an low-activated/low- polarized naïve-like (HLA-DR^{low} $CD14^{+}CD16^{int}$) phenotype compared to the HLA-DR^{high} $CD14^{+}CD16^{+}$ phenotype of mature macrophages shown by MDMs, where HLA-DR stands for human leukocyte antigen – DR isotype [8]. These naïve-like iPSCs-derived macrophages were transcriptionally similar to pulmonary macrophages and restricted *Mycobacterium tuberculosis* growth *in vitro* by >75% higher phagocytic potential than MDMs [8]. Similarly, a study from Hong et al also showed that iPSCs-derived macrophages perform the immunological functions in response to Bacillus Calmette-Guérin a vaccine against *M. tuberculosis*, similar to MDMs by undergoing apoptosis, increased production of nitric oxide and elevated expression of Tumor necrosis factor (TNF)-α, thus demonstrating their suitability as a potential drug target [55].

Viruses require a specific cellular host for replication and so the readily available supply of infectable cells is crucial in viral research. iPSCs are ideal for this purpose because they can be differentiated into the specific cell type associated with an infectious agent, including endothelial cells for cytomegalovirus, neurons for herpes simplex virus, hepatocytes for hepatitis viruses, CD4 T-cells for human immunodeficiency virus (HIV) [56]. The field of HIV research has used iPSCs-derived macrophages widely. Using various genetic editing techniques, Kambal *et al* and Ye *et al* introduced mutations into C-C chemokine receptor type 5 (CCR5), the major coreceptor required for macrophage trophic strains of HIV and demonstrated that monocytes and macrophages differentiated from CCR5-mutated iPSCs were resistant to HIV-1 challenge [57, 58]. Kang et al confirmed this study by demonstrating that CCR5-mutant iPSCs derived macrophages showed unique and enhanced resistance

to CCR5-tropic HIV challenge but were susceptible to CCR4-tropic viruses [59]. One of the most elegant studies that utilized human PSCs-derived macrophages characterized the molecular and cellular basis involved in both Zika and Dengue viral infections [60]. Using macrophages derived from both human ESCs and iPSCs, Lang et al showed that though both these viruses are closely related, their mechanism of infection was different. Zika virus disrupts the nuclear factor κB (NF-κB)-migration inhibitory factor (MIF) positive feedback loop by inhibiting the NF-κB signaling pathway and thus the infected macrophages exhibit prolonged migration but expressed low levels of pro-inflammatory cytokines and chemokines. In contrast, Dengue virus strongly activates MIF secretion and results in decreased macrophage migration. In summary the characteristics of iPSCs-derived macrophages together with genetic editing tools such as CRISPR/Cas9 has significantly enhanced our ability to study host–pathogen interactions as well as the role of human genetic variations in influencing the susceptibility to specific pathogens and disease outcomes.

2.5 iPSCs-derived macrophages in cell and regenerative therapy

Several studies performed in the last decade identified macrophages to have a prominent role in tissue repair and regeneration by their injury response features including clearing cell debris by phagocytosis, activating and resolving inflammation and promoting fibrosis by providing growth factors [61, 62]. For example the transplantation of mouse bone marrow-derived macrophages into a CCL4 mediated advanced liver injury mice model resulted in the reduction of fibrosis by increased recruitment of host effector cells such as neutrophils and secretion of regenerative factors such as matrix metallopeptidase 9 (MMP9), insulin-like growth factor 1 (IGF-1), M-CSF, vascular endothelial growth factor (VEGF) and IL10 [63]. Similarly exogenous macrophage treatments were shown to promote injury resolution in a several murine models of inflammatory and degenerative diseases including pulmonary fibrosis and osteochondral defect [64, 65]. This successful demonstration of macrophage therapy in pre-clinical models led to the use of autologous macrophages in therapeutic interventions in clinical studies against chronic liver injury and neurodegenerative diseases [66, 67]. It is thought that these repair functions are performed by the anti-inflammatory or resolving M2-polarized macrophages and several studies identified iPSCs-derived macrophages to be able to be polarized into an M2-phenotype similar to MDMs [30]. The polarization potential together with their ability for unrestricted production makes the iPSCs-derived macrophages ideal candidates for future cell therapies. Some of the studies highlighting these *in vivo* studies using iPSCs-derived macrophages as exogenous interventions are summarized in **Table 2**.

