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STEM CELLS AND VIRAL INFECTIONS: RELEVANCE TO COVID-19

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ABSTRACT

Stem cells currently offer new hope for treating a great number of diseases. MSCs have immunosuppressive and immunomodulatory properties enabling them to have many clinical and therapeutic applications. They exerted broad-spectrum destroying function covering a wide range of bacteria, protozoa, parasites, and viruses that caused diverse clinical problems. But, there is still limited available data on how far MSCs have effective deleterious activity against viruses. Covid-19 (coronavirus infectious disease) is menacing the world and not hesitating. the viral load in COVID-19 causes a state of hyperinflamation and uncontrolled reaction of immune cells. Many researchers tried to test MSCs dual effect on immune cells. In this review, we summarize the role of stem cells in dealing with viral infections with special relevance to novel COVID-19.

KEYWORDS: Stem cells, regenerative medicine, viral infection, immune cells, COVID-19.

INTRODUCTION

Stem Cells Basic Concepts

New Therapeutic approaches based on stem cells currently offer new hope for treating a great number of diseases. This includes muscular degenerative disorders, diseases due to degeneration of nervous system; cardiovascular diseases; disorders of the blood and immune system; metabolic disorders; injuries of the hepatic system; cancers and more diseases in the body of patients are taking advantage of stem cell therapy.[1] Stem cells are progenitors of every tissue in our body. By definition, *stem cells are cells in the undifferentiated state, having the potential of dividing and giving two daughter cells , one similar to the immature parent and the other in a differentiated state*.[2] Stem cells can be subdivided into: a) cells of embryonic origun which are isolated from the inner layer of blastocyst. They have a great power of multilineage differentiation. b) Cells of somatic origin, sheltered inside niches in almost every tissue in our body. They are restricted in their ability to proliferate and differentiate. They may be derived from bone marrow mainly as well as from other organs and tissues as skin, orofacial region and adipose tissue. Bone marrow derived stem cells are divided in HSCs for blood constituents replenishment and MSCs. c) Somatic cells after induction of stemness genes to mimic embryonic stem cells. They are able to differentiate into cells of the three germ layers and this makes them a good choice to study drug development as well as to transplant organs.[3]

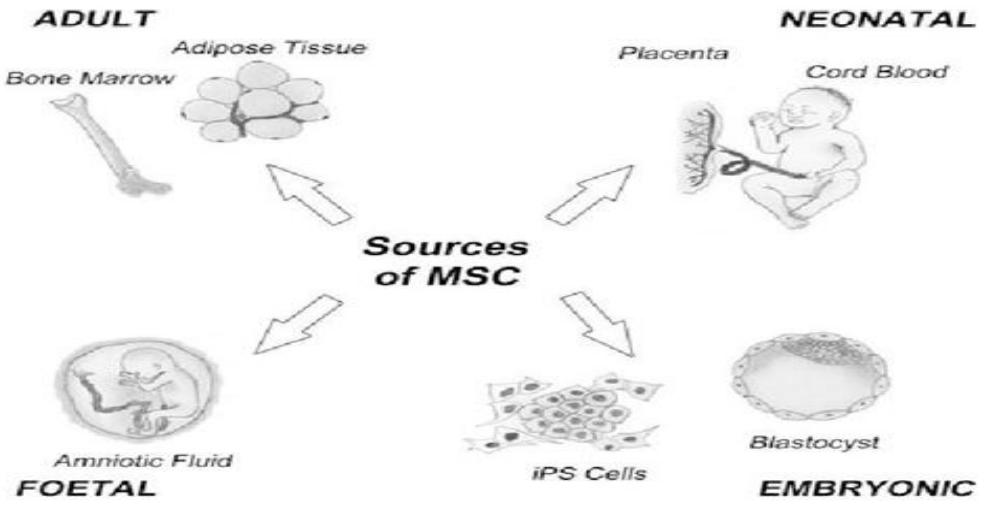


Figure: 1. Sources of stem cells *{ Advances in biochemical engineering/biotechnology 2013*}.

