Placenta stem cells:

Biology and clinical applications

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Key Points of the Chapter

- Placenta-derived stem cells are investigated in many clinical trials for malignant and non-malignant disorders.
- Several placenta cell subpopulations have been identified including stromal cells, trophoblastic cells, amniotic cells, chorionic cells, and mesenchymal stem cells.
- The human placenta is also significantly enriched with exosomes carrying micro-RNA molecules and other compounds.
- Results from clinical trials have provided evidence that placenta-derived stem cells are better suitable and more robust for cell therapies compared with mesenchymal stem cells derived from bone marrow or cord tissue.
- Cell preparations can be used for tissue repair, cancer treatment, and the correction of genetic disorders.
- If these early clinical data are confirmed in larger randomized trials a paradigm shift in cell therapy approaches can be expected.

Abbreviations

ALS:	amyotrophic lateral sclerosis
AKI:	acute kidney injury
AKT:	protein kinase B
BMP-7:	bone morphogenetic protein 7
CCL2:	cellular chemokine ligand 2
CD:	cluster of differentiation
CMV:	cytomegalovirus
CTL:	control group
CXCR-4:	cellular chemokine receptor 4
DMD:	Duchenne muscular dystrophy
DMSO:	dimethylsulfoxid
DNA:	desoxyribonucleic acid
EDSS:	Expanded Disability Status Scale
EGF:	epidermal growth factor
ER:	endoplasmatic reticulum
ERK:	extracellular regulated kinase
ESCs:	embryonic stem cells
EV:	extracellular vesicle
GATA4:	GATA binding protein 4
G-CSF:	granulocyte-colony stimulating factor
GFR:	glomerular filtration rate
GMP:	good manufacturing practise
GvHD:	graft-versus-host disease
HCG:	human chorionic gonadotropine
HGF:	hepatocyte growth factor
HLA:	human leukocyte antigen
HLSCs:	human liver stem cells

HSCT:	haematopoietic stem cell transplantation
IBD:	inflammatory bowel disease
IGF:	insulin-like growth factor
IGFBP-1:	insulin-like growth factor binding protein 1
IL:	interleukin
ISCT:	International Society of Cellular Therapy
MAP2:	microtubulin-associated protein 2
MCDD:	Methionine-Choline Deficient Diet
MCP-1	monocyte chemoattractant protein 1
MHC:	major histocompability complex
MIP:	macrophage inflammatory protein
miR:	micro-RNA
MMP:	matrixmetalloproteinase
MSCs:	mesenchymal stem cells
NAD:	nicotinamide
NANOG:	stem cell transcription factor
NASH:	non-alcoholic steatohepatitis
NES:	nestin
NF:	nuclear factor
Nm:	nanometer
OCT:	octamer-binding transcription factor
PAI:	plasminogen activator inhibitor
PGF:	poor graft function
PSC:	placenta-derived stromal cell
REX1:	reduced expression 1 (pluripotency marker)
RNA:	ribonucleic acid
SIRT-1:	Sirtuin-1

SOX2:	sex determining region box 2
SSEA:	stage-specific embryonic antigen
STAT:	Signal Transducer and Activator of Transcription
TGF:	tumour growth factor
TRA:	tumour rejection antigen
UTR:	untranslated region
VEGF:	vascular endothelium growth factor
v/v:	percentage of volume

Abstract

Cell therapy strategies are currently being investigated in many clinical trials for malignant and non-malignant disorders. During the last decade promising preclinical data have sparked considerable interest in the human placenta, and several cell subpopulations have been identified with stem cell properties (*i*) stromal cells, (*ii*) trophoblastic cells, (*iii*) amniotic cells, (*iv*) chorionic cells, and (*v*) mesenchymal stem cells. In addition, the human placenta was found be significantly enriched with exosomes carrying micro-RNA molecules and other compounds. All these cell fractions and the exosomes have in common that they do not express HLA class II molecules (DR, DQ, DP) enabling them to be immune evasive, a finding that could form the basis for the development of allogeneic off-the-shelf drugs in the near future. In addition, depending on the experimental conditions placenta-derived cells can be trams-differentiate in other tissues making them an ideal cell source for regenerative applications ("tissue repair"). Results from several early clinical trials have provided significant evidence that placenta-derived stem cells are better suitable and more robust for cell therapies compared with mesenchymal stem cells derived from bone marrow or cord tissue. If these early clinical data are confirmed in larger randomized trials a paradigm shift in cell therapy approaches can be expected.

Keywords:

Biology – Clinical Applications – Human Placenta – Stem Cells

1. Introduction

The human placenta is a disc-shaped organ that develops during pregnancy. It is cpmprised of a maternal part (pars materna) and a fetal part (pars fetalis) (Figure 1).

At the end of pregnancy, the placenta has the shape of a disc with 15-20 cm in diameter, a thickness of about 3 cm, and a weight of 500-600 g. At birth, the placenta first separates from the uterine wall and is expelled about 30 minutes after the birth of the child. The close connection created by the placenta of the fetal tissue with the uterine mucosa of the mother is used for the controlled exchange of substances between fetal and maternal blood (so-called placental barrier). The structures of this placental barrier separate the maternal from the fetal circulation. The maternal blood flows in the intervillous space, while the fetal blood flows in the blood vessels of the villi. With increasing duration of pregnancy, the placental barrier becomes "thinner", which ensures a more effective exchange of metabolites. Furthermore, the placenta is able to produce hormones (e.g., HCG, estrogens, progesterone, human placental lactogen) (Aumiller et al. 2017).

However, the placental barrier is not an impenetrable barrier, and many toxic compounds (e.g., alcohol, nicotine, drugs, etc.) can penetrate the fetus. This primarily affects the first trimester when the placental barrier is not yet fully developed (Sadler et al. 2020).

The umbilical cord (funiculus umbilicalis) is the connection between the fetus and the placenta and is about 50 cm long at birth. It contains a vein and two arteries that connect the fetal vascular system with the fetal part of the placenta. It is able to transport blood to the site of metabolic exchange. The mature umbilical cord is covered with amniotic epithelium (Aumiller et al. 2017).

2. Human placenta – Anatomy

From the 4th month onwards, the placenta has its mature structure which consists of a total of four parts (Figure 2):

1. Chorionic plate
2. Villous trees (about 50% of the volume weight)
3. Intervillous spaces
4. Basal plate

The chorionic plate forms the border of the placenta to the amniotic cavity and is of fetal origin. It consists of trophoblast cells, extraembryonic mesoderm and amnion (Sadler et al. 2020). Within the chorionic plate, the umbilical cord vessels divide and extend radially in the connective tissue of the chorion to the border of the placenta. The gap between the chorionic plate and the basal plate represents the intervillous space and maternal blood flows through it. This inflow, however, is limited because the intervillous space is severely restricted by branching villous trees and only about 30 µm wide gaps remain for the maternal blood to flow through, thus larger proteins cannot pass through them (Aumiller et al. 2017).

The intervillous spaces of the mature placenta contain about 150 ml of blood, which is renewed about three to four times per minute. This blood surrounds the chorionic villi, whose surface area is about 4-14 m^2 (Amann et al 2020).

The basal plate forms the bottom of the placenta. It consists of the decidua basalis, which is interspersed with an extra villous trophoblast. The term "decidua basalis" describes the layer of the decidua of the uterus which is located between the germ and the myometrium. It consists of decidua cells which develop from the stromal cells of the zona compacta of the endometrium (Sadler et al. 2020).

The basal plate consists of fibrinoids, an extravillous trophoblast, and endometrial tissue are embedded in it, the latter means maternal leucocytes and decidua cells (Sadler et al. 2020).

During birth the basal plate detaches from the uterine wall along a preformed zone within the decidua. This is a "predetermined breaking point". Immediately after birth the placental bed (part of the decidua) remains in the uterus. It is expelled postnatally through postpartum haemorrhages (lochia) (Aumiller et al. 2017). The area where the placenta detaches is characterised by about 10 to 40 bulges, the cotyledons. For every cotyledon of the mother, there are one to four villous trees. This interlocking of bulges and villous trees is similar to a zipper system (Sadler et al. 2020).

2.1 Human placenta – Placental cell populations

Within the macroscopic structures of the human placenta, different cell subpopulations can be histologically distinguished, all of which have a therapeutic potential (Pethe et al. 2021). These are (*i*) stromal cells, (*ii*) trophoblast cells, (*iii*) amniotic cells, (*iv)* chorionic cells, and (*v*) mesenchymal stem cells. In addition, very high concentrations of extracellular vesicles (mostly exosomes) are found in human placental tissue (Guttmacher et al. 2014, Klyachko et al. 2020)

Stromal cells are the precursor of decidua cells and are localised in the zona compacta of the endometrium. The decidua is the restructured uterine mucosa due to the implantation of the oocyte. A number of proteins are expressed at an increased level by the endometrial stromal cells during decidualisation and are secreted into the lumen have been detected, such as the insulin-like growth factor binding protein-1 (IGFBP-1) (regulates trophoblast functions), prolactin as well as interleukins (IL-11, IL-15), growth factors and VEGF, and others (de la Torre et al. 2021, Deus et al. 2020).

Placental stromal cells are closely related to mesenchymal stem cells. They express typical mesenchymal markers (e.g., CD29, CD73, CD105), but can be reliably identified by their specific markers CD56, CD90, CD166 and CD223. They do not express any HLA class II molecules on their cell surface (e.g., DR, DQ, DP) and also no co-stimulatory markers (CD40, CD40L, CD80, CD86), which explains their non-immunogenicity. In contrast to MSCs from other tissues, they have only a very limited capacity to differentiate into other tissues. Their

main function is the secretion of specific proteins and cytokines (e.g., CCL2, IL-6, IL-8, G-CSF etc.) (Amann et al. 2020, Fuentes et al. 2021).

Trophoblast cells form the fetal surface of the placenta with direct contact to the maternal blood. These cells separate the fetal compartment from the fetal tissues of the placenta from the maternal compartment. Shortly after fertilization cells blast elements of the trophoblast invasively grow into the endometrium, in that the cells concerned lose their borders and form irregularly interconnected trabeculae and lacunae (Aumiller et al. 2017).

The cell material required for the process is supplied by the cytotrophoblast, these are the parts of the trophoblast lying around the embryoblast. The cells of the trophoblast are neither permanently integrated into the maternal nor into the fetal organism. They simply mediate between the two biological systems (Knöfler et al. 2019). Since half of the trophoblast contains paternal antigens, it is a potential target for defense reactions of the maternal immune system.

However, trophoblast cells do not carry classical MHC class I molecules (HLA-A or HLA-B) on their surface (Knöfler et al. 2019). Although there are numerous natural killer cells in the placenta that respond to the elimination of cells without HLA-A and HLA-B molecules, trophoblast cells are selectively spared. This is mainly due to the non-classical MHC class I molecules that some of these cells express: HLA-C, HLA-E and HLA-G. HLA-E and HLA-G, in particular, are recognized by inhibitory receptors of the natural killer cells and thus prevent cytolysis. In addition, regulatory T-cells in the placenta create a microenvironment of immune tolerance which supports implantation (Aumiller et al. 2017, Knöfler et al. 2019, Chang et al. 2017).

Amniotic cells form the thin, transparent, vessel-less inner part of the amniotic sac. The middle layer is comprised by chorionic cells, while the decidua forms the external envelope (diameter: approx. 250 µm). The amniotic epithelium produces the amniotic fluid (Aumiller et al. 2017). Conventional chromosome analysis from amniotic cells after amniocentesis is the standard method for examining the fetal karyotype. Amniotic cells are pluripotent and show

very low immunogenicity (hardly any HLA-DR and HLA-DQ on the cell surface) (Qiu et al. 2020).

Chorionic cells are involved in the formation of villi (finger-shaped protrusions) and thereby increase the contact surface with the maternal blood. These cells infiltrate the uterine mucosa of the mother's uterus and, together with it, form the placenta. Chorionic cells are not part of the fetus, but contain its genetic information. They are also pluripotent and do not express any HLA-DR markers on their cell surface (Koo et al. 2012, Gupta et al. 2015).

Mesenchymal stem cells (MSCs) were originally discovered in bone marrow. But they can also be detected in a variety of other tissues (e.g., placenta, umbilical cord blood, umbilical cord tissue, fatty tissue, muscles, etc.). However, it is not yet fully clear if cells from these tissues really correspond to MSCs of the bone marrow or are at least similar to them (Pethe et al. 2021). These are stem cells of the connective tissue that are known to differentiate into fat-, muscle-, cartilage-, bone-, and bone marrow-stromal cells. They have a particular relevance for the control of the development of haematopoietic stem cells (Amann et al. 2020, Keshtkar et al. 2018). MSCs possess a high proliferation and differentiation potential, being able to differentiate into tissues of all three germ layers (de la Torre et al. 2021) (Figure 3).

In terms of their surface antigen expression, MSCs are positive for CD13, CD29, CD44, CD73, CD90, CD105, CD106, CD166 and the MHC class I. In contrast, they are negative for other surface antigens such as CD14, CD34, CD38, CD45, as well as HLA-DR (MHC-II), CD80, CD86, CD40 and CD40L (Amann et al. 2020). Within the placental structures, MSCs are primarily present in the umbilical cord tissue as well as in the amniotic tissue (Amann et al. 2020, Zhang et al. 2020). This large number of different placental cells which have so far not been exposed to toxic environmental influences can be regarded as genetically intact and represent a highly attractive cell pool that can be used for a wide variety of therapeutic applications ("Placenta: A gold mine"; Pethe et al. 2021). Of particular relevance is that all cell subpopulations of the placenta are non-immunogenic, which would enable low-risk allogeneic application (de la Torre et al. 2021).

