



Cell Therapy: Types, Regulation, and Clinical Benefits

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OPEN ACCESS

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Specialty section:

This article was submitted to
Gene and Cell Therapy,
a section of the journal
Frontiers in Medicine

Received: 09 August 2021

Accepted: 01 November 2021

Published: 22 November 2021

Citation:

El-Kadiry AE-H, Rafej M and
Shammaa R (2021) Cell Therapy:
Types, Regulation, and Clinical
Benefits. *Front. Med.* 8:756029.
doi: 10.3389/fmed.2021.756029

Cell therapy practices date back to the 19th century and continue to expand on investigational and investment grounds. Cell therapy includes stem cell- and non-stem cell-based, unicellular and multicellular therapies, with different immunophenotypic profiles, isolation techniques, mechanisms of action, and regulatory levels. Following the steps of their predecessor cell therapies that have become established or commercialized, investigational and premarket approval-exempt cell therapies continue to provide patients with promising therapeutic benefits in different disease areas. In this review article, we delineate the vast types of cell therapy, including stem cell-based and non-stem cell-based cell therapies, and create the first-in-literature compilation of the different “multicellular” therapies used in clinical settings. Besides providing the nuts and bolts of FDA policies regulating their use, we discuss the benefits of cell therapies reported in 3 therapeutic areas—regenerative medicine, immune diseases, and cancer. Finally, we contemplate the recent attention shift toward combined therapy approaches, highlighting the factors that render multicellular therapies a more attractive option than their unicellular counterparts.

Keywords: cell therapy, FDA regulations, multicellular therapies, regenerative medicine, cancer, immune diseases, bone marrow aspirate concentrate (BMAC), mesenchymal stem cells

INTRODUCTION

Cell therapy refers to the transfer of autologous or allogeneic cellular material into a patient for medical purposes (1, 2). The year 1889 witnessed the first practices of cell therapy by Charles-Édouard Brown-Séquard—pioneer in hormone therapy at the time—who attempted to suppress the effects of aging using injections of animal testicle extracts (3). Today, cell therapy continues to evolve with ongoing investigations for clinical safety and efficacy, and with a global market size estimated to expand from USD 9.5 billion in 2021 to USD 23.0 billion in 2028 (4). Cell therapy combines stem cell- and non-stem cell-based unicellular or multicellular therapies. It typically employs autologous or allogeneic cells; might involve genetic engineering or manipulations in formulation; and can be administered topically or as injectables, infusions, bioscaffolds, or scaffold-free systems (5–9). Cell therapy spans multiple therapeutic areas, such as regenerative medicine, immunotherapy, and cancer therapy. Currently, most cell therapies are in early stages of development (phase 1/2), with several exceptions being either a current best practice in specific

settings (e.g., bone marrow/stem cell transplants, hepatocyte transplantation, skin equivalents), or approved for specific indications, such as PROVENGE[®] (sipuleucel-T), LAVIV[®] (azficel-T), MACI[®] (autologous cultured chondrocytes on porcine collagen), and KYMRIAH[™] (tisagenlecleucel) among others (Table 1) (5, 9, 27). Herein, we describe the different types of cell therapies, including stem cell-based and non-stem cell-based cell therapies, providing an overview of their nature as well as isolation and characterization techniques. We further create the first-in-literature portfolio for the different “multicellular” therapies, delineating their different cellular components and areas of use. In addition to reviewing the FDA’s regulatory guidelines governing their use, we dive into the pros of cell therapies reported in regenerative medicine, immune system disorders, and cancer. Finally, we give our perspective on why multicellular therapies could contribute to more beneficial clinical outcomes compared to unicellular therapies, and how their development could be optimized for faster commercialization.

STEM CELL-BASED CELL THERAPIES

Overview of Stem Cells

Stem cells can be found in an organism in embryos and adult cells; they are a type of unspecialized, self-renewable cells prepped to differentiate into any cell type and/or as many cell types (28). What dictates how many cell types stem cells can differentiate into is their developmental potency. Developmental potency represents a differentiation continuum starting with totipotency (i.e., highest differentiation potential; e.g., zygote) and dwindling to pluripotency (e.g., embryonic stem cells), multipotency (e.g., hematopoietic stem cells), oligopotency (e.g., myeloid stem cells), and unipotency (i.e., least differentiation potential; e.g., dermatocytes) (29, 30). During passage along this continuum of potency toward mature/specialized cells, stem cells lose their self-renewal and differentiation potential (30). However, this hierarchy can be artificially reversed by nuclear reprogramming methods, including the use of transcriptional factors, which can eventually induce pluripotency in any cell type (31, 32). Stem cell specialization is influenced by external signals (e.g., physical contact between cells, paracrine secretions of nearby tissue, and tissue type), internal signals (e.g., genes), and epigenetics (embryonic cell origin) (29, 33). Depending on the type of stem cells, stem cell specialization can be detected by *in silico* gene expression analysis [e.g., PluriTest bioinformatic assay (34)] and validated by several techniques, including microarrays, polymerase chain reaction (PCR), and immunocytochemistry (35–38). Specific surface markers, molecular markers (e.g., transcription factors, microRNAs, transcription regulators, histone modifiers, DNA methylation state, X chromosome functional state, key molecular signaling pathways) (39–45), functional assays (e.g., teratoma formation assay, *in vitro* differentiation assay, blastocyst chimerism) (46–48), and culture characteristics (e.g., morphology, tolerance to single cell dissociation by trypsinization) (33) also help guide the evaluation of developmental potency.

Stem Cells Used or Targeted by Cell Therapy

Stem cells used or targeted by cell therapy can be grouped into three categories: pluripotent stem cells (PSCs), adult stem cells (ASCs), and cancer stem cells (CSCs).

PSCs: Types and Use in Cell Therapy

PSCs give rise to all cell types except extraembryonic placental cells; they include embryonic stem cells (ESCs), found in the inner blastocyst cell mass of preimplantation embryos; epiblast stem cells (EpiSCs) and embryonic germ cells (EGCs), found in postimplantation embryos; and induced pluripotent stem cells (iPSCs), derived from direct reprogramming of postnatal/adult somatic cells *in vitro* (30, 33, 49). In 1981, Evans and Kaufman established the first murine PSC lines in culture following isolation of ESCs from mouse blastocysts *in vitro* (50). In 1998, Thomson and colleagues established the first human ESC line from *in vitro*-fertilized human embryos (51). In 2006, Yamanaka and Takahashi generated artificial PSCs (i.e., iPSCs) from adult and embryonic mouse somatic cells (fibroblasts) by induction with transcription factors (Oct-3/4, Sox2, KLF4, and c-Myc) (52). In 2007, Takahashi and colleagues used the same four transcription factors to generate iPSCs from adult human somatic cells (dermal fibroblasts) (32). Although ESCs and iPSCs have been proven to be molecularly and biologically equivalent, the use of ESCs is restricted due to ethical obligations related to endangering fetal lives (33). Generally, the clinical use of PSCs (elaborated further in section Clinical benefits of cell therapy by select fields) lacks therapeutic evidence and is limited to investigational regenerative medicine, with the rationale of cell differentiation/tissue repair in different diseases, including macular degeneration and heart failure (53–59).

ASCs: Types and Use in Cell Therapy

Somatic or ASCs are rare, undifferentiated cells distributed among differentiated or specialized cells in organs of a developed organism (60). With more limited self-renewal and differentiation potentials than PSCs, ASCs replenish lost cells or contribute to the healing or growth of cells by giving rise to precursor or progenitor cells and ultimately differentiated cells (61). ASCs include hematopoietic stem cells (HSCs), skin stem cells (SSCs), neural stem cells (NSCs), and mesenchymal stem cells (MSCs) (62). HSCs are mostly found in the bone marrow (BM) and give rise to all mature blood cells: red blood cells, white blood cells, and platelets (63). SSCs, such as epidermal stem cells and hair follicle stem cells, maintain skin integrity (64). NSCs are self-renewable stem cells found in the central nervous system and can give rise to nerve cells, oligodendrocytes, and astrocytes (65). MSCs are of mesodermal, non-hematopoietic origins and are present in multiple tissues, including BM, adipose tissue, peripheral blood, and placenta (66). They can differentiate into bone, cartilage, and fat cells, as well as cells of ectodermal or endodermal parentage (67, 68). Unlike ESCs, which are mainly defined by their origin in embryos using molecular and functional assays, ASCs have varying defining criteria, with cell morphology and surface markers being the go-to in most experimental evaluations

TABLE 1 | FDA-approved cell therapy products.