Regarding cell potency, which is defined as how far a stem cell can differentiate into effector cells, cells can also be divided into totipotent (e.g., zygote or fertilized egg), which are cells that can differentiate to almost all cell types. Pluripotent stem cells (such as embryonic stem cells), which are cells that have the ability to differentiate to the majority of cell types and finally multipotent cells (such as mesenchymal stem cells [MSC]) with a limited differentiation ability limited to a small number of cell types.[4,5,6]

The hierarchy of stem cells consists of heterogeneous subpopulations of stem cells not from hematopoietic origin.[7] The International Society for Cellular Therapy (ISCT) defined mesenchymal stem cells as having the ability of adherence to culture plastic dishes as well as generation of different cell lineages in vitro as osteoblasts, adipocytes and chondroblasts. They bypass other types of stem cells by their multipotential differentiation power plus their relative accessability as well as their ease of isolation and expansion in vitro.[8] This explains why they have captured the attention of scientists all over the world as an new hope for a great number of therapeutic applications. MSCs initially were isolated from the bone marrow but this is a painful and threatening procedure. Today MSCs can be obtained from many other sources such as placenta, fat, cord blood, pulp of teeth, skin etc. This explains why new therapeutic approaches using MSCs for variable diseases has been substantially studied. This is why preclinical and clinical studies have increased either using MSCs or MSC-based extracts over the last 10 years.[8] However, comparatively, little is known their curing capability in pathogenic diseases. Even MSCs intervention in viral diseases is very little investigated . The appearance of discovered viruses as the new coronavirus disease (COVID-virus makes public health face serious threats.[9] Because of the insufficiency of drugs and vaccines capable of treating patients attacked by COVID-19; Workers of the medical field are curious to substitute from conventional medicine to the more safer and efficace therapies depending on MSCs owing to their immunomodifying and tissue restoring properties. This review will be concerned with the last newly developped MSC clinical researches and the evolving in MSC-based therapies regarding their power in diseases caused by viruses such as HIV, hepatitis and COVID-19.[10]

Can MSCs modulate Host defense mechanisms?

The most important roles exerted by BMMSCs are the ability to form the microenvironment that supports blood cells, to modulate the immune system performance and finally to control trafficking of cells. Safety of using stem cells as well as their differentiation power make them an attractive potential therapeutic approach. Nevertheless, many unanswered questions are raised when talking about stem cell potential therapy such as their ideal source and the exact dose that may be used. Also, their interaction with already used therapies and long term follow up must betaken into consideration.[11] BM microenvironment consists of MSCs as well as hematopoietic stem cells niche and it appears that they are the maestro guiding its survival and clonality.[3]

MSCs have immunosuppressive and immunomodulatory properties enabling them to have many clinical and therapeutic applications including: Bone marrow transplant, management of self-immune disorders, regenerative medicine and tissue recovery, diseases of neurological system, bone and cartilage problems and most recently treatment of several infections.[12]

Role of MSCs in human infectious diseases

Currently, Eyes are turned to MSCs aiming to explore their effect against the novel infectious disease owing to their anti-inflammation, immunoregulation and tissue repair / regeneration power.[13] Additionally, Scientists showed that MSCs can secrete LL37 (a 37 amino acid cathelicidin antimicrobial peptide), a powerful protein acting to get rid of microbes and destroying pathogens.[14] Added, MSCs inhibit dendritic cells maturation, act to suppress T- and NK-cells and promote upregulation of regulatory T-cells resulting in the exerted anti- inflammatory effects in diverse models of acute inflammation in different tissues and organs. Toll-like Receptors (TLR) are the main sensors of viral presence and are involved in many immune responses during infection. Inflammation caused by direct viral injury results in TLR-priming (TLR-agonist engagement) which can affect MSC phenotype, multi-lineage potential and their immune-modulatory capacity. TLR activation as a response to viral infection changes expression of HLA-antigens and other co-stimulatory molecules resulting in unproper function of some immune cells. In the same way.[15] Exosomes and micro-vesicles, are formed by budding from the membranes of MSCs and are released into the extra-cellular space. Evidence based data indicate that MSC- exosomes contain molecules (tetraspanins, heat shock protein, phosphatidylserine, annexins, MHC class I and II, etc.). These substances are released in response to diverse stimuli as infections by bacteria or viruses and help in controlling the degree of host response.[16]

Do MSCs Possess Antiviral Effector Function?