2.2 Extracellular vesicles

Although known for a long time, EVs are increasingly becoming the focus of preclinical and clinical research. EVs are microscopic structures that are surrounded by a lipid double membrane and are secreted by almost all cells into the extracellular space (Amann et al. 2020, Klyachko et al. 2020). EVs contain lipids, nucleic acids (DNA, micro-RNA) and proteins, play an important role for intercellular communication, for the metastasis of tumours, and they can also be used as biomarkers (Pethe et al. 2021, Klyachko et al. 2020). Currently, three different EVs are distinguished according to their size, since there is currently no specific size, as there are still no specific protein or lipid markers for the individual classes (van Niel et al. 2018) (Figure 4):

1. apoptotic vesicles (''apoptotic bodies''): 500 - 4,000 nm in diameter.
2. ectosomes (''micro-vesicles''): 100 - 1,000 nm in diameter
3. exosomes (''exosomes''): 30 - 150 nm diameter

It is remarkable that all known EVs are non-immunogenic as they do not express HLA class-II antigens (HLA-DR, DQ, DP) nor any co-stimulatory surface markers (e.g., CD40, CD40L, CD80, CD86). They can, therefore, be applied as an allogeneic cell therapy without causing GvHD (van Niel et al. 2018).

Initial studies have shown that EVs can be used in regenerative medicine (''tissue regeneration'') both in tumour therapy and the treatment of genetic defects (Keshtkar et al. 2018, Bruno et al. 2020, Fonsato et al. 2012, Lopatina et al. 2018, Brossa et al. 2020).

Apoptosis vesicles are the largest of all EVs and are usually formed in the onset of cellular apoptosis (programmed cell death) by membrane budding. They usually contain cell organelles and other cellular elements of dying cells. They play no role in cell therapy (van Niel et al. 2018).

Microvesicles (ectosomes) are much smaller than apoptosis vesicles and their vesicle content is also clearly different. Mostly cellular proteins are transported from the ER to the inner side of the cell membrane and there laced off as vesicles from the cell membrane. Many

human exocrine glands use microvesicles to deliver their secretions (e.g., mammary glands) (van Niel et al. 2018).

Exosomes are the smallest of all EVs. Exosomes can transport proteins, lipids and nucleic acids. However, the content of exosomes is not only dependent on the secreting cell, but is also influenced by the state of the cell itself. This means that depending on the health and the current status of the cell, different types of molecules can enter the extracellular space (van Niel et al. 2018). One outstanding property of exosomes is that they are able to be released after injury or in acute phases of a disease, they can stimulate the body's own regenerative processes. Studies have shown that exosomes from MSCs activate several processes that are important for the repair of bone fractures and wound healing (Klyachko et al. 2020, Keshtkar et al. 2018, Nassar et al. 2016). These exosomes are also involved in the regulation of immune-mediated reactions and anti-inflammatory processes (Nassar et al. 2016). However, it is also known that exosomes can incorporate numerous disease-associated molecules. This is due to the fact that they can also be released from cancer cells or carry neurodegenerative-associated peptides. Of note, the mechanisms by which disease-associated factors spread between the cells are not yet fully understood (van Niel et al. 2018). Of particular scientific interest is the observation that exosomes are found in very high concentrations in the human placenta (Pethe et al. 2021). These exosomes can be easily isolated using standardized methods. The preparations are subsequently frozen at -80 °C with the addition of 5% v/v DMSO (Bruno et al. 2020) (Figure 5).

2.3 micro-RNA

Of great interest is the experimental detection of micro-RNA (miR) within EVs, especially in exosomes. The existence of miR molecules was first demonstrated in 1993 (Lee et al. 1993). They are single-stranded, non-coding RNA molecules (approx. 22 nucleotides) and are responsible for over 50% of the human phenotypes (O'Brien et al. 2018). The human genome codes for more than 2,300 different miRs which are currently classified into about 90 groups. Most miRs are initially transcribed from the DNA (precursor: "primary miR") which are

then modified into active miRs. These "mature" miRs in turn interact with the 3'-UTR of the target mRNA and thereby inhibit expression. To achieve this, the mRNA-miR-duplex is bound by the RISC complex and then cut by the Argonaute 2 (a RNAse) (Ghanbarian et al. 2022). Conversely, miR molecules are also able to activate genes under certain conditions.

Furthermore, it has been shown that miRs are able to control cellular transcription and translation which requires active transport between cells. Pathological expression of miR molecules is associated with a number of malignant and non-malignant diseases. Moreover, miR has been detected in the extracellular space which opens up possibilities for using these molecules as potential biomarkers (O'Brien et al. 2018, Ghanbarian et al. 2022). In the exosomes of the placenta, a number of different miR molecules have been detected in the so-called "secretosome" of the placenta (O'Brien et al. 2018):

- miR21
- miR22
- miR23b
- miR133
- miR145
- miR146

These specific miRs could be associated with the regenerative potency of exosomes (Klyachko et al. 2020). Of particular oncological interest are three miR molecules:

- miR31
- miR223
- miR451

These molecules can act as tumour suppressors (Lopatina et al. 2018) and have already led to impressive tumour regressions in vivo (see section 4.1). The evidence that certain miR molecules can produce regressions of human tumours was first demonstrated by Brodie et al. (1995). This research group used placental exosomes which were loaded ex vivo with a specific miR (miR124) and then applied to tumour-bearing mice (gliomas), thereby a

significant and prolonged regression of tumours in almost all animals was observed (Brodie et al. 1995).

3. Placenta - Preclinical Studies

Human stem cells can generally be divided into two groups - embryonic and adult stem cells. ESCs whose experimental investigation is ethically controversial or even prohibited in many countries, are usually derived from the inner blastocyst, while adult stem cells are understood to be the stem cells of the respective tissues (e.g., haematopoietic stem cells, MSCs, stem cells from the placenta, etc.) (Aumiller et al. 2017).

Adult stem cells usually have a shorter lifespan and a reduced plasticity which may limit their application. In contrast, placental stem cells show much faster growth and a significantly higher differentiation capacity; they are not immunogenic (Pethe et al. 2021). The mature and complete human placenta after birth is a highly attractive source of different stem cells that can be used in regenerative medicine ("tissue repair"), in tumour therapy, and for the repair of genetic defects. Moreover, human placenta contains an excessive number of EVs that can be isolated and used therapeutically.

The human placenta can be obtained from all obstetric departments of hospitals or from birth centres ("waste product"), and its cellular subpopulations as well as EVs can now be isolated easily and cryopreserved under GMP conditions. The obtained cell fractions are currently being used for numerous in vitro and in vivo experiments which should elucidate the further potential of the cells for medicine. Due to the extremely encouraging results of many of these studies, a large number of early clinical trials with these cells have now been initiated which have also impressively demonstrated the clinical potential of placental cells (Pethe et al. 2021). It is, therefore, not surprising that with the placenta extract "Placentrex®" another preparation was recently approved for the treatment of wound healing disorders (Pethe et al. 2021). A summary of the preclinical and clinical development status of cells described herein is shown in Table 1.

In the following sections, the most important preclinical studies as well as the results of the first early clinical trials will be presented. Due to the large number of studies available to date, only exemplary ("pivotal") experiments and studies are described avoodig to exceed the scope of this chapter. For a more detailed list, it is referred to the relevant excellent and extensive reviews (Amann et al. 2020, Pethe et al. 2021, de la Torre et al. 2021, Deus et al. 2020, Rodríguez-Fuentes et al. 2021, Shahrbaf et al. 2022).

3.1 Preclinical studies with extracellular vesicles

Exsosomes as part of EVs have only recently become the focus of preclinical investigations, but it quickly became clear that these vesicles offer an enormous therapeutic potential. Especially in the case of diseases that are very difficult to treat, such as Duchenne muscular dystrophy (DMD), Parkinson's disease, acute kidney failure, multiple sclerosis, certain types of tumours, etc., impressive results have been obtained with exosomes in animal models, which are currently being validated in the first clinical studies. The particular advantage of using exosomes lies in the fact that these vesicles contain miRs and thus represent an excellent tool to modulate the activity and expression of human genes, especially since exosomes can also be loaded with specific miRs ex vivo (see sections 2.2 and 2.3) (Vasudevan et al. 2012).

A significant experimental observation that the miR molecules, contained in the exosomes of placental MSCs, appear to be able to modulate epigenetic processes in deficient cells to activate cellular "repair programs" (Vasudevan et al. 2012). (Figure 6).

A significant contribution to the understanding of EVs and especially exosomes comes from the Italian research group led by Professor Giovanni Camussi (University of Turin, Italy). Together with the spin-off company UniCyte AG (Switzerland), a first phase I study is currently being planned.

The regenerative potential of exosomes and their possible use in tumour therapy has been very well demonstrated in preclinical models. In a mouse model, NASH was induced using the MCDD technique. The mice were then treated with EVs of human (!) MSCs twice a

week after the development of liver cirrhosis and the liver was examined histologically and molecularly after four weeks of therapy. A significant decrease in cirrhosis and inflammation was observed compared to the control group (Bruno et al. 2020).

In addition to the significant improvement in liver function, the underlying molecular biology has also shown that treatment with human EVs was able to suppress 28 genes associated with the development of liver cirrhosis, which explicitly underlines the importance of EV therapy (Bruno et al. 2020).

Further impressive evidence for the efficacy of the therapy with EVs was demonstrated in the animal model for acute renal failure (Herrera-Sanchez et al. 2014). In a mouse model, acute renal failure was induced in the animals by intramuscular injection of glycerol. EVs were then isolated from human stem cells, purified, characterized and expanded ex vivo. The animals were then treated once intravenously with the isolated EVs three days after induction of acute renal failure. Histologically, there was a significant improvement in tubular necrosis and renal morphology. A significant improvement of the renal function results could also be demonstrated (Herrera-Sanchez et al. 2014).

In addition to their regenerative potential, EVs and exosomes also are becoming increasingly important for the therapy of malignant tumours, as first studies by Brodie et al. (1995) have shown (see section 2.3). For this reason, Professor Camussi's research group has excellently demonstrated the activity of human EVs in malignant tumours in a series of murine xenograft models (Fonsato et al. 2012, Lopatina et al. 2018, Brossa et al. 2020). It could be shown that the growth of human renal cell carcinomas in the mouse model is significantly reduced by the application of human EVs. If the EVs were additionally loaded with anti-tumour miRs (miR145, miR200b, miR200c and miR223), tumour regression was enhanced. Due to the non-existing immunogenicity of the EVs as well as the MSCs, again no rejection reaction was observed despite allogeneic application (Fonsato et al. 2012, Lopatina et al. 2018, Brossa et al. 2020).

However, it is remarkable that the activity of human EVs and exosomes (together with the miRs they contain) seems to be independent of the respective tumour entity. Thus,

previous studies (Fonsato et al. 2012) have been able to show that in murine xenograft models with lymphomas, glioblastomas and hepatocellular carcinomas, equally significant tumour regressions can be induced by EVs (Figure 7).

In summary, the role of EVs in tumour therapy is not yet fully understood, however, preclinical results so far support the core benefits of EVs such as

- ex vivo loading with anti-tumour miRs possible;
- easy to obtain from human placental tissue;
- no immunogenicity;
- cryopreservation at -80 °C possible and

and the enormous potential of EVs for modern haemato-oncology.

3.2 Placental stromal cells

PSC have been shown in vitro to secrete haematopoietic proteins, to stimulate colony formation, and to induce bone marrow migration. Previous studies in mice showed that a PSC-based product (PLX-R18: Placenta eXpanded-R18; Pluristem Ltd., Israel) responded to radiation-induced haematopoietic failure by transiently secreting hematopoiesis related proteins to enhance reconstitution of the haematopoietic system.

PSCs represent a well-characterized subtype of MSCs. They are characterized by high expression of typical mesenchymal markers (e.g., CD29, CD73, CD105) and no expression of CD14, CD19, CD31, CD34, CD45, CD71. Of particular interest, however, is the observation that PSCs do not express HLA class II molecules (DR, DQ, DP) and co-stimulatory markers (CD80, CD86, and CD40) enabling them to be immune evasive.

PLX-R18 is a 3D-expanded placenta-derived stromal cell product designated for the treatment of haematological disorders (derived from human healthy placentas). In an attempt to further investigate the effects of PLX-R18 on bone marrow recovery following irradiation, cells were administered intramuscularly to C57BL/6 mice on two doses of PLX-R18 (2 million cells/dose) on 1 day prior and 3 days post total body irradiation (8 Gray) (Kumar et al. 2022).

Under these experimental conditions PLX- R18 treatment significantly increased survival after irradiation (81% versus 23%, $p < 0.0005$) (Figure 8).

In addition, the investigators also monitored peripheral blood and bone marrow cellularity at several time points up to 30 days and could show that PLX-R18 treatment significantly increased the number of colony-forming haematopoietic progenitors in the femoral bone marrow and significantly raised peripheral blood cellularity.

Furthermore, PLX-R18 administration attenuated biomarkers of bone marrow aplasia (erythropoietin), sepsis and systemic inflammation (sPselectin and E-selectin) and attenuated radiation-induced inflammatory cytokines/chemokines as well as growth factors, including G-CSF, MIP-1a, MIP-1b, IL-2, IL-6 and MCP-1. In addition, PLX-R18 also ameliorated radiation-induced upregulation of pAKT. This seminal preclinical study provided the first evidence that PSC administration may serve as a protection measure, mitigating bone marrow failure symptoms and systemic inflammation in lethally irradiated mice. Further studies are clearly needed to fully explore the detailed underlying mechanism and cascade of events contributing to the radioprotection effect of PSCs.

Finally, it should be noted that human (!) PSCs have been administered to animals (i.e., rodents) which is the first experimental demonstration that PSCs are immune evasive and even treatments of different species are possible. This finding is clearly a paradigm shift for cellular therapies and will have, if confirmed, major clinical implications in the near future.

3.3 Trophoblastic cells

The human placenta has been described several times in the past as a "least understood organ" - this applies primarily to an essential cell component of the human placenta, the trophoblast cells (Chang et al. 2017). Half of the trophoblast cells have paternal antigens, so that they would represent a potential target for defence reactions of the maternal immune system. However, they do not express classical MHC class I molecules (HLA-A or HLA-B) on their surface. As a result they are not recognised by either natural killer cells or the immune system. However, the ability of trophoblast cells to penetrate the normal uterine

mucosa is remarkable (Knöfler et al. 2019). The underlying mechanisms are not yet fully understood, but are used as a cellular model for the metastasis of tumour cells. The complex processes of trophoblast anchorage and invasion are controlled by various autocrine and paracrine regulatory mechanisms. Numerous growth factors produced at the fetomaternal interface promote trophoblast cell invasiveness by activating critical signaling pathways such as ERK, AKT, STAT and Wingless (Aumiller et al. 2017, Chang et al. 2017).