Therapy	Description	Indication	Mechanism of action	Initial US approval date	Manufacturer	References
ALLOCORD (HPC, Cord Blood)	Consists of allogeneic cord blood hematopoietic progenitor cells (HPCs), monocytes, lymphocytes, and granulocytes	Hematopoietic system disorders	Hematopoietic stem/progenitor cells from HPC, Cord Blood migrate to the bone marrow where they divide and mature; mature cells are then released into the blood, restoring counts and functions of bone marrow cells. Mature leukocytes may also synthesize enzymes that might improve cellular functions of host tissues (mechanism of action unknown)	2013	SSM Cardinal Glennon Children's Medical Center	(10)
CLEVECORD (HPC, Cord Blood)				2016	Cleveland Cord Blood Center	(11)
HEMACORD (HPC, Cord Blood)				2011	New York Blood Center, Inc.	(12)
DUCORD (HPC, Cord Blood)				2012	Duke University School of Medicine	(13)
HPC, Cord Blood				2012	ClinImmune Labs	(14)
HPC, Cord Blood				2018	MD Anderson Cord Blood Bank	(15)
HPC, Cord Blood				2013	LifeSouth Community Blood Centers, Inc.	(16)
HPC, Cord Blood				2016	Bloodworks	(17)
BREYANZI® (lisocabtagene maraleucel)	CD19-directed genetically modified autologous T cell immunotherapy	Relapsed or refractory large B-cell lymphom	CAR binding to CD19 expressed on tumor and normal B cells induces activation and proliferation of CAR T cells and cytotoxic killing of target cells	2021	Juno Therapeutics, Inc., a Bristol-Myers Squibb Company	(18)
KYMRIAH™ (tisagenlecleucel)		Relapsed or refractory B-cell precursor acute lymphoblastic leukemia Relapsed or refractory large B-cell lymphoma		2017	Novartis Pharmaceuticals Corporation	(19)
YESCARTA® (axicabtagene ciloleucel)		Relapsed or refractory large B-cell lymphoma		2017	Kite Pharma, Inc.	(20)
TECARTUS™ (brexucabtagene autoleucel)		Relapsed or refractory mantle cell lymphoma		2020		(21)
ABECMA® (idecabtagene vicleucel)	B-cell maturation antigen (BCMA)-directed genetically modified autologous chimeric antigen receptor (CAR)-positive T cell immunotherapy	Relapsed or refractory large B-cell lymphoma	BCMA expressed by malignant plasma cells activates CAR-positive T cell proliferation, and subsequent plasma cell cytotoxicity	2021	Celgene Corporation, a Bristol-Myers Squibb Company	(22)

(Continued)

TABLE 1 | Continued

Therapy	Description	Indication	Mechanism of action	Initial US approval date	Manufacturer	References
PROVENGE® (sipuleucel-T)	Autologous cellular immunotherapy consisting of CD54 ⁺ cells activated with PAP-GM-CSF	Metastatic castration-resistant prostate cancer	CD54 ⁺ cells activated with PAP-GM-CSF take up, process, and express PAP, then induce an immune response against PAP-expressing prostate cancer cells	2010	Dendreon Corporation	(23)
GINTUIT (Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen)	Allogeneic ready-to-use cellular scaffold consisting of human keratinocytes, fibroblasts, and extracellular matrix proteins, as well as bovine collagen	Surgically created vascular wound bed in the treatment of mucogingival conditions	Does not function as a tissue graft; increases keratinized tissue at the treated site in an unknown mechanism	2012	Organogenesis Inc.	(24)
LAVIV® (azficel-T)	Autologous cellular product consisting of fibroblasts	Aesthetics of nasolabial fold wrinkles	Unknown	2011	Fibrocell Technologies, Inc.	(25)
MACI® (autologous cultured chondrocytes on porcine collagen membrane)	Cellular scaffold product consisting of autologous cultured chondrocytes on a resorbable porcine Type I/III collagen membrane	Symptomatic full-thickness cartilage defects of the knee	Unknown	2016	Vericel Corporation	(26)

(61). However, morphology and surface markers of ACSs are generally indistinguishable from those of mature cells; therefore, ASCs cannot be readily isolated from tissues, but enriched to varying degrees of purity in tissue extracts (60, 61). The use of ASCs (further elaborated in Section Clinical benefits of cell therapy by select fields) is mainly observed with HSCs or MSCs and envelops several clinical fields. For example, MSCs and HSCs are widely used in regenerative settings as, respectively, investigational and established modalities with the rationale of repopulating damaged cells or resetting tissue homeostasis (69–74). In immune system disorders, HSCs and MSCs have been generally used as investigational agents to alleviate disease activity with their vast mechanisms of action, and have shown varying success rates depending on the disease type (75–90). In cancer, HSCs have long been the standard treatment for hematological malignancies due to their regenerative potential (91); they have further been investigated in solid cancers as progenitors of immune cells, eventually driving tumor regression (92, 93). MSCs have also been investigated in cancer settings due to their anti-tumorigenic properties (66, 94, 95) yet have had only limited successes (96, 97).

Currently a hot topic in translational stem cell research, PSC- and ASC-derived organoids are highlight-worthy. Despite pending clinical investigations, these organoids hold promise as future regenerative medicine applications by offering *in vitro* three dimensional (3D) structural and functional mimicry of organs (98). Originally, these organoids are patient-derived stem cells manipulated and grown in controlled media formulations

to dictate their differentiation, then propagated into 3D structures/matrices (99). Besides their potential in organogenesis and regeneration for cell-based therapy (100), PSC- and ASC-derived organoids represent useful tools for drug screening and disease modeling (98, 101). For instance, human PSCs have been used to grow lung organoids *in vitro*, with tissular and cellular compartmentalization similar to the native lung (102, 103). Similarly, kidney organoids structurally equivalent to the human fetal kidney were derived from human PSCs (104), further demonstrating native tissue-specific functions (105).

CSCs: A Therapeutic Target

CSCs, or tumor-initiating cells, are found within solid and blood tumors and originate from normal stem cells or progenitor cells by several proposed mechanisms, such as mutations, gene transfer, epigenetic alterations, and microenvironmental factors (106, 107). CSCs possess self-renewal, differentiation, metastasis, and immunosuppressive properties and play an important role in cancer growth, metastasis, relapse, and resistance to chemotherapy and radiotherapy (107, 108). Identification criteria of CSCs generally include surface protein markers (e.g., CD133, CD44, tumor-associated antigens) and metabolic/functional properties (e.g., high metabolism, slow cell division); however, they might overlap with those of normal somatic/germ cells or of other stem cells (49). Generally, the clinical use of CSCs (see Section Clinical benefits of cell therapy by select fields) is seen in cancer settings and involves targeting CSCs by different

signaling pathway-interfering agents that subsequently prevent cancer growth and relapse (109, 110).

NON-STEM CELL-BASED CELL THERAPIES

Non-stem cell-based cell therapies are generally somatic cells that are isolated from the human body, propagated, expanded, selected, and subsequently administered to patients for curative, preventive, or diagnostic purposes (6). Non-stem cell-based cell therapies include fibroblasts, chondrocytes, keratinocytes, hepatocytes, pancreatic islet cells, and immune cells, such as T cells, dendritic cells (DCs), natural killer (NK) cells, and macrophages (5, 9, 111). Isolation techniques vary between somatic cells depending on their tissue localization and could include enzymatic digestion of harvested tissues (112) or processing of withdrawn blood (113). For example, peripheral blood mononuclear cells (PBMCs) can be collected by leukapheresis using automated systems, then cultured overnight, after which adherent cells (monocytes) can be separated from non-adherent cells (lymphocytes); DCs (HLA2DR⁺CD80⁺CD83⁺) can then be obtained by culturing adherent cells with granulocyte macrophage colony-stimulating factor, interleukin (IL)-4, and tumor necrosis factor over a week (114). Similarly variable are the characterization techniques of somatic cells, which are important to preserve a specific phenotype in sufficient yields, and can include microscopic examination, molecular analysis, immunocytochemistry, and gene expression analysis (112). Somatic cells are highly specialized (115) and can be further manipulated or treated before reintroduction into humans (116). Somatic cell-based therapies are generally employed as an *in vivo* source of enzymes, cytokines, and growth factors; as an adoptive cell therapy (ACT) to treat cancers; as transplanted cells, such as hepatocytes or pancreatic islet cells, to correct inborn metabolic errors; or as scaffold-based or -free cellular systems to treat ulcers, burns, or cartilage lesions (9, 117).

