APTI women's forum

APTI Women's Forum Newsletter

Cell-based therapies

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Editor's Note

Prof. Vandana B. Patravale

Chief Editor, APTI Women's Forum Newsletter

Dear Readers,

On the occasion of the launch of the latest issue of the APTI Women's Forum Newsletter, I am elated to welcome all our readers and authors. Technology is driving science in the present era and developments in biopharmaceutical industry are exponential. Biopharmaceutical companies are increasingly showing interest in cell and gene based therapies owing to their prospective in curing a very niche set of diseases. It has been claimed by global scientists to possess a promising potential to transform the field of modern medicine, especially across the areas needing urgent care.

Global position of cell therapies is very unique, thereby motivating, not only top-class scientific community to engage, but also industries to developing state-of-the-art research facilities around the world. Owing to the constraints in large scale manufacturing of these products, the investment in development of manufacturing facilities has been a point of focus for biopharmaceutical industry. FDA has shown a positive approach towards the acceptance of cell and gene therapies over the past couple of years with the number of approved therapies and clinical trials increasing exponentially as we discuss. It was remarkable to see an overwhelming response in the form of more than six articles in this issue on CAR T therapy by our own scientific community, further highlighting the growing interest in this field.

The dynamic nature of cell therapies has been a driving force for the conceptualization of the current issue of our newsletter. Our esteemed authors have taken great efforts to bring forth a diverse set of articles for all the readers. The articles published herein give a brief perspective on the emerging trends in cell based therapies along with their benefits and limitations. Some of our authors provide a detailed review on specific applications of cell based therapies for cancer, immunotherapy, neurological disorders, diabetes as well as infectious diseases. We also have an interesting interview lined up to tackle the most common aspects of cell based therapies. It is with pride that we present such a wide range of discussion on this emerging field of science through our articles in this issue.

The entire editorial board would like to express a deep appreciation towards all the contributing authors for their immense dedication in making this newsletter engaging for the readers. I would like to thank the editorial board and my team members including Ms. Sarika Jadhav, Ms. Anjali Pandya and Mr. Shrikant Dhage for their assistance in editing and compilation of the newsletter. I am sure that you will enjoy reading the contents of this issue of the APTI Women's Forum Newsletter as much as we liked the process of compiling it for all of you!

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Cell Therapy: Expert Perspective

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Q.1. Could you briefly describe what cell therapy is and delineate the major types of cell therapy?

Cell therapy is the use of cells as active entities to remove and/ or replace the damaged or diseased cells in a patient's body, wherein, the therapeutic cells can directly or indirectly interact with the native tissues or their designated targets to either modulate the pathological condition or completely replace their diseased counterparts. There are multiple ways of delineating different types of cell therapy. Based on origin of the cells, they can be classified as autologous- derived from the host patient or allogenic- derived from alternate source in the same species. Based on the type/ techniques for cells employed, cell therapies are classified into stem-cell, adoptive-cell transfer, cell-based vaccines and so on. Another important way of delineating cell-therapies is the application for which they are intended for- oncology, auto-immune disorders, cardiovascular diseases and so on.

Q.2. What are the key factors or opportunities that provided an impetus to the research and market growth of cell therapy?

Cells represent a unique class of therapeutic modalities. For starters, they are living and functional entities. This means that they have the potential to modulate their microenvironment and foster cultivation of entire tissue-systems. This attribute has been utilized by stem cell-based therapies for regenerative applications. Differentiated cells have highly specialized functionality. Use of such cells is an obvious choice for tissue repair and replacement applications. From a delivery perspective, specific biology of the cells- including their unique target engagement as well as natural trophism towards certain tissues makes them an attractive candidate to consider, especially given the transport related biological barriers that drug delivery strategies encounter. All these considerations and many others provided a huge impetus to research on cell therapies and its consequent market growth.

Q.3. How are cell therapies administered, and could you also comment on the use of medical devices for the safe and efficacious delivery of cell-based therapeutics?

Almost all the cell therapies that have been approved by health authorities are being currently administered by intravenous infusions. Therefore, at the moment , the use of medical devices has been is limited to intravenous administration using infusion lines driven via

infusion pumps. However, novel cell therapies being explored in the clinic are exploring alternate routes of administration such as subcutaneous, intradermal, intracranial, etc. For stem- cell therapies, accessing the bone marrow directly as opposed to intravenous is also being considered. These different routes of administration would certainly bring more medical devices into play to ensure safe and efficacious delivery of cells.

Q.4. In light of recent FDA-approved cell therapy products, what are some of the most promising clinical applications of cell therapy?

While the initial set of cell therapies were targeted against blood-based malignancies or specifically aimed at restoration after myeloablative events, their clinical success has opened avenues for other indications. In oncology itself, the ability of cells the penetrate deep within the tissues makes them an effective strategy against solid tumors. Autoimmune indications, cardiovascular disorders and trauma are important indications where there could be additional impact of cell therapy. Use of genetic engineering coupled with cell therapy has the potential to add non-druggable targets and/or manage the safety profile of existing therapies across a wide variety of indications. Another emerging set of application is the use of microbes for treating infections, metabolic disorders, and inflammation besides the already proven utility for nutraceutical purposes

Q.5. What are the challenges or restraints that hinder the growth of the cell therapy market?

As with any other therapy, a few challenges are encountered by cell therapy. Firstly, cells themselves are very potent modalities and managing the safety of such potent therapies has been a very significant challenge. Cytokine storm is a very common side effect associated with T-cell based immunotherapies for oncology indications. Safety concerns greatly impact the applicability of cell therapies as patients are generally in an immunocompromised state. Hence, the delivery route as well as the delivery mechanism for such therapies is under investigation. Secondly, maintenance of the functional and effector functions of the cell is central to a successful cell therapy intervention. Often, manufacturing processes themselves present a significant challenge to the viability of the cells and in turn their potencies as cells are used to a certain microenvironment in the body and typical manufacturing processing may not be ideal, especially for certain cell-therapies that rely on receptor-based pharmacological effects. Thirdly, manufacturing cell therapies is a significant challenge considering that many of them heavily rely on expanding cells isolated from patients. Further, management of drug product filling and shipping to the clinical sites in a way that is not compromising to the quality of cells and their potency remains a work-in-progress. Lastly, lack of universal standards of regulatory framework for cell therapies adds a logistical hurdle to fast development and deployment of these strategies.

Q.6. What are the current regulatory frameworks and regulatory challenges for the manufacture and scale-out of cell-based therapies?

Ans: There is isn't one-size fits all regulatory framework for dealing with cell therapies. FDA's Center for Biologics Evaluation and Research (CBER) and EMA's committee for advanced therapies (CAT) advise and regulate the entry of new cell therapies in the clinic.

At the moment, there is a high degree of uncertainty over manufacturing considerations – reporting of critical quality attributes and around product purity. Unlike, protein-based products where manufacturing process and the underlying must haves are clearly defined by the regulatory agencies, diverse cell therapies and their unique manufacturing processes as well as parameters to be considered for their functional effectiveness make it very difficult to have a universal standard set for advancement of such products. Sometimes economics of a rigorous regulatory requirement versus a very small market also skews companies against pursuing novel therapies. Nevertheless, it is an ongoing effort between industries and regulatory agencies to streamline this workflow and facilitate entry of novel therapies

Q.7. Could you comment on the global market size, market trends, and key market players in the cell therapy industry?

As of 2022, cell therapy was value at ~ $10.7 billion and is projected to grow with a compound annual growth rate (CAGR) of 19-20% by 2030-2032. This growth is driven by investment of big pharma into niche small or mid-scale companies who have a heavy cell-therapy driven focus. A few key market players as far as the cell therapy is concerned are- Kolon Tissue Gene, Inc., Osiris Therapeutics, Inc., JCR Pharmaceuticals Co. Ltd., NuVasive, Inc., Stemedica Cell Technologies, Inc., Cells for cells, Holostem Terapie Avanzate S.r.l., Mesoblast Ltd., and Medipost Co.,Fibrocell Science Inc., PHARMICELL Co. Ltd., MEDIPOST, Vericel Corporation, Anterogen Co. Ltd., Inc. , Celgene Corporation and AlloCure

Q.8. How is the cell therapy industry likely to evolve over the next decade?

Cell therapy has the potential to evolve both from a research and development perspective. From a research perspective, the applicability, and the multitude of cells in clinical trials are indicative of how the field continues to evolve in terms of more cell types and indications being explored. Apart from that, there is an active interest for using cell therapies in combination with other therapeutic modalities, in some cases, even using cells as delivery vehicles to improve the effectiveness of molecules which were previously hindered due to off-site deliveries. From a development perspective, a huge effort will be invested in setting up scale-out strategies for making manufacturing processes robust and easily implementable for a variety of cell types. As with protein-based therapies, understanding process parameters and their impact on product quality will be extremely crucial in making sure that quality of manufactured product is maintained. Manufacturing of allogenic therapies and innovative techniques of cell-expansion are likely to evolve over the next decade. Storage and transportation of currently existing therapies is likely to see more advancements as cryogenic storage and transport could add to logistical and product quality issues, if not well characterized. Lastly, definition of product purity will likely be consolidated and solidified with active engagement between industry leaders and regulatory agencies. All in all, scientific, process development and regulatory advancements are expected in the field of cell therapies.

Disclosure:

The view and opinions expressed within this content are solely the author's and do not reflect the opinions and beliefs of Sanofi or its affiliates.

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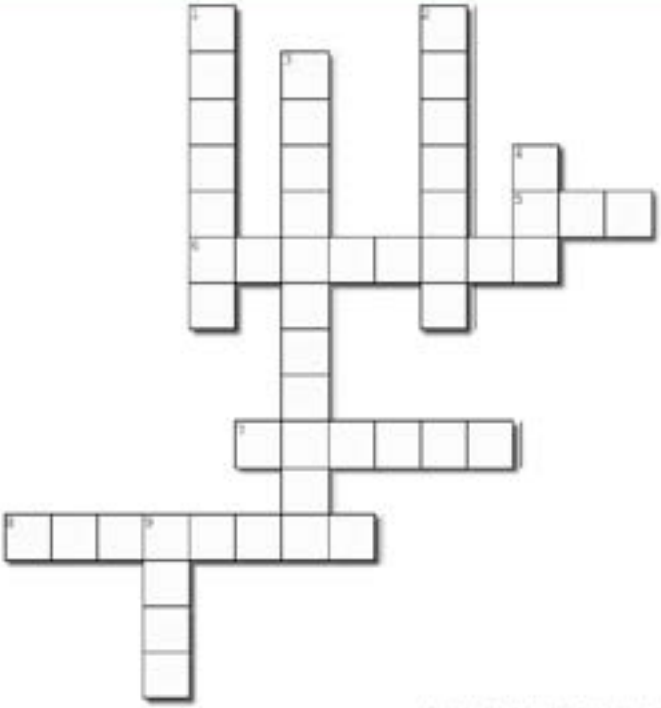
Fun & frolic - Crossword



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Across

5. Immortalized cell line
6. Cell-based therapy to treat Type 2 diabetes
7. Dermal fibroblasts for wound repair from Arita Medical
8. CAR-T therapies for the treatment of relapsed or refractory large B-cell lymphoma,

Down

1. CAR-T therapy for the treatment of acute lymphoblastic leukemia
2. Approved in vivo gene therapy
3. World's first 'personalized' cancer therapy marketed by Dendreo
4. Abbreviated Michigan Cancer Foundation Breast Cancer Cell Lines
9. Chimeric antigen receptor T-cell therapy

Solution on page 52

The Living Panacea: CAR T Cell Therapy



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Introduction

The conventional strategies for treating neoplastic diseases have shown limited efficacy with various side effects deteriorating patients' quality of life. Poor recovery rates, varying therapeutic outcomes and significant relapse rates are some of the major challenges while curing dreadful cancer. As conventional medicines are falling short of expectations, a new paradigm – 'Immunotherapy' has emerged in recent years, offering the possibility of curing cancer fully. The immune system involves potent T-lymphocytes, which can eradicate the neoplastic cells, but often several hurdles mitigate the T cell response:

1) *Lacking Co-stimulation and Tumour antigen*

Cancer cells frequently suppress the expression of Major Histocompatibility Complex (MHC) class I and Costimulatory molecules, which are critical for cancer cell recognition and antitumor response by T cells (1).

2) *Anergy*

In the malignant environment where costimulatory molecules are present in reduced numbers, T cells often loose the ability to generate cytokines, thus threatening their proliferation and cancer cell-killing activity (2).

3) *Tolerance of T cells*

Most cancer cells have self-antigens, whereas the T cells possess low-affinity T Cell Receptors (TCRs) designed to identify nonself antigens. This permits malignancies to avoid detection by immune systems (3).

Chimeric Antigen Receptor (CAR) T cell therapy helps to overcome these barriers by genetically altering T cell receptors with an antigen-binding domain of an immunoglobulin using modern gene cloning techniques. T cell receptors that have been genetically changed are known as chimeric antigen receptors (CARs), and T cells that have their receptors modified are known as CAR T cells. This revolutionary therapy was declared as 'advance of the year' by the American Society of Clinical Oncology in 2018 after the US FDA approval in 2017 for B cell malignancies by anti-CD19 CAR T cell therapy.

Structure

CAR TCells are synthetically engineered cells that combine the capabilities of B-cell antibodies and T cells, just like the chimera, a fire-breathing creature from Greek mythology. The structure of CAR as receptors shown in fig. 1 can be divided into 4 parts:

·Antigen Binding Domain (ABD):

This domain provides antigen specificity to the CARs by targeting the extracellular tumor antigens present on the surface resulting in MHC-independent T-cell activation. They are derived from variable light (VL) and heavy (VH) chains of antibodies that are joined by the single chain variable fragment (scFv) as a flexible linker. The characteristics of scFv decide the specificity and affinity of the CARs (4).

·Hinge:

This extracellular region, majorly derived from amino acid sequences of CD28, IgG4, CD8 or IgG1 connects the antigen binding domain to the transmembrane domain. They help the ABDs to access the target epitope while providing sufficient length and flexibility. Changes in them can also impact the recognition of epitopes, CAR expression and signalling (5).

·Transmembrane Domain:

Derived from natural proteins like CD4, CD28, CD3ζ or CD8α, the transmembrane domain anchors the CARs to the T cell surface. The characteristics of this domain are yet to be thoroughly studied, but some reports suggests that they might influence the stability, activity and expression levels of CAR (6).

·Intracellular Signalling Domain:

When CAR T cells recognize the antigen, the intracellular signalling domain also known as the signalling endodomain triggers an intracellular cascading signal to impart its activity (7).

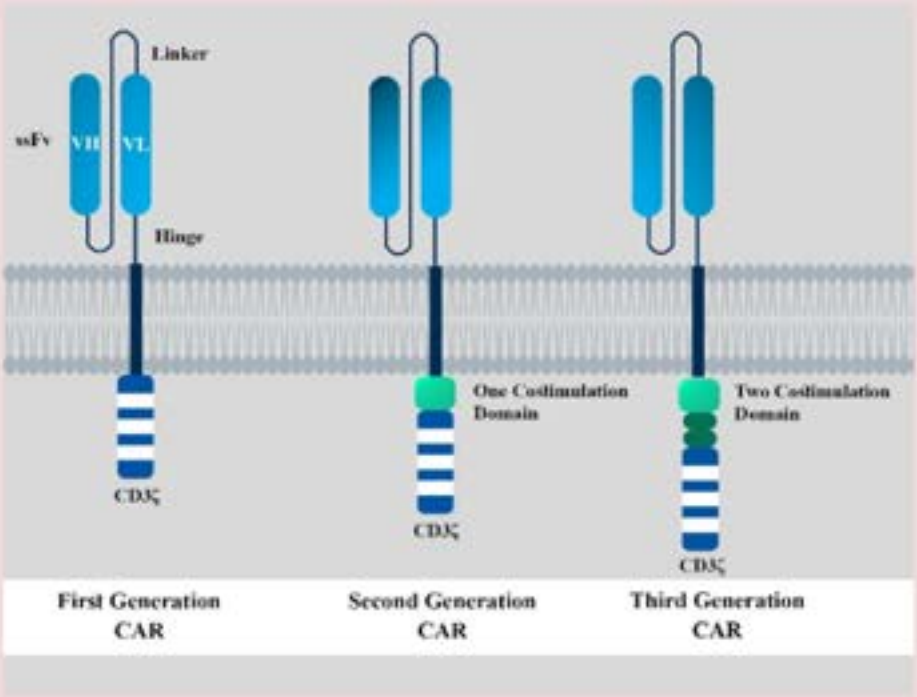

Figure 1: Structure and Generations of CARs

Generations of CARs

In the late 1990s, first generation CARs were produced with a CD3ζ chain transmitting the signals from endogenous TCR, but they did not carry costimulatory molecules. In the clinical trials, it was seen that their persistent exposure resulted in therapeutic effects, but due to lack of activation by signalling had resulted in inadequate anticancer activity (8). To enhance the safety and efficacy, second generation CARs are designed with dual signalling – activation via CD3ζ and costimulation by integrating costimulatory molecules like inducible T cell costimulator (ICOS), CD28, CD137, OX40 or 4-1BB to the tail of the CAR. Adding such costimulatory domains not only helps to produce a significant complete response for B-cell malignancies but also enhances the T cell accumulation and effector cell functions (9). In order to increase efficacy, two signalling domains are introduced in the third generation CARs with a CD3ζ chain. Infiltrating and lysing the tissues in chronic lymphocyte leukemia, CARs with CD-28 and 4-1BB molecules showed complete remission rates. They aid in prolonged proliferation, enhanced signalling and increased production of cytokines (10).

Productions of CAR T Cells

At the clinical level, the production starts with the recruitment and screening of patients with stage IV cancer as shown in fig. 2 and then they undergo apheresis so that Peripheral Blood Mononuclear Cells (PBMCs) can be isolated. Ex vivo cultures activate and increase the PBMCs until the required T cell number is attained. Further, the T cells are modified by infecting them with CAR-retroviral particles and these CAR-modified T cells are then expanded in an ex vivo culture while monitoring their changes. Finally, the prepared CAR T cells are reinfused into the patients and their antitumor response is monitored (11).

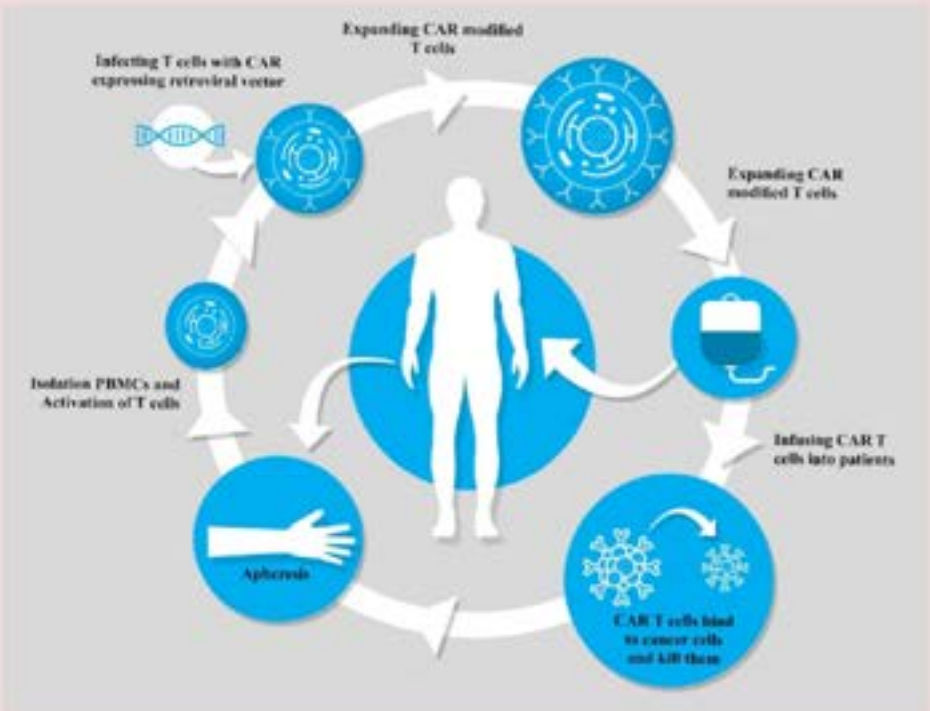

Figure2: CAR T cells production at clinical level

CAR T cell Therapy Advantages

The biggest advantage of this treatment is the single infusion of CAR T cells which impart their action over decades after 2-3 weeks of sufficient care and observation and can destroy the tumour even duringrelapse. It is also seen that refractory blood cancer comes back despite various transplants, the CAR T cell therapy successfully eliminated the disease. Moreover, maintaining the patients' quality of life, they provide a living without any significant risk of relapse (12–14).

Limitations of CAR-T Cells

On-target off-tumour effects –

It is seen that antigens expressed on solid tumors are also present in normal tissues at different levels. CAR T cells targeting such antigens can cause an on-target off-tumor effect by attacking healthy cells. Thus, to avoid such toxicity, tumor-restricted post-translational modifications like overexpressed truncated O-glycans for solid tumors can be targeted (15).

Antigen escape –

In this phenomenon, CAR T cells showing high response rates for a target antigen can lose their activity by tumor resistance development. In such cases, malignant cells can show partial or complete antigen expression loss which were targeted by CAR T cells. Recent studies demonstrate the development of resistance in patients with recurrent disease after the treatment by downregulation of CD19 antigen in 30%-70% of patients. Furthermore, downregulation or deletion of BCMA (B-Cell Maturation Antigen) expression has been reported in multiple myeloma patients receiving BCM targeted CAR T cells. Thus it is essential to select a target antigen which can reduce the antigen escape (16,17).

Immunosuppressive Microenvironment -

Many cell types that cause immunosuppression can infiltrate solid tumors, including Tumour-Associated Macrophages (TAMs), Myeloid-Derived Suppressor Cells (MDSCs) and Regulatory T Cells (Tregs). Such tumour cells and infiltrates stimulate tumor generation, facilitating chemokines, growth factors and cytokines. Also, the immune checkpoint routes act to decrease anticancer immunity. It's been proposed that co-inhibitory pathways cause the development of T cell exhaustion. As a result, combined immunotherapy with CAR T cells and checkpoint blockade is anticipated to be the next immunotherapy frontier since it delivers both the infiltration and the PD-1/PD-L1 blockage required for significant immune responses (18). Other than this, CAR T cell toxicities and trafficking are some of the other problems that had to be resolved for safe and effective tumor treatment (19,20).

Future Prospects

FDA approved CD19-specific therapies in the form of Yescarta (axicabtagene ciloleucel) by Kite Pharma/Gilead Sciences and Kymriah (tisagenlecleucel-T) by Novartis. More than 220 trials have been conducted to identify CAR T cell therapy targets. It is marking a paradigm shift for curing cancer using a self-replicating and living drug, but the overall cost for the treatment and hospitalization is around $1,500,000 per cancer patient which is significantly high. Thus, research must be conducted to optimize the treatment and find solutions to reduce the costs. Other than that, problems associated with the treatment, transportation, manufacturing and storage of these living drugs possess a big challenge which have to be overcome in the near

future. Effective preclinical models are also needed to evaluate the efficacy and safety of medications before human studies. CAR T cell therapy has shown effective treatment in hematological cancers, but this is just the beginning. Several features of this therapy are yet to be uncovered and its potential to cure recurrent non - hematological, metastatic and resistant cancers is yet to be explored.

Abbreviations:

CAR - Chimeric Antigen Receptor
MHC - Major Histocompatibility Complex
TCR - T Cell Receptors
scFv - Single Chain Variable Fragment
ABD - Antigen Binding Domain
PBMC - Peripheral Blood Mononuclear Cell
BCMA - B-Cell Maturation Antigen

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Cell Therapy: An Emerging Treatment Strategy of the Current Era for ophthalmic diseases

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Cell therapy has emerged as a hope for incurable diseases, which aims to replace disordered cells with able cell populations to repair the underlying cause of disease initiation and progression. Over the past century, average human life expectancy has increased due to the advancement in medical science in the diagnosis and treatment of diseases as well as the accessibility of healthcare. However, with the increasing lifespan, age-related disorders have increased drastically. There is an unmet need to develop effective treatment strategies for age-related disorders, including neurological, autoimmune, skeletal, renal, liver, and ophthalmologic diseases. The current treatment strategy for chronic degenerative diseases only relies on palliative treatments that aim to delay the disease's progression. Organ transplantation is one of the options with limited success many times due to the immune response, rejection, shortage of available donors, and chronic immunosuppressive treatment following surgery. Therefore, regenerative medicines using various types of cells, including primary, progenitor, or stem cells, have emerged as promising treatment strategies. In recent years, stem-cell based therapies have found success in the clinical trials (1, 2). Stem cells have the limitless replicative potential, and the ability to generate all types of cells. Many cell therapies that reach advanced clinical trials are based on mesenchymal stem cells. However, pluripotent stem cell research has demonstrated great potential in regenerative medicines.

With the aim of repairing diseased organs or tissues previously thought to be irreparable, cell therapy involves the replacement of aberrantly functional or injured tissue by engrafting, stimulation of the self-healing process of existing endogenous tissues by releasing growth factors and cytokines, and use of genetically modified cells to deliver therapies. Several cell types are used for the purpose, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), adult/fetal SCs, including mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), mononuclear cells (MNCs), endothelial progenitor cells (EPCs), and neural stem cells (NSCs), and several tissue-specific primary cells. ESCs are isolated from the inner cell population of the blastocyst. ESCs have unlimited differentiation potential, which is highly beneficial for the success of cell therapy. However, the challenge lies in isolating a pure population of differentiated cells. iPSCs are of stem cell origin and programmed to exhibit pluripotency by introducing several factors. Long-term genetic stability is a challenge for this class of cells. Adult/Fetal SCs are isolated from specific tissues, including bone marrow, placenta, umbilical cord, blood, adipose, or other specialized tissues of interest.

Cell-based therapies have shown promising implementation in ophthalmic diseases (3). Cell replacement has been found to be beneficial for the treatment of retinal cell death associated with multiple ocular dysfunctions, including age-related macular degeneration (AMD), glaucoma, retinitis pigmentosa (RP), and juvenile macular degeneration or Stargardt's disease (SD). The irreversible loss of retinal cells initiates the disease. The underlying cause of the disease progression is genetic abnormalities in the retinal pigmented epithelial cells and photoreceptors. The damage in retinal pigmented epithelium (RPE) cells and photoreceptors causes central vision loss in SD. On the other hand, the degradation of retinal ganglion cells (RGCs) is found in glaucoma patients. There is no treatment to reverse the loss, and transplantation-based therapeutic approach has only shown the ray of hope. Various cell types of retina derived from pluripotent stem cells such as retinal pigmented epithelium cells, photoreceptor cells, RGCs, and retinal organoids provide cell source for transplantation.

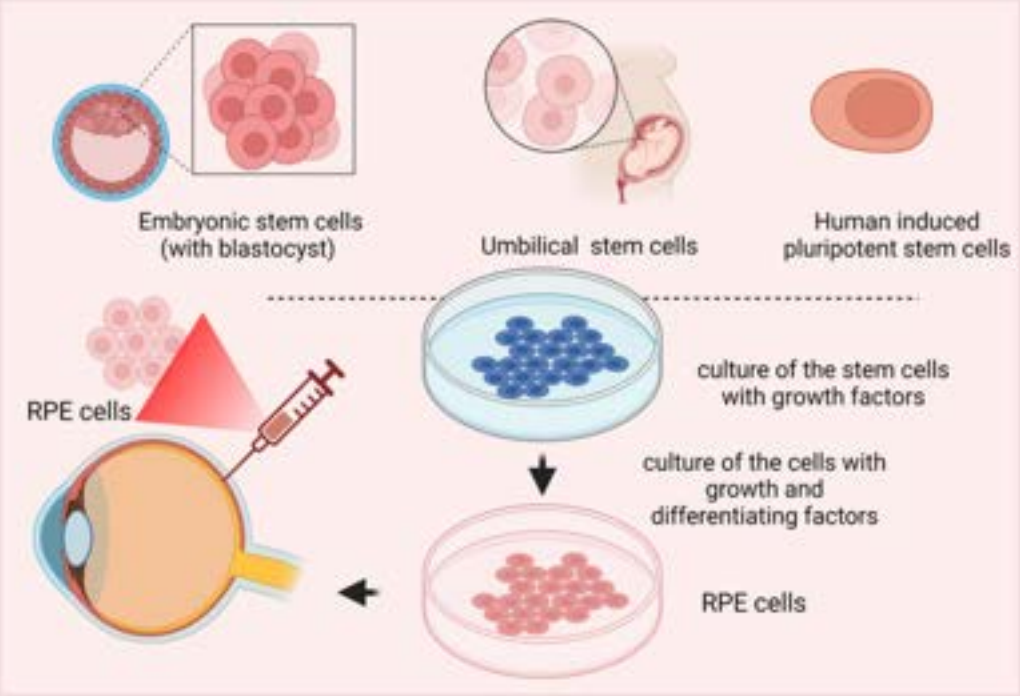

Figure 1. Stem cell-based therapy for retinal degenerative diseases.

Human embryonic stem cells (hESC) and induced pluripotent stem cells have been explored to correct ocular dysfunctions (4). The RPE replacement is evolving as a feasible approach for treating age-related macular degeneration. In several preclinical studies, RPE cells were transplanted as a cell suspension into the immunosuppressed animal eyes. The transplant effect were monitored on a short-term basis. In a study, hESC cells were directly differentiated towards RPE cells under certain cell culture conditions (5). The general protocol has been shown in Figure 1. The hESCs differentiated in presence of nicotinamide, which upregulated transforming growth factor, TGF-β, resulting in development of RPE cells during embryogenesis. The hESC-derived cells showed morphology, expression of markers, and functions of authentic RPE cells. Transcriptome analysis was used for comparative assessment of putative RPE cells obtained from hESCs and human fetal RPE (6). The hES cell derived RPE cells expressed molecular markers, could perform phagocytosis, and were able to differentiate into cells of Royal College of Surgeons (RCS) rats (7). Cultures of 67-passages established from 18 different hESCs and RPE cells derived from NIH-approved hESCs were able to rescue

Cultures of 67-passages established from 18 different hESCs and RPE cells derived from NIH-approved hESCs were able to rescue photoreceptors in animal model of retinal disease. The results were highly promising with 100 % improvement in visual performance compared to untreated controls. In a recent study, long-term transplant effect of iPSC-RPE cells grown in monolayers was assessed in immunocompromised RCE rats (8). In this study, the RPE cells of iPSC origin were cultured on a nanoengineered ultrathin parylene C scaffold, which was transplanted into the subretinal space of immunocompromised RCS rats pups and evaluated 1,4, and 11 months following transplantation. Interestingly, transplant remained as a monolayer and expressed RPE-specific markers, performed phagocytosis, and preserved the vision. The RPE survival of only 50% of the eyes were observed after 11-months post-implantation. Loss of the cellular characteristics could be due to the immune reactions via macrophage activation, fibrosis in peri-membrane, and transition of cell's fate towards mesenchyme (as judged by the upregulation of mesenchymal markers). Mesenchymal stem cells exhibit benefits towards neuroprotection, however, their sub-optimal capacity to differentiate in the in vivo condition reduces their therapeutic activity (9). A study reported direct conversion of human umbilical cord mesenchymal stem cells into RPE cells for the treatment of retinal degeneration (9). A cocktail of 5 transcription factors, including CRX, C-MYC, NR2E1, LHX2, and SIX6 were used to differentiate the stem cells to RPE cells. The cells demonstrated epithelial-to-mesenchymal transition (EMT)-inhibitory ability. Moreover, grafting of the cells in the subretinal space in rat induced with AMD demonstrated reversal of AMD pathophysiology.

There is a recent report of a phase I clinical study, where an RPE patch consisting of a fully differentiated RPE cells grown in monolayer on a suitably coated synthetic basement membrane were administered using a microsurgical tool into the subretinal space of an eye in two patients (1). The endpoints were the sign of adverse effects and the proportion of subjects with improved visual acuity of 15 letters or more. Visual acuity was found to be 29 and 21 letters in two subjects, respectively over 12 months. The patients received immunosuppressive drugs during the 12 months to avoid implant-related immune reactions. Long-term safety of the transplantation of human retinal progenitor cells were evaluated in retinitis pigmentosa patients (10). The RPE cells transplantation in retinitis pigmentosa patients did not cause tumorigenesis when immunosuppressive agents were not administered. Moreover, a significant improvement in visual acuity ($P<0.05$) were registered in patients.

The stem cell therapy shows efficacy in the in vivo systems and clinical trials, however, it is challenging and requires investigation to determine the optimal stage for cryopreservation of the hESC-derived RPE cells. A recent study published in 2022 proposed that the differentiated RPE cells could be frozen during their exponential phase, which resulted in a best post-thawing outcome in terms of cell viability and RPE cell properties and functions (11). While cryopreservation of the cells in optimal condition is important for the cells viability and performance, the delivery protocol is also another concern in the whole therapeutic regimen. The cells would be delivered to the subretinal space through a surgical procedure through a microcatheter to be inserted through sclera using a viscoelastic material (12). The device with a lighted tip is used to guide the application inside the eye.

The delivery to the suprachoroidal space other than subretinal space is needed for precision. However, this task is challenging with the possibility of bleeding from the highly vascular choroid.

Future perspectives:

The stem cell therapy has emerged as a promising strategy to restore vision in retinal degenerative diseases. A decade ago, this idea was limited to the laboratory level. Thanks to the continuous advancement in this area of research, which paved the way for this treatment to go to the human clinical trial. There are few major concerns which needs to be addressed. The cell source is an important factor in the cell replacement therapy. The quality, type, and the viability of the cells are concerns. Even though, so far, in the completed clinical trials, there was no tumorigenicity of the implanted cells. However, tumorigenicity of the stem cells are a great concern. RPE cells survive better as a sheet form than as a cell suspension. However, it is challenging to maintain a sheet form during transplant surgery, needing skilled surgical intervention. There is a huge immunological concern which cannot ignored. Stem cell research has given a ray of hope for ocular degenerative diseases, and successfully translated to human clinical trials. Therefore, beyond all doubts, the effort of the research community would fully harness the power of stem cell therapy in neuro-retinal regeneration in near future.

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The Chimeric Antigen Receptor Macrophages (CAR-M): An Immunotherapy Against Solid Tumors

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Introduction

Cancer still tribulates death in different population sectors worldwide. Around 10 million deaths occurred due to cancer, as per the World Health Organization (WHO) 2020 statistics (1). The solid tumor treatment encompasses major solicitude than the liquid tumor. Existing chemotherapeutic strategies include novel drugs and nanoparticles targeting checkpoint proteins and receptors causing downregulation of immune reactions, immunotherapy, and monoclonal antibodies. However, existing treatment is associated with drawbacks including a rise in immunosuppressive cells including tumor-associated macrophage (TAM), myeloid deriver monocyte (MDM), and regulatory T-cells along with death receptor suppression onto cancer cells, low chemokine expression, inefficient drug concentration due to phagocytosis, p-glycoprotein efflux, hostile tumor microenvironment, tumor vasculature barrier, and T-cell exhaustion (2,3). This indeed paves a way for the scientific community worldwide towards the development of novel translational anti-cancer therapy.

Novel cell-based therapy includes a strategy to educate and instigate the body's defense cells against cancer. Chimeric antigen receptor-T cells (CAR-T) were widely developed in this regard. The USFDA approved three CAR-T products in 2020, including Tecartus®, Breyanzi®, and Carvykti® for the treatment of liquid tumors. However, the therapy failed to penetrate and survive within a solid tumor. The development of chimeric receptor-based other immune cell therapy may serve as a suitable alternative.

Although macrophages are inconspicuous but can be lucrative cell-based strategies against cancer. The macrophages encompass the ability to penetrate the stromal tumor tissues and proliferate within solid tumors. The functional plasticity of macrophages may lead to various anti-tumor and pro-tumor activities under different conditions. The macrophage exists in varying phenotypes and expresses various receptors including but not limited to the C-C chemokine ligand-2 (CCL-2), colony-stimulating factor (CSF-R), and a cluster of differentiation 206 which are engaged in tumor proliferation (4). The expression of the phenotypes and receptors could be modulated by the delivery of chimeric antigen receptor-expressing macrophages (CAR-M) (Figure 1). The succeeding section includes the significance of macrophages in cancer, various efforts directed toward the *ex-vivo* genetic integration of a few anti-tumor genes within macrophages, its advantages, and its translational potential.

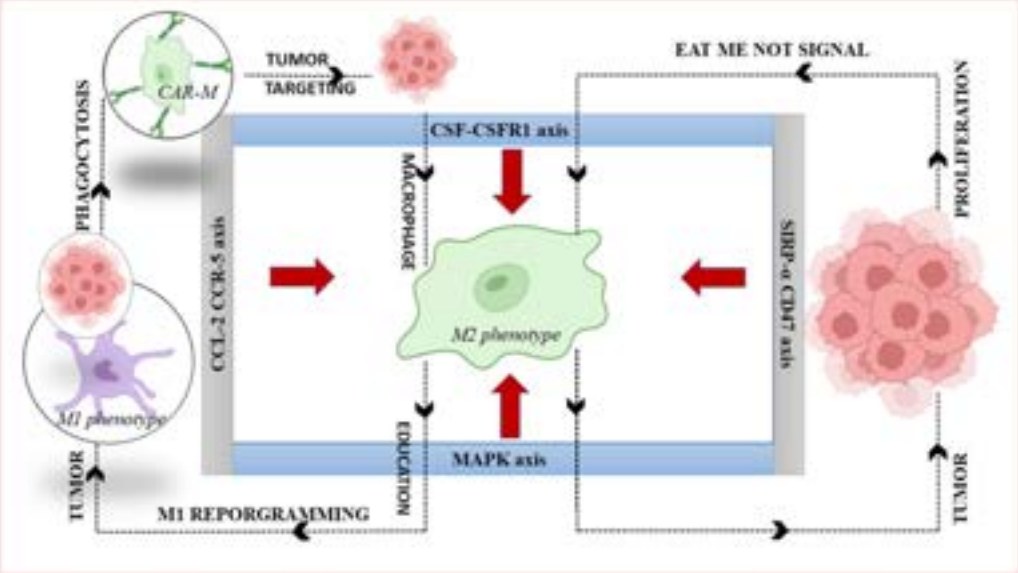

Figure 1. Pathways inhibiting macrophage repolarization and CAR-M therapy for macrophage education in solid tumor

Macrophages in solid tumors

Due to the secretion of cytokines (IL-6), tumor growth factor-β (TGF- β), and the low oxygen environment of tumor tissues, the macrophage mistakenly recognizes the tumor cells as damaged tissues and initiates its repair. Moreover, macrophage exists naturally in two phenotypic forms which function as an anti-inflammatory (M1 type) and pro-inflammatory (M2 phenotype). The M1 phenotype is activated by the Interferon- γ (IFN-γ), CSF, and lipopolysaccharide (LPS). While the M2 phenotype can be activated by Interleukin-4 (IL-4), bacterial LPS, and glucocorticoid. The M1/M2 ratio varies based on the disease condition (4). The M2 polarized state is responsible for the 'eat me not' condition in the case of solid tumors. The tumor cells secrete CSF which binds to the MCSF-receptors (MCSF-R) on myeloid cells and causes the downregulation of signals including the mitogen-activated protein kinase pathway (MAPK)(5,6), polarizing the M2 and generating the TAM through a variety of soluble and mechanical factors. Moreover, the cancer cells express the CD47 proteins which are generally recognized by the signal regulatory proteins (SIRP-α) expressed by myeloid cells and activate the Src homology region 2(SH2) phosphatase activating the 'eat me not' signal (7). The expression of antigen presentation, activation of the inflammatory response, and CD4+ and CD8+ activation are widely reduced in TAM. The CCL2 expressed by TAM can target C-C receptor 2(CCR-2) and instigate tumor invasion and metastasis (8) (Figure 1).

Therefore, the inhibition of the CSF-CSFR axis, SIRP-α-CD47 axis, MAPK axis, and CCL2-CCR2 axis would repolarize the M2 into M1 and diminish the M2 cells. Moreover, the CD40 cells expressed on monocytes, macrophages, and B-cells and the TLR activation might enhance the antigen presentation and pro-inflammatory signals, thereby repolarizing M2 to M1. Additionally, the TAM is infiltered into the tumor through CCR5 and CCL5 receptors. While the trafficking of myeloid cells to the tumor is caused by IL-8 via the CXCL-8 receptors. Therefore, blocking the CCL5, CCR5, and CXCL8 may enhance the anti-tumor efficacy (9). Various small molecules and antibodies have been developed targeting single or combination pathways mentioned (Table 1). However, the therapy suffered from drawbacks including tumor relapse, subject variability, and inconsistency of M1 or M2 state in various solid tumors which coax to

limited application.