During the invasion of trophoblast cells into the vessels as well as into the extracellular matrix of the decidua, the expression of adhesion molecules such as various integrins (heterodimeric glycoproteins that form specific bonds with proteins of the extracellular matrix) play an important role. In addition, the activation of proteases of the MMP family or plasminogen activators are of high importance for the process of migration (Amann et al. 2020, Chang et al. 2017).

Macrophages stimulate trophoblast invasion by remodeling the extracellular matrix and eliminating apoptotic cells. On the other hand, TNF-α from macrophages has been shown in in vitro studies to have an inhibitory effect on trophoblast invasiveness by inducing apoptosis and by activating PAI (Chang et al. 2017).

Although the importance of human placental trophoblast cells for cell research and therapy is of great importance, many mechanisms of placental development and trophoblast formation are still not fully understood. This is mainly due to the fact that no suitable cell models are been available so far, and the transfer of data from the murine placenta to the human placenta is not readily possible (Knöfler et al. 2019). In recent years, however, it has been possible to isolate human placental trophoblast stem cells and permanently propagate them in cell culture systems. Nevertheless, it remains a challenge to study the differentiation of human trophoblasts in cell culture. Although it has been possible to isolate multipotent trophoblastic stem cells, which can differentiate into all other cell subtypes of the trophoblast from the mouse blastocyst, this has so far not been possible. However, this has not yet been achieved with human trophoblast cells (Chang et al. 2017).

Reduced invasion of the extravillous trophoblasts into the uterine mucosa and a resulting insufficient remodeling of the blood vessels is associated with various pregnancy complications (e.g., preeclampsia, diabetes mellitus type growth retardation etc.) (Chang et al. 2017). Correction of the dysfunctional trophoblast cells with allogeneic human trophoblasts therefore opens up a possibility to treat these complications. Thus, the future role of trophoblast stem cells in the therapy of placental dysfunction should be further evaluated.

3.4 Amniotic cells

Compared to the other placental cell subpopulations, amniotic cells have the advantage that they can be easily isolated in higher cell numbers (no complicated isolation techniques are required). Furthermore, they are not immunogenic and do not induce tumours. In addition, they show great plasticity (differentiation into multiple cell types) as well as a large homing potential, and they can secrete exosomes in a paracrine manner. Furthermore, in cell culture, there is a certain tendency of amniotic cells to epithelial-mesenchymal transition, which is attributed to the paracrine secretion of TGF-β (Yang et al. 2018, Zhou et al. 2011, Luo et al. 2011, Zhao et al. 2012).

The identification and characterization of amniotic cells is readily obtained by their cell surface markers: SSEA-3, SSEA-4, OCT-4, TRA1-60, and TRA1-80 (Zhou et al. 2011). Although amniotic cells have characteristics of a classical stem cell, they are not able to proliferate unlimitedly because they lack telomerase activity (Zhao et al. 2012). However, they are able to differentiate into a number of different cell classes.

Remarkable is their lack of immunogenicity; they do not express HLA-A, HLA-B, HLA-C and HLA-DR molecules on their cell surface. It could be demonstrated in a clinical study that intravenously applied amniotic cells neither led to haemolysis nor to allergic reactions (Yang et al. 2018). In fact, numerous animal studies (Qiu et al. 2020) have demonstrated the potential of amniotic cells in a number of different diseases:

- Alzheimer's disease
- Parkinson's disease

- Stroke
- Lung injury
- Liver damage
- Type 1 diabetes mellitus
- Acute kidney failure
- Myocardial infarction
- Wound healing disorders
- Ovarian insufficiency

In an animal model of Parkinson's disease, it was shown that the amniotic cells had migrated specifically into the striatum which was associated with a significant improvement in the clinical outcome. Similar results were reported for ischaemic brain lesions, where the immigration of amniotic cells occurred via a CXCR-4-dependent mechanism (Zhang et al. 2021).

A rapid differentiation of amniotic cells into cardiomyocytes was also demonstrated in myocardial infarction in animal models, leading to a significant improvement in cardiac performance (Evans et al. 2018).

It should be noted that amniotic cells have little homing ability and most cells after intravenous administration remain trapped in the lung filter so that alternative routes of administration are the subject of current research.

A number of preclinical studies have demonstrated that amniotic cells secrete exosomes that promote fibroblast migration as well as proliferation and upregulate collagen synthesis. This explains the excellent effect of amniotic cells in wound healing. Of particular importance is also the extremely high plasticity of human amniotic cells which differentiate into a number of different cell types under different experimental conditions (Figure 9). Dexamethasone, HGF, IGF and other cytokines can achieve differentiation into liver cells (Luo et al. 2011), while poly-L-ornithine and EGF enable differentiation into pancreatic cells (Zhao et al. 2012, Balaji et al. 2016) which could be successfully used in animal models to treat type

1 diabetes mellitus. Differentiation into corneal and Schwann cells, neurons, cardiomyocytes, etc. has also been demonstrated (Miki et al. 2018). Moreover, differentiation into osteoblasts was obtained by the addition of osteopondin, TGF-β and BMP-7 (Zhou et al. 2011).

3.5 Chorionic cells

The placental membranes (amniotic and chorionic membranes) are of extra-embryonic origin. The fetal side of this structure separates the fetus from the endometrium. Of note, the chorionic membrane is comprised of 6-8 cell layers and is connected loosely to the amniotic membrane by collagen fibres. Therefore, it can be isolated easily (Figure 10). Components of the extracellular matrix comprise proteioglycans, fibronektins, laminins, and collagens (type I, III, IV, V, and VI) (Koo et al. 2012).

Chorionic stem cells are characterized by a homogenous morphology (similar to fibroblasts) and express CD29, CD73, CD90, and CD105, but no markers of the haematopoietic system. In addition, they express markers of pluripotency such as OCT4, SOX2, NANOG, and GATA4 which are strongly associated with a myocardial differentiation potency. Furthermore, markers associated with neurogenesis (e.g., MAP2, NF, NES) are also expressed on the cell surface of chorionic stem cells.

Moreover, they are immune evasive since almost no HLA-DR expression is detectable and therefore they can be used in allogeneic settings. Chorionic stem cells also show huge pluripotent properties and are less differentiated compared with bone morrow stem cells (Walentin et al. 2016).

Several lines of preclinical research have provided significant evidence that chorionic stem cells under certain experimental conditions can be trans-differentiated into adipocytes, osteoblasts, chondroblasts, and other cell types (Figure 11, Table 2) (Koo et al. 2012).

It should be noted that the potential of chorionic stem cell to differentiate into neuronic tissue is two-fold higher compared with bone marrow-derived MSCs or amniotic cells. Furthermore, some genes which are normally expressed in embryonic stem cells are still active in chorionic stem cells (e.g., NANOG, OCT4, and REX1), but not in MSCs (Koo et al. 2016).

Finally, chorionic cells were found to have very high levels of telomerase. Telomerase activation is required for cellular immortalization and is found in most malignant tumours. Normal somatic cells are generally telomerase-negative, except for stem cells in renewing tissues. During pregnancy, the human trophoblast continues to proliferate and acts as proliferating stem cells for the development of chorion and the formation of placenta. In an earlier study Kyo et al. (1997) examined 105 chorions from human placentas. A total of 33 (76%) normal early chorions at 5 to 9 weeks gestation were telomerase-positive. Chorions from early spontaneous abortions also exhibited telomerase activity but at a low level. In contrast, only 4% of late chorions at 34 to 41 weeks gestation expressed telomerase activity. The findings support the emerging concept that normal somatic cells with stem cell-like characteristics can express telomerase activity and explains, at least in part, the high pluripotency and proliferation rate of chorionic stem cells – an observation which is of great importance for the future clinical applications.

3.6 Mesenchymal stem cells

MSCs are found in a variety of human tissues and are easy to isolate (Figure 12). In contrast to bone marrow and adipose tissue, the placenta is significantly enriched with MSCs (amnion, chorion, umbilical cord) (Amann et al. 2020).

According to the original definition of the ISCT from 2006 (Dominici et al. 2006), MSCs are plastic-adherent cells and carry the following markers on their cell surface: CD73, CD90, CD105 (≥ 95% of cells). However, MSCs do not express CD45, CD34, CD14, CD11b, CD79a, CD19 and no HLA markers (HLA-DP, HLA-DQ, HLA-DR (≥ 98% of cells). Furthermore, MSCs are reported to be differentiable in vitro into adipocytes, chondroblasts and osteoblasts (Amann et al. 2020, Dominici et al. 2006) (Figure 3).

As stem cells, MSCs are multipotent and they can still differentiate into a variety of other cell types (see above). As a result, they can compensate for cell loss and thus replace old, defective cells or cells that have been lost through trauma. In addition, they possess immunomodulatory properties (Amann et al. 2020).

MSCs are able to respond to signals of inflammation and tissue damage and act at the site of action by releasing growth factors, anti-inflammatory, anti-apoptotic, anti-fibrotic, immune-modulatory and chemotactic factors, as well as EVs. MSCs prevent excessive inflammatory responses and promote the formation of new vessels, proliferation and differentiation of progenitor cells, modulate immune responses and inhibit the formation of fibrosis or scarring during wound healing. Due to their low expression of HLA class II, co-stimulatory molecules (CD40, CD40L, CD80, CD86) and their immunoregulatory effect, allogeneic transplantation is also possible. Nevertheless, MSCs could be recognized by the recipient's immune system and trigger allo-sensitisation (Amann et al. 2020).

Despite possible allo-sensitisation, they are well tolerated by the recipients and do not appear to be any less effective. For this reason, MSC preparations are largely considered safe with a low side effect profile. MSCs are also able to differentiate into certain cell types, e.g., bone cells and possibly endothelial cells or skin cells, and thus replace defective cells. The currently assumed mechanisms of action are shown in Figure 13.

A large number of animal studies have impressively demonstrated that MSCs can contribute to the repair of cartilage and bone tissue in osteoarthritis (Wang et al. 2019), to the amelioration of the effects of cerebral ischaemia (Vu et al. 2014) and to the improvement of rheumatic (Tyndall et al. 2015), cardiovascular (Kim et al. 2015), respiratory (Matthay et al. 2010) and metabolic diseases.

4. Placenta stem cells – Clinical studies

The aforementioned promising preclinical data have sparked considerable interest in further research and paved the way for many clinical trials in early and late-phase development which is clearly reflected in the exponentially increasing number of research reports listed in PubMed (Figure 14).

Results from several clinical trials have provided the first evidence that the regenerative capacity ("tissue repair") of placenta-derived MSCs is significantly better and more robust that the capacity of MSCs derived from human bone marrow or cord tissue. This significant observation is, at least in part, due to the fact that placenta-derived stem cells are young and healthy with no genetic abnormalities since they have not been exposed to environmental toxins (de la Torre et al. 2021).

4.1 Extracellular vesicles

MSCs have been studied extensively in several preclinical and clinical experimental systems, and a growing body of evidence has demonstrated that their observed regenerative properties (i.e., tissue repair) is due to the paracrine potency of releasing EVs.

The field of EV research has developed rapidly over the last decade from the study of fundamental biology to a subject of significant clinical relevance. The potential of harnessing EVs in the diagnosis and treatment of diseases (including cancer, neurological and cardiovascular disorders) is now being recognized. Accordingly, the applications of EVs as therapeutic targets, biomarkers, novel drug delivery agents and standalone therapeutics are being actively explored. The therapeutic potential of EVs is an area of intense research as these lipid-bound particles can facilitate transfer of protein, lipid, and miRNA cargo to target cells, thereby enabling utilization of their inherent therapeutic characteristics or their use as potential vehicles for drug delivery. In this regard, EVs possess distinct advantages, including less immunogenic potential than parental cells (e.g., lower expression of surface human leukocyte antigen compared with cell-based therapies), intrinsic ability to cross cellular barriers, no replication potential and thus less risk of tumour generation, lower toxicity

compared with synthetic drug carriers, and inherent targeting characteristics that may enable minimization of off-target effects.

The first phase I trial with EVs has already been conducted in 2014 (NCT02138331). A total of 20 patients with diabetes mellitus type 1 were enrolled and treated with EVs (day 0) and micro vesicles (day 7). Interestingly, the required insulin dose per day was found to be significantly reduced during three months after treatment suggesting that EVs may be an attractive treatment strategy for diabetes. Recently, these results have been confirmed by several other studies with EVs (Hu et al. 2020). It has been shown that EVs released by stem cells and immune cells can regulate gene expression in recipient cells, a finding that adds weight to the proposal that EVs may represent a novel opportunity to treat diabetes and its complications.

In a case report a patient with a refractory GvHD was treated with four applications of EVs derived from MSCs (Kordelas et al. 2014). No adverse events were observed and a significant improvement of the clinical symptoms was recorded after two weeks after treatment. In another larger randomized and placebo-controlled trial (N = 40) patients with chronic kidney failure (stage III and IV) were treated with EVs derived from MSCs (Nasser et al. 2016). Patients received EVs (100 µg/kg body weight) on day 1 and day 8. Again, no significant side effects were observed. Following 12 weeks after treatment creatinine values were reduced by 50% and a two-fold GFR increase was seen. This trial clearly demonstrated that the administration of two doses of cell free cord-blood mesenchymal stem cells EVs is safe and can ameliorate the inflammatory immune reaction and transiently (3–6 months) improve the overall kidney function in grade III-IV patients. Moreover, it has been shown that cryopreserved EVs can be injected directly and repeatedly. It is, therefore, conceivable that this approach may also be applied to a wide variety of autoimmune diseases as a means of therapy of diseased cells (i.e., reprogramming) and not cell therapy.

The vast majority of clinical trials with EVs are early phase I trials recruiting patients with degenerative disorders (reviewed by Keshtkar et al. 2018). EVs are also attractive candidates for drug delivery, and various engineering strategies are being investigated to alter

their cargo and increase their efficacy. However, rigorous standardization and scalable production strategies will be necessary to enable the clinical application of EVs as potential therapeutics.