The application of cell grafts, such as hepatocytes, has only been slowly progressing due to technological hurdles and limited data supporting clinical efficacy and durability (111). For instance, hepatocyte transplantation has not yet been able to replace liver transplantation, due to limitations in post-transplant histological assessment and engraftment, despite limited clinical data showing its potential for being a future alternative to organ transplantation in treating patients with hepatic disease (118, 119). Contrarily, pancreatic islet cell transplantation for the treatment of insulin-deficient diabetes and pancreatitis has shown more promising potential (120), with clinical outcomes being dependent on islet availability and engraftment success rates, and limited with non-specific inflammatory/thrombotic mechanisms post-transplant. Indeed, in Canada, Australia, and several European countries, islet transplantation has become a standard of care for select patients (121).

ACT involves the intravenous transfer of modified peripheral or tumor-resident immune cells into patients to mount an immunologic reaction against tumors. Modified immune cells

used in ACT include tumor-infiltrating lymphocytes (TILs), tumor-specific T-cell receptor (TCR)-modified T cells, and chimeric antigen receptor (CAR)-T cells (122). TILs can be grown from different tumor types under standard culture conditions *ex vivo*. Prior to TIL infusion, patients undergo lymphodepleting chemotherapy, and shortly after, they are administered high-dose IL-2 to amplify the therapeutic potency of TILs (123–125). T cells isolated from peripheral blood by leukapheresis can be genetically engineered *in vitro* to express modified TCRs that can be directed against specific tumor antigens, such as melanoma differentiation antigens and cancer/testis antigens (126); however, the downside of this TCR gene therapy remains its evasion by tumor cells, which can downregulate their major histocompatibility complex (MHC) expression (127). TCR gene therapy also generally involves patient preconditioning with lymphodepleting regimens and IL-2 support (128). CAR-T cells employ synthetic antibody-based CARs, which can be of a proteinaceous, carbohydrate, or glycolipid nature (124). The transfer of CARs to T cells can be performed by various techniques, including retroviral infection. The genetic construct of CARs encodes the single-chain variable fragment (scFv) of a monoclonal antibody (serves as the extracellular antigen recognition domain), a CD3 ζ chain (serves as the intracellular signaling domain of TCR), and a co-receptor, such as CD28, for co-stimulation (129, 130). Upon tumor antigen binding by the scFv domain, CD3 ζ is phosphorylated, resulting in downstream signaling that is further amplified by co-receptor signals and that culminates in induction of cytotoxic activity (131). CAR-T cells are functionally similar to TCR gene therapy yet function in a non-MHC-restricted manner (132). Since their discovery in the 1980s (133), CAR-T cells continue to evolve. In 2003, second-generation CAR-T cells were redesigned to target CD19 in the setting of B-cell malignancies (134). Today, next-generation CAR-T cells explore innovative strategies aiming to improve antigen recognition, enhance cell proliferation and persistence, and evade the immunosuppressive tumor microenvironment (135, 136).

Other ACT strategies include lymphokine-activated killer (LAK) cells, cytokine-induced killer (CIK) cells, $\gamma\delta$ T cells, and NK cells (137). LAK cells are PBMCs derived from patients by multiple leukaphereses and incubated with IL-2; they were first demonstrated in 1984 to possess antitumor properties (138). CIK cells are also a heterogenous mixture of lymphocytes (mostly CD3⁺CD8⁺CD56⁺ T cells) with natural killer T (NKT) cell phenotype generated by incubation with various types of molecules, such as IL-2, IFN- γ , and CD3 monoclonal antibodies; their antitumor capacity can be further elevated by incubation with other cytokines, including IFN- γ and IL-1 β (139). $\gamma\delta$ T cells constitute 5% of peripheral blood T-cell counts and are characterized by their expression of the $\gamma\delta$ TCR instead of the more conventional $\alpha\beta$ TCR (140); following their *ex vivo* expansion, $\gamma\delta$ T cells become tumor-reactive with strong, non-MHC-restricted cytotoxicity (141). Like $\gamma\delta$ T cells, NK cells possess the ability to kill tumor cells in a non-MHC-restricted manner. Immunophenotypically, NK cells are CD3⁻CD56⁺ (137, 142). To enhance their antitumor activity, NK cells are

expanded by IL-2 incubation and/or co-administration (143, 144).

MULTICELLULAR THERAPIES

The term “multicellular therapies” is coined by few sources and can be defined as therapies containing at least two stem cell and/or non-stem cell types cultured from isolated cells or tissue extracts (145–147). The generation of multicellular therapies involves selective phenotypic expansion, rather than purification or enrichment processes, and can exploit automated cell-processing technologies (145, 147, 148). The distinct cell constituents of a multicellular therapy possess a broad range of biological activities, which contribute to its typically abstruse mechanism of action (26, 145). Therefore, the composition and/or functional intricacies of multicellular therapies might mirror those of normal tissues (148). Examples of these therapies include ACT products (149), scaffold-based or -free cellular products (9), stromal vascular fraction (SVF), stem cell transplant (150), and bone marrow aspirate (BMA)-derived therapies (151).

ACT Products

Among ACT products, TILs is a multicellular therapy that includes different lymphocyte lineages, including T cells and B cells (149). In cancer biology, lymphocytes recognize growing cancer cells and infiltrate the tumor. Once in the tumor, TILs try to initiate cancer killing. However, cancer cells can inactivate TILs to evade immunosurveillance by ligating their checkpoint receptors [e.g., programmed death 1 (PD-1), cytotoxic T lymphocyte-associated protein 4 (CTLA-4)], which are normally bound by specific ligands (e.g., programmed death-ligand 1 (PD-L1), B7) to control immune activity (152). Many tumors express PD-1 receptor ligands, such as PD-L1, permitting the mitigation of anti-tumor immunity (153, 154). Therefrom, immune checkpoint inhibitors were devised to lift off immune cell suppression and promote anti-cancer immunity (155). Indeed, it has been shown that TILs can promote tumor invasion and metastasis through several mechanisms, including cancer cell-leukocyte fusion and recruitment of regulatory T cells (Tregs) (156). The 1970's recorded the first attempts of lymphocyte isolation from tumor tissues (157, 158). In the next decade, IL-2-expanded isolated TILs showed significant antitumor activity *in vivo* (159), as opposed to TILs alone (160). TIL preparation involves tumor excision, digestion, culture with IL-2, and assessment for specific tumor recognition; tumor-specific TIL cultures are then expanded using anti-CD3 monoclonal antibody, high IL-2 concentrations, and irradiated allogeneic feeder cells (161). Characterization methods of TIL cellularity include gene expression analyses and analytical tools, such as CIBERSORT (162, 163). TIL products are heterogenous in terms of CD8⁺/CD4⁺ T cell ratios and T-cell differentiation stage and can be impacted by tumor biology (164). For an endowment of resistance to tumor suppression and/or enhanced tumor homing, TILs can be genetically modified either using different types of vectors or *via* gene editing technologies (165).

LAK cells are another ACT product composed of IL-2-activated PBMC's, mainly NK cells, NKT cells, and T cells, with

non-specific cytotoxicity and non-MHC-restrictive cytotoxic effects (137). The use of LAK cells is limited to few cancer types due to their difficult amplification and associated adverse effects (166, 167). LAK cells have been reported to induce tumor cell killing by releasing cytolytic mediators, including perforin and granzymes (168).

CIK cells are a subset of T lymphocytes with an NKT cell phenotype, and can be expanded *ex vivo* from PBMC's or BM mononuclear cells. When activated, CIK cells stimulate the immune system to recognize and eradicate tumor cells in a non-MHC-restricted manner (137, 139).