The CAR for macrophages-treatment against solid tumors

Genetically engineered educated macrophages would be advantageous compared to existing chemotherapy against the solid tumor. The synthesis of CAR-M involves a week-long process. Subcutaneous administration of G-MCSF leads to the mobilization of the monocytes. Later, leukapheresis is done and appropriate CD14+ monocyte cells are selected and separated. The monocytes are differentiated into macrophages ex-vivo. Lastly, the CAR-M is synthesized by mRNA or viral transfection using lentivirus or adenovirus to express antigen-specific against cancer cells or train the pluripotent stem cells by stimulation into M1 macrophages which enhances the anti-tumor effect.

Breast cancer relapse is majorly associated with overexpression of anti-human epidermal growth factor receptor-2 (HER2) expression. Targeting the HER-2 using CAR-M could be an effective therapy to eradicate breast cancer (10). To this end, the differentiated human CD14+ macrophages from human monocytes were transduced using an adenovirus vector (Ad5f35) to elicit the M1 activity of the macrophages specifically in HER2+ expressing solid tumors. The CD14+ CAR-M were active against GM-CSF and M-CSF-expressing tumors and were devoid of M2 phenotype and immune suppression. Moreover, the developed CAR-M did not cause phagocytosis of normal human tissues. In vivo studies revealed persistent CAR expression in NSG and NSG-S mouse models and were effective in the treatment of HER+ solid tumors (11). Moreover, the anti-HER-2 CAR-M (CT-0508) induced MHC-II and TNF-α through pro-inflammatory signals in human TME. It induced the activation of markers on immature dendritic cells and activated T-cells against the HER-2+ solid tumors (12). Due to its enormous potential observed during the pre-clinical trial, the CT-0508 has been translated to the first in a human trial in patients with over-expressed HER-2 solid tumors (13).

Table 1: Recent clinical trials of small molecules and monoclonal antibodies against solid tumors*

Sr no	Molecule/Monoclonal antibody	Clinical stage	Target
1	BI 765063+ BI 754091	Phase I	SIRP-α PD-1 receptor antagonist
2	ALX148+Doxorubicin liposome+ Pembrolizumab	Phase II	CD47 inhibitor
3	IB1322	Phase I	
4	TTI-621	Phase Ia/Ib	SIRP-α-CD47 axis inhibition
5	TTI-622+Doxorubicin	Phase I/II	
6	JSI-1187	Phase I	MAPK inhibitor
	Trametinib	Phase II	
8	Carlumab	Phase II	CCL-2 inhibitor

*All the clinical trial information has been adapted from ClinicalTrials.gov

Klichinsky et. al. transduced THP-1 cells with CD3ζ to develop CAR-M. The developed CD3 ζ CAR-M elicited polyphagocytosis of antigen-positive target cells. Additionally, they developed anti-HER-2 CAR-M against HER2+ metastasized lung cancer. In-vivo studies in NSGS mice revealed consistent tumor growth in the control group as compared with the HER2+ CAR-M treated mice where a significant increase was observed in the survival rate of the mice with decreased tumor progression. Moreover, the treatment was nontoxic with no change in the body weight of the mice. The CAR-M-activated inflammasome, and induced M1 phenotype, antigen-presenting ability, MHC class I/II expressions, and interferons due to the use of an adenovirus vector. Moreover, the M1 phenotype was maintained upto 40 days after transduction. The expressed phenotype was consistent despite M2 stimulations induced using IL4 and IL13 (14). In yet another study, CAR-M (CT-1119) is under pre-clinical development targeting the overexpressed mesothelin receptors onto solid tumors. The CAR comprised human scFv transduced using adenovirus onto human monocytes. The CT-1119 demonstrated robust killing of A549 tumor cell lines expressing MES-OV mesothelin, M1 polarization, dose-dependent pro-inflammatory TNF-α release, and tumor reduction in the murine xenograft model in lung cancer(15).

The CAR-M would emerge as a personalized therapy with the transduction of CAR onto the specific human monocyte based on the overexpressed receptors onto tumor macrophages. The immune response may not be triggered against the CAR-M but, a specific tumor. Tumor relapse shall be a rare occurrence due to prolonged expression of the CAR. Unlike the T-cells, the macrophages have low graft versus host disease and can be prepared in advance for administration. Nevertheless, an uncontrollably triggered immune response due to insertion or mutation of the gene through viral transduction and the safety and efficacy in humans still needs to be thoroughly evaluated for the clinical success of CAR-M.

Conclusion

Despite of tremendous cost of chemotherapy, the treatment regimen was prolonged due to limited efficacy until their in-vivo administration. The surface-modified nanoparticles actively targeting cancer cells could target only specific pathways which led to incomplete eradication of cancer cells. The lack of wholesome treatment and rising deaths due to cancer requires consolidated treatment measures without off-target side effects and selective instigation of the immune response against the solid tumor. Education of macrophages against specific inhibition of cancer cells would be a boon to the current stumbling block. Moreover, CAR-modified other adaptive immune cells including T-cells, natural killer cells, and lymphocytes were ineffective due to the hostile tumor environment and failed to penetrate within the solid tumor. On the contrary, the genetic modification of host monocytes sustains the hostile conditions, penetrate the solid tumors, and antigen presentation potential stimulating the cytotoxic T cell response. However, the extent of proliferation of the CAR-M post-injection, their efficacy against the target tumor in humans, migration of CAR-M to the target site compared with their residence site in the liver, and optimal expression of the target receptors onto cancer cells to elicit appropriate CAR-M efficacy in humans remains elusive.

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Cell Based Therapy for Infertility: A Review




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1. Introduction

Cell Based Therapy (CBT) is transfer of cells of human with the aim to restore the damaged tissue or cells. With the augmentation of newer technologies, innovations, ideations, many different types of cells are being used for the treatment of diverse disease conditions and disorders. Cells that may be employed are pancreatic islet cells, skeletal muscle stem cells, dendritic cells, mesenchymal stem cells, blood forming cells/ hematopoietic stem cells (HSC). Bone marrow transplant also called HSC transplantation is the most widely used CBT and is utilized in treatment of hematologic conditions and many types of cancers. Impending applications of CBT are numerous for example cancers, urinary problems, repair of spinal cord injuries, immune system improvement, neurological disorders like Alzheimer, Epilepsy etc (1, 2).

2. Need for CBT for Infertility

Worldwide large numbers of couples have problems conceiving. Because of this there is deprivation in having offspring that are genetically related. The treatments to overcome this issue are many like drugs inducing ovulation, Assisted Reproduction Technology (ART). But these treatments develop the risks of multiple pregnancies. To overcome the drawbacks of the traditional treatment methods the basic ability of stem cells to replenish damaged tissues and their ability to differentiate the multiple lineages, the CBT is considered to be a novel technique to deal with the reasons of infertility (3).

3. Causes of Infertility

The age of the woman is the most common risk factor for infertility. If the female's age is more than 35 years the evaluation for the infertility must be started as early as 6 months from the realisation of unable to conceive, whereas if the females age exceeds 40 years immediate evaluation is recommended. However, there are potential contributing factors that need to be evaluated before narrowing down the diagnosis and a therapeutic approach (4). The causes of infertility are summarised in Fig.1.

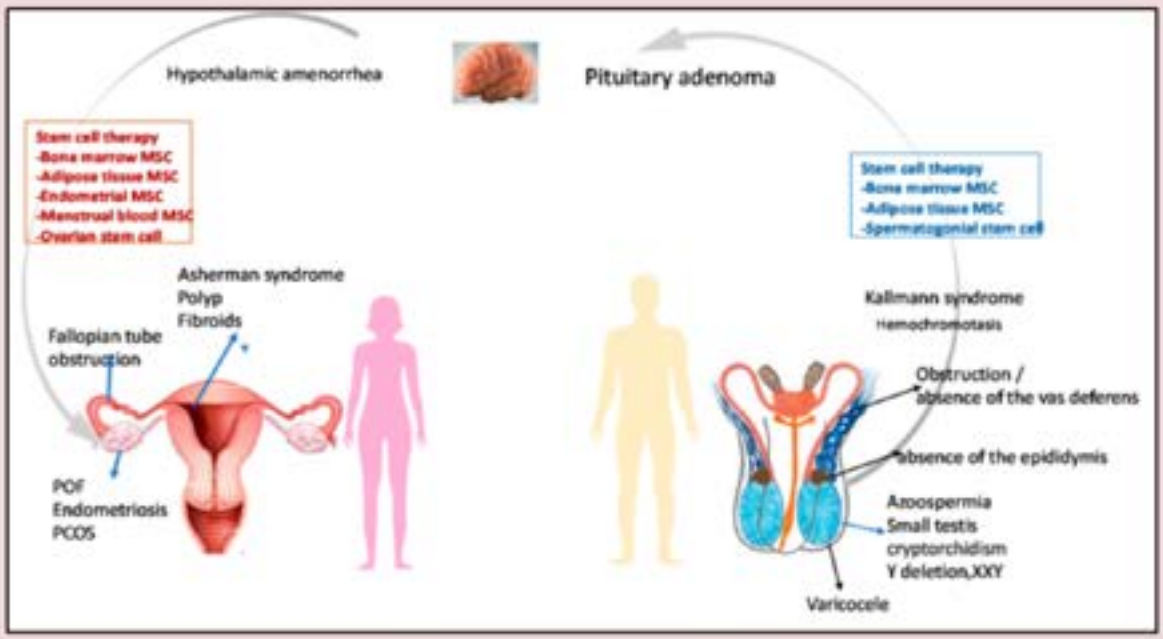

Figure 1: Some causes of infertility

4. Conventional Treatment

To treat male infertility, various measures are incorporated like increase the sperm quality, the causes of endocrinopathy are identified and treated accordingly. In case of Varicocele, surgery interventions are preferred (6).

To treat the infertility in females, ovulation inducing drugs are used based on the diagnosis done. In contrast to conventional treatment, ART improves the rate of fertilization. ART includes several steps in a particular order Ovarian Stimulation, Oocyte retrieval, Invitro fertilization and Embryo culture, Embryo transfer (7,8,9,10,11,12,13,14,15).

Unfortunately, even after the substantial progress of ART, many couples are still unable to be a parent to healthy babies. Moreover the cost involved in ART is high and is an invasive process. CBT presents a new hope to overcome the issues related to above mentioned methods and have success to resolve the infertility of couples.

5. CBT for treating Infertility

A subtype of cells called Stem Cells remain in embryos and human tissues in an undifferentiated form. They can renew on their own and differentiate when needed. In differentiated organs stem cells restores the function of organ damage repair. Based on their origin, these stem cells are of various types. The embryonic stem cells, adult stem cells which are mesenchymal stem cells, induced pluripotent stem cells, spermatogonical stem cells and ovarian stem cells (3).

5.1 Embryonic Stem Cells (ESC)

ESC has an immense capacity of self-renewal, able to differentiate into three segments ectoderm, endoderm and mesoderm, able to maintain the specific karyotype at the time of growth. In humans they are derived from inner blastocysts and transcription factor Oct 4. There is documentary evidence of feasibility of development of functional sperm using gene repaired ESC. Hence we can say ESCs have a promising tool to address the cause of infertility. But due to ethical concerns it is not so commonly used technique (16).

5.2 Induced Pluripotent Stem Cells (iPSC)

The cells derived from skin or blood cells and are reprogrammed back to a pluripotent state is called iPSC. They enable development of boundless source of any type of cell required for therapeutic purpose. For instance, an iPSC can be stimulated to become a beta islet cell to treat diabetes and so on. These iPSCs are considered to be better than ESCs in CBT for the reasons that they originate from adult cells unlike the ESC hence avoids the ethical issue of using embryos and another reason is iPSCs are developed from the patients' somatic cells, therefore comparatively less chance of immune rejections (17, 18). Generation and projected therapeutic suggestions of iPSCs in infertility are presented in Figure 2.

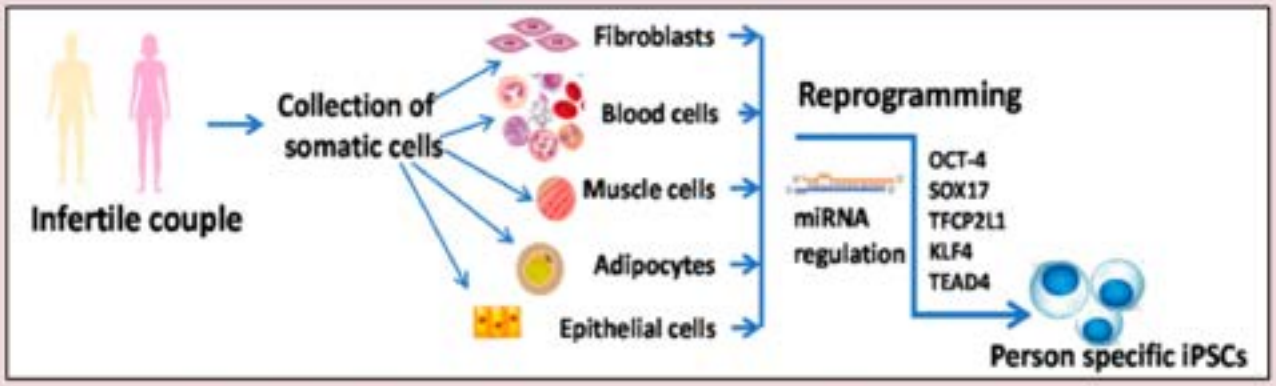

Figure 2: Role of stem cells in generation of patient-specific gamete cells (35).

KLF4: Kruppel-like factor 4; miRNA:Micro RNA; OCT-4: octamer-binding transcription; SOX17: endoderm regulator SRY-box 17;TEAD4: trophectoderm regulator TEA domain transcription factor 4; TFCP2L1: TFCP2L1: pluripotency factors transcription factor CP2-like 1. Reprinted with permission (43)

Though the iPSC technology does not abolish the embryos, the chances to exploit embryos generated from gametes established after iPSCs reprogramming mandate ethical consent from the institutional review board and the assembly of informed consent from the cells or tissue donor previous to obtaining sample for development of iPSC for research purpose. For their solicitation in animal models, consent is essential from IACUC.

5.3 Mesenchymal Stem Cells (MSCs)

They are also known as mesenchymal stromal cells or medicinal signalling cells are multipotent stromal cells. They have plastic-adhesion properties; can segregate into various types of cells, involving osteoblasts (bone cells), chondrocytes (cartilage cells), myocytes (muscle cells) and adipocytes (fat cells which give rise to marrow adipose tissue). Based on their origin they are of many types like

1. bone marrow stromal cells
2. adipose-derived stem cells
3. menstrual-blood-derived MSCs
4. umbilical-cord-derived MSCs
5. amniotic-fluid-derived MSCs
6. placental-tissue-derived MSCs
7. salivary-gland-derived MSCs, and
8. dental-pulp-derived MSCs

The MSCs work by travelling to the damaged ovary and there they help in restoring the ovarian function by the release of various cytokines by paracrine mechanism. The cytokines induces the formation of newer vessels, prevents apoptosis, fibrosis and therefore ameliorate ovarian dysfunction. The MSCs also helps in endometrial regeneration through release of many bioactive molecules which modulate inflammation and other immunological reactions. They also help in activating tissue-specific progenitor cells. The potential therapeutic use of MSCs for the treatment of infertility caused by ovarian and endometrial abnormalities has been reported in a number of preclinical and clinical trials. According to reports, as people become older, MSCs become less potent and abundant as well as less able to divide and differentiate into distinct lineages (19, 20, 21).

5.3.1 Bone Marrow Mesenchymal Stem Cells (BMSC)

They are separated from one another using a density gradient centrifuge, and after that, the incubation for growth and expansion procedure is performed. In rat models, the BMSC were proven to be effective in endometrial and follicular cell growth, endometrial mending, hormonal resuscitation, and conception. Infertile patients' endometrial thickness, the quantity of developed blood vessels, and the kind of menstruation they had all showed improvement. There is proof that BMSCs may offer renewed hope for individuals suffering from ovarian or uterine disorders(22).

5.3.2 Menstrual Blood Mesenchymal Stem Cell (MB-MSC)

These cells may be obtained by non-invasive methods that are simple, secure, and free from moral dilemmas and minor immunological responses, allowing for therapeutic use. According to study, angiogenesis and the production of anti-inflammatory factors by MB-MSC can restore fertility in animals with damaged uterine walls (23, 24).

5.3.3 Endometrial Stem Cells (EndSCs)

Stromal cells, epithelial progenitor cells, and endothelial cells constitute the EndSC microenvironment. The distinct stem cells are present in the endometrium in a resting, undifferentiated state when there is no lesion. Mesenchymal stem cells (MSCs), epithelial stem cells (ESCs), endometrial side population cells (ESPs), and endometrial regenerative cells are all descended from endometrial stem cells (ERC). ERCs, like MSCs, may differentiate into diverse tissues, including adipose tissue, neural tissue, bones, and cartilage. In an animal transplantation model, it was shown that insufficient neovascularization led to the endometrium's thinning, probably as a result of the vascular endothelial growth factor's defective discharge. Therefore, the therapeutic use of endometrial stem cells for endometrial regeneration may involve their angiogenic potential (25). The Figure 3 encapsulates the various mechanisms for restoring the endometrium.

5.3.4 Umbilical Cord Mesenchymal Stem Cells (UC-MSCs)

UC-MSCs have emerged as a common cell-based therapy technology for the restoration of fertility due to their low tumorgenicity, quick capacity for self-renewal, ease of resource availability, lowest ethical issues, and low immunogenicity. Due to their antiapoptotic action over granulosa cells and manipulation of hormone levels, UC-MSCs restored ovarian function in multiple experimental animals who had early ovarian impairment (26, 27).

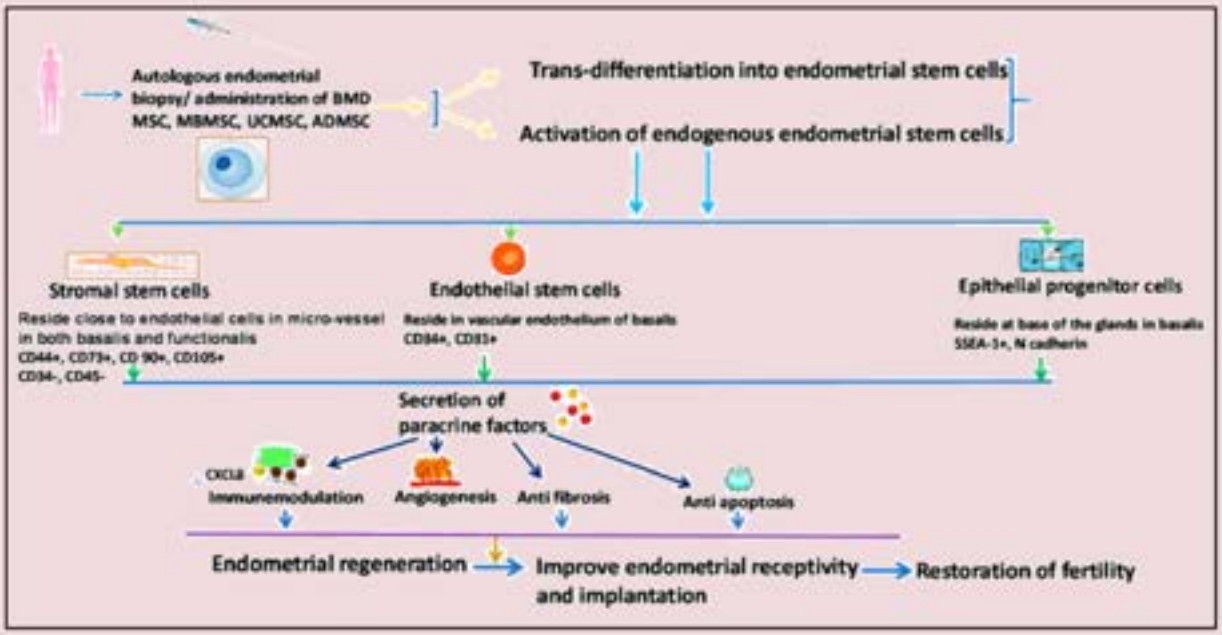

Figure 3: The explanation for restoration of fertility via endometrial regeneration; ADMSC: adipose-tissue-derived mesenchymal stem cell; BMDMSC: Bone-marrow-derived mesenchymal stem cell; CD: Cluster of differentiation; CXCL: C-X-C motif chemokine ligand; MBMSC: menstrual blood mesenchymal stem cell; SSEA-1: Stage-specific embryonic antigen-1; UCMSC: umbilical cord mesenchymal stem cell. Reprinted with permission (43)

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5.3.5 Amniotic Fluid Stem Cells (AFSCs)

The amniotic fluid, which serves the foetus's nutritional needs, is a significant source of MSCs. There are no strict regulatory requirements or ethical considerations in the AFSCs purchase. Additionally, it has the innate capacity to develop into a variety of cells, including osteocytes, muscles, and adipocytes, making it a very valuable source for regenerative medicine. Studies have demonstrated that AFSCs may aid in the treatment of infertility problems by restoring the activities of the ovaries through the activation of several signalling molecules, including bone morphogenic protein (BMP), epidermal growth factor (EGF), and many more (28, 29, 30).

5.3.6 Amnion-Derived Mesenchymal Stem Cells (AmDMSCs)

The thin membrane forming a closed sac about the embryos is called amnion. The amnion serves as a hopeful source of CBT. The studies are performed for treating the ovarian dysfunction in chemotherapy based animal models. AmDMSCs work through paracrine strategies to enhance the granulosa cells in the physical vicinity while also minimising apoptosis. (31, 32).

5.3.7 Placenta-Derived Mesenchymal Stem Cells (PDMSCs)

The studies conducted on animal models have revealed that PSMSCs act by improving folliculogenesis through cytokine modulation and elevation of hormone levels such as estradiol, LH, FSH and their receptor expressions (33, 34).

5.3.8 Adipose-Tissue-Derived Stem Cells (AD MSC)

ADMSC is a unique form of MSC; while coming from distinct origins, they share comparable biologic characteristics. Because ADMSC may be produced using less traumatic methods and in greater amounts than BMSC, they offer comparative benefits over the latter. These characteristics increase the acceptability of ADMSC for a variety of clinical diseases in regenerative medicine. In an animal model of Asherman syndrome, ADMSCs with hormone treatment might successfully reduce fibrosis and trigger endometrial rejuvenation by angiogenesis (35, 36, 37).

5.4 Ovarian Stem Cells (OSC)

The idea of the existence of OSCs was first suggested by observations of the pace of follicular atresia, associated with the mortality of oocytes and loss of ovarian reserve in mice. It has been proven that these cells were capable of initiating follicle production. However, these cells were hidden for a very long period, most likely as a result of their extremely small number, which made up just 0.0145% of the entire cell population in the ageing mouse ovary. Additionally, similar to their male counterparts, these cells require a longer extent for differentiating in in vitro culture. For people suffering from idiopathic premature ovarian failure, OSCs may provide new hope. Revival of fertility and live births are two examples of OSCs' proven therapeutic uses in age-associated infertility (38, 39).

5.5 Spermatogonial Stem Cell (SSC)

Through self-rejuvenation and limitless segmentation into spermatogonia and haploid spermatozoa, SSCs play a crucial part in maintaining the highly efficient complicated procedure known as spermatogenesis in the seminiferous tubule. Figure 2 depicts the creation of them. These differentiating spermatozoa fertilise the oocytes. Infertility may result from any deviation in these carefully timed stages of spermatogenesis. SSCs are a highly effective method for treating infertility, but due to the small amount of them in testes and the difficulty in recognizing them to gradually separate and grow them, they are not employed as regularly in regenerative medicine. Technology advancements have made it possible to extract and describe SSCs using their distinct, species-specific identifying markers. Rather than sending SSC to testicles, they can be cultivated. SSC may be employed in unique situations such male infertility brought on by chemotherapy since it has been shown to induce spermatogenesis and produce functional sperm. The biggest drawback of this CBT in reproductive medicine is that it may disrupt the testis' natural environment, making SSC transplantation unacceptably risky and ultimately leading to therapeutic failure (40, 41).

6. Conclusions

Across the board, 15% of couples struggle to become pregnant. Infertility cases are on the rise as people are waiting longer to start a family. Infertility can be caused by a variety of reasons, which can affect either the female or the male, or a combination of the two. A thorough investigation of infertility, including measurements of blood hormone levels and semen, may help in identifying the main factors at fault. A significant proportion of couples reportedly experience infertility even after ART. CBT may offer relief in these situations for couples hoping to conceive genetically related children. ESC transplantation has fallen out of favour due to ethical concerns and immunologic interference, leading experts to learn about other stem cell

possibilities. iPSCs are the subject of extensive study since they have little to no ethical concerns and can produce findings that are satisfactory. The use of MSCs is also growing due to their low ethical issues and ease of access to a variety of readily available sources, including bone marrow, adipose tissue, menstrual blood, amnion, amniotic fluid, and placenta. Numerous animal and human research have examined the effect of MB-MSCs in endometrial regeneration and the recovery of ovarian function. Understanding of the role of microRNAs in stem cell differentiation is essential for gaining a thorough grasp of the methods by which stem cells function to restore fertility. Large-scale clinical trials are still necessary to validate the security and effectiveness of stem cell-based treatment in the area of human reproduction, nevertheless.

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Cell based therapy for the treatment of neuropsychiatric disorders


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Introduction

Worldwide, neuropsychiatric illnesses have a significant negative impact on the economy, health care system, and wellbeing of affected patients and their dependents (1). Neuropsychiatric disorders, such as major depressive disorder (MDD), schizophrenia (SZ), or bipolar disorder (BD), is thought to affect at least one in four members families. Mental illness and substance use disorders accounted for a combined 173.1 million disability-adjusted life years (DALYs), or roughly 7.1% of the total disease burden worldwide (2). Due to the COVID-19 pandemic, the number of people who suffer from anxiety and depressive disorders significantly increased in 2020 (3). According to studies from India, depression and anxiety are very common in both the general population and COVID-19 infected patients as well as in the post-COVID-19 infection stage (4).

The term "potency" describes a stem cell's capacity to multiply and differentiate into various cell types. In many ways, stem cells have revolutionized how we study and treat diseases (5). Due to the central nervous system's (CNS) poor capacity for regeneration, stem cell therapies are widely used in regenerative medicine, particularly in neurological pathologies. Numerous pre-clinical animal models have demonstrated the safety and efficacy of stem cell therapy, spurring an increase in clinical trials (6). Stem cell (SC) therapies benefit the CNS through a number of mechanisms, including cell replacement, inflammation control, and neuroprotection. Depending on where stem cells are coming from, these mechanisms change. Human pluripotent stem cells (hPSCs), fetal-derived neural progenitor stem cells (fNPCs), and mesenchymal stem cells (MSCs) are the three types of stem cells that are frequently used (7).
Human Pluripotent Stem Cells

Stem cells, like hPSCs, are helpful in regenerative medicine because they can self-renew and differentiate into specific tissue types. Human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are the two types of hPSCs that are most frequently used. In the clinical setting, cell expansion and replacement are the main uses of hPSCs. Due to the high risk of cancer caused by mutations accumulated during the proliferation of undifferentiated tissues as well as the effects of the local microenvironment, this cannot occur by direct implantation.

Prior to transplantation, the cells should ideally be cultured and differentiated into tissue (2).

Fetal-Derived Neural Progenitor Stem Cells

Cells from the fetal brain and spinal cord are used to create fNPCs. These stem cells are advantageous for treating CNS pathologies because they can differentiate into different CNS cell types, including neurons, glial cells, and neuroectodermal cells. The availability of fNPCs is a significant limiting factor, though; for some transplantations, ten aborted fetuses may be needed for each patient. Cell replacement is the main therapeutic strategy for treating fNPCs, with encouraging outcomes in rat models of Parkinson's disease, Huntington's disease, traumatic brain injury, and stroke (7).

Mesenchymal Stem Cells

Multipotent self-renewing stem cells known as mesenchymal stem cells (MSCs) can also be found in the umbilical cord, peripheral blood, and adipose tissue. MSCs are typically taken from bone marrow. Animal models of pathologies of the heart, liver, eye, and blood have been used primarily in MSC testing. A meta-analysis found that there was no increase in acute infusional toxicity, organ system complications, infection, death, or malignancy when MSCs were administered intravenously (8).

Cell therapy employed for neuropsychiatric disorders

Depression

Stem-cell therapy targets the hippocampus in an effort to improve neuronal plasticity. The hippocampal grey matter is severely reduced in patients with depression. In depressed people, this diminution results in fewer neurons and decreased brain communication. Many of the symptoms of depressive episodes are caused by the loss of interneurons in the hippocampus' anterior cingulate cortex (1). The outcomes of various experimental studies strongly support stem cells' potential therapeutic use in the treatment of depression. Although data from experimental models has demonstrated beneficial effects in treating depression, there are still open questions (8).

Autism Spectrum Disorder

The basis for the potential use of stem cells in ASD therapy has been provided by the molecular and cellular changes that underlie ASD. Most importantly, the paracrine effects and immunomodulatory properties of SC have made it possible for clinical applications in the treatment of ASD. SC option therapy may offer significant advantages for restoration therapy through the secretion of, secretome, a complex tool of macromolecules (interleukins, growth factors, and extracellular vesicles) which is responsible for the recovery of damaged tissues, and plays a significant role in the paracrine effect of stem cells (27).

Schizophrenia

iPSC may be a more effective option in schizophrenia. Interneurons, which are damaged in schizophrenia, are created using embryonic stem cells in several studies to serve as a disease model. In addition, stem cell-derived interneurons may one day be used to treat schizophrenia in patients by being transplanted into their bodies. Patients with schizophrenia have fewer GABAergic cells in their prefrontal cortex (PC) therefore implantation of interneurons in PC region could improve connectivity (1).

Bipolar Disorder

Neuropsychiatric disorders including bipolar disorder, have been linked to microRNAs (miRNAs), which play a critical role in brain development and plasticity. Additionally, it has been reported that changes in neural stem cell numbers, distribution, and differentiation capacity have a significant impact on brain homeostasis and neuroplasticity. Therefore, it may be important to identify target miRNAs to understand the future prospects of neural stem cells in maintaining brain homeostasis as a promising therapeutic tool for stem-cell-based therapy and as a promising potential therapeutic tool suitable for BD therapy (28).

Obstacles in bench to bed progress of cell therapy

Efficacy issues

Numerous cells are being used for cell therapy, however, the therapeutic effectiveness is one of the important factors of any transplanted cell. Cell therapy alone is not sufficient to produce desired therapeutic effects, therefore, supportive treatments such as rehabilitation, pharmacological agents, and electrical/magnetic stimulation are required to enhance the effectiveness of cell therapy (29).

Safety issues

Safety issues are not the problem in case of autologous patient-derived cells. Reprogrammed or genetically modified cells have increased stemness potential but these cells are associated with the risk of uncontrolled proliferation. As an alternative, allogenic transplants from healthy donors may avoid the cell proliferation issues, however, these cells have a risk of rejection from immune system. Failsafe procedures should be verified using a variety of techniques in clinical settings even after extensive testing (29).

Evaluation procedure issues

Several methods are employed during the clinical testing to ensure the safety and efficacy of the cell therapy. The significant criteria of the stem cells, quality and well-defined characteristics, should be checked before their clinical use. Dose, route, time, and non-invasive procedure to administer cells, and well-designed clinical trials play significant role in ensuring the safety and efficacy of cell therapy (29).

Patient's rights issues

Privacy of the patients, involved in the testing of cell therapy, must be protected. Problems may arise from the transplanted cells, cell associated factors, and cell therapy procedure during the clinical trials. Patient complications should be monitored in invasive procedure, as well as during transplantation and post-transplantation period to ensure long term safety (29).

Conclusion

Cell based therapy is the promising therapy for various neuropsychiatric disorders including depression, ASD, schizophrenia, bipolar disorders. Stem cells including hPSCs, fNPCs, and MSCs are frequently used, and provide their effects through neuroestoration (release of neurotransmitter), activation of neurogenesis, anti-inflammation, and neuroprotection mechanisms. Despite of several advantages, cell therapy also has numerous obstacles such as poor efficacy, safety issues, investigational problems, and patient's rights issues which hinder

translation of safe and effective cell-based therapies from bench to bed. Further, cell-based therapies might be good therapeutic options for the treatment of neuropsychiatric disorders, however, extensive research in this area is required.

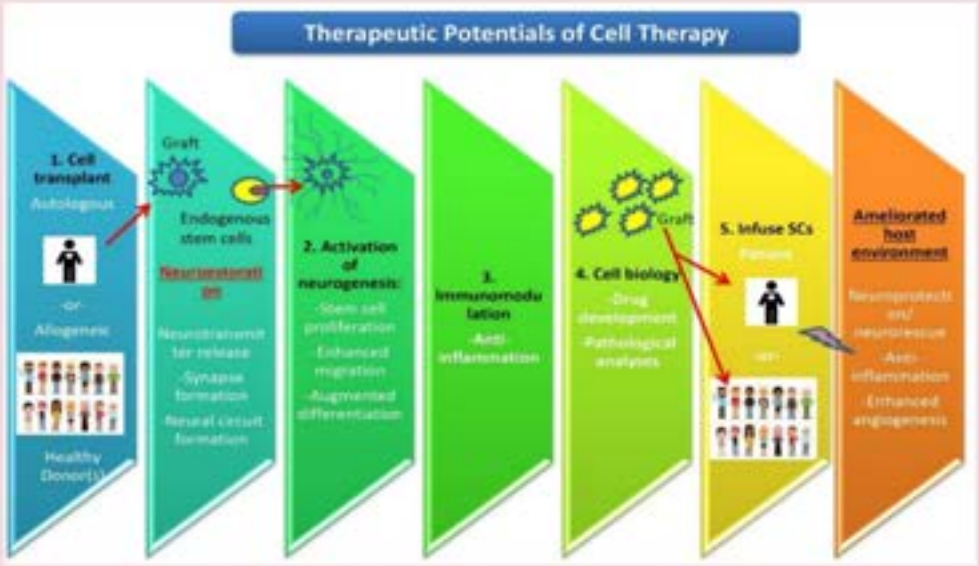

Figure 1: The therapeutic potentials of cell therapy

Table 1. Stem cell models to study neuropsychiatric disorders

S. No.	Neuro-psychiatric Disorders	Animal model details	Major findings	Refer-ences
1.	Depression	Wistar rats; depression, induced with Chronic mild stress (CMS)	Anti-inflammatory effects; ↓pro-inflammatory cytokines; ↑expression of anti-inflammatory cytokines; BMMCs ↓ 8'2-deoxyguanosine level	(9)
		C57BL/6 mice; depression-induced with CMS	Improves depressive-like behaviors. ↓expression of inflammatory factors in the serum. ↓microglial activation in the hippocampus	(10)
		Wistar Kyoto rats model of treatment-resistant depression	MSCs encapsulation ↑treatment effects	
		Mice model depression induced by CUMS	The hUC-MSCs treatment improves anxiety-like behaviors, ↓pro-inflammatory factor levels, and ↑anti-inflammatory factor levels. Inhibit microglial M1 polarization and the level of inflammation factors. C3a-C3aR signaling inhibition alter polarization of microglia results ↓neuroinflammation. ↓neuronal damage and synaptic deficits.	(11)
		Sprague Dawley rats; Depression model by corticosterone injection	BMSCs-derived exosomes improved hippocampal neuron injury of rats with depression by upregulating miR-26a.	(11)

Table 1. Stem cell models to study neuropsychiatric disorders (continued...)

		Male BALB/c mice depression, induced by CS	miRNA ↓levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNFα) released by astrocytes *in vivo*; Exosomes with ↓miR-207 levels showed ↓antidepressant activity *in vivo* experiments. ↓antidepressaet activity MiR-207 could ↓release of pro-inflammatory cytokines *in vitro*.	(12)
		iPSC-derived serotonergic neurons	Treatment of serotonergic neurons with short interfering RNA (siRNA) targeting PCDHA6/PCDHA8 ↑neurite length in serotonergic neurons.	(13)
2.	**Autism Spectrum Disorders (ASD)**	BTBR mice; MSC derived exosomes	Ameliorating autistic-like behaviors.	(14)
		BTBR mice and control wild type C57BL mice; MSC derived exosomes	↑Social interaction and ↓repetitive behaviors; ultrasonic vocalization and maternal pup retrieval were also improved	(14)
		iPSC-derived telencephalic organoids	↓ levels of GABAergic neuronal differentiation	(15)
		iPSC-derived NPCs and neurons	↓Proliferation; neurons showed a rescue of network defects	(16)
		iPSC-derived neurons and astrocytes	↑Synaptic puncta	(17)
		iPSC-derived excitatory neurons	↓Synapsin 1 puncta, synaptic connections, dendrite length, branch complexity	(18)
3.	**Schizophrenia**	Rodent; phencyclidine (PCP) model of schizophrenia	Improved novel object recognition and normalized pre-pulse inhibition	(19)
		MAM rodent model	Normalized latent inhibition, ↑social interaction time, ↑reversal learning and extradimensional set shifting, normalized dopamine population activity, ↓hippocampal hyperactivity, ↓amphetamine-induced locomotor activity, ↑contextual fear conditioning normalized dopamine population activity, and ↓hippocampal hyperactivity.	(1)
		Cyclin D2 knock-out	↓Amphetamine-induced locomotor activity, ↑contextual fear conditioning normalized dopamine population activity, and ↓hippocampal hyperactivity	(20)
		iPSC-derived neurons	Neurons showed an ↑connectivity and expression of glutamate receptors	(21)
		iPSC-derived NPCs, excitatory neurons	FURIN rs4702: control and CRISPR-Cas9-edited excitatory neuron FURIN RNA expression made more similar	(22)
4.	Bipolar Disorder	iPSC-derived hippocampal dentate gyrus (DG) granule-like neurons	LiCl responder neurons exhibited ↓number of action potentials and ↓hyperexcitability	(23)
		iPSC-derived hippocampal DG granule-like neurons and CA3 pyramidal neurons	Treatment with a-dendrotoxin, Tetraethylammonium chloride, or 4-aminopyridine: ↓neuronal excitability in LiCl responder and non-responder lines treatment with LiCl: ↓ excitability of LiCl responder neurons	(24)
		iPSC-derived hippocampal DG-like neurons	↑Wnt/b-catenin signaling activity, ↓hyperexcitability	(25)
		iPSC-derived astrocytes and neurons	Recovered neuronal activity	(26)

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Cell Based Treatment Approaches in The Headway for Neuroblastoma: An Outline




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Abstract

Neuroblastoma is specifically the cancer of sympathetic nervous system rarely seen in neonates, hence called embryonal cancer. It usually occurs due to mutations observed in gestation period of child growth. Holistic approaches have been reported for the pharmacological management of the same. Both the approaches focus on the etiopathogenesis of the disease which is seen due to poor functioning of a noncoding mRNA (microRNA) which is regulated by MYCN transcription factor in mutant neuroblastoma cell. Recent approaches being developed are signaling the interactions of signaling proteins with the cellular processes. Pharmacological approach focuses on the nanomolecules targeting the mutant protein of neuroblastoma. Research suggests the MYCN oncogene contributes to high risk of neuroblastoma tumors in neonates which paves a way for advancements in the treatment of the disease both from the therapeutic and pharmacological perspective. Targeting the MYCN oncogene followed by inhibiting the proliferation of residual cells in the haemopoietic compartment can serve as an effective approach for the treatment. Advancements in the myeloablation chemotherapeutic regimen are also under trials. Chimeric anti-GD-2 monoclonal antibody has also shown anti-tumor activity in the initial phases of neuroblastoma which exhibits additive effect when given with granulocyte- macrophage colony stimulating factor (GM-CSF) which is usually considered a combination therapy for the treatment. The article presents the approaches in targeting the MYCN oncogene and the advancements in the myeloablation chemotherapy in combination with GM-CSF.

Keywords: Neuroblastoma, MYCN, oncogene, nanomolecules, GM-CSF, myeloablation

Introduction

Neuroblastoma is a malignancy in children which generally accompanies no symptoms initially, but when the child attains a considerate age of five, symptoms like incompetence in daily activities and vigorous behavior are commonly observed. The extra-cranial tumor in the children is targeted by tumor initiating cells (TIC) to provide durable metastasis cure. The NB TICs withstand various characteristics of cancer stem cells of self-renewal. Though conventional therapies have not been successful in the treatment of NB, systemic study of cell-based treatment targeting SKPs promises to provide potent and less toxic approach for the NB treatment.(1,2,3,4) This article focuses on the approach to target NB TICs for the treatment of NB without injuring normal non-cancerous cells. Neuroblastoma is the cancer of sympathetic nervous system of the body which generally occurs when the fetus is in the development phase during the gestation period. It starts in the neuroblasts which are commonly understood as immature nerve cells that are on the verge of maturity.

The primary site where this disease develops is the supra-renal gland often referred to as adrenal gland.(5,6). The cancerous cells proliferate to other parts of the body like lungs, liver, lymph nodes, etc. Studies suggest that this fatal disease occurs in infants below 5 years of age (Fig.1). Irritability, pain, constipation, swollen belly, dark circles, weakness, fever are the common symptoms of this disease which are often misunderstood as other common diseases, and therefore it is very difficult to diagnose. The neuroblastoma have genetic predisposition. This type of cancer is well understood by classifying into various stages:

L1: In this stage, the tumor cells are confined to one place.

L2: In this stage, the tumor starts its proliferation.