Studies from our lab demonstrated that *in vitro* generated macrophages from mouse ESCs had the capacity for repair *in vivo* in a murine model of liver fibrosis [68]. Using a chemokine (C-C motif) ligands 4 (CCL4)-mediated liver injury model, the effect of injecting ESCs-derived macrophages or bone marrow derived macrophages (BMDMs) were assessed. ESCs-derived macrophages had a higher capacity to repopulate the Kupffer cell compartment of injured liver compared to BMDMs, supporting the theory that PSC-derived macrophages have a phenotype more akin to tissue resident macrophages. In addition, markers of liver damage were significantly lowered in mice that received ESCs-derived macrophages compared to controls indicating their reparative capacity. Another study assessed the effects of human iPSCs-derived macrophages polarized to an M1 or M2 phenotype on CCL4-induced fibrosis

Studies demonstrating iPSCs derived macrophages as therapeutic interventions		
Disease	**Research findings**	**References**
Liver Fibrosis	Mouse ESC-derived macrophages showed repair capacity in CCL4 murine model.	[68]
	M1 and M2 polarized human iPSCs-derived macrophages ameliorated fibrosis in an immunodeficient CCL4 murine model.	[69]
Pulmonary Alveolar Proteinosis (PAP)	Mouse iPSCs-derived macrophages gained an alveolar phenotype lung of PAP model and improved alveolar protein deposition.	[34]
	Human iPSCs–derived macrophages transplanted into the lung of humanized PAP mice showed *in situ* differentiation to an alveolar macrophage-like phenotype and disease remission.	[70]
	Human iPSCs-derived macrophages engrafted into the lung of the PAP mouse model differentiated into alveolar macrophages and eliminated disease associated surfactant proteins.	[5]
	Macrophages derived from gene corrected PAP patient-derived iPSCs showed restoration of normal phenotype.	[71]
Inflammatory Bowel Diseases (IBDs)	Very early onset of IBDs in patients leads to decreased bacterial killing ability in macrophages, which was reverted by the pharmacological inhibition of PGE2 synthesis and PGE2 receptor blockade.	[72]
	Genetic correction of patient iPSCs-derived macrophages *in vitro* led to reinitiating of the anti-inflammatory response and reduction of IBD associated traits.	[73]
Solid Tumors	Engineered iPSCs-derived CAR-macrophages with antigen-dependent anti-cancer functions demonstrated pro-inflammatory/anti-tumor state, enhanced clearance of tumor cells by phagocytosis.	[74]
	Designer iPSCs-derived macrophage cell line to secrete IFN-β (opinion)	[75]

Table 2.
Summary of studies that have used iPSCs-derived macrophages as a source of therapeutic cells and/or to study mechanism of disease.

of immunodeficient recombination activating gene knock-out (*Rag2*$^{-/-}$ $\gamma c^{-/-}$) mice [69]. These human iPSCs-M1 (in presence of IFN-γ and LPS) and M2 (in presence of IL-4 and IL-13) macrophage subtypes demonstrate distinct pro-inflammatory and anti-inflammatory phenotypes at both gene and protein level, which was confirmed by their RNA-seq analysis. Interestingly administration of both M1 and M2-polarized macrophages led to reduced liver fibrosis and inflammation. iPSCs-M2 as expected demonstrated a stronger downregulation of clinically relevant fibrotic markers. However, the profound antifibrogenic potential and resolution in the presence of iPSCs-M1 was unexpected based on currently thinking on the reparative phenotype of macrophages. The scenario of iPSCs-M1 showing a resolving phenotype could be corroborated by a previous study reporting the therapeutic potential of M1 polarized BM-derived macrophages to ameliorate fibrosis by the recruitment of endogenous macrophages as well as promote apoptosis through mediators of inflammation including MMPs, transforming growth factors (TGFs) and TNF related apoptosis inducing ligand (TRAIL) [73]. This could be the situation as the iPSCs-M1 macrophages in this study were shown to secrete MMP9 and TGF-β [69]. However more detailed study is required to understand the mechanisms underlying this resolving capacity

of iPSCs-M1 macrophages. Nevertheless, the study clearly confirms the potential of iPSCs-derived M2 macrophages as a therapeutic option against liver fibrosis.