Scaffold-Based or -Free Cellular Products

Scaffold-based cellular products are engineered technologies that deliver different cell types (e.g., fibroblasts and keratinocytes) seeded within 3D biocompatible tissue analogs (9). Traditionally, scaffold-based cellular products employ biodegradable natural or synthetic polymers (e.g., bovine collagen, hydrogels, sponges) with sophisticated porous networks through which oxygen, nutrients, and metabolites can be exchanged (169, 170). Current scaffold-based cellular products with FDA approval are used for the treatment of diabetic foot ulcers (e.g., Apligraf[®], Dermagraft[®]), burns (OrCel[®]), and mucogingival conditions (GINTUIT) (9, 24, 171, 172). Scaffold-free cellular products are tissue analogs that are densely populated with cells carried and protected by their secreted, tissue-specific extracellular matrix (ECM) (9). This biotechnology can employ temperature-responsive polymers (e.g., pNIPAM, PVME) that transition between hydrophobic and hydrophilic states at certain temperatures, allowing the control of cell culture and growth and subsequently the deposition of ECM and the formation of cell sheets that adhere to biological surfaces (173–175). Several automated technologies (e.g., robots, bioreactors) can also be used to enhance the scalability, elevate the architectural biomimicry, or allow for the perfusion of these tissue analogs (176–178). An example of commercially available, FDA-approved scaffold-free cellular products is Epicel[®] (cultured epidermal autografts), a petrolatum gauze composed of sheets of autologous keratinocytes and proliferation-arrested murine fibroblasts and indicated for deep/full burns (179). Generally, the specific mechanisms of action of scaffold-based or -free cellular products are unknown, but are surmised to involve the production of cytokines and growth factors similar to healthy human skin (180). Although these products represent important advances in regenerative medicine, they are still limited by their high costs and non-regenerative outcomes, including their inability to fully reconstitute the damaged skin architecture (181).

SVF

SVF is a heterogeneous mixture of stromal and vascular cells, including ASCs, granulocytes, monocytes, lymphocytes, pericytes, and endothelial progenitor cells (EPCs), obtained from the processing of adipose tissue (e.g., lipoaspirate, excised fat) (182–185). Besides its use as an investigational product in different clinical settings (186–188), SVF is used as a source to isolate ASCs (i.e., adipose-derived stem cells,

or ADSCs), which can constitute up to 10% of its fraction depending on the processing technique, usually involving serial straining and centrifugation of SVF and cell culture in growth media (189). SVF composition can be identified immunophenotypically by flowcytometry, for example for the presence of ADSCs (CD45⁻CD235a⁻CD31⁻CD34⁺), which share several surface markers with BM-MSCs but are CD36⁺CD106⁻. Other techniques used for identifying the cellular composition of SVF include lineage-specific differentiation assays and biochemical or PCR evaluation (190). Due to its heterogeneous composition, SVF functions in various mechanisms, including paracrine signaling through cytokines, chemokines, and growth factors and cell-cell interactions, ultimately promoting neovascularization, cell repair, and immunomodulation (191).

Stem Cell Transplant

Stem cell transplant is performed in settings that damage the body's stem cells, including hematologic malignancies (e.g., leukemia, lymphoma, multiple myeloma, neuroblastoma) or cancer therapy (e.g., high-dose chemotherapy, total body irradiation) (192). Stem cell transplant relies on 3 stem cell sources: bone marrow, peripheral blood, and umbilical cord blood (193).

Stem cell transplant with bone marrow as the source of stem cells is known as bone marrow transplantation (BMT), which has been in practice since the 1960's (194). BMT entails BM aspiration (195) for harvesting HSCs (196) as well as progenitor cells, MSCs, lymphocytes, neutrophils, platelets, red blood cells, eosinophils, basophils, and monocytes (197, 198) (see Section BMA-derived therapies). Peripheral blood stem cell transplantation (PBSCT) is another type of hematopoietic stem cell transplantation (HSCT) that uses peripheral blood-derived HSCs (199). PBSCT came forth in the 1990's (196) as an alternative to bone marrow transplantation (BMT) (194), due to easier stem cell collection, higher stem cell yields, and faster patient recovery post-transplantation (150). In this procedure, autologous or HLA-matched allogeneic peripheral blood stem cells (PBSCs) are infused into the patient's bloodstream following a preparative conditioning regimen consisting of chemotherapy with/without total body irradiation that ensures immune tolerance of the engraftment. Once in the blood, PBSCs home toward the BM to repopulate lost blood cells or allow cancer remission (150, 200). PBSCs are collected by continuous-flow apheresis after mobilization using medications including granulocyte colony-stimulating factor (G-CSF) agents and chemokine receptor 4 (CXCR4) blockers (e.g., plerixafor); chemotherapy can also be used for mobilization (i.e., chemoembolization) (200). PBSCs are generally identified and quantified using flowcytometry *via* their immunophenotypic patterns (e.g., CD34⁺CD38⁻) (192). Besides CD34⁺ HSC subpopulations, PBSC grafts contain nucleated cells including DCs, T cells, B cells, NK cells, and monocytes (201). Compared to BM-derived stem cells, PBSCs express more lineage-specific differentiation antigens, are less metabolically active, and show higher clonogenicity (202). However, the clinical benefit/risk ratio of PBSCs vs.

BM-derived stem cells is disputed, with the preference being dependent on the type of hematologic disease, the age of donors/recipients, and whether the HLA-matched donor is related or unrelated—factors which influence the incidence of graft vs. host disease (GVHD) or the patient's quality of life (196, 199). The differences in cellular composition (e.g., CD34⁺ and lymphocyte numbers) between both stem cell grafts is also associated with differences in their clinical outcomes (203).

Another source of stem cell transplantation is cord blood (CB), whose HSCs and hematopoietic progenitor cells are observed to differ from those of peripheral blood and BM in terms of surface markers, recovery speed of blood cells post-transplant, clinical outcomes, and GVHD incidence (204, 205). As of 2011 to date, eight allogeneic cord blood products have gained FDA approval for the treatment of hematopoietic system disorders. These products mainly contain HSCs and hematopoietic progenitor cells, which migrate to the BM where they divide, and their progeny cells mature and subsequently replace lost blood cell reservoirs. Notable, these products are also composed of monocytes, lymphocytes, and granulocytes, which render their mechanisms of action only partially known (10–17).

BMA-Derived Therapies

BMA-derived therapies are commonly termed concentrated bone marrow aspirate (cBMA), bone marrow concentrate (BMC), or bone marrow aspirate concentrate (BMAC) (151). BM aspiration is a procedure performed under local or general anesthesia, in which a liquid sample is collected from the BM of usually the anterior or posterior iliac crest among other bones (195). Since the early 1960's, the BM has been the chief source for harvesting HSCs for BMT procedures (196); however, its use in this context has diminished following the emergence of PBSCT (206, 207). BMA includes various cell types including HSCs, progenitor cells, MSCs, lymphocytes, neutrophils, platelets, red blood cells, eosinophils, basophils, and monocytes (197, 198). The processing of BMA to concentrate nucleated cell yields, such as MSCs which represent 0.001% of the non-hematopoietic, multipotent cellular portion of BMA, yields BMAC (198). The concentration of BMA can be performed by different techniques, including automated centrifugation systems (208) or cell filtration systems (209). In BMAC, concentrations of nucleated cells become 5-fold higher, and concentrations of MSCs 6-fold higher (210, 211). The composition of BMAC also includes HSCs, progenitor cells, white blood cells, platelets, and cytokines/growth factors (212, 213) and can be characterized by microscopy, flowcytometry, cytogenetic and molecular analyses, and cytochemical staining (214). Generally, the clinical application of BMAC spans orthopedic settings, in which it can be sterilely injected intra-articularly under the guidance of fluoroscopy or ultrasonography (215). Like SVF, BMAC functions in various mechanisms involving paracrine signaling by MSCs and nucleated cells that drive tissue repair and immunomodulation (66, 209, 210) and by growth factors and cytokines that induce tissue growth and promote reparative processes (198, 216).