4.2 Placental stromal cells

HSCT procedures (i.e., allogeneic, autologous) are the only curative treatment for some malignant (e.g., leukaemias) and non-malignant (e.g., genetic disorders) haematopoetic diseases. Many patients can achieve a complete haematopoetic recovery following myeloablative therapy, however, in a small proportion of patients a graft failure is seen. This is caused primarily by either graft rejection and/or PGF.

PGF following HSCT is a life-threatening complication, and with rapid development of allogeneic HSCT (especially haplo-identical HSCT) it becomes a growing concern. The incidence of PGF is approximately 5-27%, but numbers are gradually increasing. Risk factors for PGF include low dose of infused CD34-positive cells, CMV infections, GvHD, donor-specific antibody development, iron overload, splenomegaly, amongst others (McLornan et al. 2021) Currently, two main treatment opportunities are widely used for the treatment of PGF: *(i)* $CD34^{+}$-selected stem cell boosts (SCBs), and (ii) infusion of (donor-specific) MSCs. In this regard, MSCs appear to be more convenient as they are without any immunogenicity, and can be collected from a third-party donor.

Unlike MSCs, PSCs have only limited capacity to differentiate into other tissues (→ no tissue repair or regeneration). Their proposed modes of action are cytokine secretions. Based on these molecular findings, Lazarus et al. (2020) have demonstrated in a phase I dose-escalation trial (NCT03002519) that administration of PSCs can increase the number of colony-forming haematopoetic progenitors in the bone marrow and can regenerate bone marrow function rapidly by releasing several cytokines (e.g., CCL2, IL-6, IL-8, G-CSF and others). A total of 19 patients were enrolled, amongst them 17 patients had prior allogeneic HSCTs and two patients underwent autologous HSCT. For all patients enrolled, PGF was documented at day 90 after transplantation.

For this trial PSCs from human allogenic placenta tissue were isolated, characterized and then expanded in a bio-reactor for clinical application. Patients were treated in three cohorts: (1) low dose (1 million cells/kg); (2) intermediate dose (2 million cells/kg), (3) high dose (4 million cells/kg), and PSCs were administered intramuscularly on days 1 and 8. Shortly after PSC application a significant increase of haemoglobin, neutrophils, and platelets were observed with a maximal effect seen in the high-dose cohort. Treatment was reported to be safe and well-tolerated, with no rejection reaction (GvHD) seen in patients seen so far. Due to these encouraging results a subsequent phase II has been conducted and is currently recruiting patients (NCT03797040).

The study reported by Lazarus et al. (2020) is the first clinical trial demonstrating a clear clinical benefit of PGF patients, a finding that, if confirmed in larger randomized clinical study, will have major clinical implications for patients undergoing myeloablative therapies.

4.3 Trophoblastic cells

As described above, preclinical studies with human trophoblastic stem cells are currently in a very early stage of research. However, initial premature results suggest that these cells may have a putative clinical role for the treatment of preeclampsia and fetal growth retardation. Clinical trials, however, have not yet been conducted so far.

Furthermore, trophoblastic cells represent an attractive model for investigating the underlying mechanisms of cancer invasion and metastases.

4.4 Amniotic cells

Human amnion-derived stem cells including human amniotic MSCs and human amniotic epithelial stem cells have shown considerable advantages over other stem cells (for details see paragraph 3.4). The paracrine effects-related immunosuppressive and immune-stimulatory features of amniotic stem cells play a key role in treating various diseases, in which these cells were able to secret a variety of growth factors including angio-modulatory cytokines,

anti-bacterial peptides, and anti-inflammatory agents, exhibiting their angiogenic, cyto-protective, immunosuppressive, anti-inflammatory, anti-scarring, and antibacterial properties.

The first evidence that amniotic stem cells can increase wound healing came from a small interventional trial (NCT02959333). In this study amniotic cells were used to treat bronchial fistulas by bronchoscopic application. Another initial trial has shown that the application of amniotic stem cells can significantly reduce GvHD symptoms (reviewed by Amann et al. 2020). Finally, allogeneic amniotic stem cells have been approved as second-line treatment for the local treatment of fistulas associated with Crohn's disease with low to moderate activity (Alofisel®: single dose 120 x 10^6 cells). Selected ongoing early phase trials with amniotic stem cells are listed in Table 3.

Collectively, amniotic stem cells have the great advantages over other stem cells in team of abundant sources, no ethical and moral disputes, no tumourigenicity and low/no immunogenicity. Moreover, the pluripotent properties and paracrine effects of these cells significantly enhanced their applications in experimental research and clinical practice. All these properties make them a promising source of stem cells for cell therapy and regenerative medicine.

4.5 Chorionic cells

Based on the current preclinical data it quickly became clear that chorionic cells due to their molecular properties will have a huge potential for future cellular therapy studies. To date, no clinical study using chorionic cells is active, but several trials are planned.

4.6 Mesenchymal stem cells

The multipotency property of MSCs has attained worldwide consideration because of their immense potential for immunomodulation and their therapeutic function in tissue regeneration. MSCs can migrate to tissue injury areas to contribute to immune modulation, secrete anti-inflammatory cytokines and hide themselves from the immune system.

In December 2022 more than 1.400 clinical trials with MSCs were listed at www.clincialtrials.gov which represent a dramatic increase compared with 2012 (220 studies). Several clinical trials have reported that both autologous and allogeneic MSCs are valuable sources for tissue forming. Particularly, autologous MSCs signify the chief sources examined safe for administration and minimization of immunological threat, regardless of the lack of reported grievances concerning allogeneic MSC-based therapy. According to the studies published so far (summarized in Table 4), administration of MSCs appear to be more effective and the usefulness of MSC therapy in bone and heart disorders, arthritis, autoimmune disorders and others has been broadly established (for excellent reviews see Merimi et al. 2021 and Ringdén et al. 2022) and has resulted to several approvals in the past (Table 5).

In terms of placenta-derived MSCs the most recently published study of Dr. Olle Ringdén (Stockholm, Sweden) should be mentioned (Ringdén et al. 2022). In an attempt to evaluate MSCs from placental tissue (decidua) these researchers treated a total of 44 patients who had undergone an allogeneic HSCT procedure with human placenta-derived decidua cells (median dose: 1.5 x 10^6 cells/kg, median applications: 2 doses). The basis for this trial was the observation that placenta-derived MSCs have a much greater immune suppressive potential compared with MSCs derived from bone marrow. Overall, cell transfusions were well tolerated (3/44 patients with transient infusion reactions). The OS was found to be 67% after one year which is much better compared with historic controls.

Collectively, numerous studies have assessed MSC therapies for treating a wide array of disorders owing to their potent immunomodulatory and regenerative properties. Typical clinical indications for MSC therapy include musculoskeletal repair, neurological and cardiovascular pathologies (e.g., stroke), cancer treatment, hematological and auto- or allo-immune complications (e.g., GvHD), and more recently also complications associated with coronavirus disease 2019 (COVID-19), such as acute respiratory distress syndrome (ARDS) and sepsis. The growing number of clinical studies with MSCs clearly indicate that MSCs will have a great promise for clinical use.

4.7 Stem cell expansion

Overall, transplantation of cordblood stem cells and other perinatal stem cell remains limited by the lower number of haematopoietic stem and progenitor cells, as well as the preponderance of naïve B and T cells, present in particular in cord blood compared with mobilized peripheral blood or bone marrow sources. This had led to higher rates of transplantation-related mortality owing to delayed engraftment and infectious complications. This is the main reason why stem cell preparations derived form cord blood can only be used for children up to a body weight of 5-10 kg.

In order to overcome this bottle neck, omidubicel (Omisirge®) is a novel umbilical cord blood-derived advanced cell therapy product comprising an ex vivo nicotinamide-expanded and enhanced $CD133^+$ stem cell fraction and a non-expanded $CD133^-$ fraction containing mature lymphoid cells (Lin et al. 2023).

CD133 (MW: 120 kDa) is a transmembane glycoprotein, expressed on haematopoietic stem cells and cancer stem cells. It is the commonly used marker for the isolation of cancer stem cells. Nicotinamide (NAD) is a vitamin B3 analogon that inhibits differentiation and enhances outgrowth of CD34+/CD38- cells. Moreover, it is a SIRT-1 inhibitor and thereby blocking NAD+ hydrolysis with sirtuin being a class III NAD+-dependent-histone-deacetylase (also regarded to be an “anti-aging enzyme“).

Stem cell expansion with omidubicel has been reported to be a three-step process (Figure 15): (*i*) immunomagnetic bead selection for $CD133^+$; (*ii*) $CD133^-$ and T cells are re-cryoconserved; (*iii*) $CD133^+$ cells are then cultured with Flt-3 ligand, NAD^+ and thrombopoietin for three weeks. The final product is again cryoconserved (“Omisirge”) and shipped to the treating physician (Gamida Ltd., 2023). Culturing stem cells with nicotinamide has been shown to inhibit stem cell differentiation and improve bone marrow homing and results in approximately 1×10^8 CD34 positive cells (Horwitz et al. 2021).

FDA approved omidubicel in April 2023, EMA approval is expected soon (CHAP votum was positive) (manufacturer: Gamida Cell Ltd., Jerusalem, Israel). The drug is indicated for allogeneic use and patients over 12 years of age.

5. Future Directions

The recent advances of regenerative approaches (“tissue repair”) is of outstanding interest for the treatment of many human disorders and injuries. Initially, stem cells which are necessary for this concept have been almost exclusively derived from human bone morrow during the last two decades. However, with a better and increasing understanding of the underlying molecular biology and properties of different human stem cells, it is now feasible to generate these cells from different organs (e.g., connective tissue, placenta etc.).

During the last years researches extensively studied placenta-derived stem cells since these cells have a bundle of biological and manufactural advantages which clearly translates into superior therapeutic properties compared with bone marrow-derived stem cells.

At the end of 2022 more than 1,400 clinical trials with MSCs (allogeneic and autologous settings) have been reported to enroll patients, and in approximately 180 of these trials placenta stem cells were used. In the vast majority of the studies, anti-inflammation, angiogenesis, and trans-differentiation properties of placenta-derived stem cells were defined as clinical endpoints.

Of specific interest in the experimental finding that human placenta-derived progenitor cells, stem cells, and epithelial cells (amniotic) can be differentiated under certain experimental conditions into other tissues (e.g., bone, fibroblasts, neurons, cartilage, pancreas, liver, etc.) which represents a land of opportunity for future clinical trials. In addition, placenta-derived cell-free vesicles (i.e., exosomes) have been found to play a significant role in the field of tissue repair and cancer therapies in preclinical systems. Based on these finding, phase I trials have most recently been conducted.

The exceptional advantage of the placenta (“waste product”) as a cell reservoir is based on the fact that several different cell subpopulations are located closely together in a reasonable amount and can be isolated easily. Of particular interest, however, are two experimental observations: (*i*) invariably all placenta-derived stem cell subpopulation have demonstrated the same biologic features as stem cells derived from bone marrow or other organs, however, placenta progenitor cells are much more potent; (*ii*) all placenta-derived stem

cells are immune-evasive since they do not express CD34, CD11d, CD19, CD45, HLA-DR, and HLA-G. Due to their immunologic properties (no HLA expression), placenta stem cells and exosomes can be administered without HLA matching (allogeneic) and without immunosuppression – a finding that could form the basis for the development of allogeneic “off-the-shelf“ drugs in the near future.

6. Summary

Collectively, the currently available early clinical data add weight to the hypothesis that the human placenta increasingly will become a “gold mine” for stem cell research and tissue repair in the near future. If these early clinical data are confirmed in larger randomized clinical trials a paradigm shift in cell therapy approaches can be expected. However, to achieve this goal, it is critical to establish placenta banks to provide researchers with sufficient tissue for ongoing studies.

7. References

1. Amann E, Rojewski MT, Bieback K, et al. Klinische Einsatzmöglichkeiten von mesenchymalen Stammzellen. Hämotherapie 35: 4-14 (2020).
2. Aumüller G, Aust G, Engele J, et al. (Hrsg.). Duale Reihe: Anatomie. 4. Aufl., Thieme, Stuttgart, 119-123 (2017).
3. Balaji S, Zhou Y, Ganguly A, et al. The combined effect of PDX1, epidermal growth factor and poly-L-ornithin on human amnion epithelial cells' differentiation. BMC Dev Biol 16: 8 (2016).
4. Brodie C, Buchris E, Finniss S, et al. Placenta-derived mesenchymal stem cells and their secreted exosomes inhibit the self-renewal and stemness of glioma stem cells in vitro and in vivo. AACR Meeting 1995, #2327.
5. Brossa A, Fonsato V, Grange C, et al. Extracellular vesicles from human liver stem cells inhibit renal cancer stem cell-derived tumor growth in vitro and in vivo. Int J Cancer 147: 1694-1706 (2020).
6. Bruno S, Kholia S, Deregibus MC, et al. The role of extracellular vesicles as paracrine effectors in stem cell-based therapies.Adv Exp Med Biol 1201: 175-193 (2019).
7. Bruno S, Pasquino C, Herrera-Sanchez MB, et al. HLSC-derived extracellular vesicles attenuate liver fibrosis and inflammation in a murine model of non-alcoholic steatohepatitis. Mol Ther 28: 479-488 (2020).
8. Chang CW, Parast MM. Human trophoblast stem cells: real or not real? Placenta 60 (Suppl1): 57-60 (2017).
9. Cudkowicz ME, Lindborg SR, Goyal NA, et al. A randomized placebo-controlled phase 3 study of mesenchymal stem cells induced to secrete high levels of neurotrophic factors in amyotrophic lateral sclerosis. Muscle Nerve 65: 291-302 (2022).
10. de la Torre P, Flores AI. Current status and future prospects of perinatal stem cells. Genes. 12: 6-30 (2021).
11. Deus IA, Mano J, Custódio CA. Perinatal tissues and cells in tissue engineering and regenerative medicine. Acta Biomater 10: 1-14 (2020).