M: Here, the tumor becomes malignant and proliferates to different parts of the body.

MS: This stage, being the last of above all stages, where the tumor reaches bone marrow of the child who is under 18 months of age.

The gene responsible for onset of neuroblastoma is MYCN gene. MYCN class of gene is the master gene that decides the fate of any cell. (6,7)

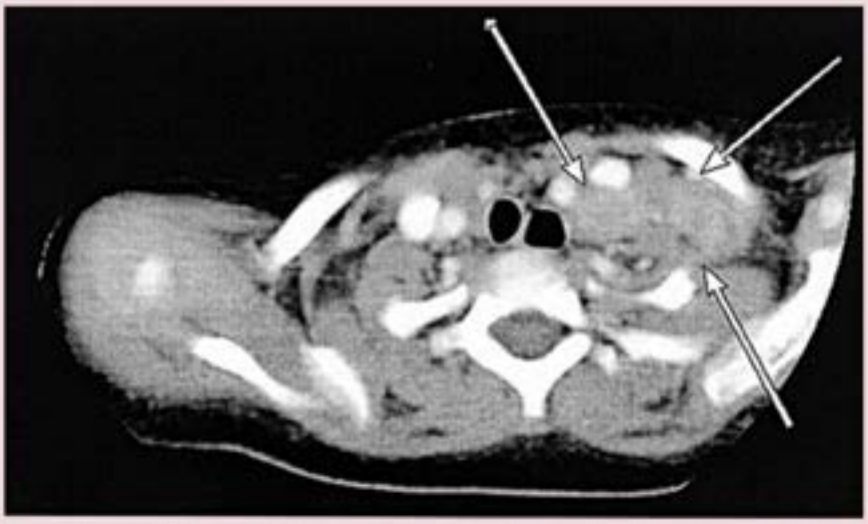

Figure1: Computed tomography scan of neuroblastoma in a child

Cell based approaches in the treatment of neuroblastoma

1.1 Anaplastic lymphoma kinase (ALK) targeted therapy in neuroblastoma:

The ALK is a receptor of tyrosine kinase family which is mainly observed in neonatal brains but its expression is least seen after birth. The ALK protein when dimerised, is responsible for the occurrence of neuroblastoma in children. This ALK protein binds with ALK inhibitors to decrease the dimerisation of ALK with each other producing oncogenic response in the infants. The current approach in the control of cancers like neuroblastoma is by binding to the ATP pocket of ALK protein which would block the proliferation of neuroblastoma onccogene.(8,9) Some approved ALK inhibitors are classified under first generation (Crizotinib), second generation (Alectinib) and third generation (Loriatinib) according to the advancements in the mechanism of action. The fusion of C-terminal region of ALK, aids in the migration and ignorance of apoptosis. The ALK pathway signals a multiple routes of signaling like ALCL, NPM, STAT-3, STAT-5.(Fig 2). Studies show that this ALK protein if given in combination with the MYCN gene approach, creates a co-amplified effect. There are some drugs that are still under clinical trials like- PLB1003, Belizatinib, Fortinib, etc. Crizotinib is found to have a semi-inhibitory effect on the action of ALK protein, thus cannot be considered as a potent drug. (8,9,10) Drugs like Belizatinib and Fortinib are more potent as compared to Crizotininb.

1.2 Expression of EZH2:

Enhancer of Zeste homolog-2 is an enzyme which is encoded by EZH2 gene. This EZH2 locus encodes gene silencing via histones. Intratumoral EZH2 inhibits the adaptive response of the immune system. In addition to the EZH2 gene, MHC-1 screening revealed that these two in coordination can be a head start in the advancement of cell based therapy for neuroblastoma. This gene and proteins are responsible for healthy embryonic development of the fetus. This EZH2 protein is responsible for the methylation of H3K27 variety of tumor suppressor gene. EZH2 gene is seen to cause oncogenic activity leading to neuroblastoma like other cancerous diseases.(10) .Over expression of the gene while coding for the protein shows onset of oncogenic activity. EZH2 is made to suppress to diminish the oncogenic activity which leads to neuroblastoma. EZH2 is made to suppress tumorogenesis thereby decreasing proliferation and motility of neuroblastoma. Also, a protein called Focal Adhesion Kinase (FAK) is also responsible in neuroblastoma tumerogenesis. Inhibition of EZH2 also helps to affect FAK expression. It helps in the suppression of MHC-1 pathway in turn inhibiting the T- cell activation thereby treating cancer to a great level. EZH2 also controls the adaptive response to the Treg activity. These are considered as the subtype of T-cells that induce immunotolerance to the cancerous cells. Treg cells are known for the activation of cytotoxic and helper T-effector cells, which triggers potent anti-tumor response. (10,11)

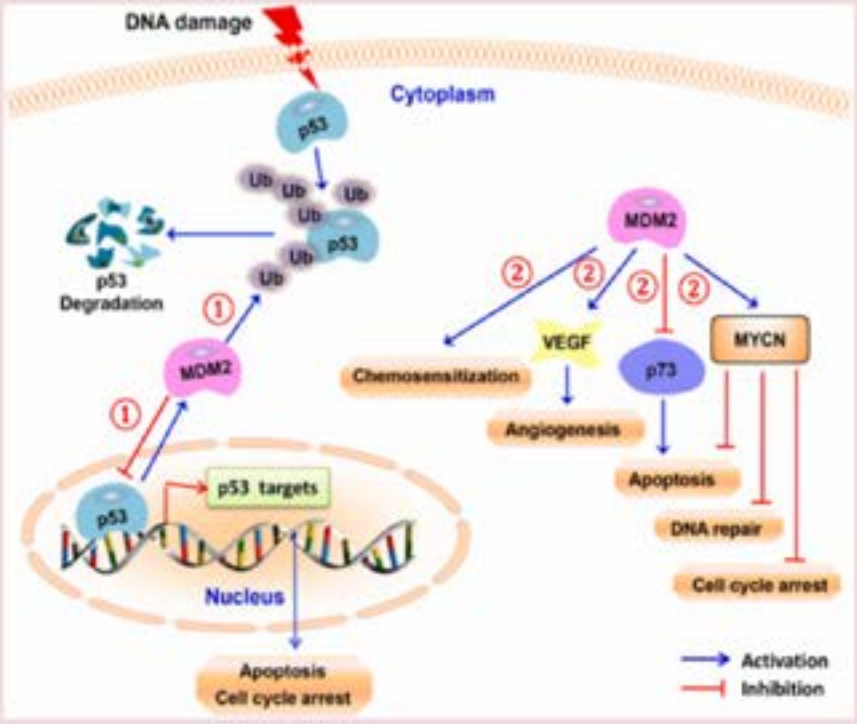

Figure 2: ALK Pathway in Neuroblastoma

1.3 Inhibiting anti-apoptic protein:

Apoptosis is referred to as programmed cell death. The cell death is delayed as in case when the anti-apoptosis proteins are given to treat neuroblastoma. To escape apoptosis, cancer cells possess high level of anti-apoptotic proteins. To get through cancer cells growth, inhibition of apoptotic protein helps to a great extent. IAP (inhibition of apoptic protein) and Bcl-2 (B- cell lymphoma-2) are very good family of proteins that help in the treatment of neuroblastoma to a great extent. These two approaches have additive effect in chemotherapy. The apoptotic protein follows a TGF-beta signaling pathway and acts in double shielding against cancer

especially in neuroblastoma, B-cell lymphoma results in the changing of the mitochondria membrane which leads to cascade of protein activation like Bcl-2, Mcl-1, etc. which classically reduces apoptosis.(11) There are various mechanisms responsible for the same. The first approach being the direction of anti-sense oligonucleotide against the mRNA of targeted protein and second being that of BH-3 proteins which mimics the anti-cancerous activity of Bcl-2 proteins, inducing programmed cell death or apoptosis. Some drugs like Abatoclax, Navitoclax are under clinical trials for the same activity. (12) (Fig 3).

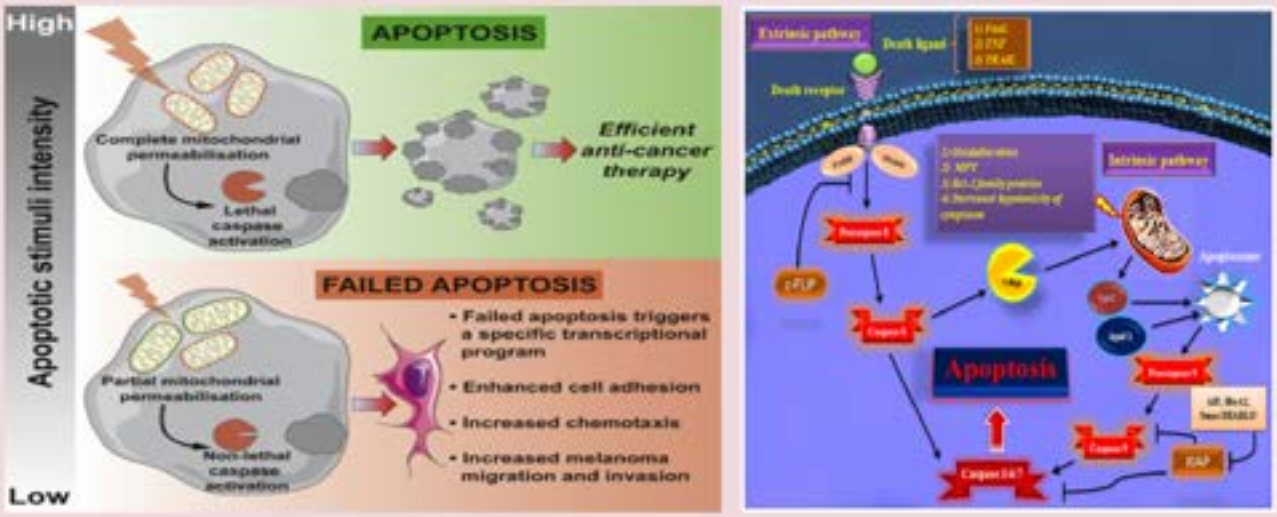

Figure 3: Apoptosis: Programmed cell death

Conclusion:

Therapeutics including different strategies have made advancements in the treatment of fatal disease like neuroblastoma. One way to improve treatment strategies is having a better understanding of the cancerous cells. If the cancerous cells can be specifically targeted for a certain therapy, with the non-cancerous cells remaining unharmed, better prospects for targeted and personalized treatments can be created. EZH2 expression of gene has led to the advancements in the science of treatment of neuroblastoma. The MYCN gene is responsible for the proliferation and the self-destruction of cancer-causing cells, nearly similar to apoptosis. Both of these approaches can be used for the effective treatment of neuroblastic cells.

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Fun & frolic - Wordsearch

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ADSTILADRIN	BREYANZI	CARVYKTI
HEMGENIX	LUXTURNA	RETHYMIC
STRATAGRAFT	ZYNTEGLO	ZOLGENSMA
TECARTUS	IMLYGIC	GINTUIT

Solution in Page 89

Use of the cell-based treatment for inflammation triggered depression



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Abstract

With a variety of immunophenotypic profiles, isolation methods, modes of action, and regulatory levels, cell therapy comprises stem cell- and non-stem cell-based, unicellular, and multicellular therapies. Antidepressant-based conventional therapy is ineffective for about one-third of depressed patients. The use of mesenchymal stem cell therapy to treat depression is one of several potential non-pharmacological treatments that have been researched recently. Since it is clinically pertinent to create novel treatments to treat psychiatric patients, these therapies are reviewed here. Based on its anti-inflammatory and neurotrophic qualities, experimental findings support the idea that mesenchymal stem cell therapy could be regarded as a possible treatment for depression. However, there are several ongoing clinical trials using stem cells to treat depression, but no findings have been made public. To determine how much mesenchymal stem cells can be used in psychiatric clinics as a technique for aiding depression treatment, this review and other upcoming clinical investigations will be significant.

Keywords: Call therapy, Depression, Inflammation, Mesenchymal stem-cell transplant.

Introduction

Cell therapy also referred to as cellular therapy, cell transplantation, or cryotherapy, is a form of treatment in which live cells are injected, grafted, or implanted into a patient to produce a therapeutic effect, such as stem cells to repair damaged tissues or T cells to fight cancer cells through cell-mediated immunity during immunotherapy. When scientists experimented by injecting animal material to treat and prevent disease in the nineteenth century, cell therapy was developed. [1] Despite the failure of these attempts, subsequent research revealed in the middle of the 20th century that human cells might be utilized to help prevent the human body from rejecting transplanted organs, eventually leading to the success of bone marrow transplantation, which is now a common procedure in treatment for patients that have compromised bone marrow after disease, infection, radiation or chemotherapy.[2] In recent decades, however, researchers have gained significant interest in stem cell and cell transplantation as a potential new therapeutic strategy for a wide range of diseases, for neurodegenerative and immunogenic pathology.

Mechanism of cell-based therapy

Cell-based therapy may be allogenic [3], autologous [4] xenogeneic [5], and the types of cells used in cell therapy are human embryonic stem cells [6], neural stem cells [7] mesenchymal stem cells [8] hematopoietic stem cells [9] differentiated or mature cells. [10] Cell therapy is targeted at many clinical indications in multiple organs and by several modes of cell delivery. Accordingly, the specific mechanisms of action involved in the therapies are wide-ranging. However, cells assist therapeutic action using two main concepts. One involves the engraftment, differentiation, and long-term replacement of injured tissue with stem, progenitor, or mature cells. In this paradigm, stem cells undergo in vitro or in vivo differentiation into a particular cell type. These cells, integrate into the wound site and help the organ or tissue function better overall. Using cells to produce cartilage matrix in intervertebral disc degeneration is an example of this mechanism. [11] Second, cells that can release soluble substances including cytokines, chemokines, and growth factors have endocrine or paracrine effects. By stimulating local (stem) cells or drawing cells to the transplantation site, these substances help the organ or area heal on its own. It has been demonstrated that early cell passes are more effective paracrine action than later passages. [12,13] The transferred cells, are viable for only a few days to a few weeks before they die. Cells that release substances that promote angiogenesis, reduce inflammation, and prevent apoptosis are examples of this. [14]

Inflammation triggered depression

Stem cell therapy has shown promise in animal trials for treating several neuropsychiatric illnesses and cognitive/social deficiencies, both during development and after neurodegeneration. Now, we have information that suggests that these stem cells may be involved in treating depression by increasing the number of neurons in the brain that can create more connections.

In recent years, there has been mounting evidence that chronic inflammatory states may play a role in the etiology of depression. This data allowed for the creation of fresh anti-inflammatory treatments that could slow the onset and progression of depression [15,16,]. One group investigated the potential application of stem cell therapy in a prior study. Their theory was based on stem cells' potential to heal the pathogenic condition that sustains depression and their anti-inflammatory and neurodegenerative characteristics. [16]

The psycho-neuroimmunological dysfunctions are emphasized as important by the inflammatory theory. This is based on a few findings: cytokines can affect neurotransmitter metabolism, neuroendocrine function, and regional brain activity, all of which are relevant to depression; acute administration of cytokines causes sickness behavior that shares features with depression; and patients receiving cytokine treatment develop depressive symptoms. Subsets of Major Depressive Disorder (MDD) patients have an altered peripheral immune system, with impaired cellular immunity and increased levels of proinflammatory cytokines. [17,18]

Stem cell therapy in depression

Neurological diseases and subacute and chronic inflammatory processes are now commonly treated using stem cell treatments. Numerous neurological disorders, including multiple sclerosis [19], autoimmune encephalomyelitis [20], Alzheimer's disease and other dementia conditions [21], Parkinson's disease [22], and epilepsy [23], may be treated with adult stem cell therapy, according to research. The encouraging outcomes of experimental investigations using mesenchymal stem cells (MSCs) and bone marrow mononuclear cells (BMMCs) in the treatment of neurological illnesses raise the possibility of creating non-pharmacological cell therapies for psychiatric disorders. The major findings from these investigations include reduced expression of pro-inflammatory cytokines, increased expression of anti-inflammatory cytokines, and decreased 8'2-deoxyguanosine in BMMCs are all anti-inflammatory effects. Treatment with adipose tissue-derived stem cells improves depressive-like behavior. By increasing miR-26a, BMSCs-derived exosomes reduced hippocampus neuron damage in depressed mice. [24]

Clinical Trials with stem cells in depression

The outcomes of various experimental studies significantly indicate stem cells' potential therapeutic application in the treatment of depression. Although data from experimental models have demonstrated beneficial effects in treating depression, there are still open questions. To determine the ideal dose, method of administration, and basic mechanisms of action, additional research is also required. Further discussion on the applicability of such experimental models to the target human population, which may include individuals who are resistive to treatment and who take multiple medications, is warranted. These deficiencies have led to the registration of the first clinical trials using exosomes or cell-based products to treat depression on international platforms. At this time, four clinical studies (phases 1 and 2) are being assessed for the security, effectiveness, and acceptability of administering MSCs and exosomes (Table 1). [25]

Table 1: Cell- therapy-based clinical trials for treating depression [25]

Study	Target population	Product	Outcomes
NCT02675556	Treatment-resistant depression; (n = 80)	Allogeneic MSCs; 108 cells. Single i.v. infusion; source not reported	Incidence of any treatment-emergent seriouss adverse events; Reduction of Inflammation.
NCT03522545	Treatment-resistant bipolar depression; (n = 30)	Allogeneic bone marrow-derived MSCs; dose not reported	Change in depression as assessed by the MADRS Scale.
NCT03265808	Alcohol use disorder and major depression; (n = 80)	Allogeneic MSCs; 108 cells single i.v. infusion; source not reported	An incident of treatment-emergent-serious adverse events
NCT04202770	Refractory depression; anxiety disorders; neurodegenerative diseases; (n = 300)	Focused ultrasound and exosomes	Beck depression inventory (BDI-II)

Conclusion

Treatment-resistant depression may benefit from cell therapy, including BMMC or MSC transplantation or the delivery of cell products such as exosomes. Many problems have not been resolved despite the small number of preclinical research, including how long the antidepressant effect lasts. It is an important query with implications for the viability of these treatments. The likelihood of an effective treatment for this chronic, severe, and common disorder must be investigated due to the difficulty that comes with the treatment of resistant depression. Preclinical research is still limited; hence it is recommended that clinical trials be extended further.

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Exploring the potential of Stem Cell Therapy for management of Rheumatoid Arthritis- Opportunities, Challenges and Future Prospective



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Abstract:
Cell based therapies bid the potential of altering and treating the diseases which cannot be resolved or eased by the conventional methods, treatments and existing pharmaceuticals. Cell therapies are diversified depending on the types of cells such as Mesenchymal stem cells (MSC's), chimeric antigen receptor (CAR-T) cells and Regulatory T-cells (Treg). MSC's offer new prospects in the management of autoimmune disorders as they exhibit unique immunomodulatory properties, thus can serve as a promising approach in stem cell-based therapies for rheumatoid arthritis (RA). RA is an autoimmune disorder that causes degradation of the synovium membrane, causing pain, inflammation, and progressive destruction of bones and cartilage. The pathogenesis of RA is linked to dysregulation of adaptive and innate immunity. The rising incidence of rheumatoid arthritis is being attributed to changes in lifestyle and food habits. In clinical practise, conventional treatments such as antirheumatic drugs, steroid drugs, and biological agents are used. However, long-term use of these drugs causes side effects, and may develop drug resistance in some RA patients. Recent research on MSC-based therapy is seen as a viable strategy in the management of RA in this regard. Current advancements in MSC based pre-clinical and clinical studies and other strategies for enhancement of MSC's immunoregulatory properties are further discussed in the article.

Keywords: Cell therapies, Rheumatoid Arthritis, Mesenchymal stem cells, Inflammation

Introduction:

Rheumatoid arthritis (RA) is an autoimmune disease that mainly affects the joints in the hands, wrists and knees. This chronic inflammation leads destruction of joints and if untreated leads to irreversible disabilities. The risk factors include genetics, environmental and socioeconomic factors. Apart from these, gender is also an important factor. Generally, RA is often two to three times as common in women than in men. The prevalence rate of RA is around 3 in 10,000 people, which rises with age and peaks between the ages of 35 and 50 (1). The annual incidence of RA is around 3 in 10,000 people, increasing with age and peaking between the ages of 35 and 50. RA affects roughly 14 million people worldwide (World Health Organization, 2021). In India, approximately 0.92% of adults have RA. Of these, 20 to 70 percent of patients become incapacitated 7 to 10 years after diagnosis (2).

In RA, the loss of cartilage over time, reduces the space between the joints and bones leading to a painful and unstable bone. This decreases joint mobility and an irreversible joint deformity (3). Conventional treatment strategies for RA include the use of Disease modifying anti rheumatoid drugs (DMARDs) and Non-steroidal anti-inflammatory drugs (NSAIDs). The first line of treatment includes a combination of Methotrexate (MTX) and glucocorticoids such as prednisolone. This combination has shown a benefit and improvement in RA. However, only 25% of patients experience remission. In addition, most of the patients develop side effects from these conventional therapies. These side effects include renal dysfunction, increased cardiovascular risk and nonspecific inhibition of immune responses, and obesity leading to further worsening of the condition. However, recent development in disease pathophysiology has led to new therapies including biological DMARDs, monoclonal antibodies, cytokine and chemokine inhibitors, several immune therapies, and stem cell therapies. Of these, stem cell therapies are gaining importance in the field of health care sector. It is the most recent and a growing area of research for the treatment of RA.

Stem cells are cells with self- renewal pluripotent ability. These cells can differentiate into several cell types depending either on the environmental factors or stimuli received. Stem cells can replace damaged and dead cells in the body. In RA, Stem cell therapy helps in reducing the inflammation of the joints and also increases the presence of healthy cells in the body. Mesenchymal stem cell (MSCs), a type of stem cell, has the ability to differentiate into bone and cartilage. Direct injection of MSCs into the tissues around the injured joints is the basis of synovial MSC therapy as shown in figure 1. MSCs can also suppress the immune system and lessen the body's inflammatory reaction. Because of this, MSC therapy represents a promising therapeutic alternative for autoimmune diseases like RA (4). Clinical proof-of concept studies have shown that management of RA with MSCs has a favourable safety profile. Stem cell based therapies may benefit patients who are not responsive to conventional therapies and are willing to lessen on their medication use, or want to try stem cell therapy first before starting a drug treatment. This article further focuses on recent developments, challenges and future perspectives of stem cell therapies for the management of RA.

Potential of MSC's for Rheumatoid Arthritis:

Previous investigations have demonstrated a dramatic reduction in disease progression using several preclinical experimental models. Of them, collagen induced arthritis mice model is one of the most widely studied models. The encouraging findings from the experimented models have cleared the path for MSCs to become a promising therapy for RA. Adult bone marrow is the most common source of MSCs, followed by adipose tissue, umbilical cord and amniotic fluid. Apart from these, MSCs have recently been found in nasal tissues, amniotic fluid, gingiva, placenta, synovial fluid or membrane. The majority of RA preclinical research carried out uses MSCs derived from bone marrow cells.

In the preclinical studies, MSCs are delivered via intravenous (IV) and intraperitoneal (IP) routes. Alternative routes such as intra-muscular, subcutaneous, intranodal, intra-articular (IA) and peri-articular deliveries have been suggested with positive outcomes.

In these experimental models, the animals are dosed either once or several times during the disease progression. A usual dose is an infusion of 2-3x106 MSCs per animal. Numerous studies have demonstrated that injecting MSCs at the early stages results in greater efficacy. These findings show a decrease in inflammatory cytokine levels along with a lowering in autoantibodies against collagen. Additionally, the MSC therapy causes an increase in the levels of chemokine receptor 3-alternative (CXCR3), IFN-induced protein 10, and anti-inflammatory cytokines such IL-10 in blood and synovium. These outcomes have been demonstrated by MSCs to be independent of tissue origin and administration method. More interestingly, MSC-based therapies have been shown to reduce inflammation by 70% in most experimental models. Autologous MSC therapy in RA patients has an acceptable safety profile and shows promising clinical efficacy in the latest clinical studies completed by various researchers worldwide (6).

Other cell therapies being researched include (Treg) regulatory T-cell therapy and (CAR-T) chimeric antigen receptor cell therapy, in addition to MSC-based therapies. These therapies have also proven their beneficial effects so far. CAR-T cell based therapy has also been explored for the management of Rheumatoid Arthritis. Rheumatoid-associated helper T-cells were able to recognise the new CAR T cells when tested by the researchers. However, it was observed that the helper T-cells could only be triggered when the CAR T-cell carried the matching antigen. The pathogenic helper T cells were efficiently eradicated by the synthetic T cells as well. This treatment delayed the onset and severity of rheumatoid arthritis on mice when given early on in disease development (7).

Safety and Regulatory requirements:

According to the "New Medications and Clinical Trial Rules, 2019" published in March by the Union Health Ministry, stem cell-derived products are to be considered as "new drugs". This implies that every physician using stem cell therapy must obtain an approval. Only bone marrow-derived blood stem cells can be used to treat various blood diseases and blood malignancies both. Studies on the clinical use of stem cells for additional diseases or stem cell types are continuously being studied (8). For the past 20 years, stem cell therapies have been under preclinical development. A constantly shifting regulatory environment has prevented the majority of these innovations from making the transition from the bench to the bedside. Despite the fact that the clinical safety of these therapies on a large scale has not yet been completely demonstrated, the overall number of patients who have safely received stem cell therapies is significant and growing. Since the safety profile of stem cells and present regulation are interwoven, understanding one requires understanding the other (9).

Challenges and Future Prospective:

There are many challenges that need to be tackled if stem cell treatment is to advance more quickly. The challenges involve the manufacturing issues, genetic instability, stem cell culture conditions, stem cell distribution after transplant, pharmacological issues and many more (10). The promising results of the completed and ongoing clinical trials on MSCs for RA, support the fact that the stem cell therapy would prove as a better alternative to the existing therapies.

The rising frequency and recurrence of the disease are the primary factors anticipated to accelerate the growth of the stem cell therapy market for RA over time. Although, precise etiology for rheumatoid arthritis is unknown, some risk factors have been linked to the disease. In Hyderabad, a 67-year-old patient with severe arthritis recently underwent stem cell therapy instead of knee replacement surgery, and the treatment was proved to be successful (11). On the other hand, a face mask was developed in the Springs Rejuvenation Centre, Los Angeles to heal skin and grow collagen and also for treatment of burns and chronic wounds, the results have been shown to be astounding in terms of all aspects, gives a hope for further research on cell based therapies for other ailments as well as cosmetics (12). The global market for management of RA using stem cell therapy is expanding as a result of the development and inclusion of novel medicines in combination with cell based therapies. In the global market for rheumatoid arthritis stem cell therapy, North America is anticipated to hold a disproportionate amount of market share due to the presence of numerous important companies. Some of the key players in the global market for rheumatoid arthritis stem cell therapy are Mesoblast Ltd., Roslin Cells, ReNeuron Group plc, International Stem Cell Corporation, TiGenix and Regeneus Ltd. (13). With the improvements in the field of stem cells and the expanding market share, it is anticipated that more businesses can enter the field of cell-based therapies.

Conclusion:

In RA, early diagnosis is necessary for prevention of severe damage of joints and loss of essential bodily functions. In-depth knowledge of disease mechanism has been made possible by advancements in MSC-based stem cell therapies, which is important for developing more effective treatment strategies. Given the complexities of the pathogenic processes underlying the disease, MSCs are a viable alternative strategy that has the potential to have significant immunomodulatory effects for the management of RA. The microenvironment, which is produced by factors secreted by immune cells both innate and adaptive, has an impact on MCSs' ability to develop either a pro- or anti-inflammatory phenotype. For better comparisons of outcomes among RA with MSC-based therapy, improvements in the harmonization of MSC treatment protocols in terms of large scale production, sources of MSCs, delivery routes and comprehensive assessment of the findings would be needed.

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The Promise of CAR T Cell Therapy




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The novel therapy of CAR T cells (Chimeric Antigen Receptor T cells) has been gaining much recognition in treating cancer along with or as an alternative to chemotherapy. They are T cells engineered with chimeric receptor proteins that increase the specificity to target a particular antigen. This artificial T cell receptor is widely used as a part of immunotherapy.

The term chimeric is used because these are the fusion proteins that are made by joining two genes that originally coded for separate proteins i.e., antigen-binding gene and T cell activating function of a gene into a single receptor. CAR T cells specifically identify cancer cells and destroy them by interacting with the Tumor-Associated Antigens (TAAs) expressed on the tumor cell surface. CAR specifically binds with TAAs then T cells get activated through the phosphorylation of immune receptor tyrosine-based activation motifs (ITAMs).[1] This consequently induces T cell proliferation, cytokine secretion, and cytotoxicity. With guidance from their engineered receptor, CAR T cells multiply in the patient's body and, recognize and kill any cancer cells that harbor the target antigen on their surfaces.

CARs comprise three parts:

1. An extracellular antigen recognition domain of the single-chain Fragment variant (scFv) obtained from an antibody), the Ectodomain
2. An anchor to the T cell membrane, the Transmembrane domain
3. An intracellular T cell activation domain of CD3ζ, the Endodomain

Each CAR bridges the cell membrane. Part of the receptor is located outside the cell and part is within the cell. The area of the CAR that protrudes out from the cell's surface is composed of fragments, or domains, of artificially designed antibodies. The internal part of each CAR is composed of signaling and 'co-stimulatory' domains. These transmit signals into the cell after the interaction of receptor and antigen. The different domains that are used can affect the cells' overall function. [2]

CAR T cells can be either derived from T cells in a patient's blood (autologous treatment) or derived from the T cells of another healthy donor (allogeneic i.e., HLA-identical donors).

Allogeneic CAR T cells therapy has moved into early-phase clinical testing anticipating that it will offer significant advantages over autologous regimens. Although there are three FDA-approved autologous CAR T-cell products, there are no allogeneic CAR T-cell products in the market. [3] The basic steps of CAR T cells production include:

1. T cells isolated from blood (autologous or allogenic)
2. A new gene that codes for chimeric antigen receptor is integrated into the T cells
3. Engineered T cells become specific to the desired target antigen
4. Tissue culture of these engineered T cells done for expansion
5. Infusion of engineered T cells into the patient [4]

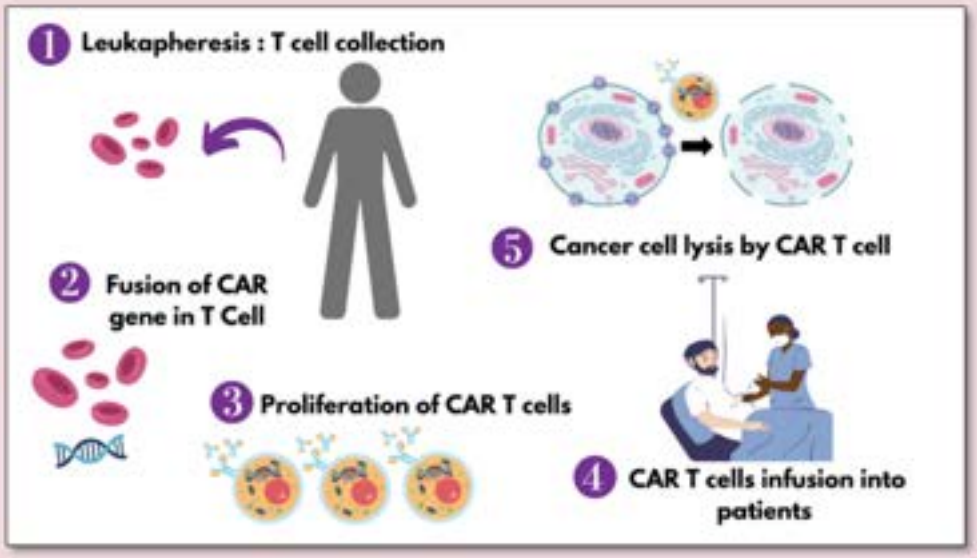

Fig. 1: Preparation of CAR T-cells for cancer treatment

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Side effects of CAR T cell therapy

1. Cytokine release syndrome (CRS)- results from the overproduction of inflammatory cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6).
2. CAR T-cell-related encephalopathy syndrome (CRES)- Symptoms of CRES include mild confusion, disorientation, fatal cerebral edema, and speech difficulties.
3. Immune effector cell-associated neurotoxicity syndrome (ICANS)- ICANS includes CRES and other neurological toxicities arising from CAR T and other therapies. Symptoms may include seizures and loss of balance.
4. On-target/off-target toxicity- Occurs when CAR T-cells attack non-tumor cells expressing the target antigen. These therapies target cancer cells and reduce the number of healthy antibody-producing B cells, thereby the patient is more susceptible to infection.

5. Allergic reactions during the infusion- A weakened immune system, with an increased risk of serious infections. Low blood cell counts, can increase the risk of infections, fatigue, and bruising or bleeding. [5]
6. Tumour Lysis Syndrome (TLS)- a group of metabolic complications that can occur due to the breakdown of dying cells which can cause organ damage and breakdown of CAR T cells. [6]
7. **FDA-approved CAR T cell therapies**

i. Abecma (Idecabtagene vicleucel)

Abecma is used to cure Multiple myeloma. B- Cell Maturation Antigen (BCMA), is expressed on the surface of both normal and malignant plasma cells implicated in multiple myeloma. Overexpression of this antigen promotes the growth of myeloma cells and cell proliferation and survival. Idecabtagene vicleucel (Abecma) is the first CAR T-cell therapy that targets BCMA. The antigen-specific activation of Abecma results in CAR T-cell proliferation, cytokine secretion as well as subsequent death of BCMA-expressing cells. [7]

ii. Kymriah (Tisagenlecleucel)

This therapy is used in the treatment of Acute Lymphoblastic Leukemia. Kymriah is genetically-modified, and CD19-directed autologous immunotherapy. The patient's T cells are programmed with a transgene which encodes a CAR to target CD19. It includes a murine single-chain antibody variable fragment which recognizes CD19, which in turn is fused to intracellular signaling domains from 4-1BB (CD137) and CD3ζ. The intercellular signaling domain CD3ζ initiates antitumor activity and T-cell activation. 4-1BB plays a role in enhancing the expansion and persistence of the CAR cells. The CAR binds to the CD19-positive cells and sends a signal to begin T-cell activation, expansion, and targeting T-cell elimination, thereby increasing the persistence of Tisagenlecleucel cells due to the property of individual components present in therapy. [8]

iii. Tecartus (Brexucabtagene autoleucel)

This combats Mantle Cell Lymphoma. It consists of two intracellular CD28/CD3 domains: CD3 ζ is a signalling domain, and CD28 is a costimulatory domain. It also has an exterior domain with a single-chain variable segment connected to the transmembrane domain by a hinge. It is introduced into T cells using a gamma-retrovirus vector. After attaching to the CD19 receptor on lymphoma cells, CAR-T cells activate T cells by stimulating signalling pathways through the CD3 ζ domain. Granzyme B and perforin are immediately released by CAR-T cells, which promotes the killing of tumors and mediates lymphoma apoptosis.[9]

iv. Breyanzi (Lisocabtagene maraleucel)

Breyanzi targets B-lymphocyte surface antigen B4 and is employed in the treatment of B-cell Lymphoma. This CAR constitutes CD28 transmembrane domain, 4-1BB costimulatory domain, and CD3ζ activation domain. The transmembrane domain is responsible for the tolerance or activity of T cells. The costimulatory domain controls interferon production and cytotoxic T-cell activity. The activation domain carries out the activation of T cells via CD2, which is a T cell surface adhesion molecule. [10]

v. Yescarta (Axicabtagene ciloleucel)

This therapy treats Follicular Lymphoma. Yescarta, which is a CD19-directed genetically modified autologous T-cell immunotherapy, binds to both, CD19-expressing cancer cells as well as normal B cells. Activation of downstream signaling cascades occurs, leading to T cell activation, proliferation, acquisition of effector functions, and secretion of inflammatory cytokines and chemokines after the engagement of anti-CD19 CAR T cell with CD19-expressing target cells, the CD28 and CD3-zeta costimulatory domains. The consequence of this is the killing of CD19-expressing cells. [11]

Scope in India

On the 4th of June 2021, the IIT Bombay team and cancer care in India carried out the first CAR-T cell therapy (a type of gene therapy) at the Bone Marrow Transplant unit at ACTREC. This gene treatment is undergoing early-stage pilot clinical studies and is a "first in India." For the purpose of performing a first-in-human phase-1/2 clinical study of the CAR-T cells, the team has been granted 19.15 crore by the National Biopharma Mission-BIRAC of the central government. The Bioscience and Bioengineering (BSBE) department of IIT Bombay, led by Prof. Rahul Purwar, created the revolutionary CAR-T cells that will serve as medications.

National Biopharma Mission has invested in the development of a Lentiviral vector manufacturing facility for packaging plasmids. The transformed T cell is then transferred into the body using this packaged plasmid. It is included in the expansion of the cGMP facility utilised for CAR T-cell production and T-cell transduction. Additionally, this was given to two other charities. The Department of Biotechnology supports the development of CAR-T cell technology for diseases like acute lymphocytic leukaemia, multiple myeloma, glioblastoma, hepatocellular carcinoma, and type-2 diabetes.

Each patient's CAR-T cell therapy costs approximately 3-4 crore (INR). The major concern is to develop this technology in a cost-effective manner so that it can be made available to a large number of patients. If the trials are successful, it may save millions of lives by making the treatment available in India at an affordable cost. [12]

The estimated market size for CAR T cell therapies in India is 20000 to 40000 patients with leukemia and lymphoma per year. Kiran Mazumdar- Shaw, Biocon founder, and managing director believes the production schedule including cell manipulation should be hospital-based unlike that in the US where a central accredited location far from the medical center is chosen. This allows earlier intervention in the patient's course of treatment and hence, increases the therapy's chances of success. [13]

Due to the limitations associated with autologous therapy, Allogeneic CAR T cell therapy is explored. Extensive manufacturing time and effort for developing personalized CAR T treatments accompanied by customization to that particular patient are significant drawbacks. As a consequence of personalized manufacturing, the prices of these therapies are exorbitantly high. However, allogeneic therapy also tends to demonstrate potential drawbacks. As they're derived from a donor, they require compatibility testing with the patient, similar to the organ transplant process. Hence they are also prone to rejection if they are not compatible. [14]

The future prospects to optimize the design and delivery of CAR T cells seems hopeful. Though it has innumerable benefits, there are a few drawbacks that need to be overcome. CAR T cells for the time being have not proven effective for a majority of people with blood cancer, due to toxicity issues or high expenses. To incorporate CAR T cells against other diseases, strategies are being developed to avoid antigen-negative relapse, control toxicity, and increase efficacy and persistence. [15]

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Fun & frolic - Crossword | solution

Across

5. Immortalized cell line (**ctx**)
6. Cell-based therapy to treat Type 2 diabetes (**apligraf**)
7. Dermal fibroblasts for wound repair from Arita Medical (**recell**)
8. CAR-T therapies for the treatment of relapsed or refractory large B-cell lymphoma, (**yescarta**)

Down

1. CAR-T therapy for the treatment of acute lymphoblastic leukemia (**kymriah**)
2. Approved in vivo gene therapy (**glybera**)
3. World's first 'personalized' cancer therapy marketed by Dendreo (**sipuleucelt**)
4. Abbreviated Michigan Cancer Foundation Breast Cancer Cell Lines (**mcf**)
9. Chimeric antigen receptor T-cell therapy (**cart**)

Emerging trends in Stem Cell Therapy: Types, Clinical Benefits, Technologies, and Future Prospects


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Introduction:

The process of introducing healthy new cells into a patient's body to take the place of diseased or damaged ones, altering the function of the patient's cells through the expression of factors or direct interaction, or removing disease-causing or dysfunctional cells with the help of immune cells is all referred to as cell therapy. Charles-Édouard Brown-Sequard, a pioneer in hormone therapy at the time, attempted to counteract the signs of aging through injections of animal testicle extracts in the year 1889. Today, cell therapy is moving forward with ongoing studies of its clinical safety and effectiveness. Stem cell and non-stem cell-based unicellular or multicellular treatments are combined in cell therapy. It typically makes use of allogeneic or autologous cells; could make use of genetic engineering or other manipulations; and can be used topically, as injectables, infusions, scaffold-free systems, or as injectables. The various types of cell therapies, including stem cell-based and non-stem cell-based cell therapies, provide an overview of their nature as well as techniques for isolation and characterization. Cell therapy spans multiple therapeutic areas, including cancer therapy, immunotherapy, and regenerative medicine.1,2

Stem-cell-Based Therapies:

Overview of Stem Cells:

Unlike the other cells in the body, stem cells have the special and unique capacity to transform into practically any form of cell. Stem cells or their derivatives are utilised in cell therapy to create new tissue or to encourage the healing response in damaged cells.

Stem cells are unspecialized, self-renewable cells that are prepared to develop into any cell type and/or as many cell types as needed. They may be found in both adult and embryonic tissues of an organism. External cues, such as physical contact between cells, paracrine secretions from surrounding tissues, and tissue type, as well as internal signals like genes and epigenetics, all have an impact on stem cell specialisation (embryonic cell origin). Depending on the kind of stem cells, in silico gene expression analysis can identify stem cell specialisation [for example. Bioinformatic test called Pluri-Test3

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
- Adult stem cells
- Cancer stem cells

Pluripotent stem cells: - PSCs 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 post-implantation embryos; and induced pluripotent stem cells (iPSCs), derived from direct reprogramming of postnatal/adult somatic cells in vitro. PSCs give rise to all cell types except extraembryonic placental cells as shown in figure 1.