The therapeutic potential of iPSCs-derived macrophages has also been assessed in lung fibrosis particularly in the case of pulmonary alveolar proteinosis (PAP). Hereditary PAP is a disorder known to be originated by a defect in the *CSF2RA* gene coding for the granulocyte-macrophage colony-stimulating factor (GM-CSF) receptor alpha-chain (CD116). Happle et al demonstrated for the first time that transplantation of human iPSCs–derived macrophages into the lung of a humanized PAP mice model led to their pulmonary engraftment and their differentiation to an alveolar macrophage like phenotype in situ [70]. They also showed that this engraftment resulted in a reduction of alveolar proteinosis as measured by diminished protein content and surfactant protein D levels, decreased turbidity of the BAL fluid, and reduced surfactant deposition in the lungs of transplanted humanized PAP model mice. Similar results were observed using the aid of wild type murine iPSCs-derived macrophage *in vivo* transplantation to a CD116 (CSF2RA) deficient PAP mice model resulted in the former's integration into the diseased lung, gaining an alveolar macrophage phenotype and improving alveolar protein deposition [34]. Kuhn et al gene-corrected patient-derived PAP-specific iPSCs carrying a defective *CSF2RA* gene using targeted insertion of a codon-optimized CSF2RA-cDNA into the *AAVS1* locus [71]. This strategy resulted in robust expression of the *CSF2RA* gene in both undifferentiated iPSCs as well as in differentiated macrophages. The authors further demonstrated that these genetically-modified macrophages showed that the exogenous CSF2RA protein was functional by STAT5 phosphorylation and GM-CSF uptake studies, supporting the idea that these functionally restored iPSCs-derived macrophages could serve as a source for an autologous cell-based gene therapy for the treatment of PAP.

Inflammatory bowel disease (IBD) is another group of inflammatory syndromes where the potential role of iPSCs-derived macrophage mediated therapy has been evaluated. Studies have demonstrated that these macrophages can be used in disease modeling and to reduce the disease pathology *in vitro*. A recent study showed that macrophages derived from an infantile-onset IBD patient iPSCs were unable to phosphorylate signal transducer and activator of transcription 3 (STAT3), and failed to reduce LPS induced inflammatory cytokines even in the presence of exogenous IL-10 [72]. These macrophages exhibited a functional defect in their ability to kill *S. Typhimurium*, but were rescued by the introduction of a functional *IL10RB* gene. The study also showed that macrophages derived from patient iPSCs produced higher amounts of eicosanoid prostaglandin E2 (PGE2) after LPS stimulation and that pharmacological inhibition of PGE2 synthesis and PGE2 receptor blockade enhanced their bacterial killing ability. This study identified a regulatory interaction between IL-10 and PGE2 and that their dysregulation contributed to IBD pathogenesis. Gene correction in an independently-derived iPSC line from another IL10RB^{-}deficient IBD patient led to reconstitution of the anti-inflammatory response - reinitiating the IL-10RB expression, IL-10-inducible phosphorylation of STAT3, and subsequent SOCS3 expression [76]. This second study also showed that LPS-mediated TNF-α secretion could be modulated by IL-10 stimulation in gene-edited iPSCs-derived macrophages. Taken together, these established iPSC-derived macrophages based IBD models provide the opportunity to identify and validate new curative molecular and cellular therapies against IBD and other inflammatory syndromes.

The iPSCs-derived macrophage strategy has also been applied to the exciting field of cancer immunotherapy. Chimera antigen receptor (CAR)-T cells and NK cells re shown to have potent cytotoxicity against tumor cells with CAR-T cell therapy

having gained great success in the clinic [77]. Recently CAR-macrophages have been developed by engineering an adenoviral vector to express a CAR targeted against human epidermal growth factor receptor 2, (a biomarker in many solid tumors) and imparted a sustained pro-inflammatory (M1) phenotype [78]. The model showed great success by demonstrating antigen-specific phagocytosis and tumor clearance *in vitro* as well as decreasing tumor burden and prolonged overall survival in two solid tumor xenograft models [78]. These successful pre-clinical data have led to an Phase-1 clinical trial using HER2 re-targeted CAR-macrophages for the treatment of solid tumors [77]. Other examples of iPSC-derived CAR-macrophages with antigen-dependent anti-cancer functions include those expressing either CD19 and mesothelin-specific fusion receptors [74]. CAR-macrophages demonstrated functions including expression and secretion of cytokines, polarization toward the pro-inflammatory/anti-tumor state, enhanced phagocytosis of tumor cells, and in anti-cancer cell activity *in vivo* when stimulated by tumor antigens. These data demonstrate the technology platform of iPSC-derived CAR-macrophage to eliminate cancer cells. These CAR engineered iPSCs-derived macrophages is a ground breaking technology in cancer immunotherapy and when combined with novel methods of bioreactor based bulk macrophage differentiation from iPSCs will provide an unlimited source of therapeutic cells [79].