12. Dominici M, Blanc KL, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8: 315-317 (2006).
13. Evans MA, Lim R, Kim HA, et al. Acute or delayed systemic administration of human amnion epithelial cells improves outcomes in experimental stroke. Stroke 49: 700-709 (2018).
14. Fonsato V, Collino F, Herrera-Sanchez MB, et al. Human liver stem cell-derived microvesicles inhibit hepatoma growth in SCID mice by delivering antitumor microRNAs. Stem Cells 30: 1985-1998 (2012).
15. Ghanbarian H, Yildiz MT, Tutar Y. MicroRNA targeting. Methods Mol Biol 2257: 105-130 (2022).
16. Gupta A, Kedige SD, Jain K. Amnion and chorion membranes: potential stem cell reservoir with wide applications in periodontics. Int J Biomaterials, ID 274082 (2015).
17. Guttmacher AE, Maddox YT, Spong CY. The Human Placenta Project: placental structure, development, and function in real time. Placenta 35: 303-304 (2014).
18. Herrera-Sanchez MB, Bruna S, Grange C, et al. Human liver stem cells and derived extracellular vesicles improve recovery in a murine model of acute kidney injury. Stem Cell Res Therapy 5: 124-135 (2014).
19. Hu W, Song X, Yu H, et al. Therapeutic potential of extracellular vesicles for the treatment of diabetes and diabetic complications. Int J Mol Sci 21: 5163-5172 (2020).
20. Keshtkar S, Azarpira N, Ghahremani MH. Mesenchymal stem cell-derived extracellular vesicles: novel frontiers in regenerative medicine. Stem Cell Res Therapy 9: 63-74 (2018).
21. Kim J, Shapiro L, Flynn A. The clinical application of mesenchymal stem cells and cardiac stem cells as a therapy for cardiovascular disease. Pharmacol Therapeutics 151: 8-15 (2015).
22. Knöfler M, Haider S, Saleh L, et al. Human placenta and trophoblast development: key molecular mechanisms and model systems. Cell Molecular Life Sci 76: 3479-3496 (2019).

23. Koo KO, Park IY, Kim J, et al. Isolation and characterization of chorionic mesenchymal stromal cells from human full term placenta. Cell Therapy Organ Transpl 27: 857-863 (2012).
24. Kordelas L, Rebmann V, Ludwig AK, et al. MSC-derived exosomes: a novel tool to treat therapy-refractory graft-versus-host disease. Leukemia 28: 970-973 (2014).
25. Kumar VP, Biswas S, Holmes-Hampton GP, et al. Pre-administration of PLX-R18 cells protects mice from radiation-induced hematopoietic failure and lethality. Genes 13: 1756-63 (2022).
26. Kyo S, Takakura M, Tanaka M, et al. Expression of telomerase activity in human chorion. Biochem Biophys Res Commu 241: 498-503 (1997).
27. Lazarus H, Escobar C, Metheny L, et al. Safety and demonstrated efficacy of placenta-derived cell therapy PLX-R18 in subjects with incomplete hematopoietic recovery following hematopoietic cell transplantation: a phase I international multi-center study. ASH Meeting 2020, #934.
28. Lee LC, Feinbaum RI, Ambos V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementary to lin-14. Cell 73: 843-854 (1993).
29. Lopatina T, Grange C, Fonsato V, et al. Extracellular vesicles from human liver stem cells inhibit tumor angiogenesis. Int J Cancer 144: 322-333 (2018).
30. Luo H, Huang X, Huang F, et al. Transversal inducing differentiation of human amniotic epithelial cells into hepatocyte-like cells. Zhong Nan Da Xue Xue Bao Yi Ban 36: 525-531 (2011).
31. Merimi M, El-Majzoub R, Lagneaux L, et al. The therapeutic potential of mesenchymal stromal cells for regenerative medicine: current knowledge and future understandings. Front Cell Dev Biol 2021; doi.org/10.3389/fcell.2021.661532.
32. Matthay MA, Goolaerts A, Howard JP, et al. Mesenchymal stem cells for acute lung injury: preclinical evidence. Crit Care Med 38 (Suppl 10): 569-573 (2010).

33. Miki T. Stem cell characteristics and the therapeutic potential of amniotic epithelial cells. Am J Reprod Immunol 80: e13003 (2018).
34. Nassar W, El-Ansary M, Sabry D, et al. Umbilical cord mesenchymal stem cells derived extracellular vesicles can safely ameliorate the progression of chronic kidney diseases. Biomaterials Res 20: 21-30 (2016).
35. O'Brien J, Hayder H, Zayed Y, et al. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol 9: 402-414 (2018).
36. Pethe P, Kale V. Placenta: a gold mine for translational research and regenerative medicine. Reproductive Biol 21: 100508 (2021).
37. Qiu C, Ge Z, Cui W, et al. Human amniotic epithelial stem cells: a promising seed cell for clinical applications. Int J Mol Sci 21: 7730-7756 (2020).
38. Ringdén O, Moll G, Gustafsson B, et al. Mesenchymal stromal cells for enhancing hematopoietic engraftment and treatment of graft-versus-host disease, hemorrhages and acute respiratory distress syndrome. Front Immunol 13: 839844 (2022).
39. Rodríguez-Fuentes DE, Fernández-Garza LE, Samia-Meza JA, et al. Mesenchymal stem cells current clinical applications: a systematic review. Arch Med Res 52: 93-101 (2021).
40. Sadler TW. Taschenlehrbuch Embryologie. 13. Aufl., Thieme, Stuttgart, 156-174 (2020).
41. Shahrbaf MA, Nouri M, Zarrabi M, et al. Extraembryonic mesenchymal stromal/stem cells in liver disease: a critical revision of promising advanced therapy medical products. Cells 11: 1074-1082 (2022).
42. Tremblay F, Ansari Y, Remaund A at al. Neurophysiological outcomes following mesenchymal stem cell therapy in multiple sclerosis. Clin Neurophysiol 136: 69-81 (2022).
43. Tyndall A. Mesenchymal stromal cells and rheumatic disorders. Immunology Lett 168: 201-207 (2015).
44. Van den Bos J, Ouaamari YE, Wouters K, et al. Are cell-based therapies safe and effective in the treatment of neurodegenerative diseases? A systematic review with meta-analysis. Biomolecules 12: 340-352 (2022).

45. Van Niel G, D'Angelo G, Raposo G. Shedding light on the cell biology of extracellular vesicles. Nat Rev Mol Cell Biol 19: 213-228 (2018).
46. Vu Q, Xie K, Eckert M, et al. Meta-analysis of preclinical studies of mesenchymal stromal cells for ischemic stroke. Neurology 82: 1-14 (2014).
47. Vasudevan S. Posttranscriptional upregulation by microRNAs. Wiley Interdiscip Rev RNA 3: 311-330 (2012).
48. Walentin K, Hinze C, Schmidt-Ott KM. The basal chorionic trophoblast cell layer: an emerging coordinator of placenta development. Bioassays 38: 254-265 (2016).
49. Wang AT, Feng Y, Jia HH, et al. Application of mesenchymal stem cell therapy for the treatment of osteoarthritis of the knee: a concise review. World J Stem Cells 11: 222-235 (2019).
50. Yang PJ, Yuan WX, Liu J, et al. Biological characterization of human amnion epithelial cells in a serum-free system and their safety evaluation. Acta Pharmacol Sin 29: 1305-1316 (2018).
51. Zeng L, Yu G, Yang K, et al. Efficacy and safety of mesenchymal stem cell transplantation in the treatment of autoimmune diseases (rheumatoid arthritis, systemic lupus erythematodes, inflammatory bowel disease, multiple sclerosis, and ankylosing spondylitis): a systematic review and meta-analysis of randomized controlled trials. Stem Cells Int 24: 9463314 (2022).
52. Zhang Q, Lai D. Application of human amniotic epithelial cells in regenerative medicine: a systematic review. Stem Cell Res Ther 11: 439-454 (2020).
53. Zhang J, Li H, Yang H, et al. Human amniotic epithelial cells alleviate a mouse model of Parkinson's disease mainly by neuroprotective, anti-oxidative and anti-inflammatory factors. J Neuroimmune Pharmacol 16: 620-633 (2021).
54. Zhao YJ, Fang N, Chen DX, et al. Study on inducing differentiation of human amniotic epithelial cells into insulin secreting cells in vitro. Zhongguo Ying Yong Sheng Li Xue Za Zhi 28: 377-383 (2012).

55. Zhou J, Yu G, Cao C, et al. Bone morphogenetic protein-7 promotes chrondrogenesis in human amniotic epithelial cells. Int Orthop 35: 941-948 (2011).
56. Lin C, Sajeev G, Stiff PJ, et al. Health-related quality of life following allogeneic hematopoietic cell transplantation with omidubicel versus umbilical cord blood. Transplant Cell Ther (2023); doi: 10.1016/j.jtct.2022.09.018.
57. www.gamida-cell.com (assessed on Jun 26, 2023).
58. Horwitz ME, Stiff PJ, Cutler C, et al. Omidubicel vs standard myeloablative umbilical cord blood transplantation: results of a phase 3 randomized study. Blood 138: 1429-1440 (2021).

Tables

Table 1: Placenta-derived stem cells and exosomes – summary of preclinical and clinical studies.

Subgroup	Development Phase	Status
Trophoblastic cells	preclinical	Trophoblast dysfunction (e.g., preeclampsia, growth retardation)
Chorionic cells	preclinical	Tissue repair and regeneration (→ most potent cell type!)
Stromal cells	II	"Pan cytokine", haematologic reconstitution after myeloablative therapies, and in PGF patients
Mesenchymal stem cells	I-III	IBDs, GvHD, arthrosis, ALS, chorea Huntington, multiple sclerosis, vascular disorders
Amniotic cells	I-III	Neuropathias, GvHD, Parkinson's disease, tissue repair, Crohn's disease
Exosomes	I/II	Diabetes mellitus Type 1, GvHD, chronic kidney failure, neuro-degenerative disorders, cancer therapy

Table 2: Differentiation of human chorionic stem cells in vitro.

Cell type	Medium	Supplements
Adipocytes	Apogenesis-differentiation basal medium	Dexamethason (1 µM) Isobutyl-methylxanthine (0.5 mM) Indometazin e(200 µM)
Chondroblasts	Chondrogenesis-differentiation basal medium	TGF-β1 (10 ng/ml) Ascorbinic acid-2-phosphat (50 nM)
Osteoblasts	Osteogenesis-differentiation medium	Dexamethason (100 nM) Ascorbinic-2-phosphat (50 µM) Human BMP-7 (10 ng/ml)

Table 3: Major clinical trials with human placenta-derived amniotic cells (listed on www.clinicaltrials.gov).

Trial	NCT number	Phase	Intervention
GvHD	NCT03764228	interventional	Amniotic cells for GvHD prophylaxis
Ovarian dysfunktion (POF)	NCT02912104	I	Amniotic cells for POF therapy
Repair of neurons	NCT04654286	interventional	Amniotic cells and mesenchymal stem cells for therapy of certain neuropathias
Uterus	NCT03381807	I	Amniotic cells can reduce refractory intrauterine adhesions
Repair of tendons	NCT04670302	I	Amniotic and mesenchymal stem cells for therapy additive therapy of a ruptured supraspinatus tendon
Ovarian dysfunktion (POF)	NCT04706312	I (not yet recruiting)	Amniotic cells may increase fertility
Leukaemias	NCT03759899	surveillance	Evaluation of the role of aminotic cells in the allogeneic stem cell transplantation
Parkinson's disease	NCT04414813	I (not yet recruiting)	Stereotactic application of amniotic cells

Table 4: Summary of relevant clinical trials with MSCs.

Disease	Approximate number of studies	Major results
Ulcerous colitis	7	Only phase I/II studies with MSCs from bone marrow, amniotic and cord tissue. Overall, the complete remission rate was higher with MSCs (alone or in combination with 5-aminosalicylic acid) was significantly higher compared with 5-aminosalicyclic alone).
Crohn's disease	30	Overall, four completed phase III stuides. NCT01541579: registrational study for Alofisel®. Significantly higher response rate of Alofisel® compared with the control group (saline solution) (64% versus 37%).
Autoimmune disorders	91	Predominantly patients with multiple sclerosis, diabetes mellitus type 1 and 2 (two phase III studies), rheumatoid arthritis (one phase III trial), systemic lupus erythematodes were enrolled. Patients with diabetes received either bone-marrow derived MSCs or MSC-derived exosomes. Patients with arthritis were treated with MSC injected intraarticularly. Treatment was well tolerated (reviewed by Zeng et al. 2022).

		A significant improvement of glucose metbolism was found in patients with diabetes mellitus type 2, results for diabetes mellitus type 1 did not reach the level of statistical significance. A total of 47% of patients with multiple sclerosis appeared to benefit from therapy (EDSS), however, results were inconsclusive (Tremblay et al. 2022).
Renal disorders	37	Only phase I/II trials have been conducted so far (acute and chronic kidney failure, diabetic nephropathia, GvHD prophylaxis following kidney transplantation). Results have not been reported yet.
GvHD	51	No late phase randomised clinical trial yet. Treatment appears to be well-tolerated, however, results are not conclusive. Data from a meta-analysis demonstrated a better mOS compared with controls (Morata-Tarifa et al. 2020).
Arthritis	97	A total of 36 trials has been completed (including eight phase III studies). Amongst the phase III trials are the two studies with registrational intent for Cartistem® (MSCs derived from cord blood) (Park et al. 2017).
Insufficient would healing	24	Currently, only phase I/II trial results are available. These trials have provided the first evidence that MSCs can support the wound healing process and are capable to reduce scar formation.

Neurodegenerative disorders	52	Predominantly phase I/II studies are ongoing or have been completed; amyotrophic lateral sclerosis: 21 studies; Alzheimer's disease: 12 studies; Parkinson's disease: 7 studies; chorea Huntington: 3 studies; ataxia and cerebral atrophia: 9 studies. A phase III trial with patients with amyotrophic lateral sclerosis did not reach its primary endpoint (Cudkowicz et al. 2022). Results for the phase III trial with patinets with chorea Huntington are expected soon (van den Bos et al. 2022).
Vascular disorders	126	Patients with myocardial infarction, peripheral arterio-occlusive disase, and stroke are currently evaluated in ongoing clinical trials. Two phase III trials with MSCs have been completed, results have not yet been published.