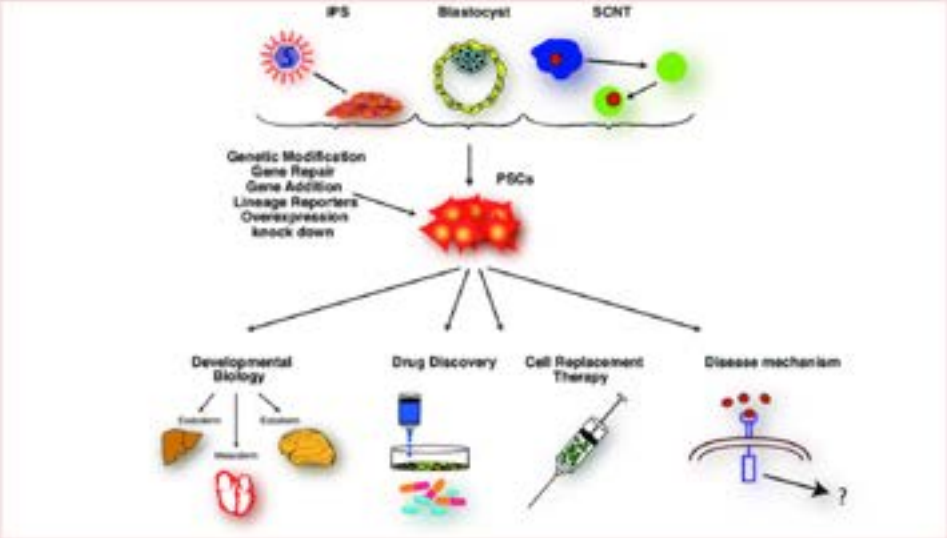

Figure 1: Pluripotent stem cell sources and their application.

Adult stem cells: Adult stem cells are uncommon, undifferentiated cells found among differentiated or specialized cells in mature body parts. ASCs, which have less self-renewal and differentiation capability than PSCs, restore lost cells or contribute to cell repair or growth by giving rise to precursor or progenitor cells and, eventually, differentiated cells. Hematopoietic stem cells (HSCs), skin stem cells (SSCs), neural stem cells (NSCs), and mesenchymal stem cells are examples of ASCs (MSCs)as shown in figure 2.

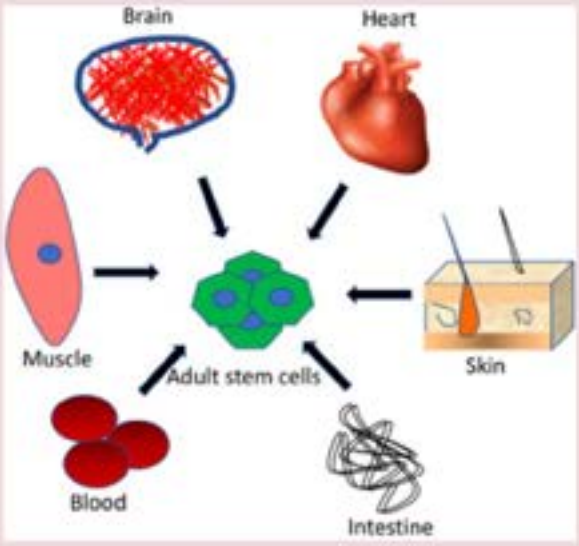

Figure 2: Adult stem cell

Cancer stem cells: CSCs, or tumor-initiating cells, are seen in solid and blood cancers and are thought to arise from normal stem or progenitor cells by a variety of methods including mutations, gene transfer, epigenetic modifications, and microenvironmental variables. CSCs have self-renewal, differentiation, metastasis, and immunosuppressive qualities and are involved in cancer development, metastasis, recurrence, and resistance to chemotherapy and radiation. as shown in figure 3.

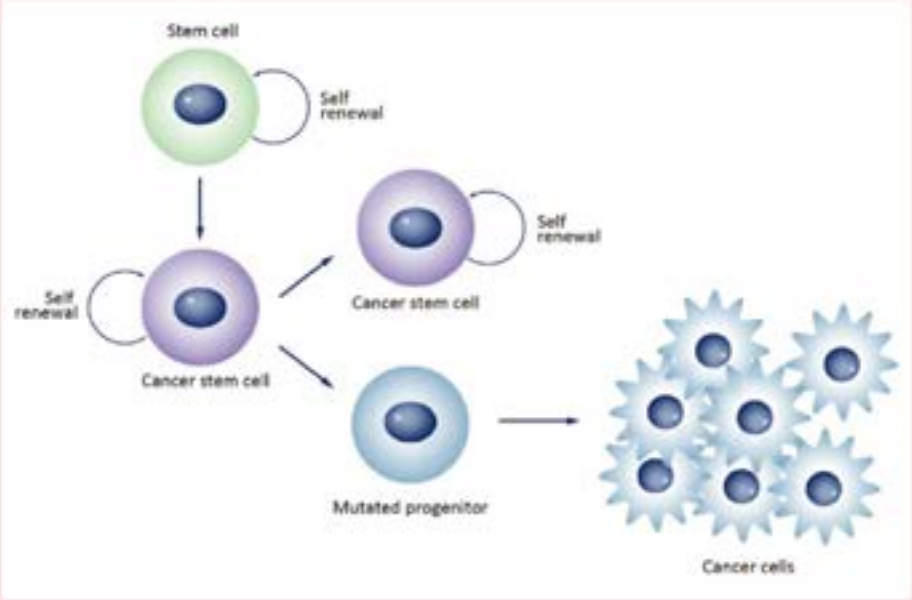

Figure 3: Cancer stem cells CSCs

Classification of Cell Therapies:

The following classifications are introduced for technologies that involve cells in various ways to treat diseases (figure 4) and a brief description of each technology is discussed in the following section.

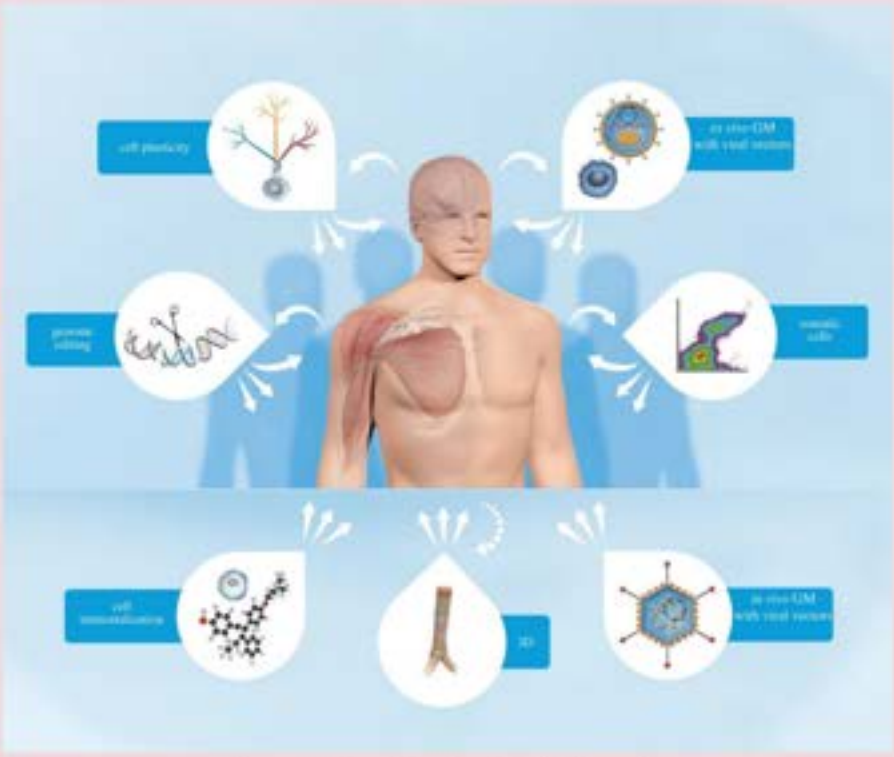

Figure 4: Classification of Cell Therapies

1. Somatic cell technologies
2. Cell immortalization technologies
3. Ex vivo gene modification of cells using viral vector technologies
4. In vivo gene modification of cells using viral vector technologies
5. Genome editing technologies
6. Cell plasticity technologies
7. Three-dimensional technologies

1. Somatic cell technologies: This technique employs human body cells that have been purified, multiplied, and/or differentiated into a particular cell product that is then supplied to a patient for a particular treatment without the need of any additional technology. Thus, despite the many cell types covered in this technology category, the translational obstacles are comparable from a technological standpoint. (Figure 5).4

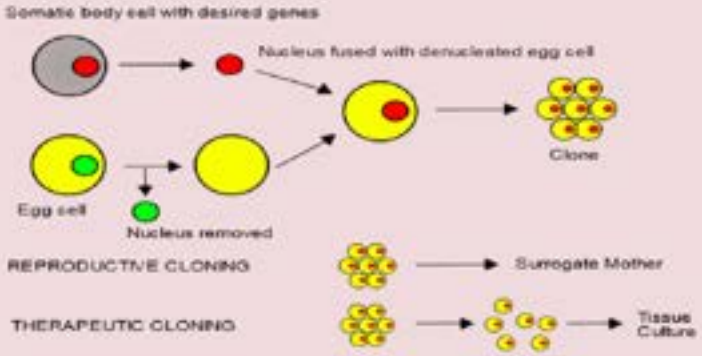

Figure 5: Somatic cell technologies

2. Cell immortalization technologies: The brain stem cell line CTX is the most well-known example of this technological field. CTX is a clonal cell line derived from foetal cortical brain tissue that has a single copy of the c-mycERTAM transgene that was spread by retroviral infection. (figure 6).5

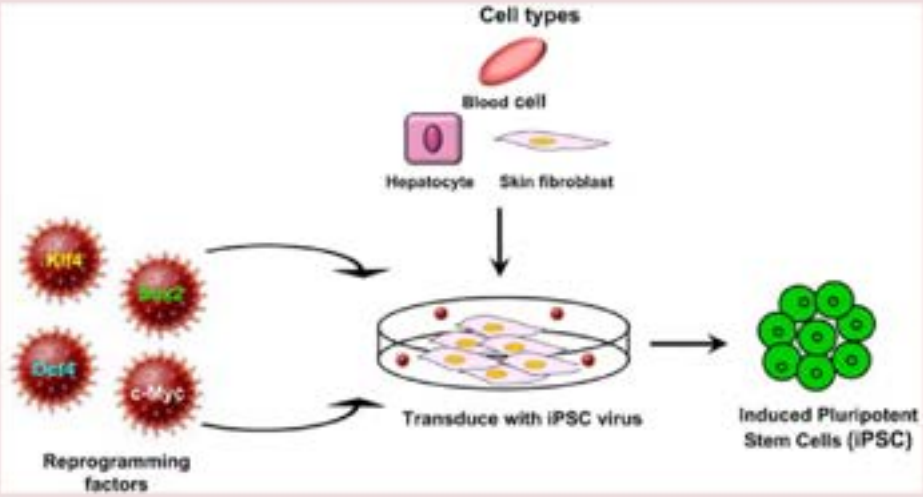

Figure 6: Cell immortalization technologies

3. Ex vivo gene modification of cells using viral vector technologies: T cells, HSCs, and MSCs are the most often employed cell types for ex vivo gene changes in cell therapy, with viral vector technology. Gene-modified MSCs are just beginning their first clinical studies for conditions like advanced cancer, whereas gene-modified HSCs have shown promise in the treatment of conditions like adenosine deaminase severe combined immunodeficiency disease (ADA- SCID). (Figure 7).6

4. In vivo gene modification of cells using viral vector technologies: Direct implantation of genetic material into a living organism is referred to as in vivo gene therapy. Although numerous delivery strategies are being developed, the most popular one involves modified viruses that contain targeted viral vectors and are infected in vivo to enter human cells shown in (Figure 7).7

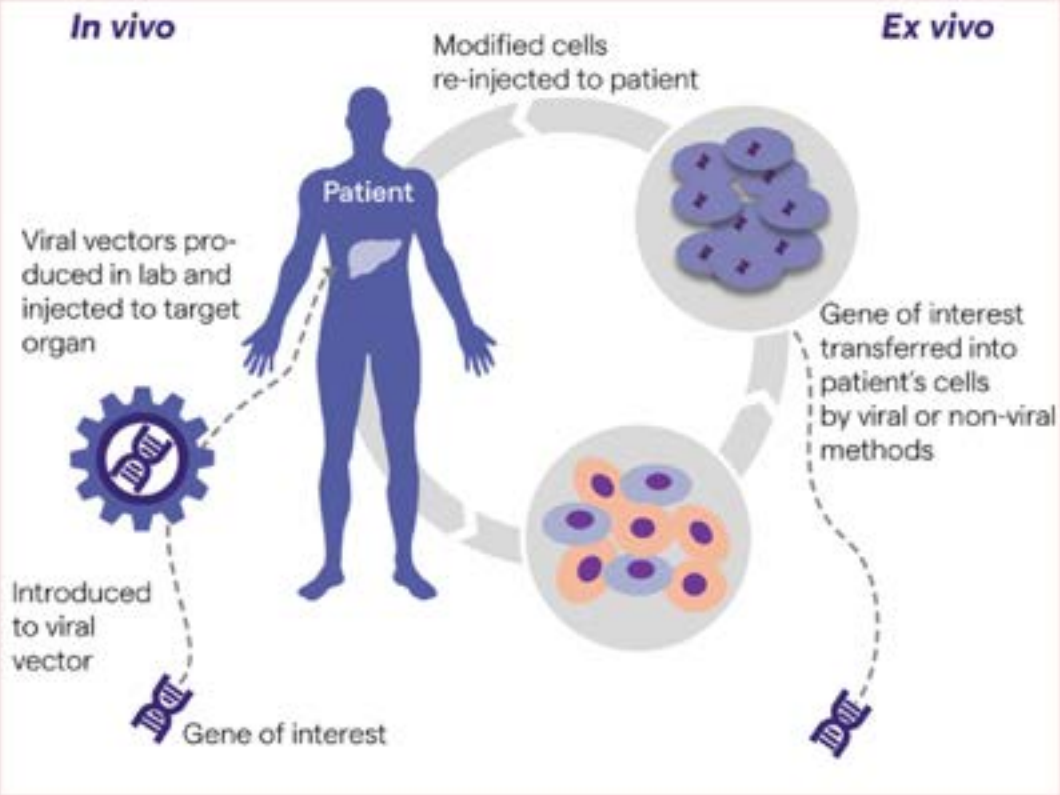

Figure 7: Ex vivo and in vivo gene modification of cells using viral vector technologies

5. Genome editing technologies: Genome editing, also known as gene editing, refers to a range of scientific techniques that enable the modification of an organism's DNA. At specific sites in the genome, these technologies enable the addition, removal, or modification of genetic material. Many diverse cell types and creatures have made substantial use of transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and mega nucleases for genome editing. shown in (Figure 8).8

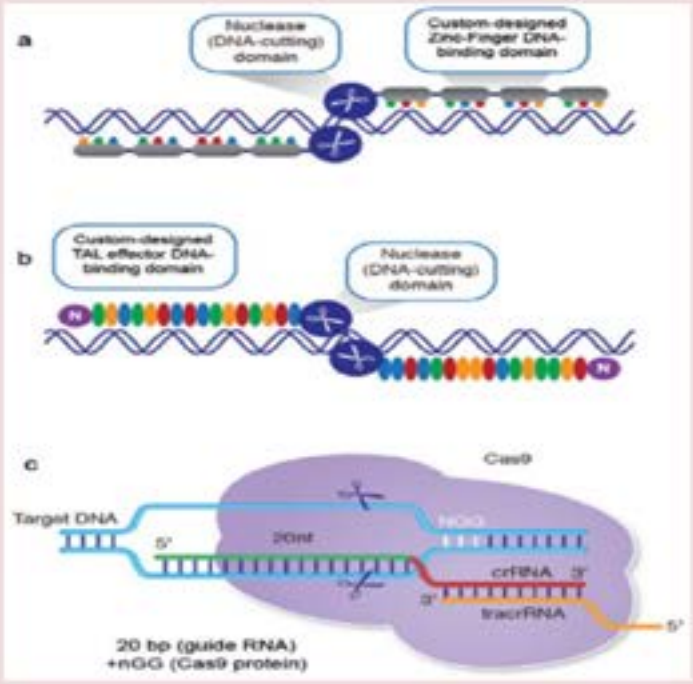

Figure 8: Genome editing technologies

6. Cell plasticity technologies: The capacity of a cell to reprogram and alter its phenotypic identity is referred to as cell plasticity, also known as lineage plasticity. Context-dependent cell plasticity takes place during embryonic development, tissue regeneration, and wound healing. (Figure 9).9

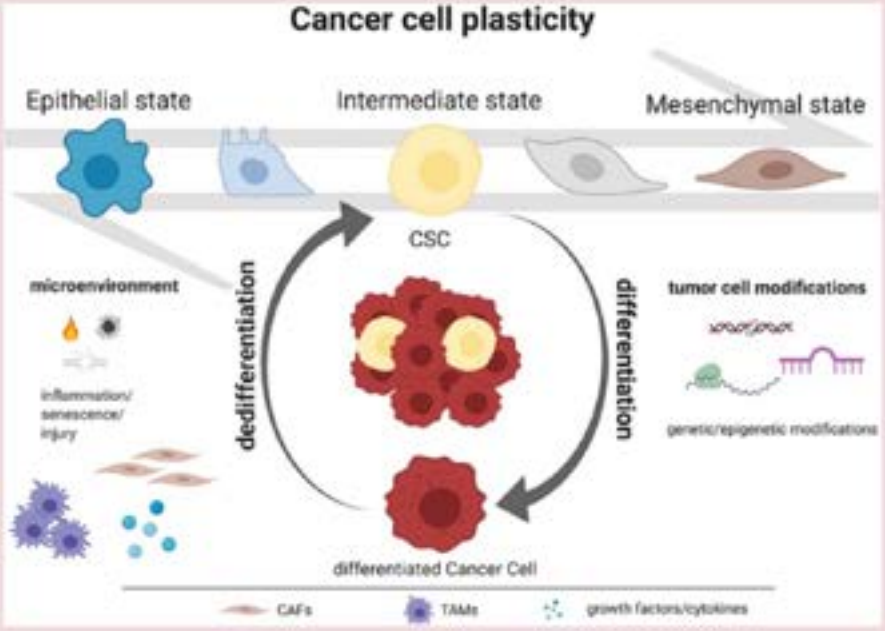

Figure 9: Cell plasticity technologies

7. Three-dimensional technologies: A 3D picture is a visual created by a computer that, when seen in computers, gives the impression of depth that an actual item would have. Movies, video games, visuals, and virtual reality (VR) initiatives like the metaverse frequently employ this technology.10

Clinical Benefit of Cell Therapy:

1. Treatment of Cardiovascular Diseases

Heart tissue can become oxygen-deprived from cardiovascular disorders, leading to the production of scar tissue that affects blood pressure or blood flow. According to study, the production of multiple growth factors enables adult bone marrow stem cells to differentiate into the cells needed to repair the heart and blood arteries.

2. Helps heal incisions and wounds

According to studies, stem cell treatment can assist promote the formation of new, healthy skin tissue, boost collagen synthesis, encourage the growth of hair following cuts or hair loss, and help replace scar tissue with freshly formed, healthy tissue.

3. Treatment of Neurodegenerative Diseases

Current advancements in the treatment of diseases like Parkinson's and Huntington's show that adult cells transplanted after brain traumas or cognitive degeneration can aid in the development of new brain neurons, cells, and synapses.

4. Autoimmune Diseases

A new treatment option for those with severe and incapacitating autoimmune diseases including lupus and rheumatoid arthritis is stem cell therapy. When your immune system assaults your body's own tissues, cells, and organs, autoimmune illnesses develop. Stem cell treatment is a minimally invasive method used at ASAP that aims to minimise recovery time.

5. Orthopaedic Conditions

This therapy can help those who are in pain due to orthopaedic disorders, spine illness, or sports injuries. ASAP addresses the fundamental causes of pain while also providing a chance for healing and long-term comfort.11

Conclusion:

Cell therapy is a growing industry that includes stem cell- and non-stem cell-based unicellular and multicellular therapies. These treatments differ greatly in terms of their traits, sources of isolation, and applications. Another crucial element that has to be taken into consideration from the beginning of the development process is the potential future cost and reimbursement of new cell treatment. Overall, the quickening pace of science in this field and the emergence of cell-based medications that are revolutionizing patient care will continue to spur development, translation, and eventually commercialization.

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Novel cell-based therapies for diabetic retinopathy


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1. Introduction

Diabetic retinopathy (DR), a common complication of diabetes mellitus, is the major cause of blindness in the middle-aged and elderly populations, affecting approximately 20% of diabetic patients [1]. The retina is a hyper metabolically active tissue that demands a dynamic interaction of cells ranging from light-sensing photoreceptors to neurons transferring electrochemical signals to the brain via glia and vascular tissue. The function of neurons is dependent on the complex interdependence of retinal cells, which includes the formation of a blood-retinal barrier (BRB). Diabetes has a negative impact on this dynamic system because it alters normal cell-cell interactions, resulting in profound vascular abnormalities, blood-barrier loss, and impaired neuronal function. The lifetime risk of developing DR in type 2 diabetes mellitus (T2DM) patients is 50-60% compared to 90% in type 1 diabetes mellitus patients (T1DM) [2].

2. Pathophysiology of DR

2.1 Inflammation, Oxidative stress, and Metabolism

Hyperglycemia is an important contributor to the development of diabetes mellitus. The hexosamine pathway, advanced glycation end products accumulation, polyol pathway, protein kinase C pathway, and polymerase activation are all identified as underlying mechanisms as to how elevated blood glucose leads to DR. These pathways cause metabolic dysfunctions, which lead to additional insults and progression of DR [3]. The pathophysiological demonstration of DR is caused by metabolic dysregulations, oxidative imbalance, and inflammatory imbalance.

2.2 Cellular degeneration

The early loss of retinal function and peripheral nerve involvement in DR patients cannot be explained solely by micro vasculopathy. Hence, further exploratory research in this regard suggests that retinal ganglion cells and amacrine cells are the first neurons to undergo apoptosis and that the apoptotic rate of photoreceptors is also increased. Since vascular and neural impairments are both present in DR pathology, the concept of the neurovascular unit (NVU) was proposed to combine all of the above theories [5]. Autoregulation serves one of the most significant physiological roles of the NVU in maintaining normal visual function by

matching changes in metabolic activity with changes in retinal blood flow. Such a phenomenon is found to be impaired in asymptomatic early-stage DR patients, implying that NVU dysregulation may be central to DR pathogenesis [6].

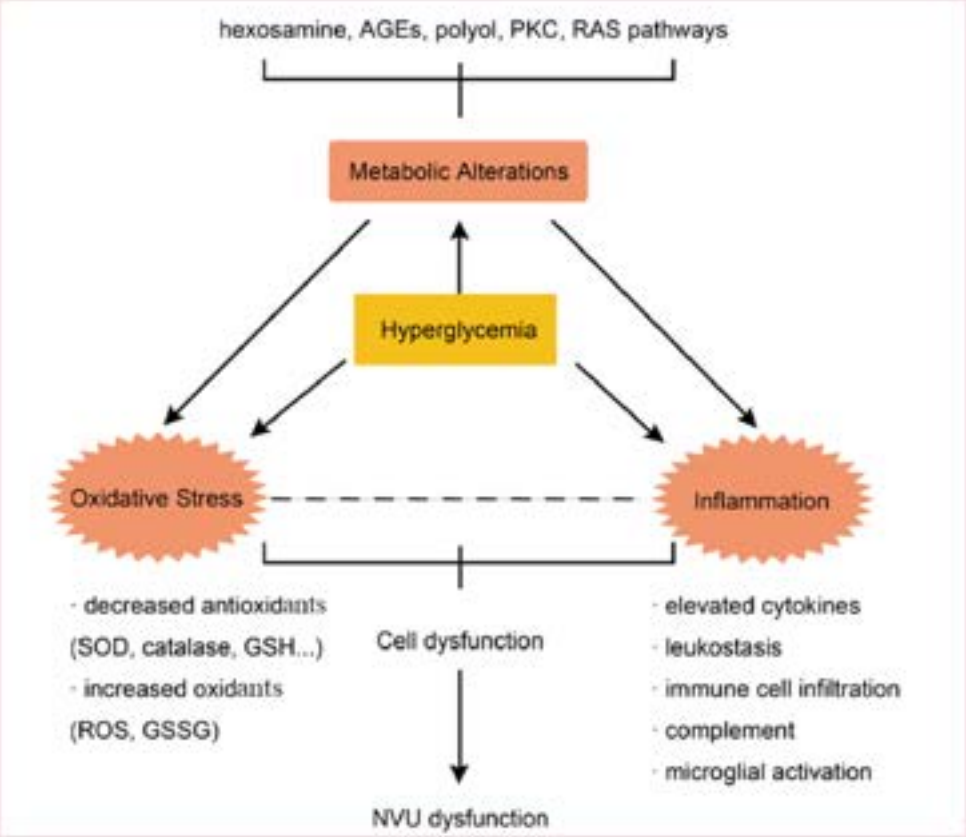

Figure 1: Metabolic dysregulation, inflammation and oxidative stress-based pathophysiology of DR [4]

2.2 Cellular degeneration

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3. Need for cell-based therapies

Processes involved in the pathogenesis of DR such as metabolic dysregulation, oxidative stress, and inflammation alter the neurovascular functions of the retina. DR interventions now include preventive strategies and interventions such as corticosteroids, anti-vascular endothelial growth factor (VEGF) agents, laser photocoagulation, surgeries, and so on. However, these strategies rarely reverse the diabetic retina's radial pathological changes, let alone the side effects of invasive surgeries and recurring injections, resulting in a poor long-term prognosis of

DR. As a result, novel treatments, primarily based on cellular and genetic interventions, with the goal of achieving long-term and effective disease reversal are being researched, providing new hope for DR treatment [4].

4. Various cell-based therapies utilized in DR

Various cells such as Endothelial Progenitor Cells (EPCs), Pluripotent Stem Cells (PSCs), Embryonic Stem Cells (ESCs), Induced Pluripotent Stem cells (iPSCs), Human Mesenchymal Stem Cells (MSCs) have been utilized in cell-based therapies for vascular regeneration owing to their peculiar characteristics.

4.1 Endothelial Progenitor Cells

Endothelial progenitor cells (EPCs) are circulating cells that are thought to play a crucial role in tissue regeneration by boosting blood vessel repair and assisting in the reperfusion of ischemic areas [7]. Asahara et al., 1997 pioneered the use of adult stem cells to regenerate blood vessels. Postnatal neovascularization was thought to be solely based on fully differentiated Endothelial Cells (ECs) derived from pre-existing blood vessels. Asahara, on the other hand, demonstrated that putative hematopoietic precursor cells (CD34+, Flk-1+/KDR+) from human adult circulating blood cells can differentiate to ECs in vitro and termed them "endothelial progenitor cells (EPCs)" [8]. These cells are more resistant to oxidative stress than other differentiated and mature endothelial cells. The elimination of retinal ischemia caused by diabetic retinopathy is likely to be part of a successful therapeutic method utilizing these cells in the treatment of DR [9].

4.2 Pluripotent & Induced Pluripotent Stem Cells

From day 5-7 of the embryo, stem cells can be obtained from blastocysts before implantation. Pluripotent cells are capable of differentiating into any type of mature cell. In recent years, reprogramming techniques have made it possible to create stem cells from somatic cells as well; these cells are known as induced pluripotent stem cells [10]. iPSC technology enables the generation of patient-specific cells, avoiding some of the ethical concerns associated with ESCs, allograft rejection, and immunogenicity, which also allows scale-up production of the desired cell lineage, generating new prospects for regenerative medicine [11]. Using hematopoietic progenitor cells generated from iPSC in vitro, researchers were able to restore multiple lineages in irradiated genetically identical adult recipient mice [12]. Damage to the tight junctions of the retinal pigment epithelium in diabetic animal models can destroy the retinal pigment epithelium barrier, indicating that cell replacement therapies using retinal pigment epithelium cells may be important for the treatment of diabetic retinopathy [13].

4.3 Embryonic Stem Cells

ESCs are deduced from the inner cell mass of blastocyst-stage embryos and have the capacity to self-renew and differentiate into all adult cell types derived from the three embryonic germ layers. Researchers found that transplanting human ESC (hESC)-derived retinal cells into the subretinal space of adult mice promoted the differentiation of hESC-derived retinal cells into functional photoreceptors and improved light responses in these mice [14]. Despite the potential benefits of ESC in retinal replacement therapies, ethical and immune rejection concerns still need to be taken into consideration.

4.4 Human Mesenchymal Stem Cells

Human adult MSCs, found in a wide range of tissues including bone marrow and adipose tissue, are a type of adult multipotent stem cell with a more limited differentiation potential and self-renewal ability. These cells have been utilized in neuro-retinal degenerative diseases such as DR owing to their ability to secrete neuroprotective growth factors such as basic fibroblast growth factor (bFGF) and ciliary neurotrophic factor (CNTF). When injected locally or systemically, both bone marrow and adipose-derived MSCs have been shown to differentiate into photoreceptors and retinal pigment epithelium in disease models [15,16]. In vivo studies have been conducted to further investigate the potential of EC-derived MSCs, which have been shown to improve muscular angiogenesis and blood perfusion restoration in a mouse hindlimb ischemia model [17].

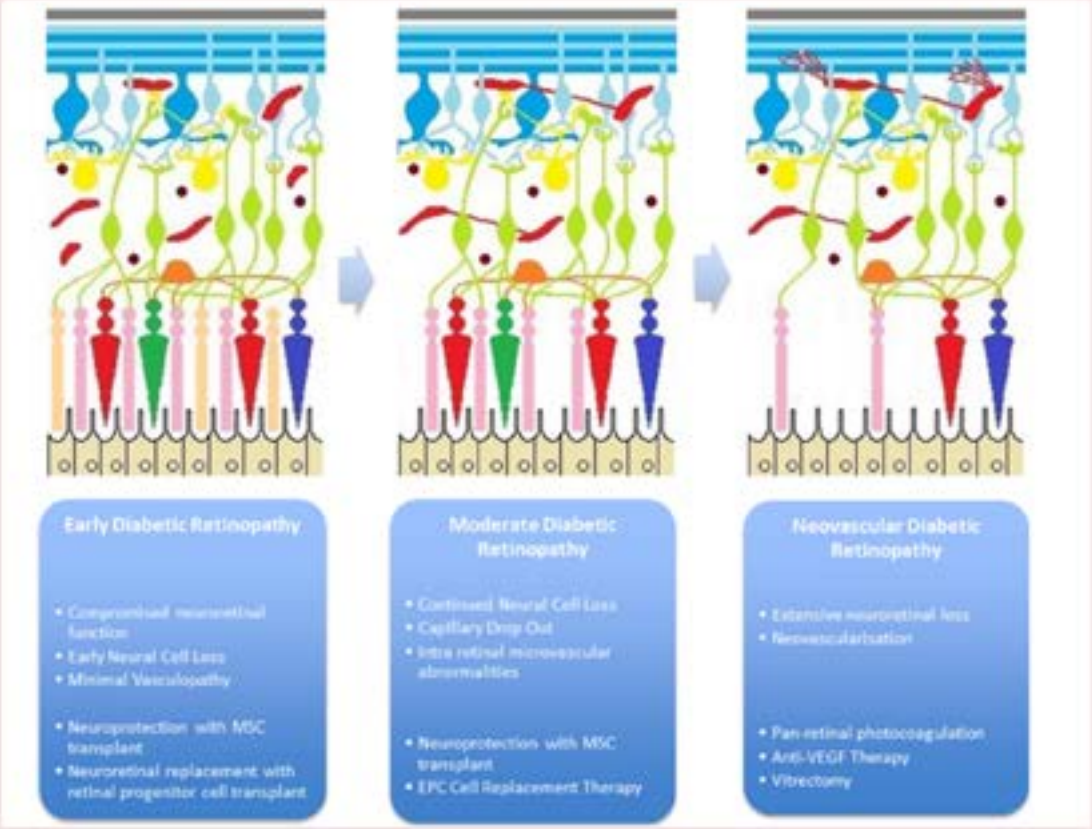

Figure 2: Use of stem cells derived at different stages of DR. Providing MSC-derived neuroprotection and neuroretinal cell replacement early in the course of the disease may be beneficial. EPC-derived vascular regeneration may be beneficial even in the advanced stages of the disease [18].

5. Conclusion

In contrast to the current end-stage approaches to diabetic retinopathy, cell-based therapy may offer an exciting new approach. In order to improve vascular repair, reverse ischemia, lessen hypoxic/inflammatory stimuli, and stop the progression of these diseases to their late, sight-threatening stages, this strategy is intended to target early/intermediate stages of vaso-degeneration. However, there remain some drawbacks to be looked after such as allograft rejection, immunogenicity, and production scale-up issues along with their inefficiency in the later stage of the disease.

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Therapies for Type 1 Diabetes Mellitus with Major Emphasis on Cell-based Therapies


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1. Introduction

Diabetes mellitus is one of the metabolic disorders characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion alone and or insulin action. The effects of diabetes mellitus are long-term damage, dysfunction, and failure of various organs. Some of the signs and symptoms of diabetes mellitus are increased thirst and hunger, frequent urination, blurred vision, frequent infections such as skin and vaginal, etc.

There are main two types of diabetes mellitus including insulin-dependent or juvenile diabetes or type 1 diabetes mellitus (T1DM) and insulin-independent or adult-onset or type 2 diabetes mellitus. T1DM is the most prevalent chronic autoimmune ailment characterized by a lack of insulin secretion due to pancreatic β-cells damage that results in hyperglycemia. Globally, around 8·4 million individuals were reported to be affected by T1DM, of which 18% (1·5 million) were younger under the age of 20 years, 64% (5·4 million) were between the age of 20–59 years, and 19% (1·6 million) were of 60 years or older (1). The International Diabetes Federation predicted the prevalence of T1DM as 643 million by 2030 and 783 million by 2045 (2).
The different kinds of treatment strategies including insulin replacement therapy, transplantation therapy, and cell-based therapies are reported for the management of T1DM. Recently, advanced cell-based therapies are found to be promising for the effective management of T1DM.

2. Treatment approaches for type 1 diabetes mellitus and associated challenges

Diabetes devoid of suitable treatment can cause various acute (diabetic ketoacidosis, hypoglycemia) and chronic (diabetic retinopathy, diabetic nephropathy, and cardiovascular disease) complications (3). The cost of diabetes treatment is anticipated to increase significantly by 2030 across various corners of the globe including low and middle-income countries (4). The different types of treatment strategies used for T1DM are clinical evidence-based and cell-based therapies (Figure 1).

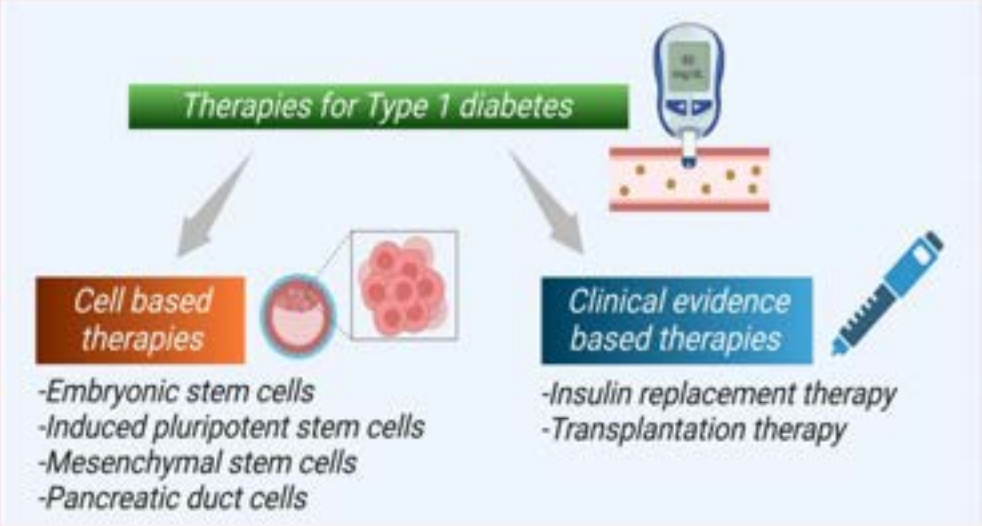

Figure 1: Different treatment strategies for type 1 diabetes mellitus

2.1. Insulin replacement therapy

T1DM is mainly associated with complete loss of insulin due to the damage of pancreatic β-cells. Inadequate islet cell repair processes are another issue that eventually compromises glycemic control. Thus, insulin replacement is a first-line therapy for T1DM. Various short and long-acting insulin preparations are widely used. However, hypoglycemia is a serious side effect associated with this therapy that can cause tachycardia, cardiac arrhythmias, sweating, coma, and death. In addition, other bottlenecks associated with insulin replacement therapy are invasive procedures, weight gain, dermatological reactions, hypersensitivity (systemic or local), and gastrointestinal distress (5). The use of artificial pancreas is also approved by Food and Drug Administration (FDA). However, excessive cost of equipment and sensor, scar tissue formation by frequent micro-needle insertion, and early sensor failure are chief drawbacks of this approach (6). Nowadays, the applicability of this therapy is enhanced via self-glucose monitoring devices and a new method of administering oral insulin using a self-orienting millimeter-scale applicator (7).

2.2. Transplantation therapy

Recently, transplantation of the pancreas and islet has shown great promise in the treatment of T1DM by restoring the normal regulation of blood glucose. However, the risk of chronic pancreatitis in pediatrics is a chief challenge associated with transplantation therapy. The clinical applications of these therapies have also been limited owing to the scarcity of pancreas and islets obtained from human organ donors, transplantation-related difficulties, huge costs, and inadequate procedural accessibility. Moreover, infusion methods of islet transplantation may cause portal vein thrombosis or intraperitoneal bleeding and some interim immunosuppression troubles (5).

2.3. Cell-based therapies

Cell-based therapies such as stem cell-based therapies have shown great potential in the management of T1DM successfully by overcoming the drawbacks of the aforesaid approaches. Stem cells serve as a potential source of providing glucose-responsive insulin-producing β-cells continuously and it also has the capacity to increase the survival and functionality of transplanted islets.

2.3.1.Human embryonic stem cells (hESCs)

The hESCs produce somatic cells in a developing embryo. These hESCs can be employed to develop new β-cells for transplantation into T1DM patients. Nowadays, it is very simple to differentiate between the insulin-producing β-cell, endocrine progenitor, and pancreatic progenitor via forced expression of pancreatic transcription factors. Thus, this strategy can be efficient to develop insulin-producing β-cells (8-9). In the United States, the phase 2 trial study is undergoing to evaluate the effect of pancreatic progenitors derived from hESCs in T1DM patients (NCT02239354). In another interesting study, Gopika et al., have developed mature β-cells from hESCs in vitro and transplanted them into diabetic mice. They observed a significant increase in metabolic activity, structural maturation of mitochondria, and a substantial decrease in hyperglycemia in diabetic mice in vivo following transplantation of matured β-cells (10).

2.3.2 .Induced pluripotent stem cells (iPSC)

These cells are generated from somatic cells. The development of autologous iPSC technology has shown the capability to separate several patient-specific iPSCs from adult somatic cells into functional β-cells. These iPSC exhibited characteristics similar to embryonic stem cells and similar pluripotent features also. Alipio and co-workers investigated the potential of pancreatic β-cells derived from iPSC in a diabetic mouse. The transplantation of iPSC caused the generation of β like cells which secreted insulin in response to glucose and corrected hyperglycemic phenotype in mouse models. Thus, the obtained outcomes revealed the clinical potential of reprogrammed somatic cells using iPSC in the treatment of diabetes T1DM (11).

2.3.3 .Mesenchymal stem cells (MSCs)

MSCs are the most generally used cell-based therapy in T1DM due to their diverse sources, simple isolation process, low immunogenicity, and self-regenerative capacity (12). Moreover, MSCs-based therapy has become incredibly popular in the treatment of T1DM owing to its capacity to control fibrosis, tissue generation, and modify immune response (13). El-Sawah et al., investigated the effect of MSCs on diabetic rats. They noticed a significant increase in the insulin and C-peptide level in serum thereby a decrease in hyperglycemia following treatment with MSCs. Additionally, MSCs caused a significant decrease in the serum level of kidney and liver function markers in diabetic rats, revealing their renal-hepato defensive benefits in T1DM (14). Thus, better effectiveness was observed with MSCs against T1DM with no sign of an adverse effect.