ACELLULAR THERAPIES WITH MULTICELLULAR COMPONENTS

Platelet-rich plasma (PRP) is an anticoagulated blood product obtained by differential centrifugation of whole blood and predominantly contains platelets in concentrations that can exceed up to 5 times physiologic platelet concentrations (217, 218). Platelets are acellular fragments derived from maturing megakaryocytes and function mainly in maintaining primary hemostasis and thrombosis to preserve vascular integrity (219). Although predominated by platelets—reservoirs of multitudinous immunologic molecules, soluble proteins, and growth factors (220)—and plasma components, PRP contains cellular components, such as leukocytes (217). Several commercially available kits can be used for PRP processing with varying outcomes in terms of platelet, red blood cell, and leukocyte concentrations (221). PRP composition can be subsequently analyzed using various analytical methods, such as automated hematology analyzers, microscopy, flowcytometry, and spectrophotometry (222, 223). Because of their unstandardized preparation protocols yielding heterogeneous formulations, PRP products can be further classified in clinical settings on the bases of platelet concentrations/activation and cellularity using various non-consensual classification systems, including the PAW, the PLRA, and the International Society on Thrombosis and Hemostasis systems (221, 224). PRP functions in several mechanisms driven by cytokines, growth factors, platelets, and nucleated cells, altogether which exert anti-inflammatory effects and promote tissue repair (225, 226).

REGULATORY CONSIDERATIONS FOR CELL THERAPY

For manufacturers, as well as researchers and clinicians, it is important to be aware of the FDA's regulatory guidance on cell therapy products. Human cells, tissues, and cellular and tissue-based products (HCT/P) are defined by the FDA under the Title 21 of the Code of Federal Regulations (CFR) Part 1271.3(d), or [21 CFR Part 1271.3(d)], as “articles containing or consisting of human cells or tissues that are intended for implantation, transplantation, infusion, or transfer into a human recipient.” Falling under this definition are several examples, including HSCs/progenitor cells derived from peripheral blood or CB, manipulated autologous chondrocytes, and epithelial cells on a synthetic matrix. If the therapy does not meet the definition of HCT/P in 21 CFR 1271.3(d), such as blood components/derivatives (e.g., PRP) and minimally manipulated BMA, the regulations in 21 CFR Part 1271 do not apply (227).

For therapies meeting the definition of HCT/P in 21 CFR 1271.3(d), the 21 CFR 1271.15(b) further guides how HCT/P are regulated. The “same surgical procedure (SSP) exception” in 21 CFR 1271.15(b) states that it is not required to comply with the requirements in 21 CFR Part 1271 if the establishment is collecting and administering the HCT/P autologously, within the same surgical procedure, and in their original form (if processed,

only rinsing, cleansing, sizing, and shaping are allowed) (227, 228). Otherwise, the algorithm progresses to the requirements of 21 CFR 1271.10(a). The criteria under this title specify that the HCT/P is minimally manipulated; intended for homologous use; not combined with other active agents; without a systemic effect; and—if with a systemic effect—administered autologously or to first-/second-degree blood relatives (227). If these criteria are met, the FDA allows the use of the cell therapy in the framework of regulatory guidelines governing disease transmission, yet without premarket approval/biologics license application, solely under section 361 of the Public Health Service (PHS) Act and regulations in 21 CFR Part 1271. Otherwise, if the cell therapy does not meet the criteria under 21 CFR 1271.10(a), it is regulated as a biological product under the Federal Food, Drug, and Cosmetic (FD&C) Act and/or section 351 of the PHS Act and applicable regulations. In this case, the cell therapy would require premarket approval, and the establishment needs to register the therapy and apply for a biologics license for lawful marketing, or have an investigational new drug (IND) application in effect if the therapy is investigational (227, 228).

The importance of being well informed about the above regulations becomes more obvious with the recent aggressive enforcement the FDA has begun to undertake to protect patients from risks of unapproved products being otherwise dispensed as HCT/P falling under section 361 of the PHS Act and regulations in 21 CFR Part 1271. In May 2018, the FDA initiated an action against a stem cell clinic for administering non-compliant autologous SVF to patients. On June 3, 2019, the Florida court ruled in favor of the FDA because, according to the FDA arguments, the isolated SVF no longer represented adipose tissue (i.e., not in its original form, or adipose tissue) after removal from the patient, and because the therapeutic use of the SVF differed from the natural function of adipose tissue (i.e., not intended for homologous use). Although the stem cell clinic argued that CFR 1271.15(b) and 21 CFR 1271.10(a) apply to their SVF product, the court saw otherwise, considering the case as a violation of federal laws and the product as a “drug” falling under the FD&C Act and necessitating extensive pre-approval. On June 25, 2019, the court ordered the stem cell clinic to cease its offering SVF services until further FDA compliance. In addition to that SVF clinic, the FDA has issued multiple warnings to other clinics working with stem cells and umbilical cord-derived products (229, 230). Counterintuitively, a California federal judge has denied a government motion initiated simultaneously with the former lawsuit, against a stem cell treatment center. In the trial, the FDA argued that the center was using illicit SVF therapies that are manipulated prior to implantation to treat degenerative diseases. The manipulation according to the FDA occurred in the removal step of the adipose tissue, thus generating an SVF product to which the SSP exception does not apply. The defendant countered that their SVF is unaltered, despite the removal of adipose tissue, and thus complies with all requirements in 21 CFR Part 1271 (231). In the hearing (232), the judge considered that the SSP exception is unambiguous and read that it does not require the reimplantation of all the removed tissue, much like coronary artery bypass procedures in which surgeons do not implant the removed blood and excess artery. Based on this logic,

the judge considered that the SSP exception applies to the SVF product, whose natural host tissue was removed as part of the collection process without further alteration to the SVF content. The court saw that the FDA's interpretation of the SSP exception is "unreasonable and creates enforcement inconsistency" and considered that "the agency's reading must fall within the bounds of reasonable interpretation... a requirement an agency can fail." (232) These incidents are indicative that the FDA regulations governing the use of cell therapy products are confusing and might not be safe from misinterpretations or dispute.

On another note, other multicellular products, such as BMAC, have not received FDA warnings and continue to be used as HCT/P under section 361 of the PHS Act and regulations in 21 CFR Part 1271.

CLINICAL BENEFITS OF CELL THERAPY BY SELECT FIELDS

Regenerative Medicine

Regenerative medicine deploys a body's own cells and growth factors to repair tissues by restoring their lost functions (111). Several cell therapies in regenerative medicine have become either established practices or commercially available with FDA approval, such as keratinocyte- and/or fibroblast-derived skin substitutes for treatment of diabetic foot ulcers (172, 233) or burns (179); keratinocyte- and fibroblast-containing scaffold products for treatment of surgically created vascular wound beds in the oral cavity (24); fibroblast intradermal injections for improvement of appearance of nasolabial fold wrinkles (25); chondrocyte-containing scaffold implants for treatment of knee cartilage defects (26); and cord blood-derived HSC/hematopoietic progenitor cell products for treatment of hematopoietic system disorders that are inherited, acquired, or result from myeloablative treatment (10–17). Although commercial cell therapies are beneficial in repairing tissues, they are unable yet to regenerate them (234). Clinical development is also an arduous process that hinders the introduction of new products into the market (235, 236). This can be seen in the proportion of approved biologics over a 9 year period, which reached 23% of all approved drugs. Additionally, biologics in the US are granted 12 years of exclusivity protection vs. ~7 years for new chemical entities (237).

In clinical investigation settings, multiple cell therapies, as well as acellular therapies with cellular components, have been assessed for their safety and efficacy in a regenerative context, including PRP, ESCs, iPSCs, SVF, ADSCs, MSCs, and BMAC (190, 234, 238, 239).

PRP is widely evaluated in orthopedics due to its enriched composition of cytokines, growth factors, and platelets, which establish an anti-inflammatory environment at the site of injection and promote skeletal and connective tissue regeneration and reconstruction (225, 226). For instance, PRP preparations have demonstrated efficacy and safety in tendon injuries (240, 241), rotator cuff tears (242), osteoarthritis (OA) of the knee or hip (243, 244), and muscle injuries (245), with benefits being mostly symptomatic relief. The cellularity of

PRP can also dictate clinical outcomes, thus classifying PRP preparations into leukocyte-rich PRP (LR-PRP)—with leukocyte concentrations exceeding baseline levels—and leukocyte-poor PRP (LP-PRP) —with leukocyte concentrations below baseline levels (241). Accordingly, it is recommended that PRP be analyzed for its leukocyte content and used in accordance with the catabolic vs. anabolic requirements of the treated condition (246).