3. Conclusion

In summary, the phenotypic, functional, and transcriptomic characteristics of iPSCs-derived macrophages share many similarities with both tissue resident macrophages and MDMs. The unlimited replication potential of iPSCs and the ease of genetic manipulation thus provides a valuable platform for disease modeling, drug screening, and studying the mechanisms of infection biology in various genetic backgrounds. Their autologous nature and polarization potential could also make them ideal tools for cell and regeneration therapy. iPSCs-derived macrophages have enormous potential in advancing our understanding of diseases that involve human macrophages and to date have demonstrated proof of principle utility in the development of disease models and in novel cell therapies. The use of iPSCs-derived macrophages does not eliminate the need for other models such as MDMs or BM-derived macrophages, but rather provides a complementary or alternative approach to further ensure validity and reproducibility. Together with genetic manipulations techniques such as CRISPR/Cas9 they can facilitate clinical and therapeutic translation for diseases such as liver fibrosis or inflammatory lung diseases where macrophages play an important clinical modulatory role. This is well highlighted by a research article under peer-review where M2 polarized iPSCs-derived macrophages are studied in context with COVID19 therapy [80]. The clinical potential of the macrophage cell therapy is highlighted by several clinical trials approved for autologous macrophages as intervention in various diseases including chronic liver injury, spinal cord injury, non-acute stroke, chronic anal fissure and as an anti-fibrotic treatment following COVID-19 infection [clinicaltrials.gov]. The future of iPSCs-derived macrophage therapy could be focused toward increasing their universality or increasing their better storage and differentiation as demonstrated by the studies of developing iPSCs-derived myeloid lines, continuous differentiation or cryopreservation [53, 81, 82]. An overview of the iPSCs-derived macrophages features and applications covered in this review is summarized as **Figure 1**.

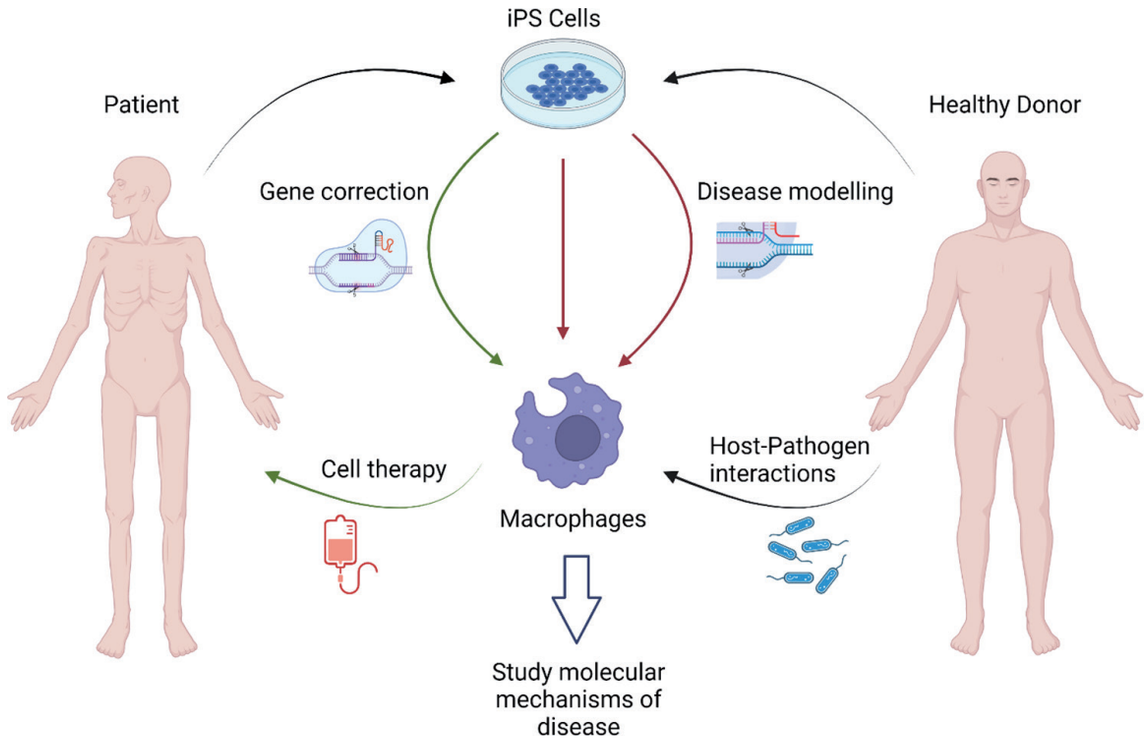

Figure 1.
Summary of iPSCs-derived macrophages attributes and applications.

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Conflict of interest

The authors declare no conflict of interest.

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