2.3.4 .Pancreatic duct cells

The exocrine cell compartment of the pancreas is composed of acinar and duct cells. It is reported that functional insulin-producing cells can be developed using rat exocrine tissue in vitro via transdifferentiation approach by the addition of epidermal growth factor and leukemia inhibiting factor to the media. The insulin-producing pancreatic duct cells developed via this approach can be useful in the efficient treatment of T1DM (15). For instance, Okuno and co-workers investigated the potential of these pancreatic duct cells obtained by the transdifferentiation process in diabetic rodents. They concluded pancreatic duct cells as a promising source of autologous transplantable insulin-secreting cells for the treatment of T1DM (16).

3. Conclusion

T1DM remains the foremost cause of kidney failure, blindness, and stroke. T1DM patient's life quality has improved with various fast- and long-acting insulin analogues, but there are still many obstacles to overcome. The huge cost and immunosuppression have limited the applications of transplantation therapy. Stem cell-based therapy has emerged as an advanced approach to the treatment of T1DM. However, the clinical trial outcomes of this approach are still lacking and a lot of issues and technological challenges need to be addressed.

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Insight view of stem cells in treating Diabetes




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Cellular therapy (CT) is an advanced therapy of transplantation of human cells to replace or repair damaged tissue or cells. In order to initiate an action, 'stem cells' should possess two essential characteristics. Firstly, stem cells should pertain an ability to unlimited self-renewal and to produce same photo copy as the originating cells. Secondly, capability of giving rise to specialized cell type which is part of the human body. CT is also termed as cell transplantation or cryotherapy. Stem cells are the master cells that act as fundamental building blocks to the human body. With the new technologies, cell therapies have advanced in innovation products and limitless imagination and many different types of cells that are injected, grated or implanted into a patient as part of therapy in a variety of diseases and conditions to regenerate diseased tissues [1].

During 19th century cell therapy was originated when scientists experimented by injecting animal material in an attempt to prevent and treat illness. Further, in mid of twentieth century positive benefit of human cell therapy led to a successful bone marrow transplantation. Now, it has become a regular practice for treatment of patients who have compromised bone marrow after disease, infection, chemotherapy and radiation. Stem cells and cell transplantation have gained interest for treating wide range of diseases, in particular to degenerative, regenerative medicine, drug discovery, toxicology and immunogenic pathologies.

The stem cells based therapy is classified based on the therapeutic indication as cardiovascular, neurological, ophthalmic; wherein the cells are taken from the donor person and administered to the same individual (autologous) or transferred to another (allogeneic) and obtained from animal source to human (Xenogeneic). The different types of cells are human embryonic cells, neural stem cells, mesenchymal stem cells, hematopoietic stem cell transplantation and differentiated or matured cell transplantation. The stem cell comprises of many distinct cell types, among animal form are embryonic and adult are two main modifiers for the development of new stem cells.

These stem cells are coined 'somatic' as they are basic building blocks of research but still lack due to differences in embryonic stem cells and adult stem cells. Based on the biological properties, stem cells are divided into two categories pluripotent stem cells and multipotent stem cells. These stem cells have definite sources, characteristics, cell differentiation and therapeutic applications. The other classification is based on the translational channels that need to overcome the therapy available to the patients. Further, to facilitate analysis the stem cell therapy is classified based on various technologies such as somatic cell, cell immortalization, ex vivo gene modification of cells using viral vector, in vivo gene modification of cells using viral vector, genome editing, cell plasticity, three-dimensional and lastly combinations of the above technologies [2].

Diabetes Mellitus (DM) is a metabolic disorder wherein body cannot take up sugar (glucose) resulting in inappropriate blood glucose levels. Glucose is required by the cells for energy production and normal functioning. This glucose enters to blood stream from the food we eat and it is further carried to cellular level by Insulin. The diabetes is developed when the body cannot prepare insulin or can't respond to the insulin. Insulin is exogenously used to control of blood glucose in diabetic patients. DM is mainly classified into two categories – Diabetes Type 1(TIDM) and Diabetes Type 2 (T2DM). The number of DM patients in the world is increasing in recent years. If untreated, diabetes is one of the direct causes for death and 49% of deaths are due to diabetes occurred before the age of 70 years [3].

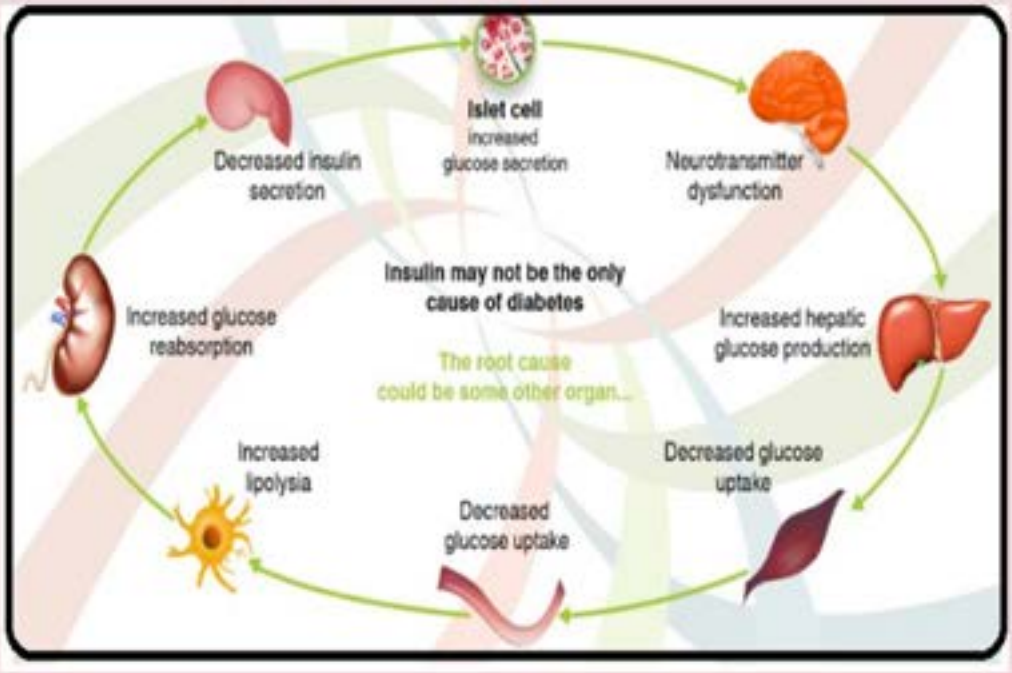

Figure 1: Causes for DM.
[Figure obtained from https://www.giostar.com/about-us/publications-information-giostar-usa/]

Stem cell therapy for DM

Stem cells therapy holds immense promise in treatment of patients with DM. The discovery of Insulin has enhanced the life span of T1DM in patients. The isolated islet/pancreas removed from a cadaveric pancreas transplantation have provided in vivo evidence in reestablishing β cells. However, the drawbacks of pancreas shortage have driven scientists to generate induced pluripotent stem cells (iPSCs).

The development of pluripotent adult stem cells into induced pluripotent adult stem cells, is more accessible method for new approaches. Much more positive results were derived from hematopoietic stem cells (hECs), embryonic stem cell (ESC) and adult stem cells. With upgrade of the research over time, scientists are now aware to persuade a cell and produce insulin with one step. The blood glucose concentration is controlled with specialized islets cells which controls the process and regulation of insulin secretion. The approaches to initiate stem cell production differ with different starting points. Differentiation is process in which stem cell reproduces itself and can then also divide asymmetrically. Stem cells were initially available only from embryos. Non-embryonic stem cells are now obtained without too much difficulty from umbilical cord, neonatal tissue and also from adult bone marrow, skin and fat. These cells are further expanded and made to different types of cells [4,5].

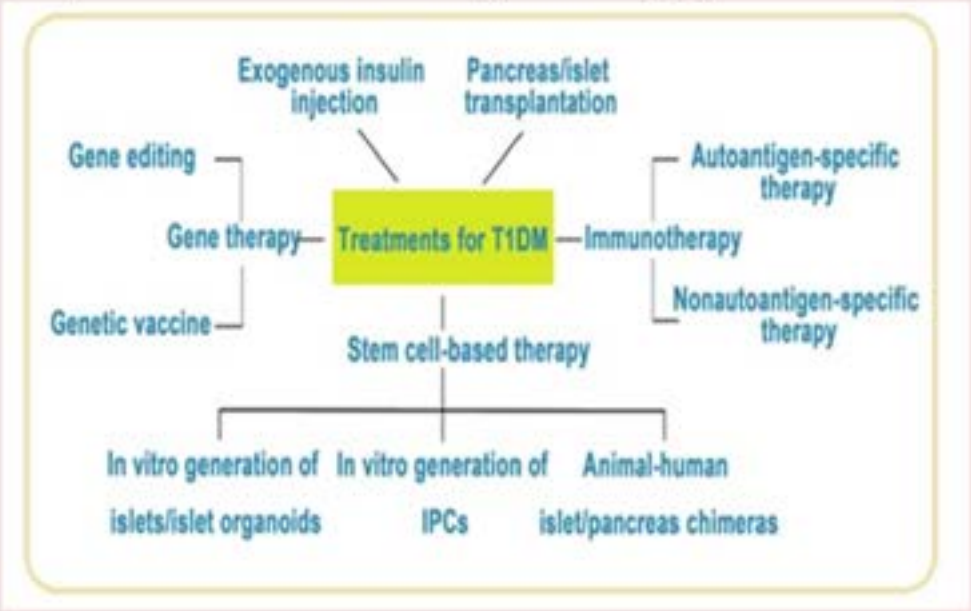

Figure 2: Treatment of T1DM.
[Figure obtained Gruessner RW, Gruessner AC. The current state of pancreas transplantation. Nat Rev Endocrinol. 2013;9(9):555–62.]

In 2006, Yamanaka was able to produce pluripotent stem cells from mouse neonatal and adult fibroblast cultures by adding a cocktail of four defined factors. This innovation led to a series for further innovations and development process which is repeatable with human tissue. Despite this, there has been very little success in directing differentiation of iPSCs to form islet beta-cells in sufficient quantity that will secrete and stop secretion in response to changes in blood glucose levels. Another approach that has been tried is combinational gene therapy with stem cells. With the use of human insulin gene construct and introduced ex vivo or in vivo into cells by direct electroporation (in ex vivo cells obviously) or by viral vectors. The adenovirus, adeno-associated virus and various retro viruses have been studied [6].

Type 2 Diabetes mellitus is characterized by insulin resistance, which may be combined with relatively reduced insulin secretion. T2DM patients who receive stem cell therapy receives various system targeted administration method like intravenously which delivers via a vein and distributed evenly and direct injection site, this delivers to the site that need repair like nerve damage or neuropathy, ischemic limbs in various areas to the body [7]. T2DM provides access to treatment that utilizes patients stem cell isolated from their own bone marrow [8].

There are multiple of inherent benefits afforded for the utilization of bone marrow component function in various diverse to innate therapeutic capacities. Hematopoietic stem cells are evident in treating chronic inflammatory and auto-immune conditions in red and white blood cells. In addition to HSCs, the mesenchymal stem cells (MSCs) are used to treat tissue damage and also exert protective cellular immunomodulatory effect [9].

Gestational diabetes resembles T2DM in several aspects wherein inadequate insulin secretion and responsiveness is seen. T2DM occurs in 2-10% in pregnancies and this may be improved or disappeared after delivery. Umbilical cord blood and hematopoietic stem cell (HSC) are used to treat total nucleated count during pregnancy abnormalities. Finally, there should be caution in selection of treatment using cell therapy. The cure of hyperglycemia, response to glucose tolerance test, evidence of appropriate insulin secretion, weight gain, prompt return of diabetes transfecting gene or insulin producing cells are removed, streptozotocin treated animals are not recommended (it may be pancreas or islet) and insulin stored stem cells. These mentioned 'Seven Pillars of Credibility' should be considered as essential criteria in evaluation and assert success in the use of stem cells or gene therapy [10].

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Engineering CAR T Cells for their Anti-tumor activity




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Introduction

Cell-based therapeutics involve replacing or repairing damaged cells by administering new, viable and healthy cells. It began in the nineteenth century and since then it has drawn significant attention from researchers as a promising treatment strategy for treating a wide range of diseases. With newer technologies and a vast imagination, its application has been wide and varied. It is generally used in the prevention of autoimmune and immunological diseases, along with regenerative medicine.(1)

CAR-T cell therapy

T cell is a part of immune system which recognizes foreign cells and kills it, thus protecting the body. This understanding of immune system can be used to fight against cancerous cells. The antigen on tumor cells gets recognized by T-cell receptors, which causes the cells to die. Certain developments have been made to increase the progression of immunotherapy one of them being CAR-T cell therapy which is also known as "a living drug". It is used for the treatment of B-cell lymphoma, follicular lymphoma, multiple myeloma, blood cancer, HIV, acute lymphoblastic leukemia, hematological malignancies etc. (2)

Structure of CAR-T cell

There are two regions in this receptor located outside the cell firstly the antigen-recognizing region, derived from monoclonal antibodies containing a variable heavy as well as light chain which interact with antigen present on targeted cells. Secondly, the hinge region present above the outer membrane of the T-cell provides flexibility to the receptor. Thirdly a transmembrane region has an α-helix structure that is hydrophobic in nature functioning to stabilize the CAR. Fourthly an intracellular T-cell messenger region which sends signals to the cell when a foreign antigen is recognized. (Fig.2)(3)

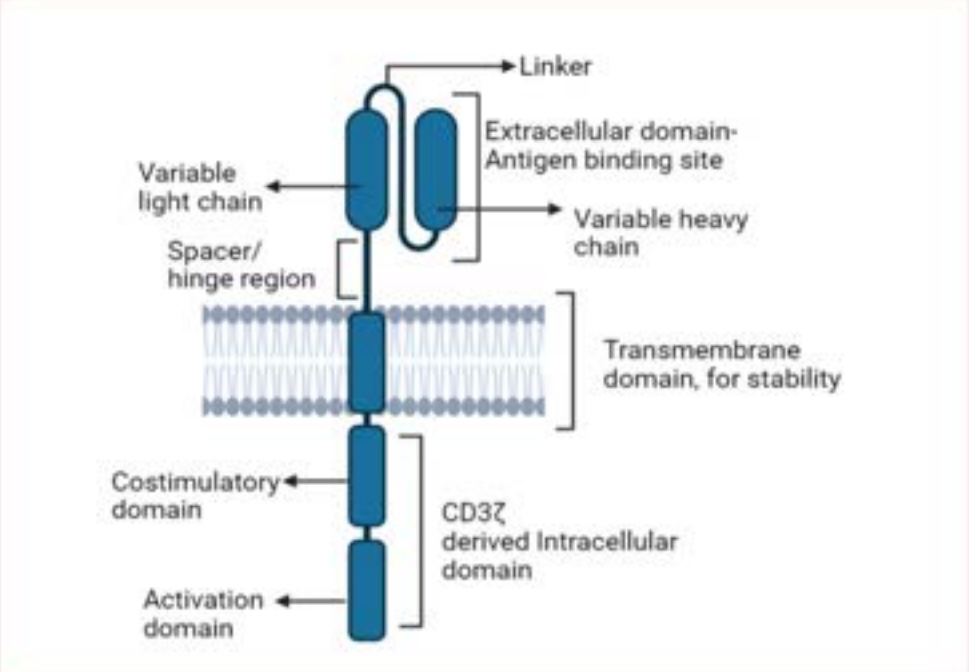

Figure 1: Structure of CAR

Mechanism

This treatment involves the modification of the T-cell, blood is removed from the patient to isolate the T-cells this procedure is known as pheresis. Then these T-cells are genetically altered with the help of a virus known as lentivirus containing the genetic information that encodes for the chimeric antigen receptor further these t cells are infected with the viral vectors which inserts the CAR gene causing the formation of chimeric antigen receptors (CARS) on the surface of T-cells. These CAR-T cells are further multiplied ex vivo and administered back to the patient intravenously. In the body these cells multiply, the receptors get attached to the target antigen and accumulation of CAR-T cells begins which leads to drying out of the tumor cell. (Fig.1) This therapy is customized for every patient according to the type of antigen present in the cancer cells. For instance, the cancer cells in the case of acute lymphoblastic leukemia have an antigen known as CD10. These CAR T-cell therapies are designed in a manner so that they attach the specific antigen only, in this case, the therapy won't work for cancer cells that don't have CD10 antigens.

The CAR T-cell therapy, not just only arms T-cells to inhibit the growth of tumor cells but also triggers T-cells to proliferate and multiply. For months, these cells stay inside the body of the patient and keep the tumor cells from growing. For improved results, it is applied in conjunction with radiotherapy and chemotherapy. It has shown promising results in patients with the reoccurrence of cancer.(4)

Side effect and treatment of CAR T-cell therapy

·Cytokine Release Syndrome (CRS)

As soon as the CAR T-cells start multiplying in the body, as a part of the immune system it releases cytokines into the bloodstream causing serious side effects including difficulty in breathing, high fever, nausea, diarrhea, vomiting, low blood pressure, etc. The emergence of CRS ensures that the therapy is 'on point' and it gets severe in case of patients with higher stages of cancer.

·*Immune Effector Cell Associated Neurotoxicity Syndrome (ICANS)*

The actual cause of these neurological side effects is yet not clear, the symptoms include twitching, impaired hearing, loss of balance, confusion etc. Other side effects include anaphylaxis, increase risk of getting serious infections due to a weak immune system, low sodium and potassium levels, and a decrease in RBC count which may elevate bleeding, infections and the patient is frequently exhausted.

·*Tumor Lysis syndrome (TLS)*

The lysis of tumor cells leads to the release of cell contents in the surroundings which cause damage to other organs like kidney failure, metabolic abnormalities, arrhythmias, etc.

·*Tumor toxicity*

The attack of CAR T-cells on the healthy cells having the same antigen as that of tumor cells causes tumor toxicity for e.g.- HER2(Human epidermal growth factor receptor), MSLN(Mesothelin), PSCA (Prostate stem cell antigen) are the antigens which are present in both healthy as well as tumor cells. The target specific approach is needed for the success of CAR T-cell therapy

The treatment strategies are still under extensive research, currently, tocilizumab (used to in the case of arthritis to suppress the activity of cytokines and IL-6) and steroids like dexamethasone are the treatment of choice. Methylprednisolone, siltuximab, and anakinra are used for severe cases of neurotoxicity associated with CAR T-cell therapy. Monitoring patients in ICU is advised for patients undergoing therapy. (5)

In cases where CAR T-cells toxicity (CRS, ICANS, TLS) is at a peak level immediate reversal of the therapy is required which is not fulfilled by the drugs used generally like rituximab. In order to address these challenges and increase the efficacy and application of CAR-T cell treatments, several new possibilities are being developed one of which being an 'OFF SWITCH'. These off switches will immediately inhibit the activity of CAR T-cells, eg- Protein derived SMASh (small molecule assisted shutoff CARs, introducing a tyrosine kinase inhibitor (Dasatinib) which prevented the activation of CAR-T cells in mice models.(6)

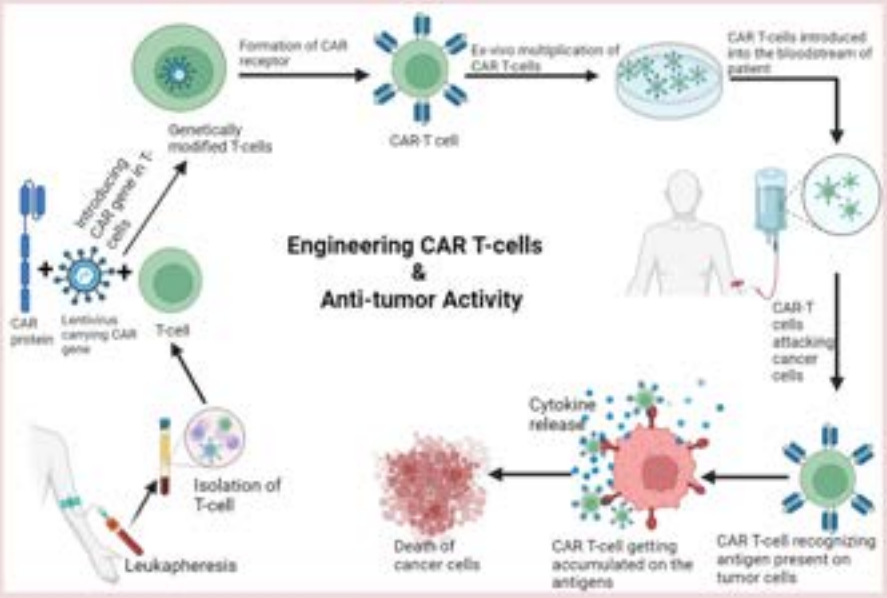

Figure 2: Engineering CAR T Cells and its Anti-tumor activity

Advancements in CAR T-cells

·Modulation in the structure of CAR T-cells

Improving the structure of CAR T-cells reduced the side effects according to a study done by National Cancer Institute, the substitution of the hinge and transmembrane regions with a protein fragment found in mice similar to that found in humans caused decreased release cytokines and was found to have the same efficiency to that of general CAR T- cells, remodeled CAR T-cells were able to attach to the antigens followed by lysis of tumor cells.

·Improving resistance to CAR T-cells

According to a study it was found that myeloma cells get rid of their surface antigens BCMA and protect themselves by getting attacked by CAR T-cells. To resolve this issue researchers introduced gamma-secretase inhibitors which increased the number of BCMA on tumor cells that were successfully killed by the CAR T- cells.

·Navigation of CART T-cells through solid tumors

The antigens present on solid tumor cells and those present in healthy cells are difficult to be distinguished. Other than this the tumor microenvironment (TME) is hostile towards CAR T-cells which makes their penetration into the cancer cells strenuous. A clinical study was conducted on the treatment of brain tumors with CAR T -cells by targeting the disialoganglioside (GD2) antigen present on tumor cells. With the subsequent treatment regimen small doses of modified CAR T-cells with GD2 were administered into the brain directly, which resulted in decreased tumor size and controlled other symptoms related to cancer. The ongoing duties of the T-cells are inhibited by the hostile tumor microenvironment due to hypoxic conditions, the release of toxic metabolites and reactive oxygen species (ROS), etc. To survive in these conditions "armored T-cells" are developed which release catalase into the TME to kill ROS.

·Controlling the metabolism of CAR T-cells

Several amino acids and their metabolites like L-arginine and ornithine respectively are required for the proper functioning of T-cells it prevents the metabolism and promotes their survival. There is an ongoing approach to developing ex vivo CAR T-cells having arginine residues, other attempts made in the same direction include genetic alteration of CAR T-cells with arginine synthesizing enzymes so that it can survive in the TME. Studies are still going based on the manipulation of glutamine metabolism in TME to promote the survival of CAR T-cells.

Vectors such as Lentivirus are used to deliver the genetic material to the T-cells but with development and research other vectors such as NK (natural killer) cells and CRISPR are under trials for their use

There are specific T-cell receptors that not only recognize antigens on the surface of tumor cells but also attack cancer cells by attaching to the antigens present inside the tumor cells. These receptors are under trials for use in conditions like sarcoma. (7)

Table 1. FDA-approved drugs under CAR T-cell-based Therapy(6)

Drug	Brand Name	Targeting antigen	Type of cancer
Lisocabtagene maraleucel	Breyanzi	CD19	High grade B cell lymphoma, Follicular lymphoma
Brexucabtagene autoleucel	Tecartus	CD19	Acute lymphoblastic lymphoma (ALL), Mantle cell lymphoma
Ciltacabtegene autoleucel	Carvykti	BCMA	Relapsed multiple myeloma
Idecabtagene vicleucel	Abecma	BCMA	Multiple myeloma
Axicabtagene ciloleucel	Yescarta	CD19	Follicular lymphoma
Tisagenlecleucel	Kymriah	CD19	Refractory B cell acute lymphoblastic lymphoma

Conclusion

Over the past few years, CAR T-cell therapy has been proven to be a highly effective treatment for patients with acute lymphocytic leukemia, hematological malignancies, HIV, etc. Several obstacles still hampering the treatment including off-target tumor toxicity, heterogenicity of tumor cells, and difficulty in penetration of CAR T-cells in solid tumor cells. Research is going on to use T-cells obtained from a healthy donor which will enhance the efficacy of CAR T-cell therapy. The newly designed CAR T-cells are taking the wheel for safer and more efficient use in treating lung and brain cancers.(8)

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CRISPR/Cas9 mediated genome editing: Applications and clinical studies





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Introduction

Eukaryotic species' genomes are made up of billions of DNA bases. The ability to modify these DNA bases at specific predetermined positions is extremely valuable not only in molecular biology, but also in medicine and biotechnology. Genome editing has long been a priority in molecular biology. The late 1970s discovery of restriction enzymes that usually defend bacteria against phages was a defining moment that fueled the era of recombinant DNA science. It was later discovered that the introduction of a double-strand break (DSB) at a target site increases the frequency of selective gene incorporation by many orders of magnitude. While artificially conceived meganucleases, followed by Zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), enhanced genome editing performance, re-design or re-engineering of a new category of proteins was needed to target different sites in the genome. The challenge in cloning and protein engineering ZFNs and TALENs hampered their widespread adoption by the scientific community. CRISPR has transformed the industry in this regard because it is as powerful as, if not more so than, existing editing tools (1). Furthermore, it is much simpler and more adaptable to use. A brief timeline of key events in the discovery and subsequent repurposing of CRISPR/Cas9 system for gene editing is mentioned in table 1.

What is CRISPR/cas9

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)–CRISPR-associated (Cas) (CRISPR–Cas) systems originate from Prokaryotes, where they serve primarily as a defensive mechanism against mobile genetic elements like phages and plasmids. It is now being utilized as potent a gene editing tool that allows highly specific and rapid modification of DNA. The CRISPR-Cas system, which has been modified for use in lab settings relies on three main components: a guide RNA (gRNA) a cas nuclease, and the target DNA. The various components are depicted in figure 1.

Table 1: A brief history of CRISPR/Cas9 gene editing system

Year	Discovery	Referenc
1987	The CRISPR sequence first identified, (not known as CRISPR)	(2)
2000	More clustered repeats of DNA identified in other bacteria and archaea, termed Short Regularly Spaced Repeats (SRSR)	(3)
2002	Term CRISPR-Cas9 published for first time	(4)
2005	Jennifer Doudna and Jillian Banfield started investigating CRISPR	(5)
2005	French scientists suggested CRISPR spacer sequences can provide cell immunity against phage infection and degrade DNA	(6)
2007	Experiments demonstrate for the first time the role of CRISPR together with Cas9 genes in protecting bacteria against viruses using the lactic acid bacterium *Streptococcus thermophilus*	(7)
2008	Created the first artificial CRISPR arrays—programming CRISPR to target four essential genes in lambda phage	(8)
2008	Proved that the target of Cas9 is DNA, not RNA. Recognized that CRISPR was essentially a programmable restriction enzyme.	(9)
2010	Showed that Cas9's nuclease activity cut DNA at precise positions encoded by the specific sequence of the crRNAs.	(10)
2011	Discovery of tracrRNA (trans-activating CRISPR RNA).	(11)
2011	Reconstitution of CRISPR in a Distant Organism.	(12)
2011-12	Reprograming Cas9 with custom-designed spacers in the CRISPR array to cut a target site of their choosing in vitro. In addition, Charpentier and Doudna showed that the two RNAs could function in vitro when fused into a single-guide RNA (sgRNA) (Lander 2016).	(13,14)
2011-13	Genome editing transferred to mammalian cells.	(15)
2018	First CRISPR-Cas9 clinical trial launched.	(16)
2020	Nobel Prize in Chemistry awarded 'for the development of a method for genome editing'. To Emmanuelle Charpentier and Jennifer Doudna.	(17)

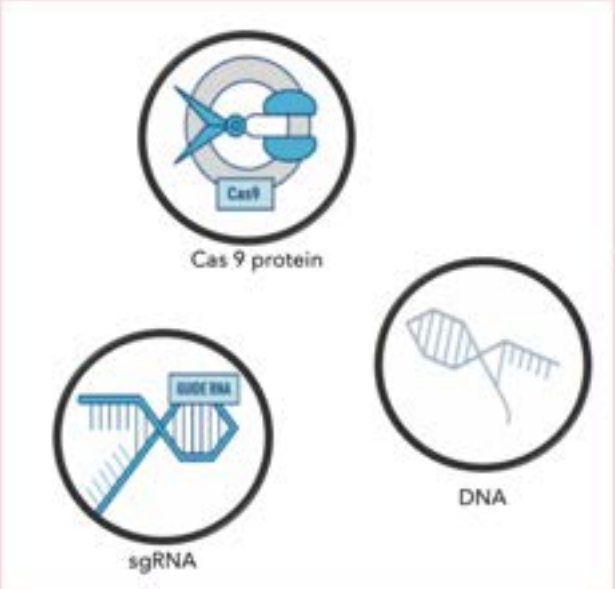

Figure 1 Main components of the CRISPR/cas9 system (19)

1. The guide RNA is a specific RNA sequence that recognizes the target DNA region of interest and directs the Cas nuclease there for editing. The gRNA is made up of two parts: CRISPR RNA (crRNA), a 17-20 nucleotide sequence complementary to the target DNA, and a tracrRNA, which serves as a binding scaffold for the Cas nuclease. The crRNA and tracrRNA can be combined to form a single guide RNA (sgRNA).
2. The CRISPR associated protein (Cas) is a bacterial RNA-guided endonuclease. It is directed to the specific DNA locus by a guide RNA (gRNA), where it makes a double-strand break. There are several versions of Cas nucleases isolated from different bacteria. The most commonly used one is the Cas9 nuclease from Streptococcus pyogenes (18).
3. The DNA sequence contains the sequence which we want to replace or the site where we want to insert our gene of interest. Once this DNA sequence interacts with the sgRNA-Cas9 complex, Cas9's HNH domain cleaves the DNA strand complementary to sgRNA and Cas9's RuvC-like domain cleaves the other DNA strand. It is necessary for this DNA sequence to show complementarity to the sgRNA and the presence of a PAM motif, for target recognition. This schematic has been diagrammatically depicted in figure 2.

Applications of CRISPR/Cas9 gene editing

Some of the many applications of this technology are as follows:

1. Generation of cellular/animal models:

The pathological analysis of different disorders relies heavily on genetically engineered animal models that mimic human diseases. Cas9-mediated genome editing has sped up the generation of transgenic models and broadened biological science beyond conventional, genetically tractable animal model organisms. CRISPR editing can be used to conveniently and rapidly model genetic disorders, research anatomy, disease development, and develop novel drugs for diseases by repeating the common mutations present in disease state populations. To prepare cell models and animal models (injection in zygote), direct transient transfection of cells with plasmids containing the CRISPR/cas9 system can be used. However, gene targeting has limited applications in some organisms due to time-consuming procedures and the lack of available embryonic stem cells (21).

2. Treatment of infectious diseases:

Given that the CRISPR-Cas system was designed to be an antiviral adaptive immune system in bacteria, it has the potential to be used to cure infectious diseases by eradicating pathogen genomes from infected individuals. According to recent research, the CRISPR-Cas9 mechanism will eliminate the HIV-1 genome and avoid new HIV infections. A sgRNA expression vector targeting the long terminal repeats (LTR, necessary for gene expression) of HIV-1 efficiently cleaves and mutates LTR target sites and suppresses LTR-driven viral gene expression when transfected into HIV-1 provirus-integrated human cells. Furthermore, this system has been shown to delete viral genes from host cell chromosomes (22).

3. Correction of genetic disorders:

This is a very exciting application of the CRISPR/cas9 technology for curing genetic diseases. the discovery and development of CRISPR/Cas9 has re-opened the door for gene therapy and changed the way scientists can approach a genetic aberration—by fixing a non-functional gene rather than replacing it entirely, or by disrupting an aberrant pathogenic gene.

This system has shown potential in efficiently correcting mutations in various animal models, and there are ongoing human clinical trials for curing genetic disorders like Beta thalassemia, Leber Congenital Amaurosis 10 (LCA 10) (22). A description of how CRISPR/Cas9 is used to treat LCA 10 is depicted in figure 3. Various genetic disorders, for which therapies based on CRISPR/Cas9 are currently in clinical trials, have been summarized in table 2.

Table 2. List of registered clinical trials of CRISPR/Cas for treatment of various diseases
HPV- Human Papilloma Virus; LNP- Lipid nanoparticle; AAV- Adeno-associated virus; HBB- Hemoglobin Subunit Beta; iHSC- induced hematopoietic stem cells; CAR: Chimeric antigen receptor

Disease condition	Ex vivo/ in vivo	Delivery system/ modification	Gene targeted	Route	Cargo	If in clinical trial-stage	ClinicalTrials.gov identifier	Sponsor
Refractory herpetic viral keratitis	In vivo	Lentivirus	Herpes Simplex virus type I	Corneal injection	CRISPR-Cas9 mRNA	Phase 1/2	NCT04560790	Shanghai BDgene Co., Ltd.
Human papillomavirus related malignant neoplasm	In vivo	Gel	HPV16 and HPV18 E6/E7	Topical application	CRISPR-Cas9 plasmid	Phase 1	NCT03057912	First Affiliated Hospital, Sun Yat-Sen University
Hereditary transthyretin amyloidosis	In vivo	LNP	Transthyretin	IV administration	CRISPR/Cas9 system	Phase 1	NCT04601051	Intellia Therapeutics
Leber congenital Amarosis 10 (LCA 10)	In vivo	AAV	Centrosomal protein 290	Subretinal injection	CRISPR/Cas9 system	Phase 2	NCT03872479	Allergan
β-thalassemia	Ex vivo	Not specified	HBB gene	IV injection	HBB gene correction in patient specific iHSCs	Early Phase 1	NCT03728322	Allife Medical Science and Technology Co., Ltd
β-thalassemia, Sickle Cell Disease	Ex vivo	Ribonucleoprotein electroporation	Enhancer of the BCL11A gene	Single infusion through a central venous catheter.	Autologous CD34+ HSPCs modified at the enhancer of the BCL11A gene	Phase 2/3	NCT03655678, NCT03745287	Vertex Pharmaceuticals Incorporated
AIDS and hematological malignances	Ex vivo	Gene knockout	CCR5 gene modification	Cell transplant	CCR5 Gene Modified CD34+ Hematopoietic Stem/Progenitor Cells	N/A	NCT03164135	Affiliated Hospital to Academy of Military Medical Sciences
Metastatic Gastrointestinal Cancers	Ex vivo	Gene knockout	CISH	Cell infusion	Tumor-infiltrating Lymphocytes (TIL),Cyclophosphamide,Fludarabine,Aldesleukin	Phase 1/2	NCT04426669	Intima Bioscience, Inc.
CD19+ Leukemia or Lymphoma	Ex vivo	lentiviral vector and electroporated (in cell), infused by IV injection	HPK1	IV infusion	XYF19 CAR-T cell,Cyclophosphamide,Fludarabine	Phase 1	NCT04037566	Xijing Hospital
Sickle Cell Disease	Ex vivo	RNP	Not specified	IV infusion	sickle allele modified CD34+ HSPCs	Phase 1/2	NCT04774536	Mark Walters, MD
Sickle Cell Disease	Ex vivo	Gene editing	HbS to HbA by SNM	IV infusion	GPH101:CD34+ Hematopoietic Stem Cells (HbS to HbA)	Phase 1/3	NCT04819841	Graphite Bio, Inc.
T or B Cell Malignancies	Ex vivo	Gene editing	CAR-T Therapy	IV infusion	CTX130: Anti-CD70	Phase 1	NCT04502446	CRISPR Therapeutics AG

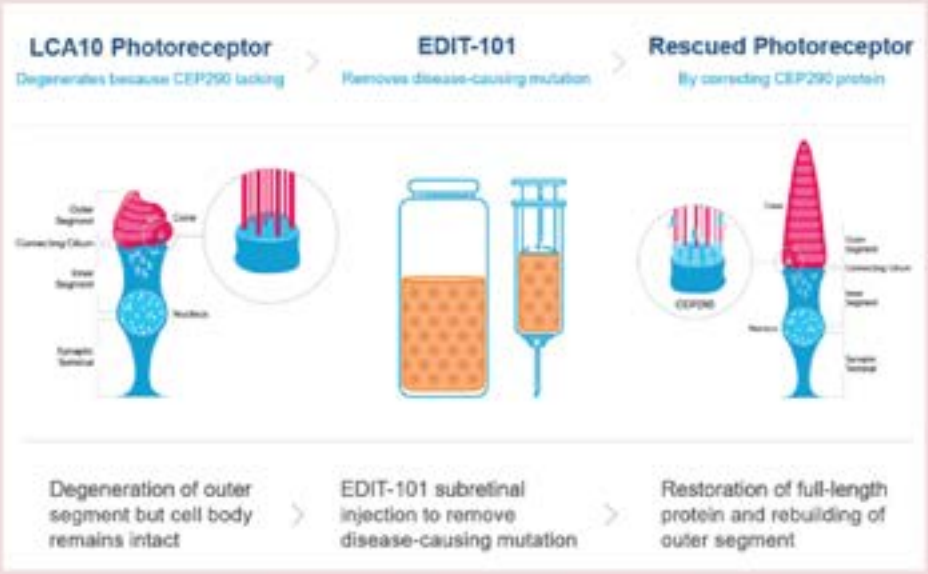

Figure 3: Successful editing by EDIT-101 restores functionality (23)

Challenges/Risks of using CRISPR/cas9 as a gene editing system

1. Off-target mutations:

The main concern of the CRISPR/Cas9 system is the problem of off-target effects, which cause genomic toxicity, carcinogenesis, genome instability, functional gene disruptions, and epigenetic alterations. Given that genomic changes caused by the CRISPR/Cas9 system are permanent, these effects should be carefully identified. The rate of off-target effects is affected by the composition and structure of the sgRNA, so shorter and unique sgRNA, which has a decreased mismatch tolerance, should show lesser off-target effects. Such a target site should be selected such that it shows no homology anywhere else in the genome. Shortening the sgRNA by 2-3 nucleotides, in the protospacer portion reduces the tolerance of the created mismatch and hence reduces the off-target effects.

2. Rate of INDEL formation

Another concern of the CRISPR/Cas9 system is the unwanted insertions and deletions (INDELs) (<20 bp) that rarely occur, but if the INDELs are too long (up to 600 bp or 1.5 kb) it can lead to pathological defects. Moreover, the size of the Cas9 protein is a key disadvantage, which is larger than a TALEN monomer and much larger than a ZFN monomer and causes Cas9 delivery by viral vectors to be challenging (24).

3. Delivery

The efficiency of delivery depends on the target cell type and the delivery method chosen. Due to the large size of the CRISPR complex, efficient and specific delivery into the cell is a major challenge. Currently, a number of viral, non-viral and physical methods are being employed for targeted cell delivery. Further development of suitable carriers will require long-term studies (25).

Conclusion

Innovation in the development of tools and technologies is indispensable for scientific progress. The CRISPR/Cas9 system is a versatile gene-manipulating tool consisting of a guide RNA sequence (sgRNA) and a DNA splicing protein complex (e.g. Cas9). The development of this

has allowed inducing DSB at selected sites determined by the modification of the guide RNA as required. Despite the availability of gene-editing tools such as ZNFs and TALENs, CRISPR/Cas9 has facilitated reaching this goal at a much faster pace, primarily owing to the ease of use and its high versatility. This technology is now available to quickly bring rapid and precise alterations to the genome. However, the road ahead is not free of obstacles. Additional research is required to better understand CRISPR/characteristics, Cas9's including its specificity, off-target effects, and delivery strategies. For example, the results of current genome-wide deep sequencing will be useful for choosing appropriate target sites and creating highly specific gRNA.

List of Abbreviations:

DNA- Deoxyribonucleic acid; DSB- Double stranded break; ZFN- Zinc Finger Nucleases; TALENs- transcription activator-like effector nucleases; CRISPR- Clustered Regularly Interspaced Short Palindromic Repeats; Cas- CRISPR associated; RNA- Ribonucleic acid; gRNA- guide RNA; sgRNA- single guide RNA; PAM- Protospacer adjacent motif; LTR- Long Terminal Repeats; LCA 10- Leber Congenital Amaurosis 10; bp- base pairs

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Bio-Engineered Cell Based Functional Systems as Next Generation for Cancer Therapeutics



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Abstract

Cell based therapy with enthusiastic capability of intrinsic homing tumor sites with noticeable on-board sensing ability helps to design the way towards the target in fatal as well as intractable disease like cancer. Structurally engineered, these cell-based systems are equipped with sensing functionality which allows them to follow the cues to reach tumor microenvironment, that potentially frames them as advanced ideal candidates in smart cancer therapeutics. The discussed review presents the highlights of all the progress and advancements on the most promising cancer cell-based therapy as targeted theragnostic including genetically engineered materials and techniques in stem cell therapy, CAR-T cell therapy, NK-cell immune therapy, as emerging era on the horizon.