The clinical application of ESCs is restricted by ethical concerns, regulatory bodies, and the lack of preclinical evidence supporting their use (53, 54). However, few successful outcomes in regenerative medicine merit acknowledgment. For example, human embryonic stem cells (hESCs) have improved the vision of patients with macular degeneration and macular dystrophy by differentiating into photoreceptors and retinal pigment epithelial cells (55). In a case report, cardiomyocytes derived from hESCs have also improved the ejection fraction of a 68 year old patient with severe heart failure and without inducing subsequent complications (56).

Despite presenting several advantages over ESCs (e.g., non-invasive collection, less immune rejection, ethically unrestricted nature), the use of iPSCs in clinical settings is still farfetched due to lack of therapeutic evidence as well as other preparation and standardization obstacles (57). Indeed, a data compilation in 2018 showed that the fraction of clinical trials investigating the aptness of iPSCs as a treatment modality constitutes only 11% of the total clinical trials of iPSCs, including those terminated (58). The first and potentially only reported clinical benefits of iPSC-based therapy are minimal and date to 2017 in a patient with neovascular age-related macular degeneration (59). On the other hand, iPSCs—like ESCs—have been extensively employed as research tools for drug toxicity testing (e.g., drug-induced QT prolongation) and—unlike ESCs—have been useful for disease modeling and drug discovery studies (247).

SVF was first clinically investigated in reconstructive surgeries with the rationale of promoting adipose tissue survival and thus provide structural tissue support (248). Multiple other studies followed, in which SVF was investigated for its healing and regenerative abilities. For example, SVF has promoted neovascularization and improved tissue hydration in patients with radiotherapy-induced lesions, owing partially to its ADSC composition (249). Few other examples of regenerative settings in which SVF has provided patients with clinical benefits include knee OA (250, 251), chronic wound healing (252), urogenital conditions (253), and systemic sclerosis (SS)-associated facial handicap (254).

In regenerative medicine, ASCs are considered the most promising among cell therapies, and ADSCs constitute an ideal option due to their ease of harvest requiring minimal invasiveness; multi-lineage differentiation potential; and anti-inflammatory and proangiogenic secretome (255). To date, there are 11 active or recruiting registered studies involving ADSCs as an intervention in conditions such as knee OA and chronic kidney disease (256). Data disclosed hitherto, mostly by pilot studies, show that—despite not living up fully to their regenerative rationale—ADSCs have shown a promising potential in multiple settings, including ischemic heart disease

(73, 74), acute myocardial infarction (257), knee OA (258, 259), peripheral vascular disease (260), SS-associated ulcers (261, 262), ischemic diabetic feet (252), urogenital conditions (263, 264), and breast cancer-associated lymphedema (265). For example, an early-phase placebo-controlled trial has shown that the intracoronary infusion of ADSCs is well tolerated, improves left ventricular ejection fraction (LVEF), and reduces infarct size after 6 months of follow-up in patients with acute myocardial infarction (257). Similarly, the intramyocardial delivery of ADSCs has been shown to significantly improve LVEF and exercise tolerance in patients with chronic ischemic heart disease (73, 74).

Among ASCs, MSCs are gaining considerable attention as a cell therapy intervention in human studies, especially with the production of good manufacturing practice (GMP)-compliant human MSCs (66, 266). To date, there are at least 180 active or recruiting registered studies involving MSCs as an intervention in various conditions (69). In regenerative settings, MSCs have exhibited a promising potential in osteogenesis imperfecta (70), Crohn's disease (71), deep burns (72), periodontal defects (267), chondral/bone defects (268, 269), and diabetic foot (270). The benefits of MSCs in clinical investigations are mostly linked, not to their multi-lineage differentiation potential, but rather to their secretome, which establishes a nutritive microenvironment, promoting autocrine and paracrine signaling that inhibits apoptosis and dictates angiogenesis, local tissue mitosis, and cross-communication with resident stem cells (66, 271–273).

BMAC has emerged as a potential alternative for regenerative therapy, owing mainly to its enriched composition of growth factors and MSCs among other cell types (216). In regenerative settings—mainly orthopedics—BMAC has demonstrated a promising potential, as it provided patients with clinical benefits and/or improved diagnostic imaging outcomes of patients (209, 274, 275). To date, there are at least 14 active or recruiting registered studies involving BMAC as an intervention mainly in orthopedic conditions (276). The mechanism of action of BMAC remains unclear, and no serious attempts have been made to delineate the interactions between the different components of BMAC, which might collectively be at the origins of BMAC outcomes (277, 278). Among the components theorized to contribute to the therapeutic potential of BMAC are MSCs, which are endowed with tissue function-enhancing regenerative and immunologic properties (66, 209, 210); growth factors, which promote tissue growth; and nucleated cells (e.g., lymphocytes), which secrete various reparative cytokines and growth factors that act via paracrine pathways (198, 216). However, the conclusions drawn by these reports about the role of BMAC components—specifically MSCs—in driving clinical outcomes are based on rather extrapolations than benchwork. Hence, further molecular investigations are necessary to fully understand the degree of contribution of each of these components in the observed orthopedic benefits. Noteworthy, BMAC could still become an established therapy even with a partially understood mechanism of action and without having to erroneously suggest that its beneficial outcomes are driven by MSCs. This possibility could be observed with HSCT, which has become an established therapy for treating

immune diseases despite its elusive mechanism of action (75, 89).

Immune System Disorders

Most immune system disorders develop due to excessive immune responses or autoimmune attacks (279). Primary treatment thus aims to alleviate inflammation, minimize symptoms, and prevent relapse (280). The rationale of exploiting cell therapy in immune system disorders extends beyond immune suppression and symptomatic relief to immune system resetting as a permanent cure (281). As of the late 1990's, BMT/HSCT has become the most established cell-based therapy for treating immune system disorders (75). HSCT has been shown to elicit durable outcomes in severe SS with acceptable rates of transplant-related mortality (76). In controlled phase 2/3 trials, HSCT has demonstrated efficacy in patients with autoimmune disorders, resulting for instance in 79% improvement in the disability status and marked improvement in disease relapse rates, MRI lesions, and quality of life in patients with multiple sclerosis (83). HSCT, including PBSCT, has also alleviated disease activity and stabilized/reversed organ dysfunction in patients with systemic lupus erythematosus (SLE) (84–86). While the benefits of HSCT in SLE are mostly reported by retrospective studies, prospective trials are limited and have not found significant benefits (83). On the other hand, HSCT has only shown transient responses or partial benefits in other immune diseases, such as rheumatoid arthritis (RA), vasculitis, and Crohn's disease (87, 88). Despite its benefits seen in most immune system disorders, HSCT's underlying mechanism of action remains elusive, with non-specific benefits being also omnipresent and pertaining to the accompanying regimen of lymphotoxic chemotherapy that reduces autoreactive antibodies (89). MSCs have also had their share of clinical successes in immune system disorders, specifically in GVHD, amyotrophic lateral sclerosis, and Crohn's anal fistula (77–81, 90). These benefits are linked to the immunomodulatory actions of MSCs originating mostly from their immune inhibitory secretome (82). Although several MSC-based therapies are approved worldwide for the treatment of immune diseases (including in Canada and Japan), they have not yet received FDA approval (66). Albeit to a less documented extent than BM-MSCs, ADSCs have also shown a promising potential as a cell therapy for the treatment of immune system disorders, such as GVHD, Crohn's disease, psoriasis, and SS (282–285). Other cell-based therapies with less reported benefits in immune diseases include PRP, which has been shown to reduce pain and inflammation with ultrasound imaging evidence in patients with RA (286), and Tregs, which have been shown to reduce the incidence of acute GVHD (287). DCs are another type of immunotherapies exploited in the treatment of patients with immune system disorders. For instance, tolerogenic DCs—a type of immature DCs that induce T-cell anergy and Treg differentiation causing peripheral tolerance (288)—have been reported to stabilize disease (289) or reduce inflammation and disease scores in RA (290). Other somatic cell-based therapies like pancreatic islet cell transplantation have resulted in substantial benefits in type 1 diabetes. For example, a single-arm phase 3 trial has shown that pancreatic islet transplantation leads to glycemic

control and protection against severe hypoglycemic events in patients with type 1 diabetes (120). Therefore, it has become recommended globally that pancreatic islet transplantation be considered for patients whose problematic hypoglycemia persists despite insulin infusion or glycemic monitoring (291). Notable, clinical outcomes with pancreatic islet cell transplantation have been found to be associated with islet availability and engraftment success rates, which are elevated for instance in allogeneic transplants, where islets can be isolated from multiple donors (121). Other immunotherapies like CAR-T cells have not yet been reported to provide clinical benefits in immune conditions (292), despite promising preclinical outcomes (293).