Table 5: Approved stem cell preparations (derived from placenta, bone marrow, adipocytes, and cord blood).

Cell type	Brand name	Indication	Approval
Human allogeneic MSC cells (derived from adipocytes)	Alofisel®	Therapy of fistulas associated with Crohn‘s disease	EMA (2018)
Human MSCs (bone marrow)	Temcell®	Steroid-refractory GvHD (children and adults)	Japan (2015)
Human MSCs (bone marrow)	Prochymal®	Steroid-refractory GvHD (children and adults)	Canada and New Zealand (2015)
Expanded MSCs (cord blood)	Cartistem®	Cartilage degeneration	South Corea (2012)
Human placenta essence	Placentrex®	Wound healing disorders	India (year not known)

Figures

Figure 1: The complete human placenta with cord after birth ("afterbirth"). Completeness is of primary importance to prevent later complications (e.g., the development of chorionic carcinoma). The fetal side is shown (the umbilical cord is in the middle).

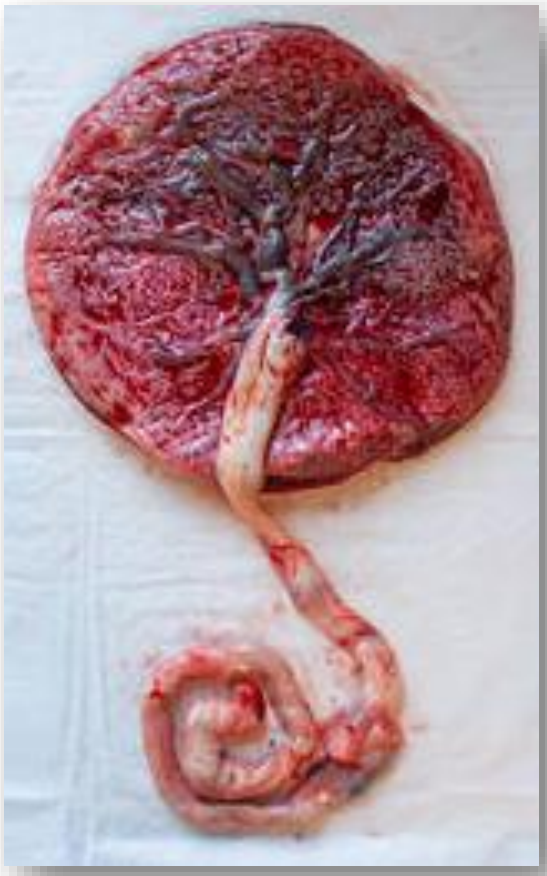

Figure 2: Schematic representation of the anatomy of the placenta and different cell subpopulations and exosomes. The mater side (uterus) is at the bottom of the picture; the fetal side is shown in the upper half of the picture.

Exosomes

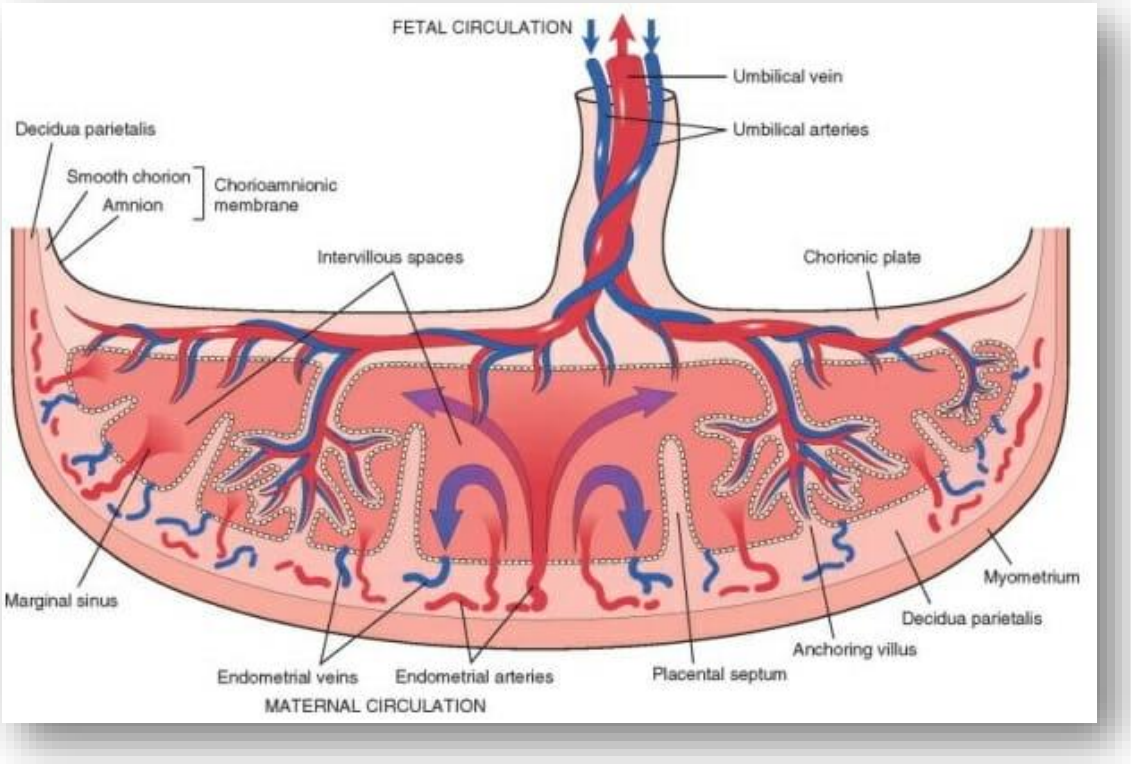

Figure 3: Differentiation of MSCs in different tissues (depending on experimental conditions).

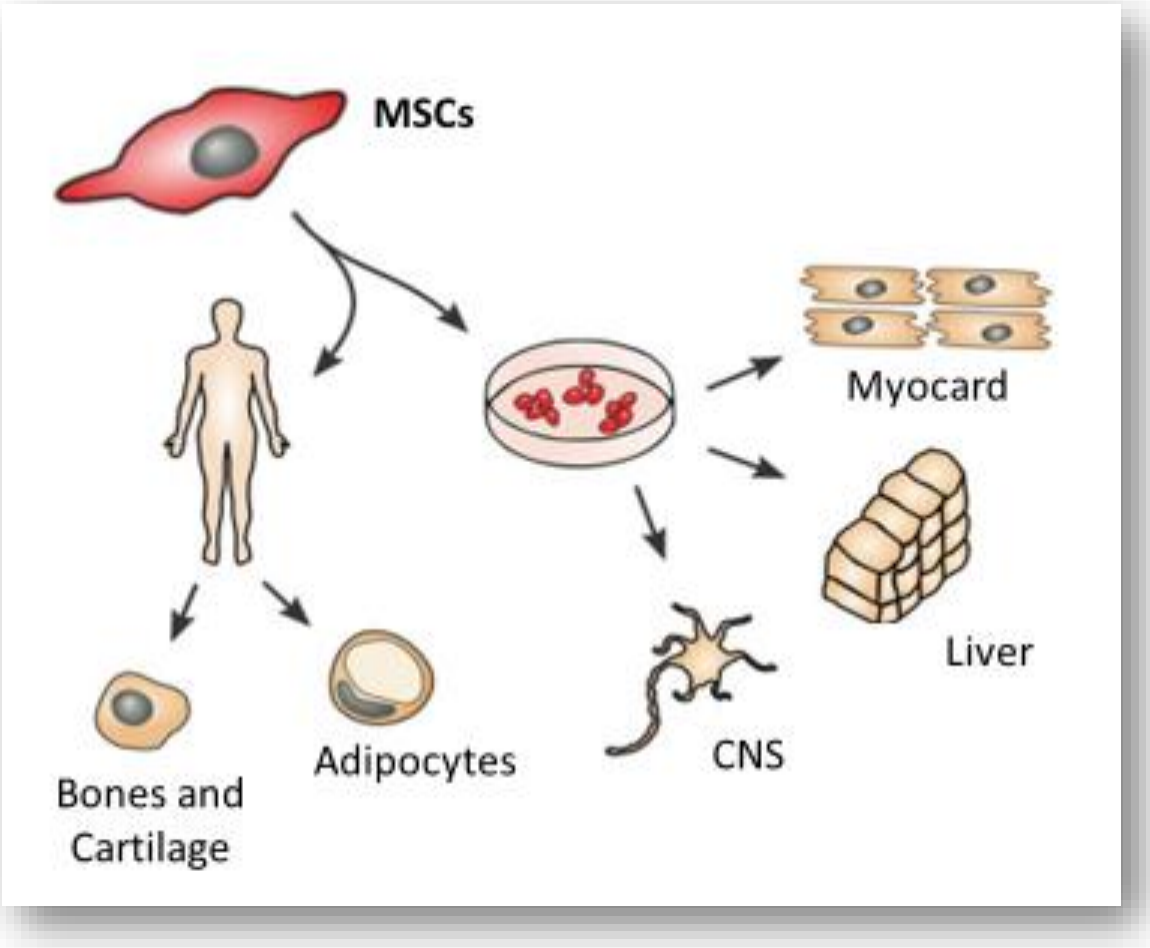

Figure 4: Multivesicular bodies (MVB) are formed during endosomal maturation, and exosomes are secreted upon fusion of the MVBs with the plasma membrane. The apoptotic bodies are derived from apoptotic cells (modified from Hu et al. 2020).

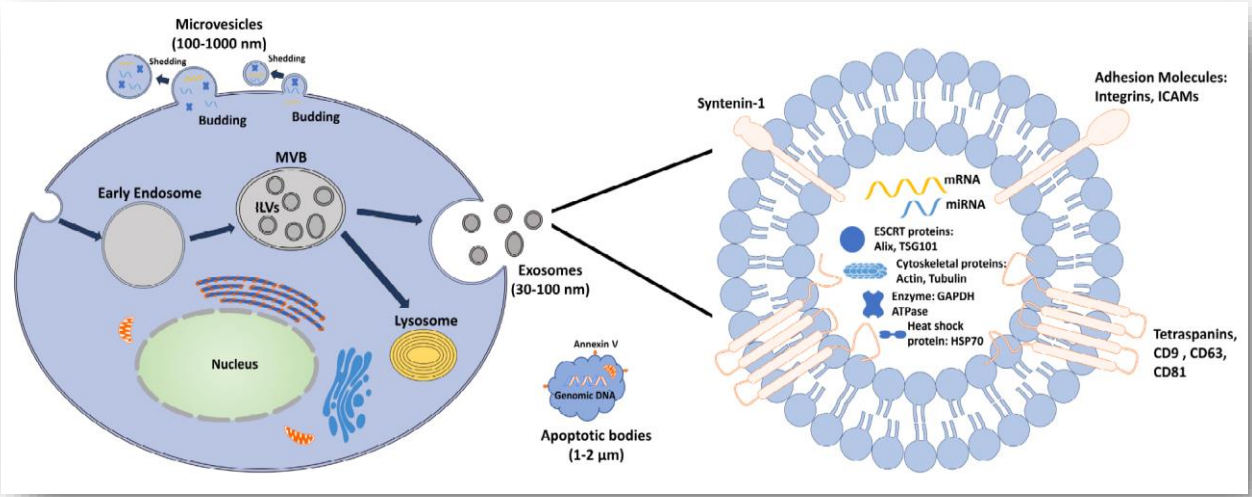

Figure 5: Schematic representation of the extraction of exosomes from human somatic cells.

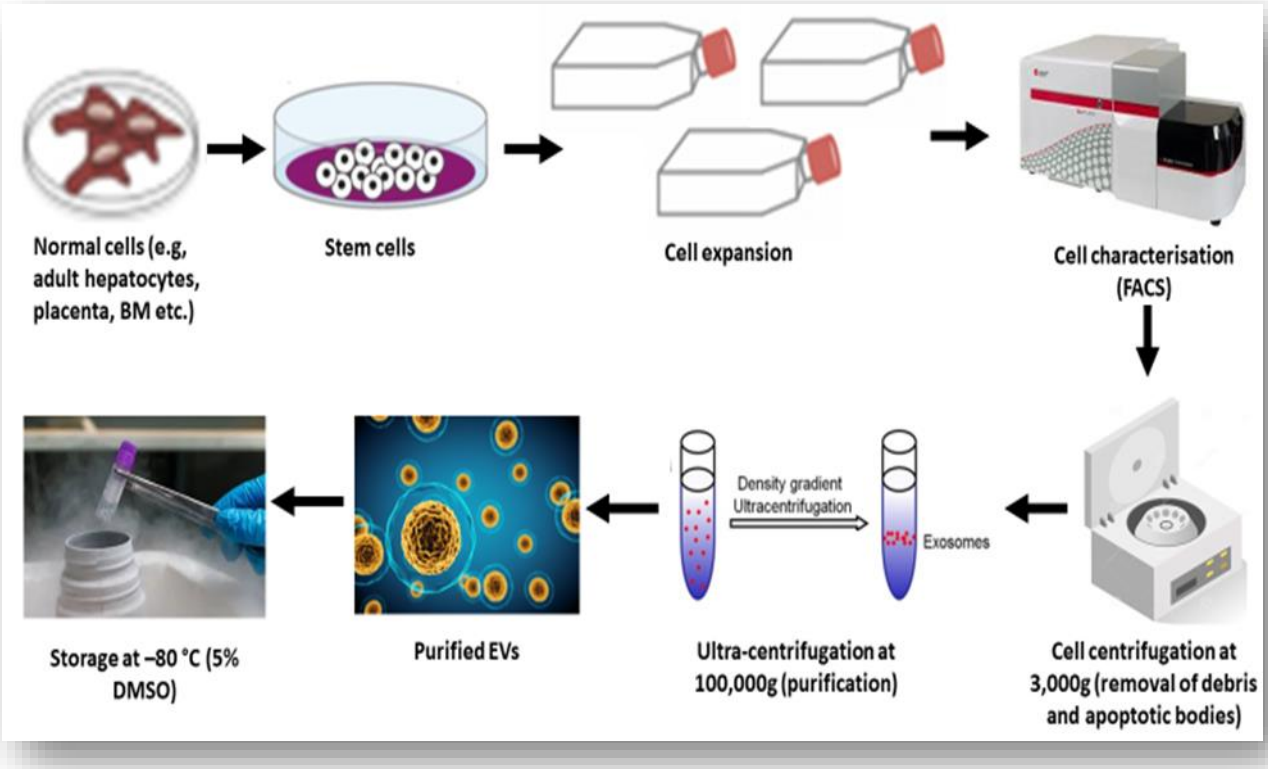

Figure 6: Exosomes of mesenchymal stem cells are able to activate cellular repair and regeneration processes (modified from Bruno et al. 2019).