Keywords: Cancer cell-based therapy, CAR-T cell therapy, NK-cell immune therapy

Introduction

Cell based therapy utilizing the programmable living vehicles and their delivery to tumor sites with contribution to communicate with faulty oncogenes in order to defraud the cancer cells as a potential to initiate the therapeutic transgenes. This may show an intrinsic immune response against the proliferating cells, with a potential to emerge as an attractive alternative to traditional resistance developing strategies for cancer targeting[1,2]. The engineered cell-based modalities can harness and mimic the proliferating cells, hence contribute for efficient biodistribution to the target site and orchestrate the complex cellular response to destroy the abnormal cells. Despite of such functionalities, the naturally derived structured tailored materials face series of challenges as discussed below, that require to be addressed[1].

- Robust cell source.
- Maintenance of viability and GRAS state.
- Relative similarity between PK/PD profile of cells and physiologic site.
- Must achieve the predicted therapeutic potency.
- Scalability considering the safety and tumorigenicity profiles of cell-based systems.

The discussion and motive of the perspective begins with landscape of cell based therapies and their advancements comprised of highly focussed CAR-T cell therapy as synthetic biology to encapsulate the CAR in T cells have risen within decades as genetic tools to introduce transgenes inside the cells, that is further highlighted briefly in recent advancement along with successful emergence of stem cells as well as NK cells as genetically engineered structural modules that can not only home and migrate to the tumor sites but also strap the tumor tropism[3,4].

Emerging strategies and recently advanced cancer cell-based techniques:

There are numerous innovations like genome-epigenome editing as computational approach, synthetic biology, that are in recent interest in order to address all the challenges for cell based therapy. When it comes to target the cancer with engineering techniques, genetic mutation based genome-epigenome editing always play a key role to target by utilizing zinc-finger nuclease (ZFNs), transcription activator like effector nuclease (TALLENS) and clustered regularly interspaced short palindromic repeats CRISPR-Cas9 system, which knockout the faulty gene, insert the correct gene and mutate the targeted gene to destroy the oncogenes in cancer. Newly derived CRISPR-Cas9 has been a lot in attention due to its practicability and reproducibility to maintain the GRAS state that is one of the challenge as discussed [5]. One such study conducted with CRISPR-Cas9 has been used as endogenous disrupting agent to mutate the resistant T cells against PD-L1 inhibition in order to produce allogenic CAR-T cells with reduced immunogenicity [6].Also, as it has been proven that the combined strategy is always a better option, one such study as combination of CRISPR-Cas9/ stem cells that is cultured with motif to mutate four colorectal cancer based genes including p53, APC, KRAS and SMAD4 with a detailed screening of mutant cells to work against the cancer[7].

Synthetic biology is an approach to introduce transgenic artificial genes inside the cells via genetic engineering, likewise the incorporation of chimeric antigen receptor CAR gene in T cells has been approached in recent times. Synthetic remote control circuit on CAR activity that follows the cues to sense the surface ligands have been a lot in attention. Synthetic sense response programmable receptor that can sense one surface ligand that enhance the target cell specificity and coupled with CAR to sense second ligand, thereby can autonomously target with no off target delivery and enabling of such Boolean gate response can enhance the uptake with destruction of cell proliferation. Numerous approaches on this like combinatorial model of split, universal and programmable CARs (SUPRA-CARs)[8] as multivalent protein scaffolds have also been constructed to enable the recognition of antigen combinations. Transfection of CAR gene in T cells is such a wide area of research, and still it is on going on fourth as well as fifth generation of CARs to overcome the toxic profiles of previous generations. This also belongs to one of very attractive model comprised of scFvs, single-domain antibodies (sdAbs) and scaffold proteins. One such preclinical study by sommermeyer and smith on scFvsCARs targeting recognized human CD-19 and BCMA specific ligands that is known to be a well-known in multiple myeloma. They reported host immune anti-murine CAR responses to limit the frequent dosing with prolonged persistence, the study priorly begins with genetic engineering of thoroughly screened BCMA clones specific CARs with rapid in-vivo expansion and infusion with T cell to eliminate the immunosuppressive cells. Still their efficiency and efficacy are on stemming trial on research[9].

tumortropic MSC delivery with the immunoapoptotin and HER2. For clinical usage, anti-HER2 antibodies were employed because of HER2 overexpression in many types of tumors such as breast cancer as an approach for tumor tropism, and resulted with synergistic effect of immunoapoptotin engineered MSC, thus provide a motivational design of dual-targeting delivery systems for a wide variety of cancer types [10].

Nano-based drug delivery in cancer cell therapy.

Identification and mutation of cells to correct the faulty genes is always challenging.

In above discussed cases CRISPR-Cas9 with high molecular weight leads to develop difficulty in targetingwithout a strategized delivery system to not only translocate but limit the off -target delivery as there are three categorized cargos of CRISPR-Cas 9 such as Cas9 endonuclease protein and its complex with sgRNA (RNP), another is mRNA, that is utilized for ex-vivo till date due to lack of stability. Hence to nano-drug delivery can enhance the stability but also improves the PK/PD profiles. Recently advancing technique to deliver Cas9 via engineering exosome vesicular drug delivery, that is explored as microinjection of exosome loaded with Cas9/sgRNA in combination against SKOV3 ovarian cancer to inhibit PARP-1 gene leading to apoptosis. Hybrid exosomes have also been studied and engineered as Cas9-loaded exosomes, likewise many of studies conducted to hybridize the exosome fusion with two cargos with increased loading efficiency as well as transfection of mesenchymal stem cells as study to provide synergistic effect t[11].

NANO-CART is strategy reported to be the alternative to sub optimal single domain CAR-T therapy associated with aggregation problem, also fence off the concern about the immune reflux. NANO-CARs have been explored as VHH (also known as nanobodies) that is better candidate than scFVs, as it quite closely resembled the human clone family of VHH, less immunogenic as variable domain heavy chain lacks the synthetic linker peptides. One study with NANO-CARs that locally secrete VHH-type immune modulators targeting CD47 or PD-L1. Another by Ackaert et al. conducted a study to investigate the possible immunogenicity of two nanobodies that are currently being investigated in Phase II clinical trials, the study reported the biological imaging of nanobodies in breast cancer to be less immunogenic that can be a potential for targeting moieties. Such nanobodies are much similar to human VH, hence a study on NANO-CAR redirected VHH based PSMA expressing genetically engineered cells incorporated that efficiently triggered the anti-tumor activity and tumor cell elimination with upregulation of immune checkpoint inhibition as well as anti-proliferating agents against the cancer [8].

MSCs have highest potential for tumor tropism, in special cases where hypoxic preconditioning is applied that improves the tumor homing as well as migration. In the field of nanotechnology MSCs have been widely explored, a study comprised the increase in expression of chemokine receptor-4 (CXCR-4) that was found to increase the tropism of MSCs via potentially using polymeric nanoparticles complexed with MSCs that shown increase in migration velocity, deep penetration, co-localization within the hypoxic tumor microenvironment [12].

The cell-based therapy can be widely used in membrane coated nanoparticles to reduce the immune reflux-based responses as eye catcher in cancer, that not only reduce the

immunogenicity but also can be biomimetic carrying device for cancer theragnostic. Genetic modification via hybridization, extrusion techniques can be efficiently utilized for surface receptor targeting. Amongst all the discussed merits of membrane coated nanoparticles, fusion of membranes and its incorporation on the surface of synthetic nanoparticles can do wonders in all the challenging situations like immunogenicity, off target delivery, receptor mediated uptake, resistance showing mutations [13]. Variety of delivery systems have been explored till date such as biomimetic RBC membrane coated nanoparticles, nucleic acid coated nanoparticles, immune cell coated nanoparticles, stem cells coated nanoparticles, neutrophil coated nanoparticles as well as platelet membrane coated nanoparticles depending on various sources as demonstrated in figure shown below:

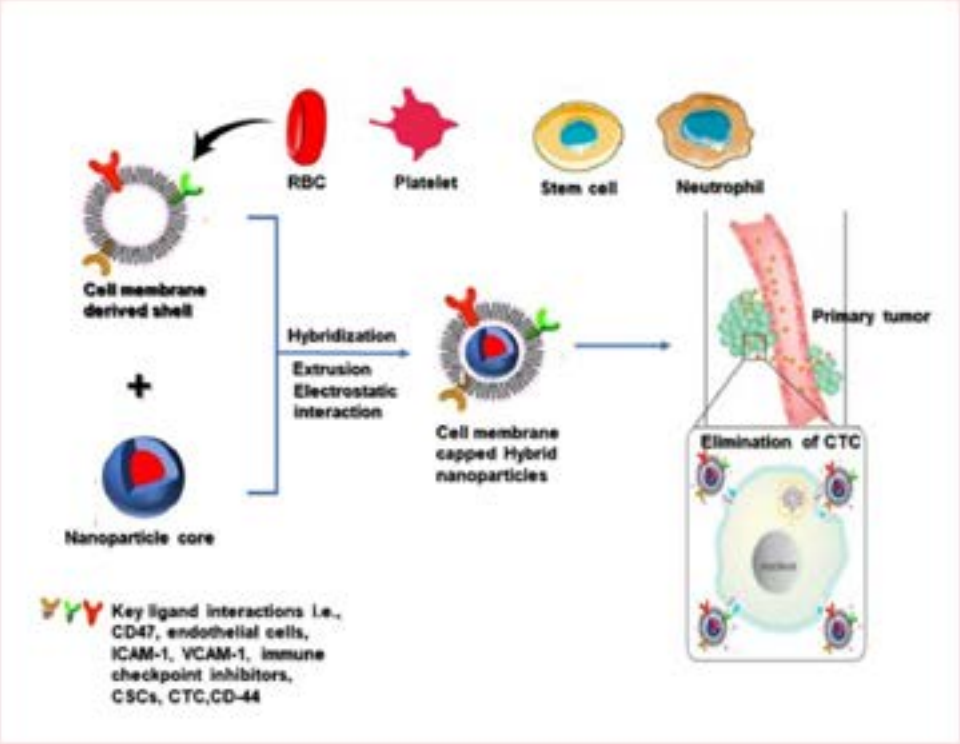

Figure 1 Coated Nanoparticle as a drug delivery system

Natural killing cells (NK cells) are the powerhouse for apoptosis as well as immune surveillance, that is known to secrete variety of cytokines once activated to regulate the immune system,as also to destroy the target cells. One such study on NK cell protein camouflaged nanoparticles as biomimetic tool using photosensitizer for cancer theragnostic by Deng's group demonstrated a successful attempt to produce anti-tumor effect via M1 polarization and cell death by photodynamic therapy (PDT) thus improving the antitumor immune workpiece ratio of the NK-cell membrane [14].

Conclusion and outlook

Significant importance of genetic engineering strategies as rising in recent advancement highlighted in the review can clarify the vast areas of cell based therapy, clear that it can be a ideal strategy for cancer targeting as well as theragnostic area of research if conducted with motive to overcome the challenges considering the safety, efficacy point of view.We anticipate that these technologies will continue to refine autologous cell therapy pipelines (for example, CAR-T therapy), offering improvements in the mode of action.

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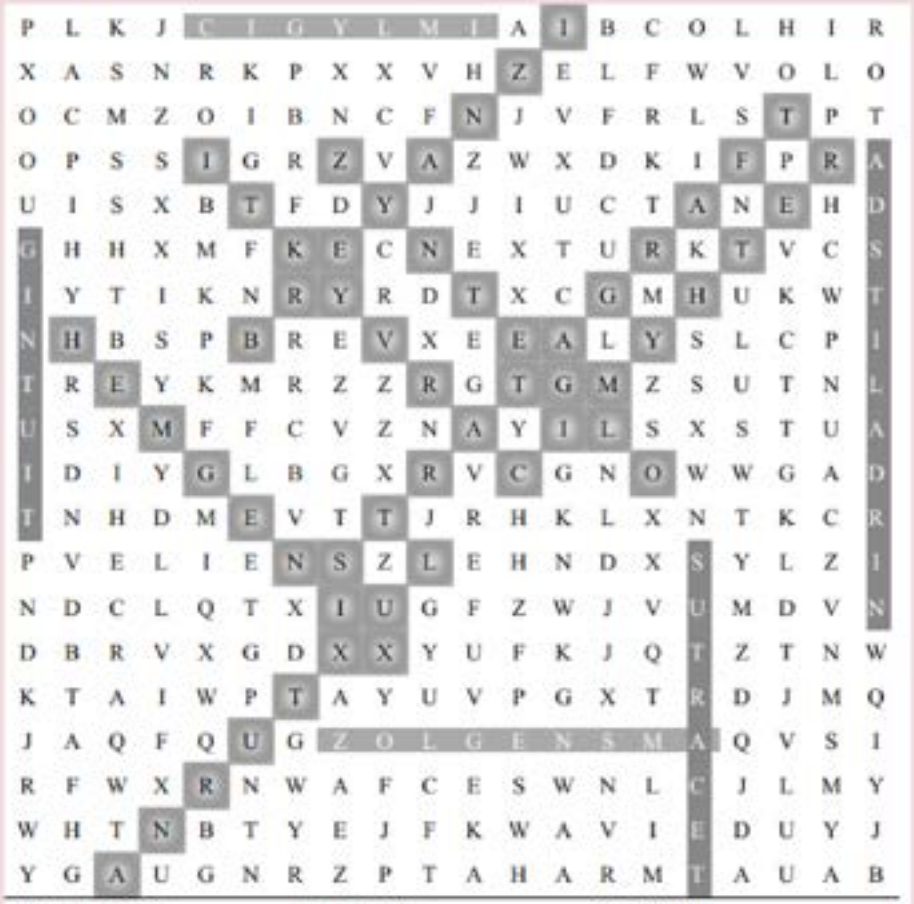

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CAR T cells: Recent Cell Based Therapy for Cancer




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Introduction

One of the main causes of death in the globe is cancer. Numerous traditional cytotoxic methods for treating neoplastic illnesses have been developed over the years. However, due to their limited efficacy in light of the heterogeneity of cancer cells, there is a continuing quest for therapeutic strategies that provide better results, such as immunotherapy, which makes use of and boosts the immune system's normal functioning (1,2,3).

Certain cancers are treated with chimeric antigen receptor (CAR) T-cell therapy, which modifies T-lymphocytes or T-cells to make them more effective cancer-fighting cells. While long-term data are still being gathered, CAR T-cell therapy is showing to be a very successful method of treating some blood cancers (4). Chimeric antigen receptor (CAR)-T cell therapy is among the newest and most effective treatments for blood cancer. In order to combat cancer, these treatments rely on your body's immune system (5).

CAR T- Therapy

A type of cancer immunotherapy known as CAR T cell therapy uses T cells, which are immune cells,that have been genetically altered in a lab to increase their capacity to recognise and destroy cancer cells (6).

CAR T-cell therapy requires platforms for quick, dependable, and secure gene transfer. Through the use of both viral and non-viral transfection approaches, ex vivo genetic alteration of autologous T-cells isolated during leukapheresis is achieved. Then, modified T-cells are raised in culture (Figure 1). Typically, after the CAR Tcell product has been produced and has passed all quality control testing, the patient will first get lymphodepleting chemotherapy, then receive an infusion of CAR T cells. The first chimeric receptor was developed in 1989 by Eshhar's group at the Weizmann Institute of Science in Israel (7)

CARTs may detect unprocessed antigens as well as glycolipid and carbohydrate structures that are frequently expressed on a tumour cell's cell surface and work as treatment of cancer.

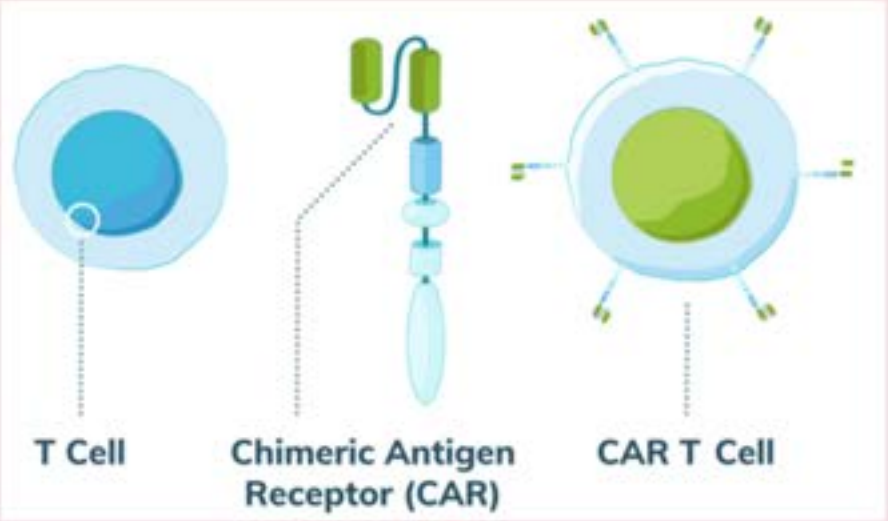

Figure 1: CAR T cell preparation

Specific cells and organs that make up the immune system guard the body against disease and cancer. These include T lymphocytes, which look for and eliminate aberrant cells, including cancer cells. The immune system needs to be retrained to recognize and kill cancer cells since cancer cells can occasionally find ways to elude it. One cutting-edge method for training the immune system to combat cancer is CAR T-cell therapy (8).

After a blood sample of a patient's T cells is taken, the cells are altered to generate distinctive structures termed chimeric antigen receptors (CARs) on their surface. The receptors on these CAR T cells may help the T cells when they are reinjected into the patient find and fight cancer cells throughout the body (9).

CAR T Therapy Process

In this process following methods are included (10) (Figure 2):

1. T cells from a patient are taken. T cells are collected via the apheresis technique, which involves drawing blood from the body and separating one or more blood components (such as platelets, plasma, or white blood cells). The remaining blood is subsequently given to the body.
2. In a lab, T cells are redesigned. The T cells are delivered to a lab or a medicine production facility where they are genetically modified by injecting DNA into them, resulting in the production of chimeric antigen receptors (CARs) on the cell surface.
3.The reengineered T cells are now referred to as "chimeric antigen receptor (CAR) T cells." Proteins called CARs enable T lymphocytes to detect an antigen on certain tumour cells.
4. The CAR T cells are then multiplied. The quantity of the patient's genetically altered T cells is "expanded" through laboratory cell growth. Once there are sufficient CAR T cells, they are frozen and sent to the hospital or facility where the patient is being treated.
5. The CAR T cells are defrosted and administered to the patient after being thawed at the hospital or treatment facility. Before receiving an infusion of CAR T cells, a lot of patients undergo a brief course of one or more chemotherapy drugs, referred to as "lymphodepletion." The patient's circulation begins to multiply with the reintroduction of 1. CAR T cells. These cells are known as "attacker" cells because they are capable of identifying and attacking cells that have the specific antigen on their surface.

6. In order to prevent recurrence, the CAR T cells might be useful. Even if the CAR T cells are given months after the injection, they may still be present in the body and eliminate all cancer cells. Some blood cancer types have seen long-term remissions as a result of the treatment.

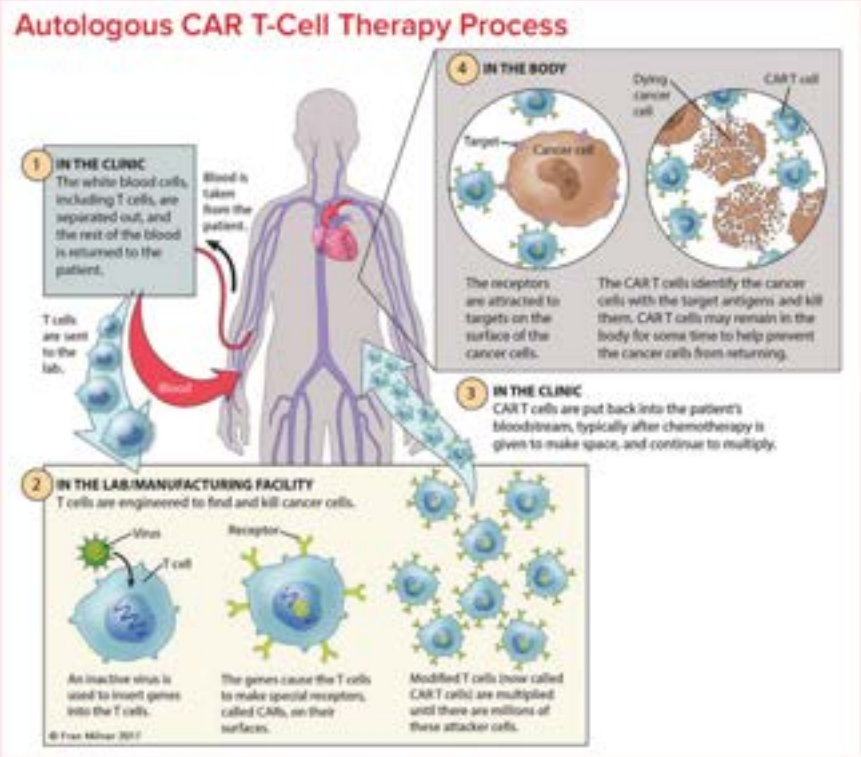

Figure 2: CAR T cell therapy process

Side Effects

Dysregulation of Cytokine Release (CRS). The use of CAR T cells is frequently associated with this potentially serious side effect. Cytokines, which aid T cells in carrying out their duties, are produced when CAR T cells multiply in the body and eliminate cancer cells.. From minor flu-like symptoms, which may include (5,9):

- Fatigue
- Nausea
- Fever
- Chills

The symptoms of CRS are:

- Cardiac Arrest
- Cardiac Arrythmia
- Hemophagocyticlymphohistiocytosis
- Low blood pressure
- Multiple Organ Failure
- Poor lung oxygenation

Biological Toxicities. Neurological side effects vary amongst CAR-T products in terms of frequency, severity, and origin. Language difficulty (aphasia), confusion, delirium, involuntary muscle twitching, hallucinations, or indifference are typical symptoms. Also mentioned are seizures.

Off-tumor, On-target Toxicity The selection of the appropriate tumor-associated antigen to target is crucial for the safe and effective usage of CAR T cells. Sadly, it is uncommon to locate such a perfect target. Numerous tumour antigens are additionally expressed on healthy tissue cells. Especially when cells in vital tissues like the heart, lung, or liver display the target antigen, damage to such non-cancerous normal tissue by CAR T cells may be fatal.

Anaphylaxis (Life-threatening Allergic Reaction) (Life-threatening Allergic Reaction). An extreme immunological reaction against the CAR itself, known as "anaphylaxis," has the potential to occur in a patient getting CAR T-cell treatment. Hives, swelling of the face, low blood pressure, and breathing difficulties are all signs of anaphylaxis. Few cases of acute anaphylaxis have been reported. For patients receiving CAR T-cell therapy, careful observation and prompt treatment of this life-threatening adverse event are essential.

Tumor Lysis Syndrome (TLS). Tumor lysis syndrome (TLS), a collection of metabolic issues that can arise as a result of the breakdown of dying cells—typically at the start of hazardous cancer treatments—is another well-known adverse effect of CAR T-cell therapy. After CAR T-cell therapy, TLS can, however, be postponed and may come around a month or more later. TLS is a potentially fatal side effect of any therapy that destroys cancer cells, including CAR T cells. It can harm organs and damage surrounding tissue. Through routine supportive care, the complication has been controlled.

Future of CAR T Therapy

Studies of CAR T-cell treatment in multiple myeloma and other blood malignancies, such as chronic lymphocytic leukaemia (CLL), also show promise. Additionally, studies investigating the use of CAR T-cell therapy in the management of solid tumours are also being conducted (3).

The majority of patients taking part in CAR T-cell trials have only been monitored for a brief period of time, but data revealing early therapeutic responses is quickly surfacing. Following a thorough analysis of trial participants' long-term behaviour, researchers will be able to forecast how long these responses will last. Clinical trials should enrol more children and adults, for many reasons. Researchers will be better able to comprehend the consequences of this kind of medication, find strategies to lessen its toxicity, and enhance the management of negative side effects with larger study populations that are assessed over longer time periods (3)

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Exosomes and their applications in cell-based therapies

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Introduction

Expanding knowledge about nanometer-sized fluid filled vesicles budding from cells, packaged with molecular cargo has placed exosomes to the stage where innovative engineering techniques translate them for clinical use. Exosomes are exploited for diagnostics and drug delivery systems. These extracellular vesicles (EVs) generated by double invagination of plasma membrane from cells and formation of intracellular multivesicular bodies harboring intraluminal vesicles which are secreted as exosomes of 40-160nm diameter (1). The heterogeneity of exosome is the reflection of its various sizes, content, functional impact and origin. The inherent cell physiology determines the content of exosomes. They may possess membrane proteins, cytosolic and nuclear proteins, nucleic acids, non-coding RNAs, and metabolites (2). At present, 9,769 proteins, 3,408 mRNAs, 2,838 miRNAs and 1,116 lipids have been reported as their composition(3). Exosomes serves as a non-canonical intercellular communication mode with the nexus of composition capable of multicellular and multi-centric targeting simultaneously. The potentiality to deliver molecular cargo to target cells and modify their intracellular mechanisms makes them ideal therapeutic agents and delivery systems for multiple diseases (4). The advantages of exosomal cargos over classical cell-based therapies that includes stem cells are lack of inherent risk, lack of replicating potential, lack of immunogenic response and site specificity(5).

Ischemia and metabolic diseases

Patients with ischemia often have diabetes. Exosomes derived from plasma of non-diabetic rats demonstrated cardioprotective signaling in cardiomyocytes from diabetic rats (6). Effective drug delivery targeting ischemic brain by engineered exosomes have been explored for the treatment of ischemic stroke(7). Another report suggests that anti-inflammatory potential of exosomes derived from human neural progenitor cells as therapeutic effectors against cerebral ischemia (8).

Cancer

Multifunctional nanoparticles as polymeric micelles have emerged as have been employed as smart drug carriers that can specifically target specific cancer sites by using both endogenous or exogenous stimuli (9). The contribution of exosomes for development of cancer vaccines can be attributed by studies such as exosomes bearing fibrosarcoma antigen induces anti-tumor immune reaction conducted in the murine models of fibrosarcoma; exosomes derived from macrophages with enrichment of HSP70 HSP70, naloxone, propranolol and/or staphylococcal

enterotoxin B exhibited anti-tumoral properties (10,11). Further, tumor cell derived exosomes serve as a drug carrier for cancer therapeutics (12). In another report, therapeutic recombinant P53 protein loaded extracellular vesicles targeted towards mitochondria drives breast cancer cells towards cell death mechanisms (13). siRNA to HER2 loaded engineered exosomes have shown anti-tumorigenic efficacy in breast cancer cells (14,15). Again, engineered exosomes loaded with triptolide suppressed the tumor progression in malignant melanoma in mice (16,17). Various studies have also identified the peptide functionalization of exosomes in glioma therapy(Table 1; 18,19).

Neurodegenerative diseases

The capacity of carrying genetic material and transfer at specific target sites by exosomes has been explored in neurodegenerative diseases(20). siRNAs loaded exosomes targeting alpha-synuclein in Huntington's disease model have demonstrated effective brain distribution following systemic injection (21) Reportedly, a study was conducted under in vivo and in vitro conditions that explored the delivery mechanisms of exosomes with a saturated solution of dopamine reported to cross the blood brain barrier and deliver into the brain for a better therapy for Parkinson's disease (22). In another study, curcumin loaded liposomes mitigated oxidative stress in neuronal cells and provided neuroprotective effect (23). In some in vitro studies, exosomes derived from bone marrow stromal cells and adipose-derived stem cells contains enzyme that can degrade Ab and attenuate neurotoxic effects (24,25).

Conclusion

Exploring new aspects of exosome biology demands both cell culture systems and animal models that can give insight into its biogenesis, trafficking, and intracellular entry. The site-specific targeting of cellular derived molecular cargo by exosomes is explored for designing therapeutics. The low immunogenicity and extreme biocompatibility emerged exosomes as an efficient drug delivery system. Although there is a long challenging way to go in commercial exploration of exosome-based drug delivery system, an understanding of the detailed biological mechanisms and clinical studies will herald them as next-generation nanoplatform for different diseases. Exploration of exosomes has opened up new possibilities for management of diseases like cancer within the purview of personalized medicine. The application potential of exosomes is anticipated while providing research support towards clinical use of exosomes for treatment of different diseases. The global burden of aging gives thrust towards the development of new therapeutics for neurodegenerative diseases in order to improve the quality of life of elderly population and thereby reducing the necessity of public management and heavy burden on caregivers. The development of cell specific therapy by employing exosomes opens new avenues for treatment of neurodegeneration considering less risk of autoimmune rejection and improved safety and biocompatibility. To our opinion, stem cell derived exosomes can form the foundation for a possible cure in various neurodegenerative conditions. Stem cell derived exosomes being superior in ability to surpass physiological barriers and effective migration to the sites of brain lesions thereby avoiding post transplant adverse events. Further, genetically or chemically engineered exosomes for directing them towards targeted cargo delivery can be employed in an advanced version of gene therapy for various genetic disorders.

Table: 1 Summary of investigating studies that reflects the therapeutic and drug delivery utilities of exosomes

Disease	Exosomal source	Exosomal product	Therapeutic potential	Ref.
Ischemia and diabetes type II	Serum	-	Cardioprotective efficacy	6
Cerebral ischemia	Engineered exosomes	Curcumin	Anti-inflammatory, anti-apoptotic potential	7
Cerebral ischemia	Human neural progenitor cell line	Recombinant protein	Anti-inflammation bioactivity	8
Sarcoma	Macrophage	WEHI-164 cell lysate, HSP70, naloxone, propranolol and/or staphylococcal enterotoxin B	Anti-tumoral properties	11
Breast Cancer	Breast cancer cell	Recombinant P53 protein	Cell death	13
Breast Cancer	Engineered exosomes	siRNA to HER2	Gene downregulation	14
Malignant melanoma	Engineered exosomes	Triptolide	Anti-tumorigenic, reduced toxicity of triptolide	16
Glioblastoma	Engineered exosomes	superparamagnetic iron oxide nanoparticles and curcumin	Anti-tumorigenic effect	18
Glioblastoma	Engineered exosomes	miR-21	Reduction in tumor size	19
Huntington's disease	Engineered exosomes	Asymmetric siRNA targeting Huntingtin mRNA	Bilateral oligonucleotide distribution and silencing upto 35%	21
Parkinson's disease	Blood exosomes	Dopamine	Increased distribution of drug in brain	22
Alzheimer's disease	Exo-liposomes	Curcumin	Reduction in oxidative stress and neuroprotective effect	23

Exosomes can be engineered to bind nucleic acids that facilitate encapsulation of plasmids carrying the desired gene sequence leading to efficient loading. Manipulation of exosomes for encapsulation of large vectors or mRNA transcripts while retaining their avoidance of immunological rejection and targeting ability will provide impetus for development in the field of gene therapy. Breakthroughs in exosome-based vector systems and optimized gene editing tools will provide the momentum necessary for establishment of exosomes as a practicable treatment for genetic diseases.

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Plant derived exosomes: an emerging frontier in endogenous drug delivery carrier



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1. Introduction:

Cell-based delivery systems are emerging drug delivery frontier exploring endogenous cellular components as carriers for proteins and molecules. Potential advantages proffered by this domain is its naturalness which enables ease in overcoming the various biological barriers in addition to conferring controlled drug release and active tissue targeting attributes. To-date, cell-based delivery systems reported in literature encompass of cellular organelles viz; Red Blood Corpuscles (RBCs), platelets, immune cells, stem cells, and tumor cells (1). In recent years, research involving exosomes is gaining momentum. Exosomes, owing to its nanosize and physiological activities, to name a few, antioxidant, anti-cancer property, anti-inflammatory action, regulation of gut microflora, prevention of pathogen attack and organ damage, have attracted attention from researchers. Historically, exosomes were considered as a cell debris with little or no biological significance (2). However, today exosomes have been recognized to play a pivotal role in array of cellular functions viz. cell communication, transport of molecules, priming of immune system by mediating the adaptive immune response towards pathogens and tumours (3).

In general, exosomes, term coined by Johnstone (1989), belong to a large family of extracellular membrane vesicles. According to International Society for Extracellular Vesicles (ISEV), extracellular vesicles are classified as microvesicles (100–350 nm), apoptotic blebs (500–1000 nm) and exosomes (30 –150 nm)(4). Exosomes are found both in mammalian cells, in lower eukaryotes and prokaryotes (5,6). Exosomes possess unique characteristic features, such as a fluid lipid bilayer, surface proteins/receptors with inner core consisting of specific proteins, transcription factors, and genetic components such as deoxynucleic acids (DNA), coding and non-coding ribonucleic acid (RNA) like messenger RNA (mRNA) and microRNA (miRNA). The lipidic components comprise of ceramide (differentiating exosomes from lysosomes), cholesterol, sphingolipids. The polar nature of lipid bilayer is contributed by carbohydrates like phosphoglycerides consisting of long and saturated fatty-acyl chains. The polar surface of exosome is ascribed to the presence of saccharide chains, such as mannose, polylactosamine,

alpha-2,6 sialic acid, and N-linked glycans (7). ISEV stated the presence of exosomal marker proteins viz. transmembrane or GPI-anchored proteins (associated to plasma membrane and/or endosomes) and cytosolic proteins (8),used to determine the extracted components are exosomes and exosomes derived from biological fluids. Exosomes are produced from either natural or artificially engineered. Figure 1B depicts the various sources of exosomes documented in the literature (9)

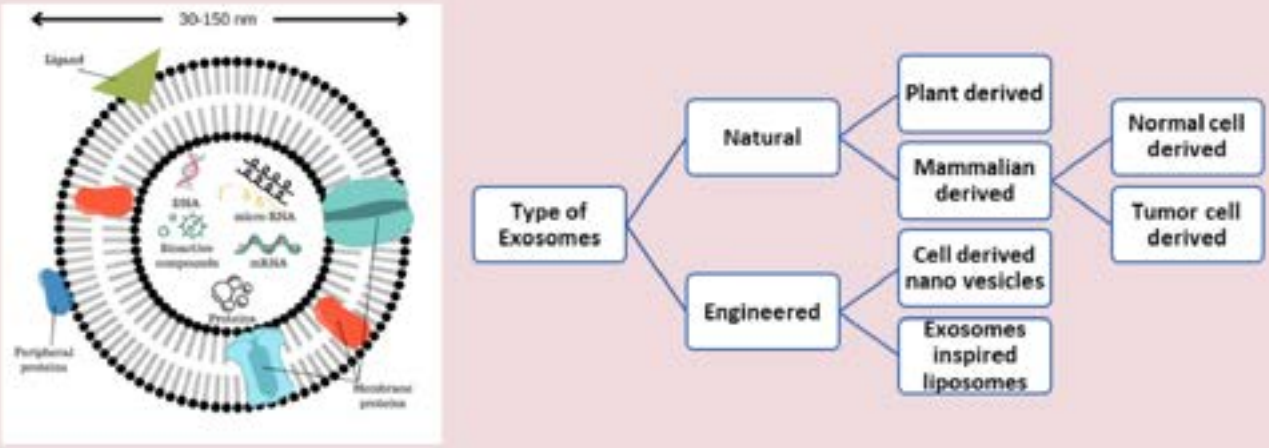

Figure 1: Overview of Exosomes AStructure and generalized composition of exosomes; B: Classification of Exosome based on sources

Based on the origin, plant derived exosomes (PDEs) have obvious advantages over mammalian cell derived exosomes i.e., low immunogenicity and absence of zoonotic infections causing pathogens. In addition to this, other advantages of PDEs include specific targeting ability, higher stability towards gastrointestinal pH, ease of synthesis, ability to be produced at large scale, easy storage, and economical and easy availability (10,11). Although research on PDEs is in nascent stage, there are few literatures citing isolation of PDEs from sources such as ginger, carrot, broccoli, grapefruit, lemon, blueberries, strawberries, shiitake mushrooms (table 1). This article will provide an overview on PDEs and its application in drug delivery.

2. Formulation of PDEs based drug delivery system

Figure 2 depicts the typically exosomes biogenesis and PDEs uptake which begins with inward budding of the cell membrane and produced by Multivesicular bodies (MVBs)/ Late endosomes. The membrane of MVBs expands inwardly to fuse with the plasma membrane to release their intraluminal vesicles into the extracellular space (called exosomes) or fuse with vacuole/lysosomes for degradation. The uptake of PDEs by recipient cell occurs by either of the mechanisms viz. membrane fusion, receptor-ligand interactions, endocytosis, macropinocytosis and phagocytosis.

2.1. Isolation and modification of PDEs

PDEs comprise of unmodified or pristine PDEs and engineered PDEs. The technique employed for pristine PDEs relies on variable size and density of exosomes. They may include ultracentrifugation, size-based isolation, immunoaffinity and precipitation. Among them, ultracentrifugation is most commonly applied extraction method. Figure 3 summaries the process parameters, advantages and disadvantages of various techniques pristine PDEs preparation. (22)

The engineered exosomes are produced by methods like incubation, transfection, physical treatments like extrusion, electroporation, sonication, freeze-thawing, surfactant treatment, dialysis, in situ synthesis. The targeting capacity is enhanced by molecular homing with receptor affinities, acidic milieu, responsivity, and magnetic features. Wang et al (2013) reported the grapefruit derived exosomes enhance in vivo targeting specificity of chemotherapeutic agents due to binding with Folic acid (13).