Cancer

The rationale of cancer therapy has evolved from the systemic targeting of tumors with chemotherapy/radiotherapy to a more targeted approach using novel biologic treatments, including monoclonal antibodies, oncolytic viruses, and cell therapy, such as antigen presenting cell (APC)-based anticancer vaccines and CAR-T cells (294). Another therapeutic approach in patients with cancer is the aforementioned regeneration of immune effectors specifically in hematological malignancies, in which case HSCT has long been the standard treatment (91). The year 2010 witnessed the FDA approval of PROVENGE[®] (sipuleucel-T), the first and only APC-based anticancer vaccine indicated for the treatment of metastatic castration-resistant prostate cancer (23). Later in 2017, KYMRIAH[™] (tisagenlecleucel) became the first CAR-T cell therapy to receive FDA approval for the treatment of relapsed or refractory B-cell precursor acute lymphoblastic leukemia and large B-cell lymphoma (19). Following tisagenlecleucel's steps, other autologous, CD19-directed CAR-T cell therapies then entered the market with the indication of treating relapsed or refractory large B-cell lymphoma or mantle cell lymphoma (**Table 1**) (18, 20, 21). In 2021, ABECMA[®] (idecabtagene vicleucel) became the first B-cell maturation antigen (BCMA)-directed CAR-T cell therapy to receive FDA approval for the treatment of relapsed or refractory large B-cell lymphoma (22), with distinctive selectivity conferred by the highly selective expression of BCMA by malignant plasma cells (295).

Besides commercial cell therapy products, a multitude of cell therapies have been investigated for treating cancer in clinical settings. Among APC-based anticancer vaccines, DC-based anticancer vaccines—either created with primary CD1c⁺ myeloid DCs or engineered by fusion with patient-derived tumor cells, pulsation with tumor peptides/lysate, or electroporation with tumor associated antigen-encoding mRNA—have elucidated promising immunologic and/or clinical responses in B-cell lymphoma (296), multiple myeloma (297), acute myeloid leukemia (298), glioblastoma (299), and metastatic melanoma (300–302). CD34⁺ HSC-derived modified/manipulated DCs have also been clinically investigated in cancer settings with promising outcomes, such as generation of tumor-specific immunity and/or induction of tumor regression in patients with metastatic melanoma (92, 93). Using chemicals like polyethylene glycol, autologous primary DCs can be fused with irradiated, resected tumor cells to create tumor-DC

hybrids whose subsequent bioengineering and administration to patients with glioblastoma receiving standard chemotherapy has been shown to improve clinical responses (303). Similarly, the vaccination of patients with acute myeloid leukemia who achieved remission following chemotherapy using autologous primary DCs fused with autologous cancer cells has led to the expansion of tumor-reactive T cell subsets and prolonged remission (304).

Investigational CAR-T cells have also shown high antitumor activity in relapsed/refractory multiple myeloma by targeting BCMA (305). Unlike CD19- or BMAC-directed CAR-T cell therapy for hematologic malignancies, CAR-T cells directed against solid tumor antigens, such as PD-L1 and prostate-specific membrane antigen (PSMA), have had less clinical success due to obstacles pertaining to the suppressive nature of the tumor microenvironment and therapy persistence within the tumor (306). In small-scale studies, the use of bispecific CAR-T cells directed against CD19/BCMA in multiple myeloma has been met with promising patient responses (307). Among ACT, TCR-modified T cells directed against tumor-specific antigens have also had promising outcomes in cancer therapy, as they induced cancer regression in patients with melanoma (308, 309) and reduced metastases in patients with synovial cell sarcoma (310, 311). Similarly, TILs and LAK cells have been reported to induce tumor regression in patients with metastatic cancers (312–314). Additionally, LAK cells have improved the survival of patients with melanoma and patients with glioblastoma (315, 316), and TILs have augmented the rates of objective clinical responses of patients with metastatic melanoma (317). CIK cells were also reported to reduce disease recurrence or improve overall survival in patients with hepatocellular carcinoma and to augment the progression-free survival and overall survival in patients with renal cell carcinoma (318–320). The mechanism of action of CIK cells is observed to involve perforin-mediated tumor killing (321). On the other hand, although few phase 1 clinical trials have demonstrated the benefits of $\gamma\delta$ T cells as a cancer immunotherapy, other studies have reported contrasting outcomes, revealing the suppressive façade of this T-cell subset and linking its presence within the tumor microenvironment to negative outcomes (137). What's more in ACT, allogeneic NK cells have only provided modest benefits to patients with acute myeloid leukemia (322) and patients with recurrent ovarian and breast cancer (323), generally due to their inhibition by host Tregs and/or the tumor as well as the high toxicity of IL-2 (137). In combined cell therapy approaches, CIK cells and tumor lysate-pulsed DCs infused intravenously at different time intervals have been shown to significantly prolong the median survival time at a rate comparable to chemotherapy in patients with colorectal cancer (114) and improve the overall survival and the quality of life in patients with advanced colorectal cancer (324). Similarly, DC-CIK immunotherapy has been reported to significantly prolong the overall survival and improve the quality of life in patients with advanced non-small cell lung cancer (325). Other combinatorial approaches include tumor lysate-loaded DC and TIL immunotherapy, which has revealed a promising potential based on evaluating objective clinical responses in a phase 1 study in patients with advanced melanoma

(326). Patients with metastatic melanoma have also experienced immunologic responses and tumor regression upon treatment with the combined therapy comprising TCR-modified T cells directed against melanoma-associated antigen recognized by T cells (MART-1) and DCs (327).

Albeit to a less documented extent compared to ACT products or other cellular therapies, genetically engineered MSCs have been investigated as a cancer treatment due to their ease of obtainment and demonstrated tumor tropism and anti-tumorigenic properties; however, no benefits have been reported to date with patient-administered MSCs (66, 94), possibly due to their insufficient cell homing to tumors as reported in a phase 1 study (95) or their paradoxical pro-tumorigenic potential seen in several preclinical studies (66, 328). Noteworthy, based on previous promising preliminary data (329, 330), the first-in-human, first-in-child clinical trial for Celyvir—an autologous MSC-based therapy carrying oncolytic adenoviruses—has reported disease stabilization in two pediatric patients with neuroblastoma (96). Following the steps of this trial, other groups are exploring the potential of bioengineered MSCs carrying oncolytic viruses—viruses that evade immune surveillance and can conditionally replicate in tumor cells, unlike traditional attenuated viruses (97)—in patients with glioblastoma (NCT03896568) and patients with ovarian carcinomas (NCT02068794). Finally, several CSC-targeting agents for cancer treatment have been approved (e.g., vismodegib, ivosidenib, venetoclax) or are still under investigation, with mechanisms of action entailing the involvement with CSC pathways (109, 110).

MULTICELLULAR VS. UNICELLULAR THERAPIES

Multicellularity in an organism enables cell-cell communication, which is crucial during the different phases of tissue development starting early in embryogenesis and persisting through later regenerative processes (331). In regenerative medicine, there is a growing notion that a mixture of cell types rather than one cell type is important to promote long-term tissue repair driven by complex, poorly understood multicellular interactions typical of the physiological nature of organisms (277, 278). Compared to ADSCs, a mixture of ADSCs, EPCs, and lymphocytes among other cell types (i.e., SVF) has received more praise in preclinical comparative studies, in part due to the heterogeneity of cellular composition perceived to exploit more physiologic properties (e.g., angiogenesis, immunomodulation, cellular differentiation) that collectively drive better outcomes (332–335). Notable, the first study to compare MSCs vs. BMAC (i.e., MSCs, progenitor cells, white blood cells, etc...) on the scale of clinical and magnetic resonance imaging (MRI) outcomes in 52 patients with chondral knee defects has found no differences in retrospect between both treatments over a 2 year follow-up (336). Similarly, a recent retrospective study found no differences in postoperative radiological findings and pain/functionality outcomes in patients who underwent high tibial osteotomy with microfracture combined with either MSCs or BMAC for medial unicompartamental knee OA (337). No further studies intended

to compare MSCs vs. BMAC have been made. On a similar note, a recent systematic review of 119 studies (clinical trials or case series) using MSCs or BMAC for the treatment of patients with different orthopedic conditions did not include any meta-analysis, possibly due to the overt disparity in study protocols and treatment regimens observed even within a single orthopedic indication (338). **Table 2** shows select studies using MSCs and BMAC for the treatment of osteonecrosis of the femoral head, with the intention to demonstrate the impracticality of head-to-head comparisons between MSC vs. BMAC benefits even with maximal control for different study factors, including study nature and disease indication.