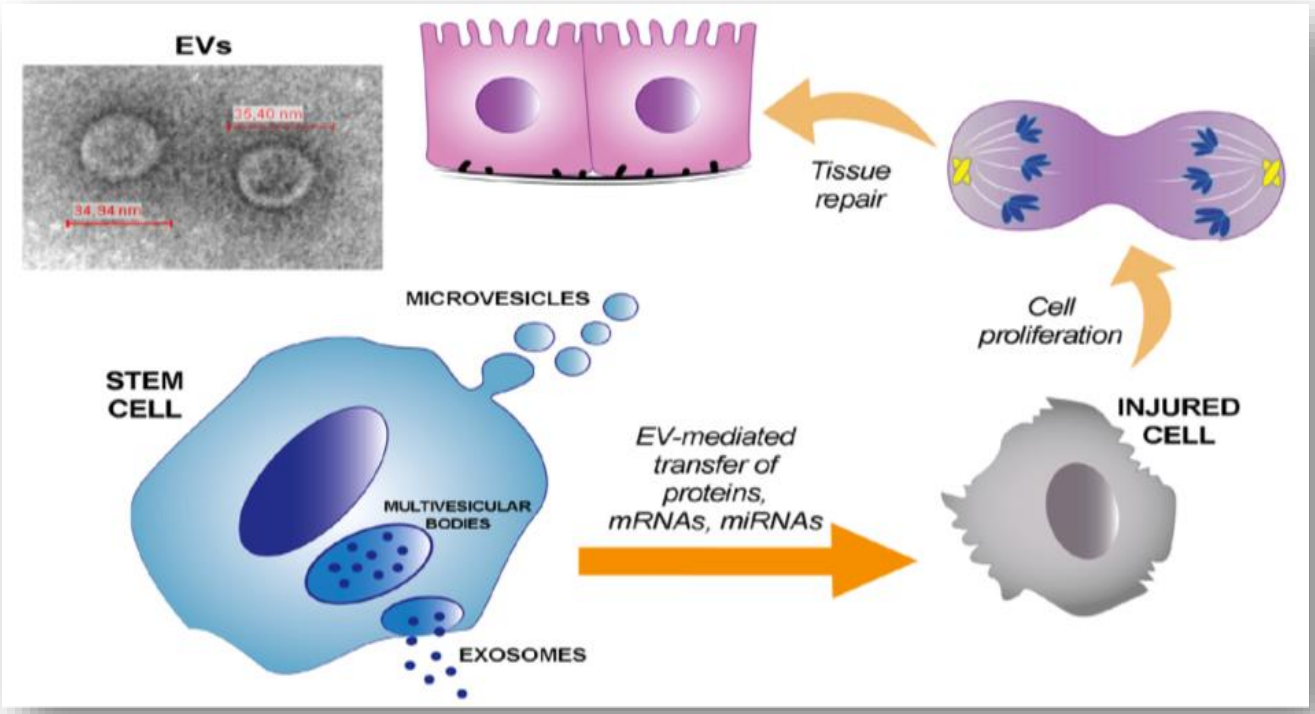

Figure 7: Tumour regressions induced by human EVs in different tumours. Green: hepatocellular carcinoma, red: malignant lymphoma; blue: glioblastoma (modified from Fonsato et al. 2012).

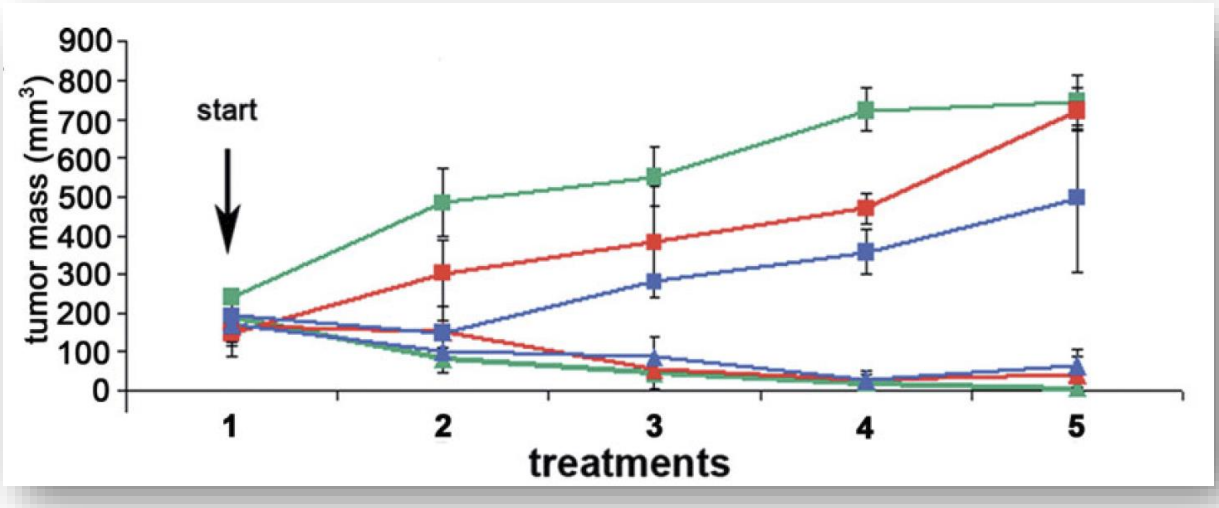

Figure 8: Survival of C57BL/6 male mice following total-body irradiation with 8 Gy and intramuscularly administration of two doses of PLX-R18 (2 million cells/dose) on 1 day prior to and 3 days post total body irradiation. Plasma-Lyte: controls (modified from Kumar et al. 2022).

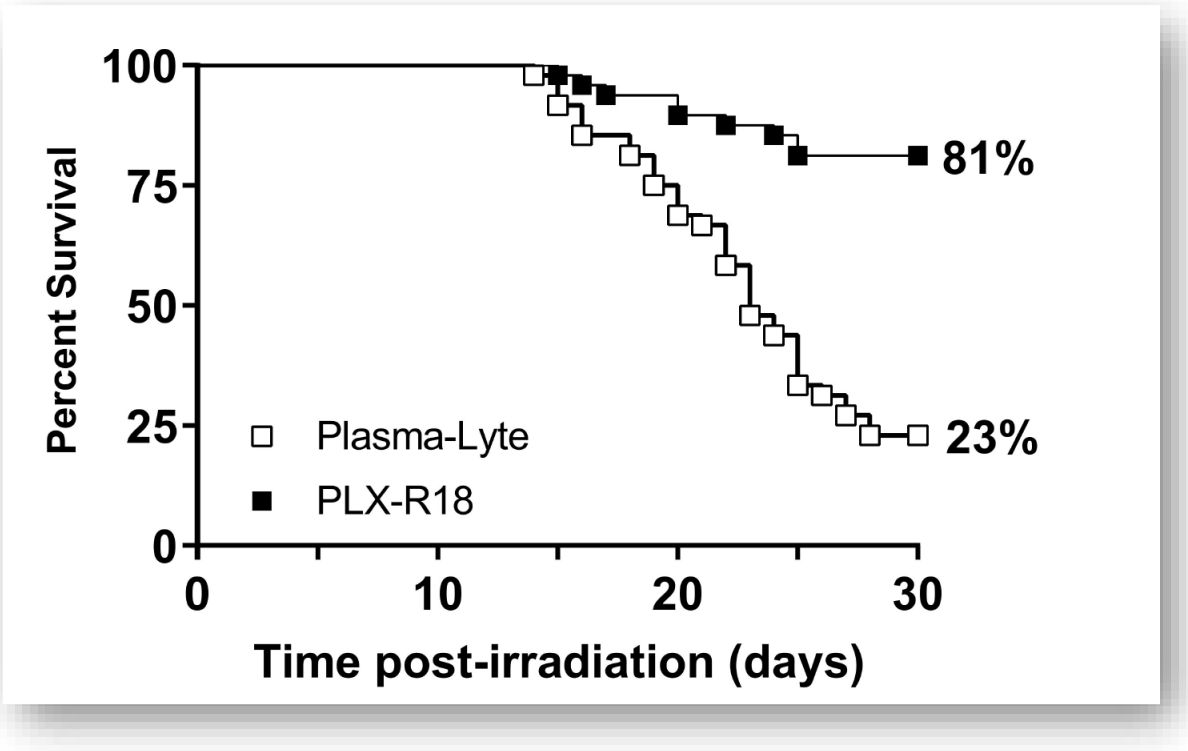

Figure 9: Plasticity and differentiation of human placental amniotic cells (examples).

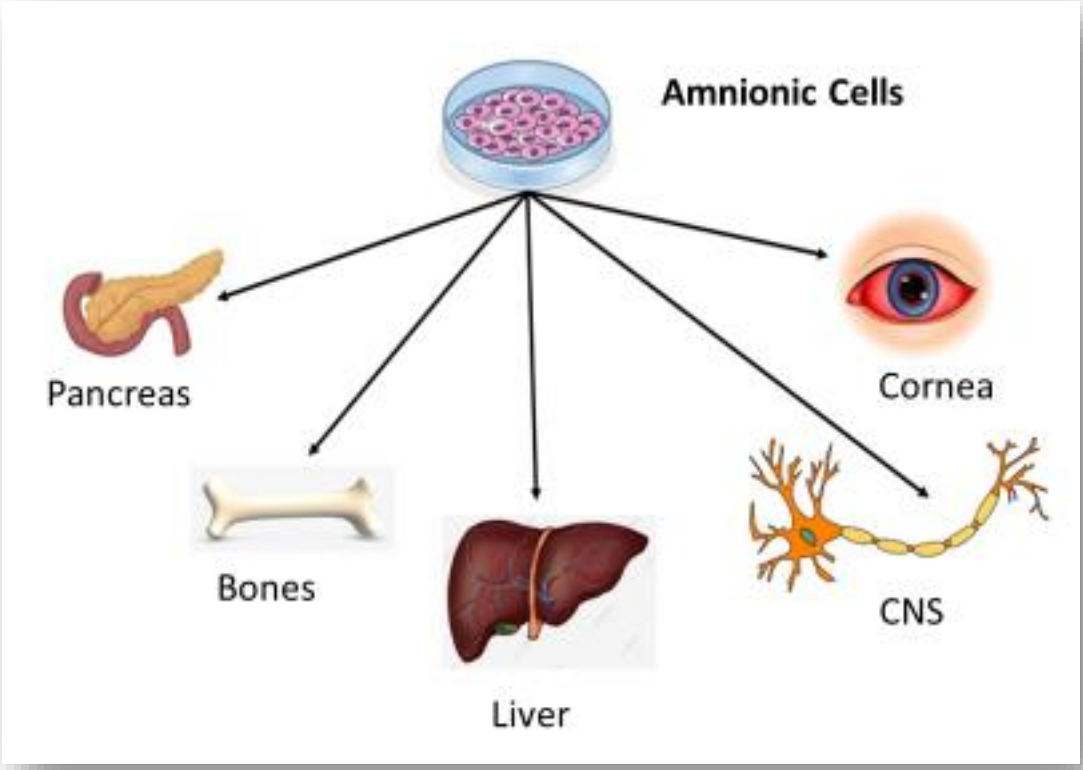

Figure 10: Isolation of chorionic stem cells from human placenta tissue. A: Tube for transportation; B: entire placenta in a petri dish; C: macroscopic excision of chorionic tissue; D: removement of the decidua part; E: manual cutting into small tissue fragments; F: single cell suspension following enzymatic digestion (modified from Koo et al. 2012).