Table 1: Overview of global research on exosomes

Country	Research Group	Study	Ref.
Iran	Moosa Rahimi Ghiasi et al	Leucine-rich Repeat-containing G-protein Coupled Receptor 5 Gene Overexpression of the Rat Small Intestinal Progenitor Cells in Response to Orally Administered Grape Exosome-like Nanovesicles	(12)
USA	Qilong Wang et al	Delivery of therapeutic agents by nanoparticles made of grapefruit-derived lipids	(13)
	Jessica Danh et al	Characterization and Uptake of Strawberry-Derived Exosome-Like Nanovesicles by Human Aortic Endothelial Cells	(14)
	Zhongbin Deng et al	Broccoli-Derived Nanoparticle Inhibits Mouse Colitis by Activating Dendritic Cell AMP-Activated Protein Kinase	(15)
USA & China	Mingzhen Zhang et al	Edible Ginger-derived Nano-lipids Loaded with Doxorubicin as a Novel Drug-delivery Approach for Colon Cancer Therapy	(16)
Italy	Baldini et al	Exosome-like Nanovesicles Isolated from *Citrus limon L.* Exert Anti-Oxidative Effect	(17)
India	Kalarikkal et al	Edible plant-derived exosomal microRNAs: Exploiting a cross-kingdom regulatory mechanism for targeting SARS-CoV-2	(18)
Thailand	Variya Nemidkanam et al	Characterizing *Koempferia parviflora* extracellular vesicles, a nanomedicine candidate	(19)
Korea	Do Kyung Kim et al	Antioxidative Effects of Carrot-Derived Nanovesicles in Cardiomyoblast and Neuroblastoma Cells	(20)
China	Wan-Jun Zhao et al	Blueberry-derived exosomes- ameliorate nonalcoholic fatty liver disease by attenuating mitochondrial oxidative stress	(21)

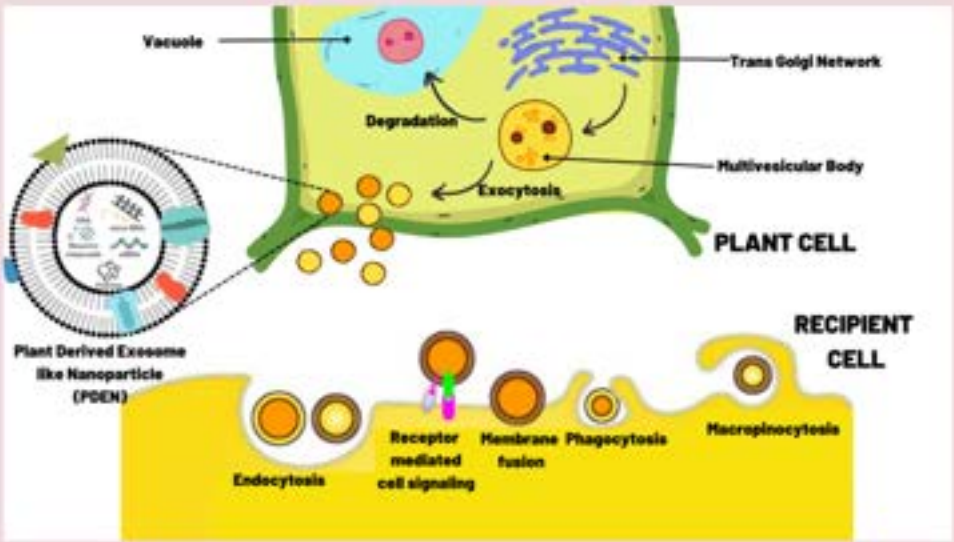

Figure 1: Exosomes biogenesis and PDEs uptake by recipient cell. (Adapted from "New insights of engineering plant exosome-like nanovesicles as a nanoplatform for therapeutics and drug delivery. Shinge et al,2022) (22)

2.2. Techniques for Cargo loading

To enhance the therapeutic effects, PDEs can be loaded with exogenous therapeutic molecules, including siRNAs, DNAs, proteins, and expression vectors, in addition to endogenous constituents. This can be done via passive and active cargo loading. The drug loading and drug release are variable with exosome types. Although, in vivo exosomal activity should be unhindered in the process (11). The loading of the drug in the PDEs can be improved by use of methods such as sonication, freeze-thaw cycle and other mechanical techniques that can disrupt the integrity of the PDE membrane. (22)

3. Characterization of exosomes

The extracted exosomes are characterized for their morphology, biochemistry, lipids, proteins and RNA profile (23). The characterization methods depending upon objective can be divided into external characterization methods (morphology analysis and particle size detection) and inclusion characterization methods (membrane protein, lipid raft). The surface and internal characteristics of PDEs can be assessed using SEM and TEM respectively, sample preparation for these techniques is tedious. Dynamic light scattering (DLS) and Nanoparticle Tracking Analysis (NTA) technology has been used to determine the size of exosome, while latter also estimates the concentration of exosomes. Western Blot (WB) and Enzyme-linked immunosorbent analysis (ELISA) detects exosomal marker proteins expression. While Flow Cytometry assist in detection of exosomal biomarkers such as exosomal marker proteins and Lipid raft-associated proteins. (9,24)

4. Applications of Exosomes

4.1. Exosomes as therapeutics

Antioxidant property is preserved in PDEs for longer duration than free antioxidant substances. Perut and co-workers demonstrated the anti-oxidant activity of Fragaria x ananassa and Citrus limon derived exosomes in Adipose-derived mesenchymal stem cells (ADMSCs). The activity was attributed to presence of Vitamin C found in Fragaria x ananassa derived exosomes (0.416 nmoles vitamin C/µg PDEs) and Citrus limon derived exosomes (0.009 nmoles vitamin C/µg PDEs (25). Liu et al reported anti-inflammatory activity of shiitake mushroom in Fulminant hepatic failure (FHF). The activity was ascribed to inhibiting formation of the NLRP3 inflammasome and blocking downstream events of inflammasome activation, including cytokine secretion, Casp1 autocleavage, and pyroptotic cell death(26). Similarly, grape derived exosomes have shown to augment the proliferation of Lgr5hi intestinal stem cell during physiological as well as pathological condition demonstrated via DSS-induced mouse colitis model. Lgr5hi intestinal stem cells yield long-lived intestinal organoid structures in vitro helping to alleviate DSS-induced colitis(27) Zhuang et al(2013) , year demonstrated hepatoprotective property of Ginger-derived exosomes enriched with 6-shogaol in alcohol-induced liver damage mice model. The underlying mechanism for this activity was believed to be via activation of nuclear factor erythroid 2–related factor 2 (Nrf2) and increasing expression of a group of detoxifying/antioxidant genes (viz. including HO-1, NQO1, GCLM, and GCLC). Further it was observed that Ginger-derived exosomes also decreased triglyceride levels and liver weight in mice. (28)

Raimondo et al (2015) showed in vivo anti-cancer of Citrus limon (lemon)-juice-derived exosomes in chronic myeloid leukemia (CML) xenograft model. The underlying mechanism was found to be inhibiting secretion of multiple angiogenesis related cytokines resulting in TNF-alpha-related-apoptosis-inducing- ligand (TRAIL)-mediated cell death and angiogenic inhibition. (29) Tajik et al (2022) extracted PDEs from two chemotypes of cannabis namely Ark-01 and Krmn-01 by ultracentrifugation method. Lack of tetrahydrocannabinol (THC) and differentiating amount of cannabidiol (CBD) lead to nomenclature of High-CBD EVs (H.C-EVs) for Ark-01 and low-CBD EVs (L.C-EVs) for Krmn-01. H.C-EVs were more efficient to decrease the viability of two liver cancer cell lines (HepG2 and Huh-7) as compare d to L.C-EVs, with exempting any effect on HUVECs' normal cells. The mechanism for the in-vitro hepatocellular carcinoma management was cell cycle arrest in the G0/G1 phase and induction of apoptosis via mitochondrial-dependent apoptosis signaling pathway. (30)

4.2. Exosomes as carrier for drug delivery:

Study undertaken by Goasdué (2016), revealed that grapefruit derived exosomes exhibited specificity overcoming brain blood barrier in comparison to placental barrier. Plant derived exosomes (PDEs) have unique advantage of the ability of the particles to cross blood brain barrier while being barred by the placental barrier. This is because of the presence of efflux transporters which identify and translocate the nanoparticles back into the circulation, making them safe to use even in case of pregnancy (31). Transdermal delivery of these vesicles was tried and was found to penetrate deeper into the dermis layer of the skin tissue. This was tried out using broccoli derived vesicles, which imparted hydrophobicity to the vesicle. The reason for efficient penetration across the stratum corneum was due to the similarity observed between the composition of vesicle surface and the skin of humans (23). Wang et al. (2022) reported that uptake of PDEs derived from grapefruit on oral administration were more effectively internalised by the intestinal macrophage as compared to commercially available liposomes loaded with methotrexate. Further studies were also carried out to investigate targetability of exosomes using folic acid as ligand. The studies revealed that the modified exosomes were localised chemotherapeutic drug (Methotrexate), it was found that these exosomes were localised more at the tumor site. Zhang et al. (2018) reported lowered toxicity for the drug Doxorubicin when loaded onto Ginger-derived exosomes functionalized using folic acid when conducted on a healthy-mice. Similarly, Ginger derived exosomes were specifically taken up by cells like hepatocytes and proteins such as albumin, whereas grapefruit derived exosomes like nanovesicles were observed to be concentrated in liver Kupffer cells. Zhuang et al. (2022) reported intranasal delivery of drug Grapefruit derived exosomes loaded with drug for brain tumors (nose to brain delivery). The effect observed was the reduced rate of growth of tumors leading to enhanced survival period. Likewise, in comparison to standard liposomes, exosomes containing miRNA were administered via the intranasal route for the treatment of glioma. It was noticed that there was enhanced delivery of exosomes as compared to standard liposomes. On comparison with synthetic liposomes, ginger derived lipid-based exosomes were observed to be a better option as a carrier for divalent metal transporter 1-siRNA to alleviate iron loading in hereditary hemochromatosis (32). Another report of studies suggested that on comparison of cationic liposomes with grapefruit derived nanoparticles, the derived nanoparticles are safe for the liver at the dose equivalent to the dose of cationic liposome. These nanoparticles were also found to have higher residence time in brain and lung after

intranasal administration. Zhang et al. (2021), reported enhanced expression of miRNA in the anti-inflammatory and cancer pathway when delivered by Ginger-derived nanoparticles. This was due to down regulation of inflammatory factors such as NF-κB, IL-6, IL-8 TNF-α. stability of exosomes was also found to conform towards gastro to be good enough to sustain the gastrointestinal pH. Similarly, lemon-derived exosomes showed more tolerance of lactobacillus towards bile. Exosomes were exploited for drug targeting and were tried out on tumor targeting. One such example is that of grapefruit-derived nanovesicles. These vesicles were loaded doxorubicin and were released only at pH around 6.5 which is closer to the acidic tumor microenvironment. (31)

Conclusion:

On comparison with the synthetic drug delivery systems (including organic, inorganic and polymeric nanosystems) plant derived exosomes prove to be more biocompatible due to natural and endogenous nature. During last decade, plant derived exosomes have been explored for their therapeutic activity as well as ability to be nano-carriers for targeted drug delivery. the reason for variability for uptake is yet to be elucidated. Further there are many technological gaps for its transition from bench to bedside. The presence of biological components in plant derived exosomes and their interaction with loaded drugs are to be examined. If interactions observed, the unloading of these biological components without changing the morphological characteristics of plant derived exosomes is needed to be established. Resolving the concerns and confirming the mechanisms of exosomes internalization and interactions, plant derived exosomes will open new doors for efficient drug delivery with complementary therapeutic benefits.

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Benefits and limitations of cell-based therapy as a novel treatment strategy for infectious diseases

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Introduction

Cell-based or host-directed or host-specific therapy has continued to gain remarkable global attention and acceptance among researchers and clinicians as a promising strategy to combat diverse diseases. Historically, this therapeutic strategy was introduced into medical research and practice lexicons in the late 19th century when the Mauritian-British neurologist and physiologist who was later known as the pioneer of hormone therapy, Charles-Édouard Brown-Séquard, attempted to slow down aging by parenteral administration of cell extracts prepared from animal testicles [1]. Some other accounts credited Ernst Heinrich Philipp August Haeckel, a German Professor of comparative anatomy, a zoologist, naturalist, geneticist, and an advocate and popularizer of Darwinism at the University of Jena, as the pioneer of cell therapy when he described the cells of all organisms using the concept of fertilized ovum in the mid-19th century [2]. Since then, cellular therapy has continued to offer numerous patients burdened with incurable and untreatable diseases the hope and opportunity to live quality life. This could be seen from the various breakthroughs recorded by many workers in diverse aspects of applications of cell-based therapies [3].

Cell therapy is occupying an important position in clinical research for disease treatment following the launch of cell-based therapeutic products with a global market share of about USD 10 billion, which has been projected to reach about USD 25 billion within the next decade [4]. These products are either non-stem cell or stem cell unicellular or multi-cellular therapies utilizing allogeneic or autologous cells which are functionalized or engineered in basal tissues for application as bioscaffolds, injections, scaffold-free products, infusions or topical devices. Though most of them are still in their early stages of clinical trial but they have received provisional approvals from regulators (mainly the European Commission and US Food and Drug Administration) for clinical use including PROVENGE® (comprising autologous CD54+ cells

primed with PAP-GM-CSF) approved in 2010 and manufactured by Dendreon Corporation for treatment of metastatic castration-resistant prostate cancer [5], GINTUIT® (which consists bovine collagen primed with allogeneic keratinocytes and fibroblasts) approved in 2012 and manufactured by Organogenesis Incorporated for the treatment of surgical-based mucogingival conditions [6], MACI® (containing porcine collagen membrane primed with autologous cultured chondrocytes) approved in 2016 and manufactured by Vericel Corporation for the treatment of defects of the knee cartilage [7], and very recently, ABECMA® (which contains genetically engineered autologous chimeric antigen receptor–positive T-cells) approved in 2021 and manufactured by Celgene Corporation for the treatment of relapsed B-cell lymphoma, and BREYANZI® (containing engineered autologous T-cells) approved in 2021 and manufactured by Juno Therapeutics Incorporated for the treatment of refractory B-cell lymphoma [8], among others.

Cellular treatment of infectious diseases

Field evidences have highlighted that the application of cell therapy in the treatment of diseases is still an emerging area of research which requires consistent iterative learning and partnership between academic researchers and hospital physicians in order to perfect existing cell-based therapies and technologies. The increasing interest in cell-based interventions in infectious diseases is due to the understanding of the diverse manipulative ways used by invading microorganisms to modulate and evade the immune cells of the host, and the consensus that cell-based therapies could be administered as immunotherapies to target specific compromised immune functions and boost the defensive responses of the host cells against invading parasites.

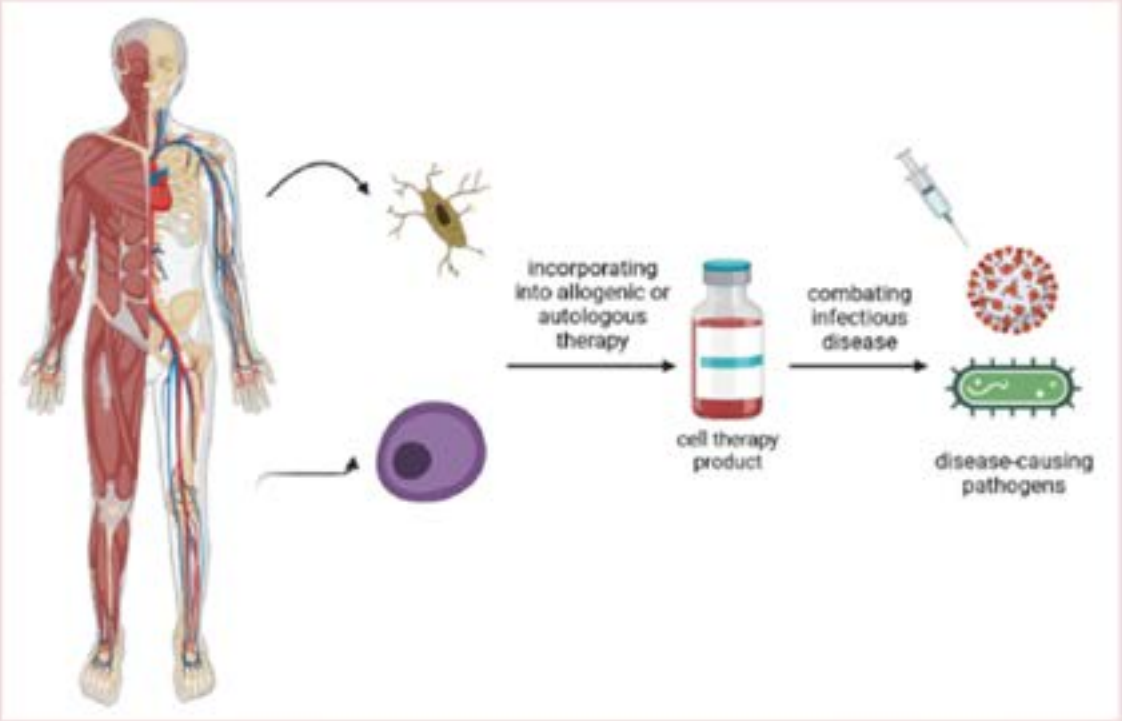

Figure 1. Mobilizing T-cells, B-cells, NK cells, DC cells, and macrophages from epithelial and ciliated tissues

The cells are incorporated into allogenic or autologous cell-based product for combating infectious diseases-causing pathogens. Cell-based products currently hold a global market share of about USD 10 billion, and are projected to reach USD 25 billion in the next decade

Human immunodeficiency virus (HIV) is a virulent pathogen responsible for the health epidemic called acquired immune deficiency syndrome (AIDS). HIV undermines the immune structures of the over 38 million people reportedly living with the virus globally [9]. Despite the innovative strategies clinically developed to treat HIV infection including the very recent combination or highly active antiretroviral therapy (cART or HAART) which has successfully suppressed viral replication, extended life expectancy of carriers and nullified the 'death sentence' tag associated with HIV, it has not completely provided the required succour intended for its introduction due to the existence of the latent memory and replication-competent CD4+ T-cells reservoir [10]. Based on the successes recorded in cell therapy of cancer, chimeric antigen receptor (CAR) T-cell therapy has been advanced as the future if total elimination of HIV is to be achieved. However, engineering the functionality of CAR T-cells using the potent and HIV-specific broadly neutralizing antibodies (bNAbs), has recorded better anti-HIV therapy efficacy [11, 12]. Before use, CD8+ T-cells are extracted from HIV carrier cells, primed with CAR genes, their anti-HIV specificity and efficacy are established in vitro followed by re-induction in carriers for clearance of HIV cells [13]. In spite of the recorded breakthroughs in clinical applications, certain limitations are associated with bNAbs-based CAR T-cell therapy including vulnerability to HIV infection by the latent virus cells reservoir, possibility of therapy-induced cytokine mobilization storm [14], off-target effects (attack on healthy cells expressing similar antigens) [15, 16], and neurologic disorders [10].

Cell therapy has been effectively utilized in treating and improving survival of patients with infectious diseases of the lungs including tuberculosis (TB), influenza, acute respiratory distress syndrome (ARDS), and influenza virus-induced acute lung injury (ALI), and more recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV 2) otherwise called coronavirus disease 2019 (COVID-19) [17 – 19]. COVID-19 is a respiratory infectious disease designated a pandemic by the World Health Organization (WHO) due to the severity and very high incidences of morbidities (>25 million), mortalities (about 900,000 deaths) and economic losses recorded globally [20]. There are recent evidences emerging from studies initiated at the epicentre of COVID-19 in Wuhan, China, that cell-based therapy, especially mesenchymal stem or stromal cells (MSC) therapy, could be the most successful approach for eliminating COVID-19 [19, 21, 22]. This is due to previous successful application of MSCs in the therapy of other infectious diseases of the lungs like TB, influenza, ARDS, and influenza virus-induced ALI owing to their immunomodulatory, anti-inflammatory, antimicrobial, and regenerative (without losing their parent pleiotropic characteristics) profiles [17]. MSCs could be harvested from the lungs, bone marrow, fallopian tube, and cord blood for reprogramming the immune responses of COVID-19 patients and decrease mobilization of pro-inflammatory cytokines which potentiate the pathogenic fatality of the infection [23, 24]. According to the International Society of Cell Therapy (ISCT), MSCs credited with remarkable properties to home and survive compromised respiratory immune structure like COVID-19 must be able to identify, isolate and differentiate osteoblasts, adipocytes, and chrondrocytes; must not express the transmembrane proteins CD-79, 45, 34, 19, 15, 11 and HLA-DR cell surface molecules; must express CD-105, 90, 73 transmembrane proteins, and must be plastic compliant [25]. Despite the expected

successes derived from MSC therapy in COVID-19, there are issues with selecting the best route of administration because the presently used parenteral route is limited by emboli formation, morphological changes, and unwanted cell loss.

Malaria is one of the foremost parasitic diseases caused mainly by the deadly protozoan parasite, *Plasmodium falciparum*. The parasite has continued to develop better strategies to evade and manipulate the immune responses, and sequester its infectious proteins within the microvasculature of the host tissues. Research for a magic bullet for total elimination of malaria is currently ongoing. Though WHO prescribed the use of artemisinin-based combination therapy (ACT) as the first line treatment for malaria but the consistent development of resistance against the ACTs has reversed the earlier successes recorded, and availability of a vaccine suitable to combat the disease in all age groups is still elusive. Recently, evidences from experimental murine malaria have revealed that cell therapy based on MSCs, the self-regenerating, expansive and highly potent stem cells, rolled back plasmodium parasitaemia by boosting secretion of interleukin (IL)-12 while down-regulating the production of T-cells and IL-10 [26]. This therapy option at conquering malaria was further tested in another murine model of CM and it was observed that MSCs isolated and differentiated from bone marrow improved survival by attenuating sequestration of parasite proteins in mouse tissues. It was shown that MSCs have the ability to enhance the immune responses of CD4+ and CD8+ T-cells as well as CD34+ haematopoietic cells [27, 28]. Expanding the antimalarial studies to include all plasmodium strains and primate models will provide better clarity on the clinical efficacy of MSCs against malaria. It could also provide important hints to improve the development of malaria vaccine.

Leishmaniasis is an important neglected tropical disease (NTD) caused by the intracellular parasite, *Leishmania spp*., and transmitted through the bite of infected vectors, sand flies. It causes both cutaneous and visceral leishmaniasis. While in the host system, the parasite sequester in vital host tissues like bone marrow, spleen and liver where it regenerates itself as amastigotes with lethal consequences [29 – 32]. Most of the early antileishmaniasis drugs introduced against the disease including paromomycin, miltefosine, and antimony, have been discontinued due to treatment failure, relapse, or drug resistance owing to the manoeuvring tendencies of the parasite against host immune microvasculature [33]. Cell therapy has been reported as an interesting option for leishmaniasis because it has been shown that intralesion administration of MSCs in mouse model of cutaneous leishmaniasis suppressed lesion propagation, elevated clearance of infectious proteins by splenocytes, and improved phagocytotic capacity of macrophages against the parasite [34]. Recent evidence has confirmed that the haematopoietic stem cells (HSC) of the bone marrow serve as the sanctuary for propagation of visceral leishmaniasis with very high parasite load [35]. This finding will open new vistas in the ongoing application of cellular therapy for eradication of leishmaniasis.

Limitations of cell therapy in infectious diseases

Clinical studies of cell therapies are done using patients instead of the conventional healthy volunteers. Though this practice reveals the real-time overall effect (efficacy and safety) of the treatment, any untoward event (off-target effect) is usually fatal because of the immunocompromised status of the patient.

Clinical trial of cell therapies for infectious diseases requires long-term (sometimes, up to 24 months) clinical monitoring and iterative interaction between the innovators of the therapy and the clinical implementers in order to obtain vital clinical data to measure progress made and areas of improvement of the developed therapy [36, 37].

Due to the profound immunosuppressed status of patients receiving cell therapy, they might be exposed to varying opportunistic infections by bacteria, fungi, and virus which elevates their systemic microbial load, worsens the pathogenesis of the disease, and prolongs recovery time [38]. Institutional, professional, and regulatory guidelines have recommended methods to prevent these setbacks but they have not been completely eradicated.

Occurrence of adverse immunogenic reactions in patients undergoing cell therapy due to the site of administration, immune competence of the patient, repeated administration of therapy, differences in allelic compositions of patient and the cells, and cell maturity prior to administration, affect the efficacy and safety of cell therapy [39].

Conclusion and future perspectives

Available evidences from molecular, cellular and tissue studies suggest that cell therapy presents an important option in the efforts to combat infectious diseases. Regulators should close regulatory loose ends by interacting and working closely with researchers and manufacturers of cell therapies to ensure that they are developed under strict quality and safety guidelines. To prevent the incidences of off-target treatment and enhance the safety of cell therapies, there is need to intensify research towards developing nanotechnology-based cell therapy products which will guarantee targeted docking and release of payload at the appropriate site for desired optimum effect, while avoiding healthy cells and tissues.

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Advances in Adoptive Cellular Therapy with special reference to Chimeric Antigen Receptor: An Overview




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Abstract

Adoptive Cellular Therapy is a potentially progressive therapy for the treatment of different types of cancers. Recently, three therapies are in considerations for the approval by the FDA; they are Tumor- Infiltrating Lymphocytes (TILs), Chimeric Antigen Receptor (CAR) and T-Cell receptor engineered T cells. Immunotherapy when taken in consideration with engineered T cell is effective have a distinct role in the cessation of cancerous cells. CAR basically finds use which grafts the immune system cells to typically T call to target its function. It has in the T cell activator domain that to induce specificity for the target cell. These approaches can create a widespread use for the treatment of malignancies when taken into consideration.

Keywords: Adoptive Cell Therapy, Chimeric Antigen Response, Tumor- Infiltering Lymphocytes, Cancer, T cell

Tumor Infiltrating Lymphocyte (TIL):

In recent years, the Adoptive Cell Therapy (ACT) has been a topic of discussion in the area of immunology where it has a diverse application in the stages of blood cancers [1]. Majorly, T cells from the peripheral blood stream are withdrawn to genetically modify the sequencing and express the new receptor as transgenic receptor which is now well understood as TIC [2]. Studies show that adoptive transfer of T cells has least differentiated phenotypes, memory cells and effector T cells. Although it is crucial for the treatment of leukemia to a considerable extent. It also withstands the memory response which is mediated by CD45RO gene in human race. [3]This gene is associated with the secondary lymphoid tissue and thus mediates the memory response, though they are examples of least differentiated memory cells like other T cell subtypes. Sometimes, T cells fail to respond to the progressive cancer and chronic infections, this produces a delayed response in the treatment of cancerous tumors [4–6].

Though human bodies predominantly have T cells which targets the cancerous cells, and thus are considered a boon in immunology. A hinderance to this mechanism arises when these T cells are bound to first get activated which would then target the cancerous cells. This roadblock takes a lot of time to induce the immune system thereby delaying the natural therapy. This form of Adoptive Cell Therapy (ACT) is called Tumor Infiltrating Lymphocytes (TIL)

that finds its importance when already activated T cells are withdrawn from the naturally occurring body part and then is infused into the patient with the same problem. (Fig. 1) This helps in cutting short of the time to get activation thereby decreasing the time of action of T cells [5].

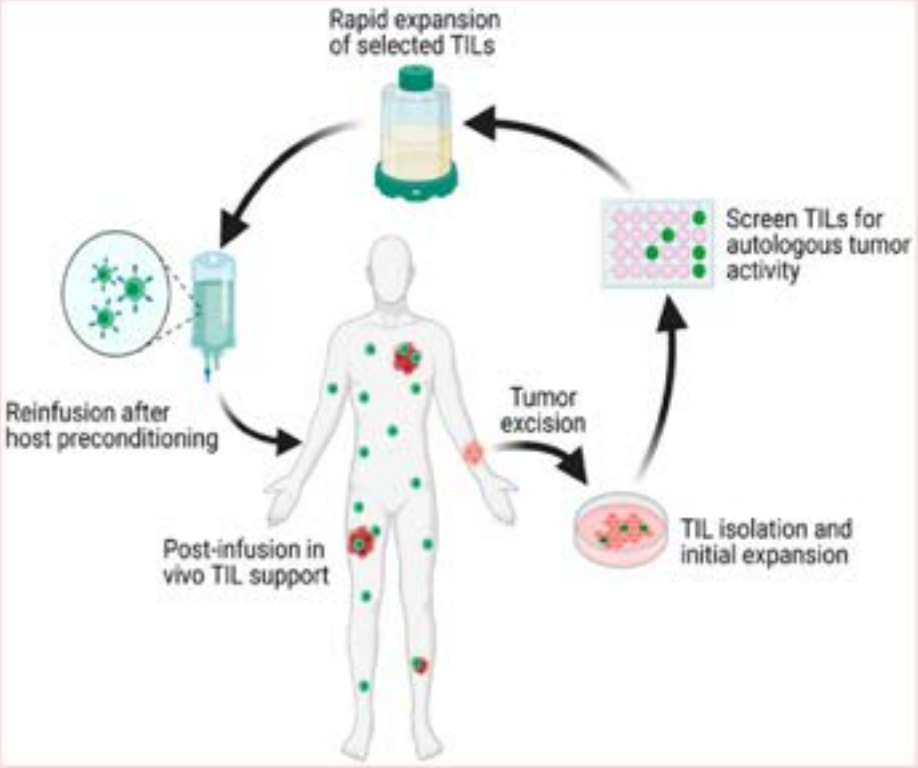

Fig 1: Mechanism of TIL

Engineered TCR (T Cell Receptor) Therapy:

Initially, from melanoma patients, the TILs isolated, showed two mutant melanocytes, MART-1 and gp100. These two genes had expressed themselves in the regions of eyes, ear and skin. But this was of great help that either of the genes did not show a toxic response when treated with TIL therapy. This paved a way for upcoming developments in the fields of TCR. With the introduction to TCR, a boon in the immunological science was made to come into account. Not every patient has their T cells activated, and for such patients, engineered T cell therapy is considered. In this, unlike the former one, the cells are extracted from the tumor inducing body and then are engineered with a new T cell that will now target the specific cancerous cell. This is a very potent technique which is now being used to a comparatively good extent and hence able to save life from a deadly disease. The mechanism behind this therapy is modification of alpha and beta chains of TCR to enhance their stability and reducing of mispairing. Moreover, TCRs have more affinity towards the tumor causing gene and easily recognizes the extracellular targets. This technique had its cons though, like it was a very difficult task to isolate a specific T cell from the cancerous cells, but clinical studies showed a significant rise in the treatment approaches by this method. (Fig. 2) Furthermore, the naïve cells undergo a multiplicative expansion to 10-20 folds after activating by the TCR. After being amplified, the activated receptors participate in the signal transduction in the plasma membrane. This transduction progresses with the channeling of calcium binding proteins, which triggers the nuclear factor of T cells [7,8]

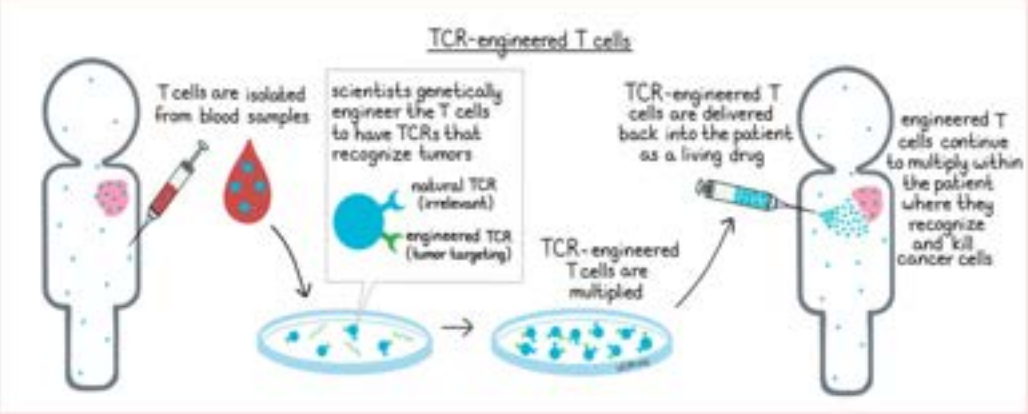

Fig 2: TCR Engineered Cells

CAR T Cell Therapy

CAR stands for Chimeric Antigen Receptor which is an artificially synthetized receptor which mimics the receptor for T cells. This is very sensitive to the cancerous cells present on the surface of the MHC. This basically targets the B- cell lymphoma till date. CAR is responsible to trigger the antiapoptotic function of the primary T cell.

CAR considers the following steps: (Fig. 3)

- Collection of T cells: The T cells are withdrawn from a body part and made to flow through the apheresis machine which filters the T cells and sends back the remaining blood back to the body through another tube.
- Modification of the cells: The CAR is engineered in the lab and made to proliferate.
- Infusion of engineered CAR cell: The engineered cell is then transferred into the body post chemotherapy, so that it may not hinder with the currently existing pathogens, if any. [9,10].

Since, CAR therapy is still an ongoing assessment program, it certainly adds some side effects like nausea, fatigue, chills, vomiting, hypotension, tachycardia, arrhythmia, cardiac arrest, renal insufficiency and so on. The CAR therapy finds use in multiple myeloma, chronic lymphatic leukemia and other certain blood cancers. Since this is a newer process and not largely used, it comes with certain limitations like the infusion of CAR antigen involves a multidisciplinary approach with sophisticated instruments and should be according to the standards specified by Foundation for the Accreditation of Cellular Therapy (FACT). For this, the nursing staff shall be well trained and has met all the necessary training needed by the FACT. The work continues with the monitoring after the CAR antigen has been fused into the patient. A constant documentation based on neurological, toxicological studies shall be maintained in case of any obligations. Both the patients and the caregiver shall be equipped with necessary resources for any emergency situations. Patients are advised to monitor their body temperature from time to time and notify the clinical team for any discrepancy.[11]Hinge region are also taken into account that extends the binding to the transmembrane region. They provide the flexibility to overcome the steric hinderance observed at the time of infusion. It allows the targeted antigen for specific domain. [12].

A combination of all the above illustrated techniques is a boon to the society for the treatment of malignant carcinoma [13].

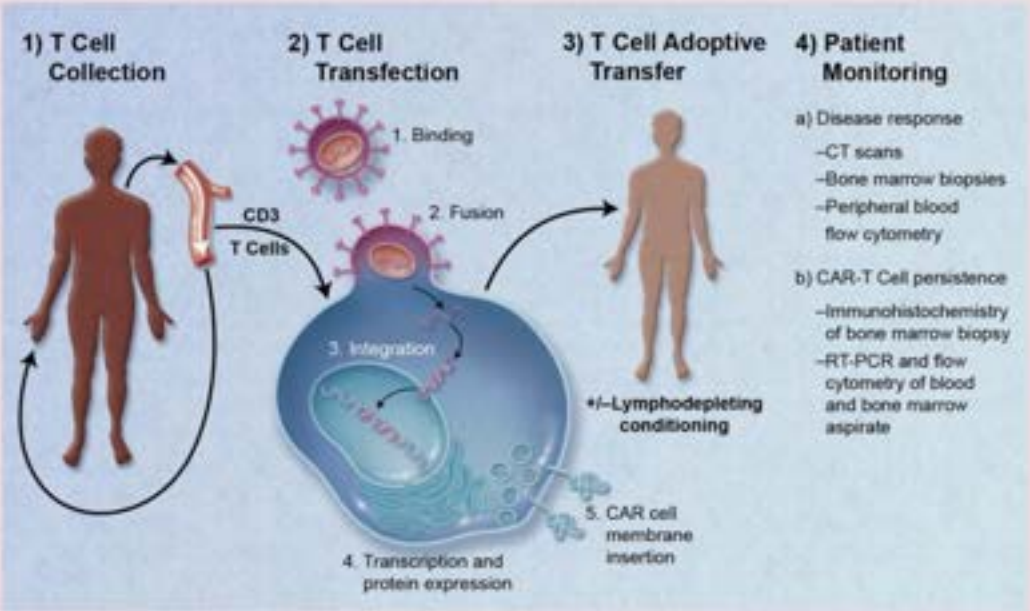

Fig 3: CAR Therapy

Conclusion

ACT is subdivided into TIL, TCR and CAR, all of which when considered in combination with each other can lead to be a boon in medical and pharmaceutical sciences. Since, this technology is just a matter in which both advancements are of their individual benefits. This technology, if cultured, will provide to be of great therapeutic help in oncogenic sciences. These combined challenges and technology require standardization; however, CAR T cells offer patients hope of advanced treatment. As the first therapy is already available in the market, there is potential for a specific and improved alternative becoming available in upcoming decades [14].

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Leveraging properties of Natural Killer cells for cell-based immunotherapy


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Introduction

Pharmaceutical Sciences provide an expanding array of novel drugs against various diseases but there are many medical conditions like cancer, heart diseases, genetic disorders etc. that remain still incurable. To alleviate all these fatal conditions researchers continued their efforts in search of cutting-edge technologies in the field of Biotechnology as well as Regenerative medicine. In nineteenth century, the knowledge of immunology led the scientists to a promising approach for immunotherapy named as Cell Based therapy or Cytotherapy wherein the viable, healthy and normal or engineered cells from self or donor person are injected or transplanted to the patient in order to make the illness less severe by facilitating the cells to exert therapeutic action (1). Cell based therapy uses vast types of cells for the treatment which are mainly stem cells, dendritic cells, lymphocytes, pancreatic cells etc. Out of these haematopoietic stem cell (HSC) based therapy is already a well-established treatment for blood related disorders whereas rest of the cells used are still in experimental phases (2). On the other hand, in recent years Autologous chimeric antigen receptor (CAR) T cell therapy is the first commercialized therapy which led to substantial breakthrough in patients with critical B cell malignancies. However, despite the success, an obstacle remains which is nothing but graft Vs host disease. Natural killer (NK) cells, furthermore, identify their targets in a human leukocyte antigen (HLA)-unrestricted manner and hence do not represent these same concern which makes them suitable components for cell-based immunotherapy (3). This article mainly focuses on the properties and mechanism of NK cells for immunotherapy against various diseases along with associated challenges and recent advances to improve the effectivness of NK cell-based therapy.

Immunological defense mechanism of Natural Killer cells

NK cells being the crucial component of innate immunity serve as the very first line of defense against tumors as well as a diverse range of pathogens mainly bacteria and viruses (4). Initial findings on NK cells highlight that the cells can discriminate between healthy cells and stressed or infected cells with the aid of inhibitory and activating receptors present on NK cell surfaces. Primarily all healthy cells possess self-major histocompatibility complex (MHC) class I

molecules on their surfaces which are detected by inhibitory receptors of NK cells and considered as self or healthy cells. According to 'self-missing hypothesis', cells lacking MHC molecules are detected by activating receptors and recognized as foreign or infected ones. Activating receptors of NK cells engage themselves by binding to the ligands displayed on pathogen infected cells consequently stimulating the activation of NK cells. Upon activation, NK cells elicit a potent response through the release of cytolytic granules and cytotoxic cytokines such as tumor necrosis factor (TNF)-α and interferon (IFN)-γ (5) . Moreover, NK cells are also capable of identifying antibody-coated cells through their FcγRIIIA (CD16) receptor and trigger antibody-dependent cellular cytotoxicity (ADCC) and cytokine production (6). In this way, NK cells exhibit immune regulatory functions which further help shape T cell and B cell responses, and impact the function of macrophages, dendritic cells, and neutrophils.

Leveraging properties of NK cells for cell-based immunotherapy

Based on the literature studies, it can be concluded that NK cells show remarkable potential to treat various diseases like cancer, cytomegalovirus infection, asthma, autoimmune diseases, and HIV-AIDS etc. They also exhibit few unique features which make them ideal and powerful immunotherapeutic agents to be used in the flourishing field of cell-based therapies. Some of the immunological salient features of NK cells are as follows:

- First line of defense: As discussed above, NK cells have the ability to recognize and kill transformed cells which are deprived of antibodies and MHC, resulting a much faster immune response without the need for prior antigen sensitization (7).
- Exhibit greater phenotypic heterogeneity: Evolution in single cell genomics and high-parameter cytometry, claimed that NK cells may demonstrate higher phenotypic heterogeneity which gives rise to diverse cell populations equipped with altered functional properties (8).
- Lacks graft Vs host disease: During the maturation of NK progenitor cells, they do not undergo clonal selection process and lack expression of the clonotypic TCR and the associated CD3 complex which are requisite components for signalling cascade. Therefore, the allogeneic NK cells do not trigger graft Vs host disease in the receiver's body.
- Memory like function: Studies reported that murine NK cells are endowed with memory like functions in case of cytomegalovirus infection. Later Todd Fehniger's group hypothesized that human NK cells should possess memory-like properties in similar manner. After consistent efforts, their study concluded that human NK cells which were preactivated with IL-12, IL-15 and IL-18, followed by 1–3weeks of resting phase, could initiate a robust immune response directed by increased IFN-γ production upon further exposure to cytokines (9) .

NK cell-based therapy and application against various diseases

NK cell-based therapy has been recognized as an incredibly promising therapeutic strategy to mitigate numerous severe diseased conditions. Effectiveness of this therapy highly relies on the source from which the NK cells are being transplanted. Predominantly, autologous strategies have been applied but in patients with cancer, NK cells denote a malfunctional phenotype marked by altered gene expression profiles which may affect the feasibility of

autologous strategy and its application by diminishing cytotoxic functions of NK cells (10),(11). Additionally, if patients are unable to provide sufficient cells, it would become more cumbersome for downstream processing and further engineering of NK cells. Therefore, NK cell-based therapy largely depends on allogeneic sources in order to overcome risks associated with autologous strategies. Apart from this, NK cells can also be derived from cord blood (9), (12), peripheral blood mononuclear cells, haematopoietic stem and progenitor cells (HSPCs) (13),(14),(15), immortalized cell lines (16), and induced pluripotent stem cells (iPSCs) (17). NK-92 is the first pioneered NK cell-based immunotherapy to receive Investigational New Drug approval by the US Food and Drug Administration (FDA) for clinical testing. The NK cell-based therapy has wide application to many diseases.

A. Cancer
B. Tumor
C. Acute myeloid leukemia
D. Hematologic malignancies

Recent Advancement to overcome few incumbrances of NK cell-based therapy

Despite numerous achievements, there are few challenges associated with the NK cell-based therapy which impact on the possible clinical benefits of the treatment. Few of the incumbrances and further recent advancements are as follows:

i)Chimeric antigen receptors (CAR) NK cells: Chimeric antigen receptors (CARs) are synthetic fusion proteins consisting of an intracellular signalling moieties and extracellular antigen-recognition domain that trigger cell activation. To reprogram the specificity of NK cells towards a particular target, CARs can be expressed on NK cells. Recent research data confirmed that these cells can target tumours with efficacy and specificity, by providing a desirable safety profile (18).

ii)Cytokine armouring: It has been observed that freshly extracted NK cells exhibit lower cytolytic potency as compared with NK cells that have been primed (19). Initiatives have been taken ahead aiming to effectively prime NK cells in vivo and/or ex vivo to preserve optimal antitumor function. During ex vivo expansion of NK cells, the cells can be combined with group of interleukin supplementation such as IL-2, IL-15 and IL-21 which demonstrated that these cytokines augmented the cytotoxic function and supported high proliferation rates while keeping the cells in a healthy and non-exhausted state (20). A new strategy has been hypothesized that cytokine armouring might be programmed in such a way that the soluble cytokines can be released into the environment or may be constructed in membrane-bound form to stimulate immune response upon cell to cell interaction (21).

iii)Overcoming immunosuppression: Tumor microenvironment (TME) comprises of a harsh metabolic landscape driven by a heterogeneous mixture of glucose and amino acid deprivation, immunosuppressive metabolites, acidity and hypoxia, which, in consequence, hinder effectiveness of antitumor immunity (22), (23). Since researchers are trying to alter immunometabolism in the TME, it is important to consider the significance of physiological balance, as few of the metabolites are requisite components of regular metabolism. Although most of the strategies are still in the embryonic phase, it has been assumed that combining TME modulation and NK cell

engineering can be plausible to reduce immunosuppression and enhance immune response of cells.

iv)Checkpoint disruption: Most often tumors have transmogrified themselves in a well sophisticated manner to enter immune surveillance, for instance the engagement of immune checkpoints, which may affect the functions of NK cells. Inhibitory KIRs and CD94/NK group 2 member A receptor (NKG2A) have been regarded as potent negative regulators of NK cell function and can revoke any concomitant activating signal when bound with HLA class I ligands. Few of the monoclonal antibodies such as Lirilumab and Monalizumab function to misrepresent the diverse signaling indications perceived by NK cells towards the activation by deactivating these inhibitory checkpoints (24). Hence, researchers claimed that monoclonal antibodies can be a reliable component to modulate the patients' immune cells, in order to maintain or extend in vivo half-life of NK cells. With further advancements in genetic engineering, the genes of NK cells can be stably altered to regulate immunological mechanisms that reinforce NK cell effector function. An example is the genetic disruption of the inhibitory receptor named as NKG2A, resulted in tumour control in xenograft mouse models which were inoculated with HLA-E+ tumours (25).