In cancer treatment, the use of multicellular therapies could also be more beneficial than biologic therapies comprising one cell type. Compared to patient-derived CIK cells, combined DCs and CIK cells have shown stronger anti-tumor effects in *in vitro* assays (343). Similarly, the addition of DC-CIK therapy has led to more enhanced immune responses and therapeutic outcomes in patients with colorectal cancer receiving routine therapy (324). This multicellular therapy has also demonstrated a better safety profile than standard chemotherapy in cancer patients (344). The clinical advantages of DC-CIK cell therapy are the result of the complex crosstalk between DCs, NK cells, and T cells, which leads to reciprocal and constant co-stimulation and initiates several immune reactions and tumor lysis mechanisms (345). Preclinical studies have also strongly suggested that multicellular therapies combining several ACT products elicit better tumor infiltration, immune responses, and therapeutic outcomes, such as tumor regression and overall survival, compared to unicellular approaches (346–350). In a clinical context, multicellular approaches could be deployed, for instance, in chemotherapy-induced lymphopenia through the infusion of DC-based vaccines followed by the adoptive transfer of naive T cells/TILs, together which trigger immune priming events culminating in more potent anti-tumor reactions with well tolerated adverse events (326, 346). From a commercial perspective, given the advantages of multicellular therapies seen with HSCT (75, 89) and other FDA-approved regenerative therapies (10, 11, 17, 24), their elusive mechanisms of action should not be an obstacle for further development, especially with the growing field of bioinformatics and computational analyses, through which simple cell-cell communication can be modeled in synthetic or digital platforms, thus providing the base for understanding more complex behaviors (351). Similarly, online platforms that map physiological networks, such as immune interactions, are available to explore cell-cell communications between different immune players. Indeed, systems immunology can be employed to study interactions within multicellular therapies or further predict their therapeutic efficacy (352), ultimately accelerating the translational pipeline between bench and bedside (353).

CONCLUSION

Cell therapy is an expanding global market encompassing stem cell- and non-stem cell-based unicellular and multicellular

TABLE 2 | Select studies using MSCs or BMAC for treatment of osteonecrosis of the femoral head.

Study nature	Patient population	Main clinical outcomes	References
MSCs			
Study 1: RCT	(N = 100) - Control group: core decompression alone - Intervention group: core decompression plus implantation of autologous BM-MSCs	- Radiological progression: 22.7% control vs. 3.7% in intervention - Greater improvement in HHS score (hip functionality) in intervention group - Significant decrease in osteonecrotic area	(339)
Study 2: Uncontrolled case series	(N = 9) Patients treated with allogeneic human umbilical cord-derived MSCs	- Reduction of necrotic area on MRI - Improvement in HHS up to 12 months post-intervention (a decrease after 24 months was reported)	(340)
BMAC			
Study 1: RCT	(N = 18) - Control group: core decompression alone - Intervention group: core decompression plus BMAC injection	- Greater improvement in VAS and WOMAC scores (hip pain and functionality) in intervention group - Significant differences in MRI outcomes: No deterioration in the intervention group and 71% worsening in control group	(341)
Study 2: Uncontrolled case series	(N = 62) Patients treated with an intra-arterial injection of BMAC	- Improvement in HHS compared to baseline on each follow-up (a decline was reported after 36 months) - Overall rate of radiological progression: 43.59%	(342)

BM, bone marrow; BMAC, bone marrow aspirate concentrate; HHS, Harris Hip Scale; RCT, randomized controlled trial; MRI, magnetic resonance imaging; MSCs, mesenchymal stem cells; N, number of patients; VAS, visual analog scale; WOMAC, Western Ontario and McMaster Universities Osteoarthritis Index.

therapies, which largely differ in their characteristics, isolation sources, and areas of use. A multitude of cell therapies have either become established practices or received FDA approval for certain indications. Other investigational and premarket approval-exempt cellular therapies have achieved a highly reputable track record in clinical settings, providing significant benefits to patients with degenerative disorders, immune diseases, and cancer. However, the clinical application of cell therapies in areas like neurodegenerative diseases still need to bypass several issues, including the standardization of cell manufacturing methods and the slow disease progression rendering clinical outcomes hard to measure (354). Other hurdles limiting the advancement of cell therapies are related to safety, which with certain products like CAR-T cells could pose life-threatening toxicities (355). Limited clinical indications, high production cost, and high patient costs are other issues associated with cell therapies that need to be addressed by ongoing and future clinical trials (356). On another note, recent investigations have surfaced a partially supported notion that a mixture of cell types, such as SVF or BMAC, more closely mimics physiological interactions and could thus be more important than a single cell type, such as ADSCs or MSCs, in regenerative medicine and cancer treatment. Although more comparative studies are required to strengthen the quality of current evidence about their

possible therapeutic superiority, multicellular therapies present with other advantages over unicellular therapies, including their lower cost (357, 358) and greater potential for exploitation by currently advancing gene engineering technologies and bioinformatic tools as a bridge to precision medicine (351–353).

AUTHOR CONTRIBUTIONS

AE-K wrote the first draft of the manuscript. RS and MR conceived the paper and wrote and edited the manuscript. All authors contributed to the revision of the manuscript.

FUNDING

This work was supported by the CCRT, Toronto ON (1027401-032017). MR holds a Fonds de la Recherche en Santé du Québec Junior I and II Awards.

ACKNOWLEDGMENTS

We thank Esteena Javier (CCRT) and Jean-Pierre Bikorimana (Université de Montréal) for their insightful criticism and review of the manuscript.

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GLOSSARY

ACT, adoptive cell therapy; ADSCs, adipose-derived stem cells; APC, antigen presenting cell; ASCs, adult stem cells; BCMA, B-cell maturation antigen; BM, bone marrow; BMA, bone marrow aspirate; BMAC, bone marrow aspirate concentrate; BMC, bone marrow concentrate; BMT, bone marrow transplantation; CAR, chimeric antigen receptor; CB, cord blood; CFR, Code of Federal Regulations; CIK, cytokine-induced killer; CSCs, cancer stem cells; CTLA-4, cytotoxic T lymphocyte-associated protein 4; CXCR4, chemokine receptor 4; DCs, dendritic cells; ECM, extracellular matrix; EPCs, endothelial progenitor cells; ESCs, embryonic stem cells; FD&C, Federal Food, Drug, and Cosmetic; GMP, good manufacturing practice; GVHD, graft-versus-host disease; HCT/P, human cells, tissues, and cellular and tissue-based products; hESCs, human embryonic stem cells; HPCs,

hematopoietic progenitor cells; HSCs, hematopoietic stem cells; HSCT, hematopoietic stem cell transplantation; IL, interleukin; IND, investigational new drug; iPSCs, induced pluripotent stem cells; LAK, lymphokine-activated killer; LVEF, left ventricular ejection fraction; MHC, major histocompatibility complex; MRI, magnetic resonance imaging; MSCs, mesenchymal stem cells; NK, natural killer; NKT, natural killer T; OA, osteoarthritis; PBMCs, peripheral blood mononuclear cells; PBSCs, peripheral blood stem cells; PBSCT, peripheral blood stem cell transplantation; PCR, polymerase chain reaction; PD-1, programmed death 1; PD-L1, programmed death-ligand 1; PHS, Public Health Service; PRP, platelet-rich plasma; PSCs, pluripotent stem cells; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; SS, systemic sclerosis; SSP, same surgical procedure; SVE, stromal vascular fraction; TCR, T-cell receptor; TILs, tumor-infiltrating lymphocytes.