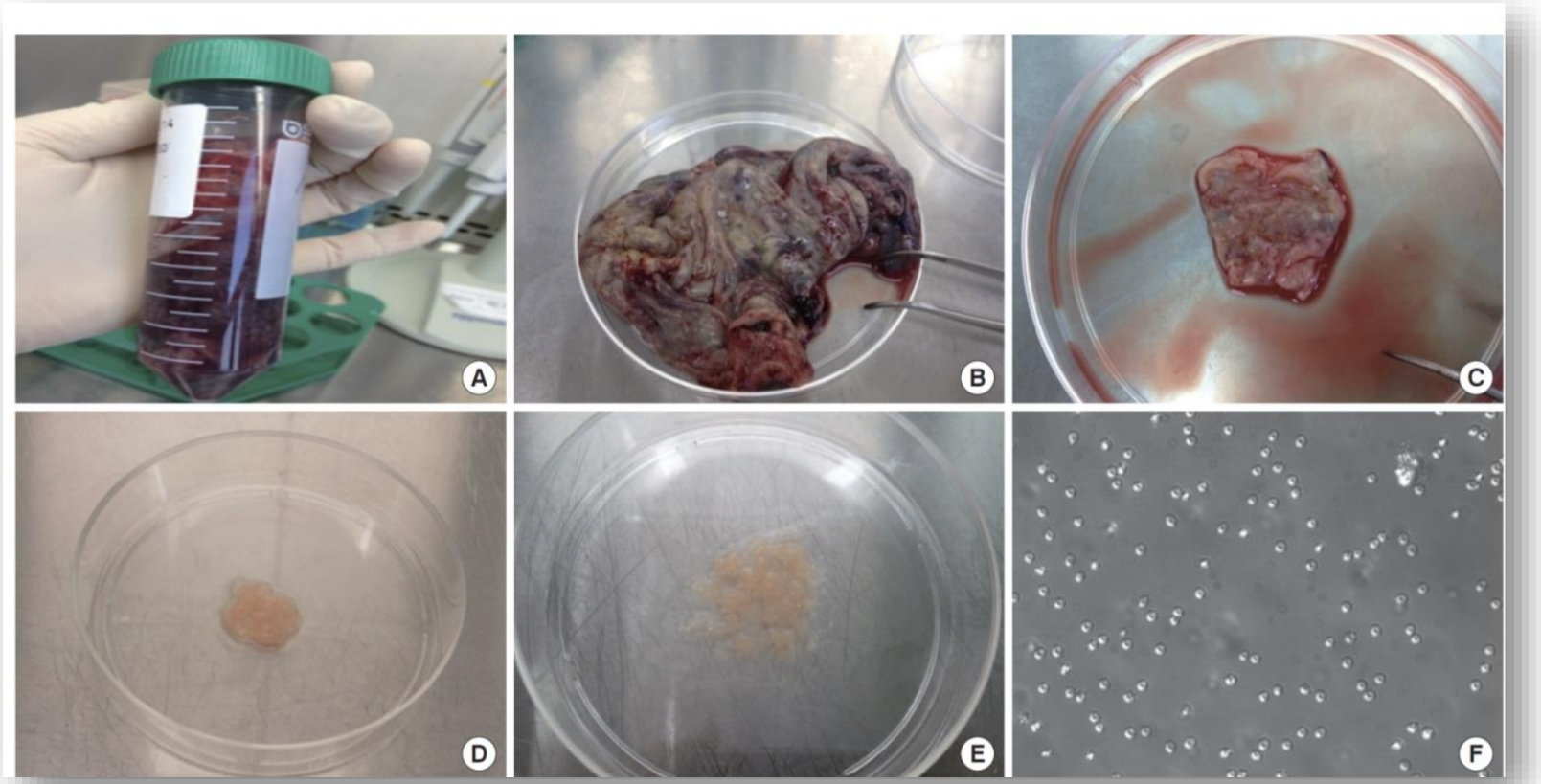

Figure 11: Histology of trans-differentiated human placenta-derived chorionic stem cells. A: adipocytes (oil-red stain); B: chondroblasts (alcian-blue stain); C: osteoblasts (silver nitrate stain) (modified from Koo et al. 2012).

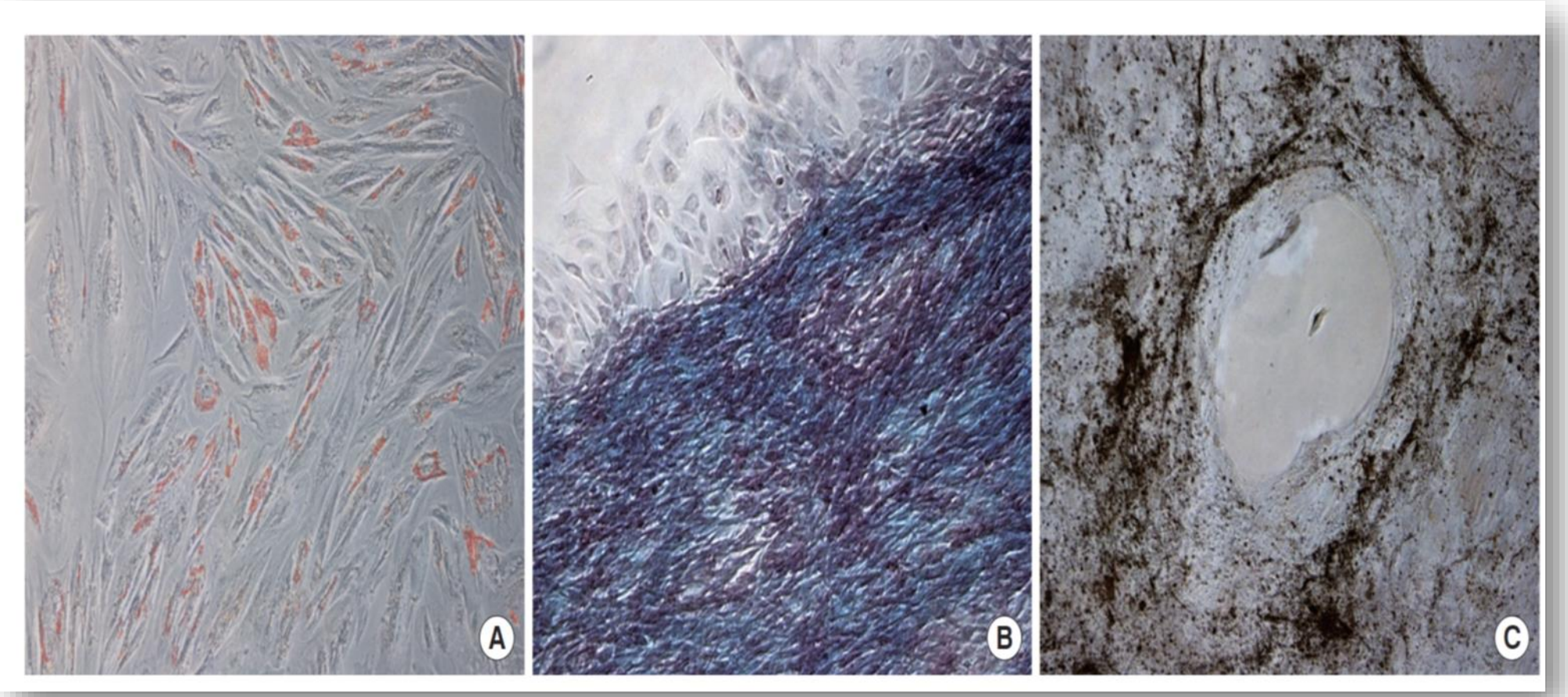

Figure 12: MSCs derived from human cord. The cells show a myo-fibroblastic phenotype.

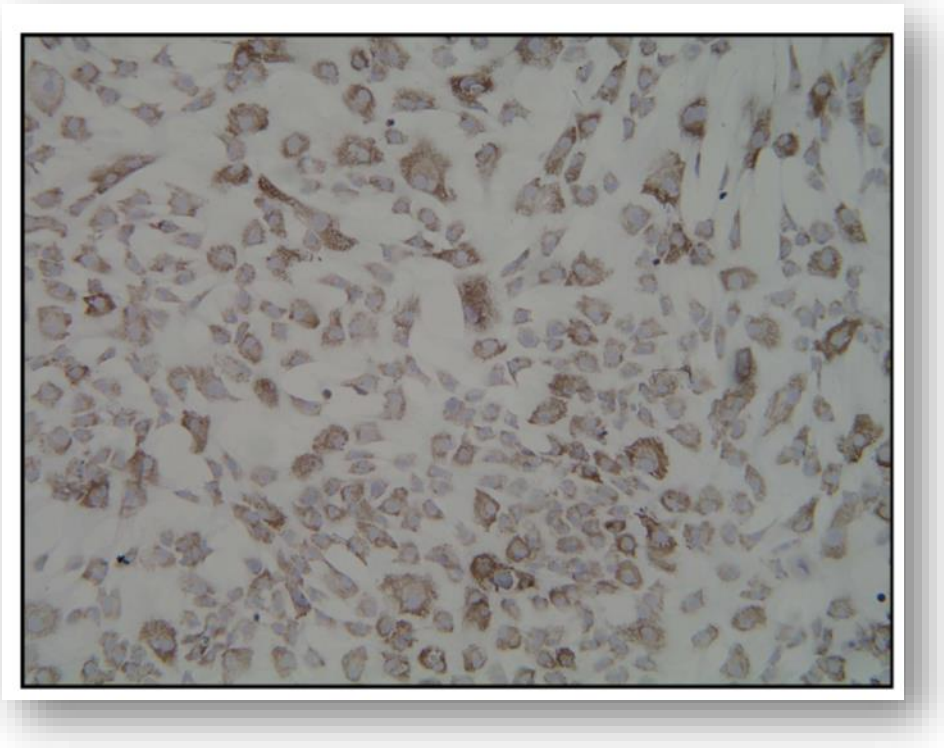

Figure 13: Postulated mode of action of human MSCs.

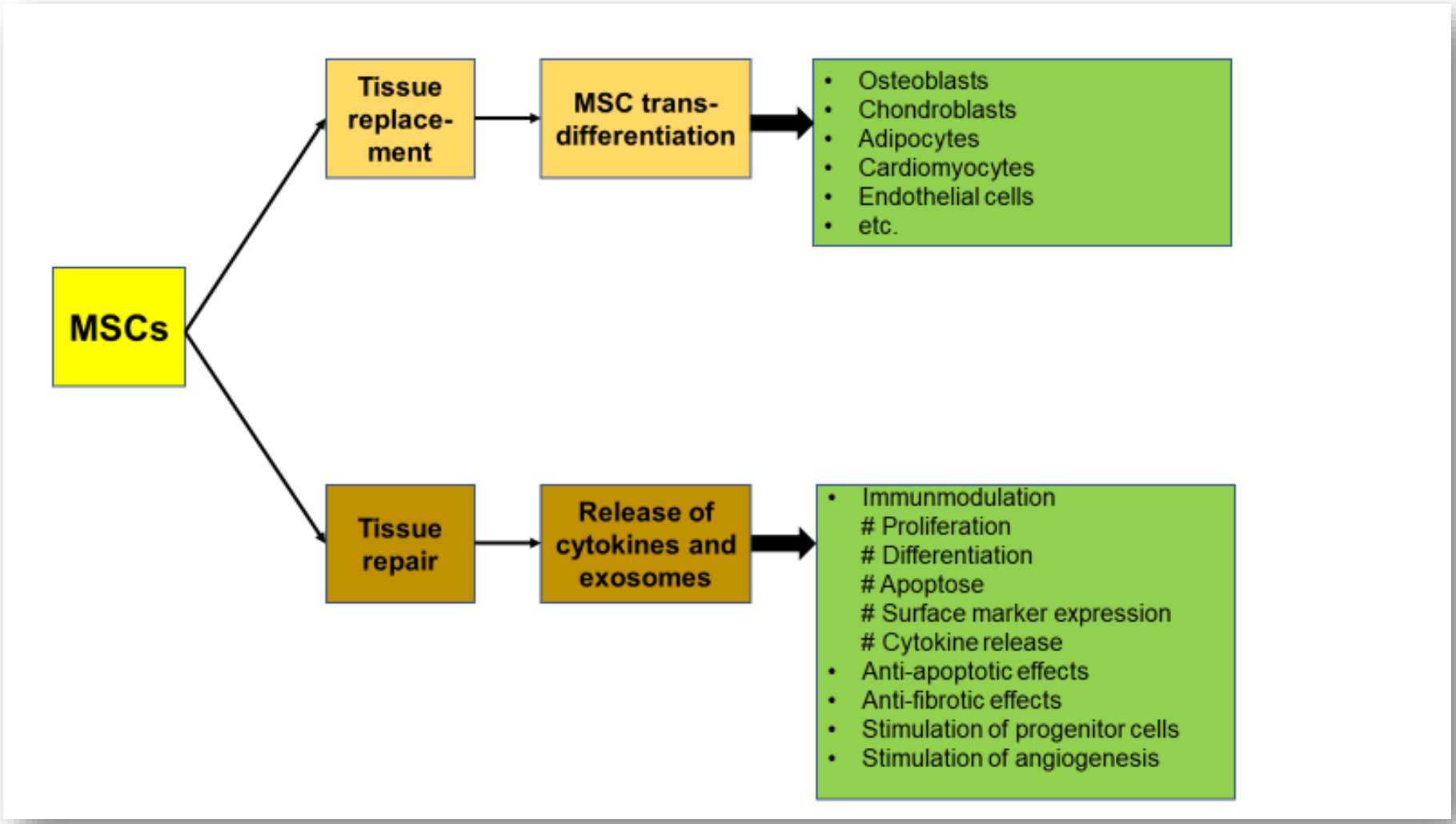

Figure 14: Exponentially increasing number of preclinical and clinical studies with human placental stem cell (PubMed search: #placental cell therapy).

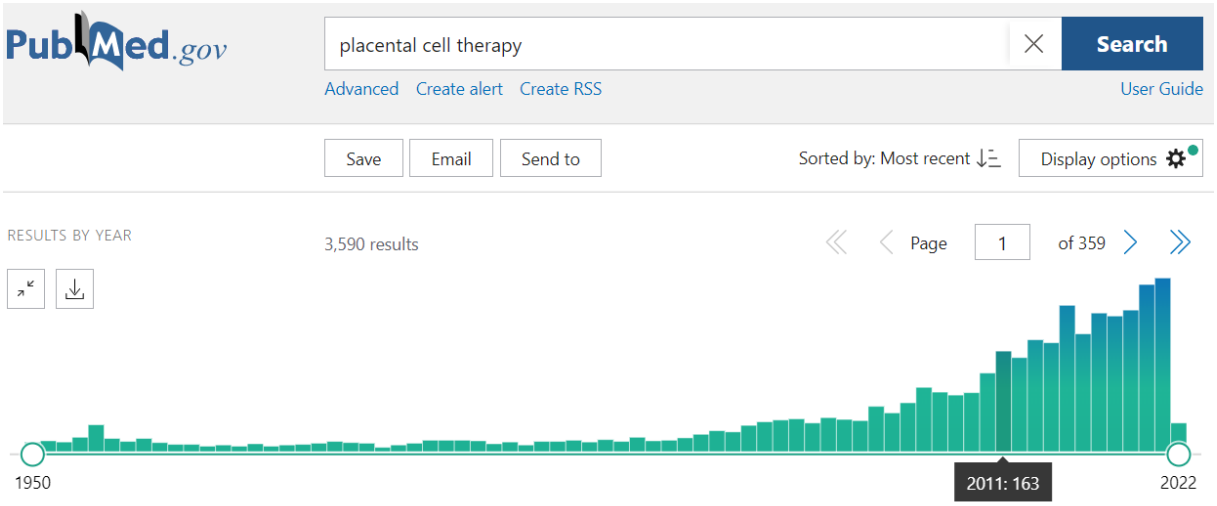

Figure 15: Processing of cord blood cells with omidubicel.