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Cell Based Therapy: An Insight into Voyage of New Generation Therapeutics



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Introduction

Cell therapy is the technique involving transfer of cells from the patient (autologous) or from a donor (allogenic) into a patient with the goal of improving a disease (1,2). The first cell treatment procedures were performed in 1889 by Charles Edouard Brown-Sequard, who was considered a pioneer in hormone therapy. He used injections of animal testicle extracts to slow down the effects of ageing (3). Cell therapy is currently evolving due to continued research on its clinical safety and efficacy. There has been constant surge of interest on cell therapy evident with the size of global market expected to 9.5 USD billion in 2021 to 23 USD billion by 2028. [4] Cell based therapy includes unicellular or multicellular therapies based on stem cells and non-stem cells. Cell therapy is widely used in various therapeutic fields, comprising cancer treatment, immunotherapy, and regenerative medicines.

Cell-based therapies have come up as a boom due to its marked potential to prevent a wide range of disorders that are presently incurable due to their remarkably effective mechanism of action. Despite major recent clinical and financial accomplishments, cell-based medicines still need to be translated and commercialised widely before they may be used in routine. These obstacles include choosing the right cell source, developing a product that is sufficiently valuable, effective, and non-toxic and meets the needs of particular patients and diseases, and creating scalable manufacturing methods. The research supported by next-generation engineering techniques, such as genome and epigenome editing, synthetic biology, and the utilisation of biomaterials, is being used to overcome these obstacles.

Treatment of multiple disorders with stem cell therapies

The initial description of "stem cells" started in 1888 marked the beginning of a significant development in the field of rejuvenation drugs. In 1902, the first recognition of hematopoietic stem cells was identified. In 1939, the first bone marrow transplant was carried out to treat aplasmic anaemia. In the intervening period, the growth of stem cell-based therapy has seen numerous breakthroughs and significant achievements made in respect to the conversion of fundamental research into pre-symptomatic investigations and clinical diagnosis. Human

pluripotent stem cells were later discovered, and their isolation in 1991 was followed by the development of stem cell-based treatment for the treatment of human diseases (Fig 1a).

Cell origins of stem cell-based treatment- undifferentiated cells such as foetal stem cells (originate from the blastocyst, an inner mass cell) and developed embryonic stem cells, give rise to a variety of human cell types, including the three germ layers, and can multiply indefinitely in vitro. Multipotent stem cells originating from mesoderm, mesenchymal stem cells have the capability to remodify itself (restricted in vitro) and have the capacity to diverge into mesenchymal lineages. Induced pluripotent stem cells are developed by reprogramming differentiated cells to return to the pluripotent stage using OSKM factors. It is crucial to remember that as compared to differentiated/somatic cells, stem cells exhibit a significant greater risk of tumour development with lesser threat of graft rejection (Fig 1b).

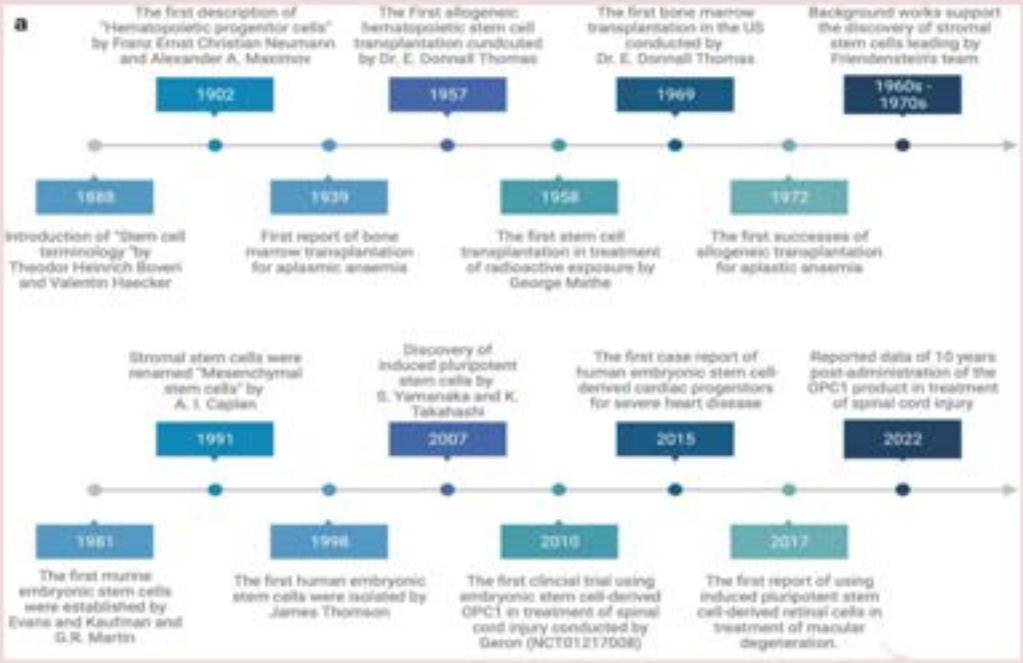

Figure 1(a) The source history of cell

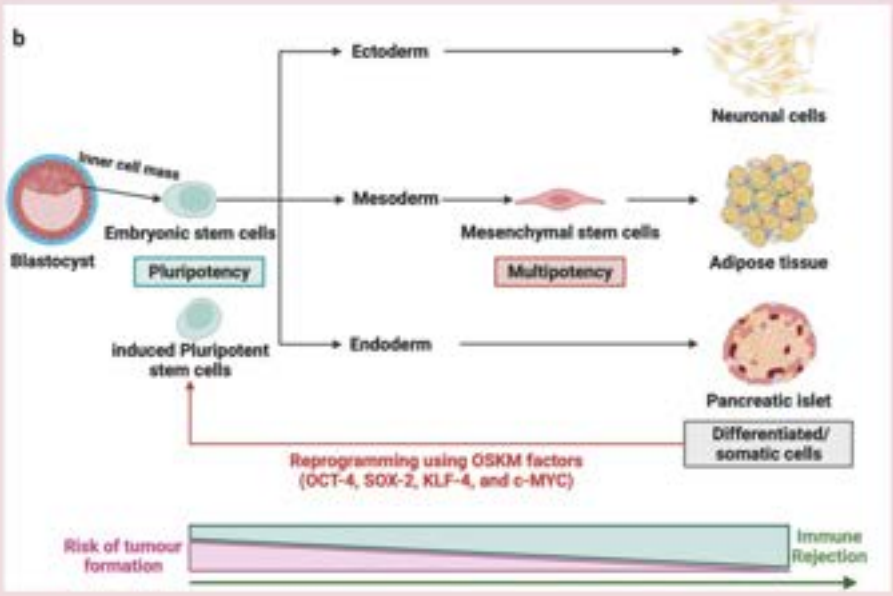

Figure 1 (b) diagrammatic representation of various cell sources that used in stem cell-based treatment

Immune system stem cell therapy

The majority of immunological system illnesses are brought on by autoimmune disorder or overactive immune responses. Therefore, the main goals of treatment are to reduce infection cause, effects, and it's recurrent nature. The goal of using cell therapy for immune system illnesses is immune system resetting as a permanent treatment, not just immune suppression and symptomatic relief (5). HSCT/BMT emerged as the most popular cell-based remedy for the treatment of immune system illnesses from 1990s (6). It has been shown that severe SS can benefit from HSCT with manageable ratio of transplant-linked death (7). In controlled phase two-third studies, hematopoietic stem cell transplantation (HSCT) has shown to be effective in treating patients with autoimmune disorders. For instance, patients with multiple sclerosis experienced 78-80% advancement in their disability status and notable improvements in the disease re-occurance rates, MRI abrasions, and quality well beings (8). The patients with systemic lupus erythematosus (SLE), hematopoietic stem cell transplantation (HSCT) and peripheral blood stem cells (PBSCT) also reduced disorder activity and improved organ failure. Platelet rich protein (PRP) demonstrated to reduce swelling, pruritis and eruption of inflamed cell by using ultrasound source imaging remarks in patients with RA (9), and regulatory T cell, which are shown to lower the prevalence of acute Graft versus host disease (GVHD), or other stem cell-based treatment besides few frequently reported benefits in immunological disorders.

Cancer stem cell therapy

The goal of melanoma therapy has changed from systemic tumour to target using chemotherapy and radiation therapy to a new focused strategy utilising new biological therapies, such as (APC)-based anticancer vaccines, oncogenic infections, and cell-line therapy, such as monoclonal antibodies and CAR-T cells (10). Numerous cell treatments have been researched for the treatment of cancer in clinical settings in addition to commercial cell therapy products. Only primary CD1c+ myeloid DCs can be used to make DC-based anticancer vaccines, or patient-derived tumour cells and tumour peptide can be combined to make them. Dendritic cell-based antineoplastic vaccines can be generated using only key CD1c+ myeloid dendritic cell s or can be created via fusing patient-derived tumour cells with tumour peptide (11).

Multicellular versus Unicellular Stem cells

Starting from the base first the unicellular stem is being used but recently it has moved towards the multicellular stem cell-based therapy. Anticoagulated blood, or platelet-rich plasma (PRP) product made by differentially centrifuging the total blood, is mostly composed of platelets in amounts that can be up to five times higher than normal platelet concentrations (12). Megakaryocytes mature to produce platelets, which are acellular fragments that primarily serve to maintain primary haemostasis and thrombosis in order to maintain vascular integrity (13). PRP comprises cellular components, such as leukocytes, despite being predominately composed of platelets, which are repositories of numerous immunologic compounds, soluble proteins, and growth factors (14). PRP can be processed using a variety of commercially available kits, with different results according to the concentrations of leukocytes, platelets, and red blood cells. Composition of PRP can be examined using a variety of analytical techniques, including microscopy, flow cytometry, and spectrophotometry (15).

PRP products can be heterogeneously formulated due to their unstandardized preparation processes. Several non-consensual classification methods, such as the International Society on Thrombosis and Hemostasis, the PLRA, and the PAW systems, are used to further categorise substances cellularity, platelet concentrations, and activation further taken into account in clinical situations (16). PRP works by a variety of mechanisms that are fueled by nucleated cells, cytokines, platelets and growth factors, which all work together to reduce inflammation and encourage tissue repair (17).

An organism's multicellularity enables cell-cell interaction, which is necessary for many stages in the process of tissue formation that begins early in embryogenesis and continues through subsequent regeneration processes. Multiple cell types, as opposed to just one, are required to enable long-term tissue regeneration, which is fuelled by multifaceted, well understood multicellular interactions typical of organisms' physiological functions, according to a growing body of evidence in regenerative medicine (18). Similar to this, a recent retrospective review of patients who had medial unicompartmental knee OA treatment with high tibial osteotomy with microfracture and either MSCs or BMAC discovered no differences in pain/functionality radiological findings and outcomes postoperative (19). Additionally, multicellular therapies may be more successful than biologic treatments comprised of a single cell type for cancer treatment. When assessed to patient-derived CIK cells, combinations of in vitro tests with DCs and CIK cells have revealed better antineoplastic activity.

Conclusion

Cell therapy is a growing industry that incorporates stem cell and the non-stem cell-based unicellular and multicellular treatments. These treatments range significantly in terms of their properties, sources of isolation, and applications. However, a number of obstacles still need to be overcome for clinical use of cell treatments for conditions like neurodegeneration illnesses in which the Clinical outcomes are difficult to evaluate due to the standardisation of cell mechanised techniques and the delayed disorder onset. Other impediments to cell development therapies are linked to safety, which with some products like CAR-T cells may present toxicities that, are fatal. Other benefits of multicellular therapies over unicellular therapies include their reduced cost and increased potential for use as a bridge to precision medicine by the rapidly developing fields of gene engineering and bioinformatics.

Abbreviations: BMT/HSCT-(bone marrow transplant/hematopoietic stem cell transplant), PBSCT(peripheral blood stem cells) CAR-T(chimeric antigen receptor cell), DC (dendritic cell), MSC (mesenchymal stem cell), CIK(cytokine induced killer), PRP(platelet rich protein)

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Role of gut microbiome on CAR-T therapy


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Keywords: Cell based therapy, CAR-T therapy, gut microbiome, gut-cancer axis

1.Introduction

Cell based therapies have been used widely for the treatment of various diseases which involves engineering of the stem cells taken either from the patient or any donor (1). Out of different types, Chimeric antigen receptor T cell therapy (CAR-T) has been used nowadays for the treatment of cancerous conditions. This technique involves the attachment of CAR receptor on the patients T cells and re-inserting it back into the patient body. Due to its several advantages over the chemotherapeutic agents, this has even been approved by FDA. But, in some cases either the failure of this strategy or side effects have been observed which points out to some other factors governing it (2,3). Nowadays, the role of intestinal microbiota have been explored widely either for the development of some disease or treatment of it. This is most probably due to the fact that gut microbiome have a huge impact on human immune system. Thus, this might also point out that unhealthy gut might have the probability for impacting the efficacy of CAR-T therapy (4).

2.Cell based therapies

The stem, primary, progenitor or the genetically modified cells from either the patient or the donor are used for the treatment of various health complications. This therapy of treating the patient with their own cells or the donor cells is called as cell-based therapy (2,3). These modified cells can be inserted into the body mainly through two mechanisms i.e., a) direct transplantation into the diseased area, or b) by injecting intravenously (5). Insertion of these cells can help in either inhibiting the initiation or facilitating the regeneration of damaged cells by two different mechanisms a) by replacing the damaged cells with the modified cells or b) enhancing the self-repair mechanism in the body. This second mechanism was mediated by the release of different growth promoting factors or cytokines (3).

The stem cells which are mostly used in this therapy include a) Embryonic stem cells derived from inner cell mass of the blastocyst, b) Foetus stem cells or c) Adult stem cells. This concept

of cell-based therapy has been depicted in Figure 1. Out of different types of cell-based therapy, use of Chimeric Antigen Receptor T cell therapy (CAR-T) has been widely explored for the treatment of cancer, mainly blood cancer. So, in the next section we are going to discuss the CAR-T therapy (3).

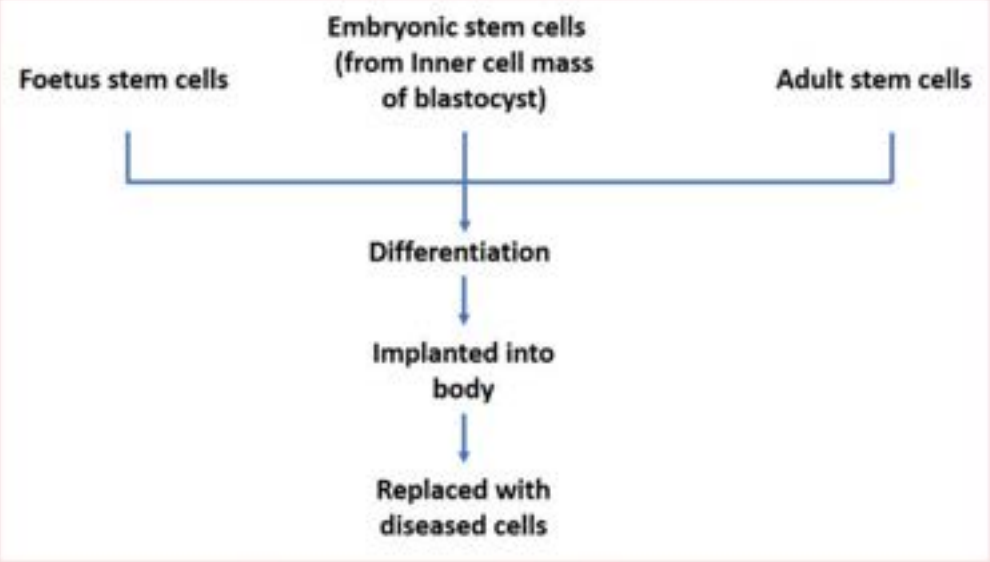

Figure 1: Concept of cell-based therapies

2.1 CAR-T therapy

Nowadays, CAR-T therapy is used widely for the treatment of refractory C cell mediated haematological cancer (6,7) in which host immune system itself is used. In this therapy, the T cells from the patients itself are removed and CAR receptor is attached on them to make a genetically modified T cells (4). In ideal scenario, the body immune system is trained in such a way that it can differentiate between the self and non-self-antigens in the body. But, in cancerous condition, it was observed that sometimes the immune system is not able to identify the cancerous cells. Thus, these T cells are engineered outside to identify these cancerous cells (8). In an NCI-funded first human trial targeting the use of CAR-T therapy for 3 patients suffering from cancer , it was shown that this strategy is safe for human use and also has targeted delivery (1). Therefore, this therapy has been approved by FDA for treating cancer. The procedure for development of CAR-T cells has been illustrated in Figure 2. Though useful, the therapy has also shown some contrasting results. Gut microbiota was found responsible for these results. Hence, in the next section we are going to discuss this factor (9).

Figure 2: Development and insertion of CAR-T cells

3.Gut microbiota

GM is a cluster of trillions of microbes that reside in our gut as commensal, symbiotic or in a pathogenic relationship. They usually start developing after the birth of child, but, the initial entry takes place through the vertical transmission of the microbes from the mother. In case of C section, the microbes on the skin of the mother are transmitted to the child. The microbes include bacteria, fungi, viruses, etc., but the major chunk is occupied by the bacterial species (10). These microbes have found to have multiple effects on the human body i.e., helping in digestion of non-digestible fibres, interacting with human immune system and many others (11). They not only have the impact on intestine but also have impact on multiple organs. This is due to the involvement of different axis like gut-lung axis, gut-liver axis, gut brain axis etc (12). This has been depicted in Figure 3.

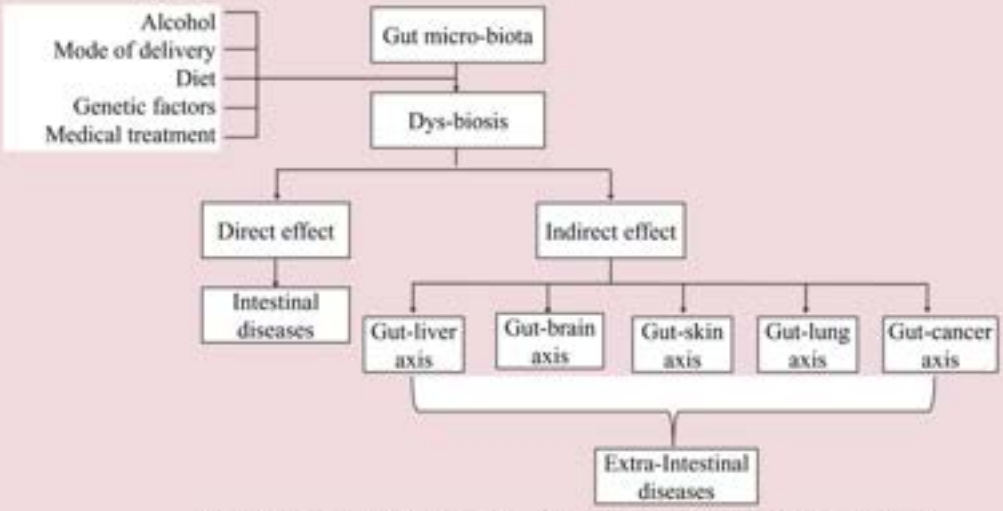

Figure 3: Different gut axis involved in disease development and progression

4.Impact of gut microbiota on CAR-T

The intestinal microbiota have found to be a key factor which might potentially impact the responses obtained by using different immunotherapies. Some studies have shown that the patients with diverse and abundant gut microbiota have better survival rate than the patients with less diverse microbes (4,9). Thus, this points out a high probability of relationship between gut microbiota and CAR-T therapy.

Andrea Facciabene, a cancer immunotherapy researcher, has very well pointed out that "The gut microbiota is a little bit like the thermostat of the immune system." Thus, understanding the role of gut microbiota in CAR-T therapy is very important for understanding why different results were obtained by the usage of CAR-T therapy in some patients (13). Hence, to find the significance of GM, the group of Facciabene et al., have found that administering vancomycin before CAR-T therapy to the mice suffering from blood cancer, have shown lesser tumour size than the one which were not administered with vancomycin. Whereas, contrasting results were shown in the study by Smith et al., where patients suffering from leukaemia or lymphoma administered with antibiotics before the CAR-T therapy. In those patients, antibiotics decreased the gut diversity which in turns causes the imbalance in immune system and thereby increases the chances of failure of the CAR-T therapy (13). These contrasting results obtained in two studies necessitates the need to develop an in depth understanding of the relationship

between the two. If the relationship between these two would be established, this would in turn help in getting better outcomes from the use of cell-based therapy.

5.Conclusions

In some studies, it have been shown that the diversity and abundance of gut microbes present in the intestine have found to have an impact on the side effects and efficacy which was seen with the usage of CAR-T therapy. The most probable reason for the involvement of gut microbe is due to the gut-cancer axis. Thus, there is a need to understand the relationship between the gut microbiota and the usage of CAR-T therapy. Hence, more studies are needed in future to understand the mechanistic basis behind it.

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Cell based therapy



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Introduction:

Cell-based therapy embodies the latest aspect phase of the biotechnology outbreak in medicine. By means of countless countermeasures, cell therapies are founded in trailblazing scientific innovations and upgrading technology (1,2). Cell therapy holds up the possibility of mitigating and reversing the progression of illnesses that cannot be satisfactorily treated by current medications by using cells as living agents. A broad category, cell treatments encompass a range of cell types and therapeutic uses. They have stood as the focus of active research for many years, but they are only just beginning to make a strong comeback through effective commercialization and patient access (3,4). Cell therapies can be categorised on the basis of treatment such as neurological, cardiovascular, or ophthalmological. The European Union categorises cell-based therapies into two groups: those regulated as medicines, which should comply with quality, safety, and efficacy guidelines in order to acquire marketing approval until being made available commercially (advanced therapy) and those regulated as marginally altered cells for homologous use (transplants or transfusions). Commercially available cell-based therapy has received growing civic recognition in medication of acute lymphoblastic leukaemia and large B cell lymphoma. There is cell-based therapy available using cell transplant of reprogrammed T cells, called CAR-T therapy, which led to the approval of tisagenlecleucel and axicabtagene ciloleucel in the year 2017 by the FDA (5–7). Adult stem cells, popularly recognised as mesenchymal stem cells, are used in stem cell therapy as they have the following favourable characteristics including self-renewal, immunomodulation, anti-inflammatory, signalling and differentiation (8). In contrast to current pharmacological therapy, neural stem cell (NSC) grafts offer a more lifelong solution for numerous central nervous system illnesses, including Alzheimer's disease (9).Glial cell lines engineered to release derived neurotrophic factor, new-born brain cells known as neural progenitor cells obtained from human foetal tissue were able to survive, migrate, and cause functional recovery of Parkinsonian symptoms (10). Patient-derived limbal stem cells for the healing of injured corneal epithelia is another approved treatment (11). Adult stem cells can mitigate fistulas associated with Crohn's disease. Stem cell based therapy replaces the mutant gene with a corrective gene (8, 11, 12).

Chimeric Antigen Receptor T-Cells (CAR-T cell)

The CAR T cell therapies currently available in the market are unique for each patient. These

are produced from the T cells of patients and which are genetically modified so that they produce chimeric antigen receptors, or CARs, on their surface. Specific proteins, or antigens, on the surface of cancer cells are identified by the CARs which bind to them. CARs are synthesised receptors bearing an extracellular target binding domain comprising of variable light (VL) and variable heavy (VH) range of an antibody (scFv), contrary to the cancerous cells. The extracellular domain and transmembrane are joined by a hinge region that also serves as a spacer. The transmembrane domain, which is a cell membrane lipid bilayer, interacts with CARs to form homodimers or trimers based on the intracellular protein complex. Subsequently, one or more intracellular signalling domains are present (14). In hematologic malignancies, acute lymphoblastic leukaemia (ALL), non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukaemia (CLL) B cell marker CD19 have been targeted by CAR-T cells (15).

Table 1: Six FDA approved CAR-T cell therapy (13)

CAR- T cell Therapy	Brand name	Target antigen	Cost of therapy	Approval date	Therapeutic Indication
Tisagenlecleucel	KYMRIAH™	CD-19	475,000 dollars	May 1, 2018	R/R-ALL (in children's and adults of Age 3-25 years)
Axicabtagene ciloleucel	YESCARTA™	CD-19	373,000 dollars	October 18, 2017	DLBCL and R/R aggressive B-NHL, MCL and FL
Lisocabtagene maraleucel	BREYANZI™	CD-19	432,055 dollars	February 5, 2021	R/R B-NHL, DLBCL,FL
Idecabtagene vicleucel	ABECMA™	BCMA	441,743 dollars	March 27, 2021	R/R MM
Ciltacabtagene autoleucel	CARVYKTI™	BCMA	465,000 dollars	February 28, 2022	R/R MM
Brexucabtagene autoleucel	TECARTUS™	CD-19	373,000 dollars	Jul 24, 2020	R/R B-ALL, MCL

R/R: Relapsed or refractory; ALL: Acute lymphoblastic leukaemia DLBCL: Diffuse large B-cell lymphoma; B-NHL: B-cell non-Hodgkin lymphoma; MCL: mantle cell lymphoma; FL: follicular lymphoma; MM: multiple myeloma

Chimeric Antigen Receptor T-Cells (CAR-T cell)

The CAR T cell therapies currently available in the market are unique for each patient. These are produced from the T cells of patients and which are genetically modified so that they produce chimeric antigen receptors, or CARs, on their surface. Specific proteins, or antigens, on the surface of cancer cells are identified by the CARs which bind to them. CARs are synthesised receptors bearing an extracellular target binding domain comprising of variable light (VL) and variable heavy (VH) range of an antibody (scFv), contrary to the cancerous cells. The extracellular domain and transmembrane are joined by a hinge region that also serves as a spacer. The transmembrane domain, which is a cell membrane lipid bilayer, interacts with CARs to form homodimers or trimers based on the intracellular protein complex. Subsequently, one or more intracellular signalling domains are present (14). In hematologic malignancies, acute lymphoblastic leukaemia (ALL), non-Hodgkin lymphoma (NHL) and chronic lymphocytic leukaemia (CLL) B cell marker CD19 have been targeted by CAR-T cells (15). First-generation CAR T cell therapy mimics T cell receptors using the intracellular domain. But the cytokinin secretion was so weak that the first-generation CAR-T cells had minimal or no benefit clinically.

Recent CAR T cells with FDA approval are of the second generation and use the CD28 or 4-1BB co-stimulatory domain. This CAR-T cell had noticeable effectiveness and greater persistence in cytokine release with an elevated proportion of complete response. OX40, CD27 and inductive T cells are additional co-stimulators (ICOS). If more than two co-stimulatory domains are used, then the third-generation design of CAR-T cells is formed. The third generation bears good anti-tumour function and increased T-cell perseverance in the in vivo study. In the tumour microenvironment, there is an abundance of immune cells which is responsible for the secretion of cytokines and transforming growth factor which suppresses and shrinks the anticancer immune response. Certain cells produce prostaglandin which does not trigger the immune response. To overcome this drawback, fourth-generation CAR-T cell therapy, called armoured CAR-T cells are formed. They are universal cytokine-killing, cytokine-modulating and antibody-secreting constructs (16,17). The fifth generation of CAR has experienced significant modification in the intracellular domain of the pathway of signal transduction, and stimulator of the transcription-3 (STAT3) transcribed factor's binding site. Furthermore, the interleukin-2 (IL-2) receptor has a second binding site.(18) FDA-approved CAR-T cell therapy as shown in Table 2

Table 2: CAR-T cell therapy - Generations (16–18)

Features		First	Second	Third	Fourth	Fifth
Extracellular domain		+	+	+	+	+
Transmembrane domain		+	+	+	+	+
Intracellular domain	CD3signalling	+	+	+	+	+
	Costimulatory domain	-	4-1BB or CD28 or 0X40	CD28 and 4-1BB both	4-1BB or CD28 or 0X40	4-1BB or CD28 or 0X40
	Interleukin 2 inducer	-	-	-	Interleukin 12 (IL-12) inducer	-
	Interleukin 2 receptor	-	-	-	-	IL-2 receptor beta chain domain
	Transcription factor for STAT3	-	-	-	-	JAK/STAT pathway activation
Key symbols	"+": Presence of feature			"-" : Absence of feature		

Manufacturing of CAR T cells:

GMP-compliant facilities must be used for the manufacture of CAR T cells. The final CAR T cell product cannot be sterilised by filtration, so it is essential that the vector is sterile. This is achieved by manufacturing the vector under controlled, clean ambient conditions with limited open production, and a sterile purification process during the finished aseptic stages of the process, all supported and verified by various safety tests. Four main processes are required for the manufacturing of CAR-T cells. In this process, T-cells are separated from blood, and the remaining blood components are returned back to the patient. Healthy donor cells and umbilical cord cells can be used to prepare CAR-T cells. In the lab, T cells are engineered by inserting genes that code for special receptors called CARs and are allowed to multiply until there are millions of cells formed as shown in figure 1.

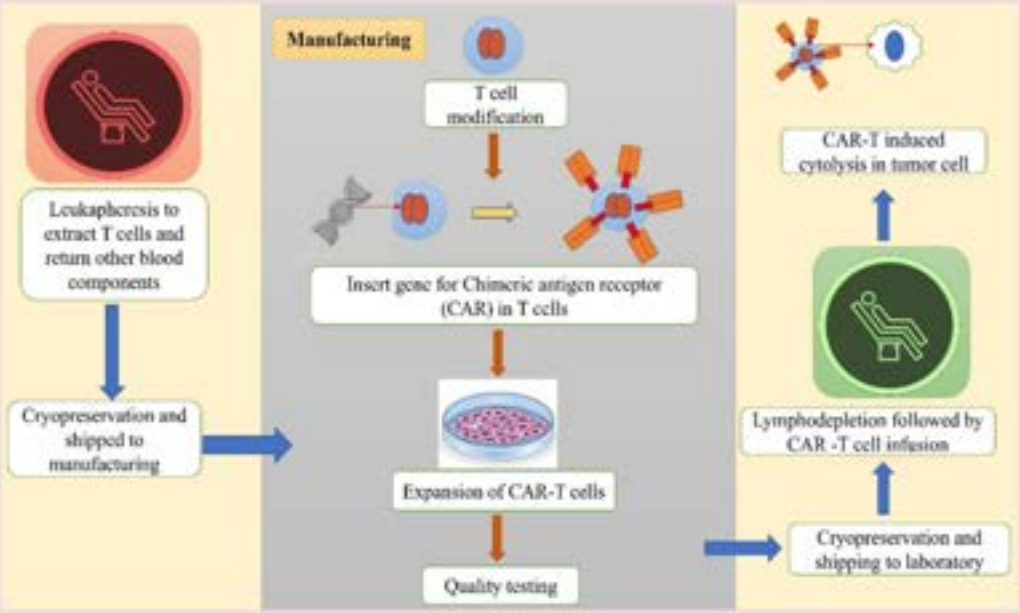

Figure 1: Manufacturing process of CAR-T cell therapy

CAR- T cells are then put back into patients' bloodstream. Inside body the CAR-T cells will identify and attach to the surface of cancer cells, killing them. CAR-T cells can remain in body for some time to prevent cancer relapse (19,20).

Clinical manifestation of CAR T Cell therapy:

There are two hallmarks of CAR-T therapy; that is cytokinin syndrome and neurotoxicity. The first symptom in clinical trials of anti-CD19 is fever while infusion, which starts from the day of infusion and can last longer, till almost 9 days. Hypotension might impose vasopressor care and low cardiac function where arrhythmia and QT prolongation are also observed. Additionally, pulmonary oedema is reported which leads to dyspnoea and hypoxia. Certain patients may require ventilation due to failure in respiration (21). This all occurs due to over secretion of cytokinins. Fatigue, headache and myalgias are additional adverse effects. Organ-related toxicities have been reported, which were reversible. Anti-CD-19 CAR T cells can deteriorate normal B cells along with malignant cells due to which B-cell aplasia and hypogammaglobulinemia were also commonly observed. Haemorrhagic events have been observed which led to patient death. Neurological toxicity is not limited to one region in the brain hence patients experience delirium, dementia, hallucinations sensory nervous defects, motor defects and seizures. Renal toxicity includes increased serum creatinine, renal insufficiency and hypokalaemia. Elevated levels of ALT, AST and direct bilirubin has also been reported (22).

Challenges with CAR T cells:

There is an allogenic constraint of CAR-T cell product i.e. graft versus host disease (GvHD), where host allorejection occurs leads to anti-tumour activity and fatal outcomes. GvHD ascends where there is an incompatibility between donor and recipient leukocyte antigen. This issue is solved by gene editing tools eliminating the expression of T cell receptors and the elimination of leukocyte antigens. Another challenge comes with the lack of efficacy in treating

solid tumours (23). There are certain physical constraints present in infiltrating solid tumours and maintaining function inside tumour cells. Hence CAR-T cells are only engaged to treat blood disorders. Tactics to improve tumour penetration include the expression of the enzyme heparanase, which degrades the primary component of a solid tumour. One other challenge that comes across is that the hostile and immunosuppressive environment of tumour cells can stop T cells from carrying out their function. The process to overcome this issue is the production of genetically engineered "armoured cells" and the blockage of immune checkpoints (24). Manufacturing challenges include supply constraints, scalability issues, storage issues and logistics.

Current clinical trials on CAR targets:

Allogeneic CAR-T cell therapy has undergone more than a thousand preclinical and clinical studies globally. Most of these are used to treat haematological malignancies, where CD19 is the most frequently targeted protein, along with other well-known targets like CD20, CD22, and BCMA. There are also some emerging targets like CD70, CD7, and CD5 (25). Tumour differentiation antigen mesothelin (MSLN), which is typically only found on the mesothelial surfaces of the body, is markedly overexpressed in a variety of solid tumours (26). Clinical studies are shown in table 3.

Conclusion:

For patients with aggressive lymphomas CAR-T cell therapies are now available in USA and some the other developed nations and thereby it is now establishing itself as one standard care. CAR-T cell are comparable to a living drug. As their name suggests, T-cells assist to organise the immune response and kill cells infected by pathogens, which is the main backbone of CAR-T cell therapy. With hundreds of current clinical trials, CAR-T cell research is moving forward rapidly. This increase is partially due to the discovery of new tumour cell antigens by researchers that may become suitable targets for CAR-T cells. CAR-T cell therapies have been developed targeting extra antigens, which are often seen in blood malignancies. Thiscomprises of treatments that target various antigens concurrently, despite the fact that CD19 and BCMA are the lone antigens that are FDA-approved CAR T-cell therapies. Many researchers have attempted to investigate antigens found on the surface of solid tumours' and not on healthy cells but have failed. Clinical trials using CAR T-cells have produced excellent preliminary results in treating individuals with blood malignancies. According to certain studies, CAR T-cell therapy has resulted in remission in up to 90% of children and adults with particular tumours who had relapsed or responded poorly to conventional treatments. Relapses may be brought on by tumour cells losing the ability to produce the CD-19 antigen, CAR T cells becoming less persistent, or CAR T cells becoming less active. Other blood malignancies like multiple myeloma and chronic lymphocytic leukaemia (CLL) have also showed promise for CAR T cell therapy. Cell therapy has recently emerged as a novel treatment possibility. How we handle the problems posed by CAR-T cell therapy will determine its future. The need of the hour is to develop standard procedures for tackling the high cost of it with innovative technological solutions like off-the-shelf therapy, enhancing the efficiencies of manufacturing engineering, supply, distribution and quality; and regulatory compliances for this therapy.

Table 3: List of 2nd, 3rd and 4th CAR generation under Clinical Trials (27,28)

Generation of CAR	Cancer type	Target of CAR	Objective Patient Group	Clinical Trial Phase	Status of recruitment (Yes/No)	Clinical Trial Identifier
2nd	B cell Acute Lymphocytic Leukemia	CD19	Adults	I	Yes	NCT02965092
2nd	T cell Acute Lymphoblastic Lymphoma	CD5	Adults	I	Yes	NCT03081910
2nd	B cell Lymphoma, Acute Lymphoblastic Leukemia	CD22	Adults	I	Yes	NCT04007978
3rd	Acute Myeloid Leukemia	CD123	Adults	I	Yes	NCT04014881
3rd	Acute Lymphoblastic Leukemia	CD19	Children and Adults	I/II	Yes	NCT03676504
4th	Solid Tumor	CD276	Children and Adults	I/II	Yes	NCT04432649
4th	Refractory Relapsed Ovarian Cancer	MESO	Adults	I	Yes	NCT03814447

Abbreviations: NSC: Neural stem cellR/R: Refractory or Relapsed; ALL: Acute lymphoblastic leukaemia ; DLBCL: Diffuse large B Cell Lymphoma; B-NHIL: B-cell non-Hodgkin lymphoma; MCL: Mantle cell Lymphoma; FL: follicular lymphoma ; MM: multiple myeloma; NHL: Non-Hodgkin Lymphoma; CLL: Chronic lymphocytic leukaemia ; CD28:Cluster differentiation 28 ;CD3: Cluster of differentiation 3 ;IL-2: Interleukin-2 ;STAT3: Signal transduction pathway and activator of transcription-3 (STAT3); ALT: Alanine transaminase ;AST: aspartate aminotransferase ; GvHD: graft versus host disease

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Pharma News Round-Up

05 Aug, 2022: AstraZeneca and MSD's Lynparza (olaparib) has been granted approval by European Union (EU) to be used as monotherapy, or in combination with endocrine therapy for the adjuvant treatment of germline BRCA1/2 mutations (gBRCAm). The therapy is recommended for adult patients who have human epidermal growth factor receptor 2 (HER2)-negative high-risk early breast cancer previously treated with neoadjuvant or adjuvant chemotherapy.

30 Aug, 2022: US Food and Drug Administration (USFDA) has granted tentative approval for generic equivalent of Sprycel Tablets marketed by Bristol-Myers Squibb Company Abbreviated New Drug Application (ANDA), Dasatinib Tablets marketed by Lupin, in partnership with Pharmascience Inc.

02 Sep, 2022: Indian Institute of Technology-Kanpur (IIT-K) has collaborated with Niche Agriculture & Pharmaceuticals Limited (NAPL) for research in the field of hemp cultivation and formulation of advanced medicines for treating chronic medical conditions such as cancer, epilepsy, migraine, chronic pain, arthritis, and insomnia in India.

16 Sep, 2022: USFDA has approved first of its kind Mallinckrodt's Terlivaz (terlipressin) injection to improve kidney function in adults with hepatorenal syndrome with rapid reduction in kidney function, an acute and life-threatening condition requiring hospitalisation.

19 Sep, 2022: The European Commission (EC) has granted approval to first of its kind injectable Roche drug Vabysmo (faricimab) for the treatment of neovascular or 'wet' Age-Related Macular Macular degeneration (nAMD) and visual impairment due to Diabetic Macular

Related Edema (DME). Vabysmo restores vascular stability by independently blocking angiopoietin-2 (Ang-2) and vascular endothelial growth factor-A (VEGF-A) pathways. This stabilises blood vessels, reduces inflammation, leakage and abnormal vessel growth (neovascularisation).

19 Sep, 2022: USFDA has approved Bluebird's beti-cel therapy, Eli-cel to treat a rare blood disorder, CALD. CALD is characterized by mutations in a ABCD1gene leading to the buildup of very long-chain fatty acids in the brain and spinal cord of boys between the ages of three and 12 years. The therapyEli-cel adds functional copies of the ABCD1 gene in a patient's stem cells to help produce a protein required to break down the long-chain fatty acids.

18 Nov, 2022: USFDA has granted approval to first of its kind, SEZABY™ (benzyl alcohol-free and propylene glycol-free phenobarbital sodium powder for injection) for treating seizures in neonatal patients. SEZABY is granted orphan drug designation by the US FDA for the treatment of neonatal seizures and is licensed by Sun Pharma Advanced Research Company to Sun Pharma.

26 Dec, 2022: USFDA has approved Roche's therapy Lunsumio (mosunetuzumab) for treating a type of rare cancer called follicular lymphoma. Follicular lymphoma is a slow-growing type of non-Hodgkin lymphoma, a cancer that starts in body's white blood cells called lymphocytes. The drug does not require extended hospital stays and can be administered at a hospital's outpatient center.

30 Dec, 2022: Gufic Biosciences has launched Zarbot' (Botulinum Toxin Type A) injection indigenously produced using purified HALL strain and proprietary technology which results in thin film formulation and better stability compared to other botulinum toxin brands.

Source:

1. https://www.biospectrumindia.com/category/segments/pharma-biopharma
2. www.expresspharma.in

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LOTUS LOGO STORY

As a lotus is able to emerge from muddy waters un-spoilt and pure it is considered to represent a wise and spiritually enlightened quality in a person; it is representative of a woman who carries out her tasks with little concern for any reward and with a full liberation from attachment. Lotus-woman in the modern sense of women's qualities: she is superbly intelligent, highly educated, and totally committed to individualism. She is politically astute and works incessantly for a better and more humane society. She is exquisite in her taste for music, art and culture, abounds in social graces and performs brilliantly in communication.