MSCs are appealing implements for the management of health problems caused by disturbed immunity as GvHD. This is one of the most dangerous conditions associated with an elevated opportunity for infection after transplantation.[17]

MSCs effect against microbes has been studied to reach a more advanced evaluation regarding their power of therapy. Interestingly, MSCs are able to produce and secrete considerable amount of a peptide having an antimicrobial function, *human cathelicidin hCAP- 18/LL-37*. This substance helped in getting rid of bacteria *in vitro* as well *in vivo.*[18] Also, human MSCs secreted tryptophan-catabolizing enzyme which was triggered by stimulation with inflammatory cytokines. It exerted

broad-spectrum destroying function covering a wide range of bacteria, protozoa, parasites, and viruses that caused diverse clinical problems . But, there is still limited available data on how far MSCs have effective deleterious activity against viruses, in particular human herpes viruses, which is a famous pathogenic candidate in the scenario of transplantation of allogeneic hematopoietic stem cell.[19]

On the other hand, Merisel et al. proved HSV-1 and CMV replication in MSCs declined. This may be attributed to the continuous, slow release of IFN-γ. This may explain why MSCs may have a negative effect on viruses. At this time and because the available data is limited, the benefits from the antiviral activity of MSCs cannot be defined yet. Antimicrobial activity depends on different factors as virus type as well as preclinical and clinical studies. Further investigations are mandatory to explain the proper antimicrobial effective function exerted by MSCs.[20]

Established therapeutic modalities using MSCs over the last decade

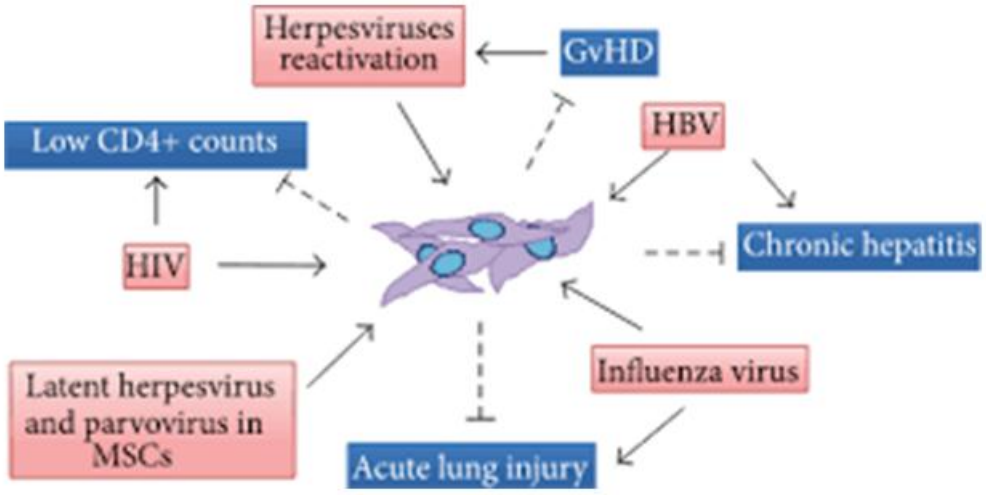


Figure 2: The suggested paired-edge sword activity of MSCs as a remedy for viral infections. Plenty of complications due to transplantation and diseases related to viruses as GvHD, decline in CD4+ numbers, Acute liver injury and chronic hepatitis have been profitably mended by MSCs. {*Maytawan Tet al. Mesenchymal Stromal Cells and Viral Infection: Stem Cells International 2015; 6: 1-8.*}

1. MSCs and human immunodeficiency virus (HIV)

From the date of HIV discovery in 1983, researchers worldwide are still doing their best to reveal an effective treatment for HIV patients. HIV pathogenesis after entering the host is marked by a discerning and a continuous damage of CD4 T cells subset. This brings about to the deleterious deficiency in immune system in patients infected by HIV.[21] The well known *Highly Active Anti-Retroviral Therapy*, known as *HAART*, is very efficient and effective enough to decrease and control viral load in patient plasma and this restores a symbolic immune function leading to a remarkable decline in mortality and morbidity in patients with chronic HIV infection. Unfortunately, there is a section of HIV patients who are non-immune responders (NIRs). They are unable to shift the immunodiscrepancy state even though the virus load declines. This is why they are very liable to opportunistic infections with a decline in their survival rate compared to candidates of immune responders section. This makes clear why exploring treatment for NIRs has become an emergency. Different treatment modalities are needed. Recently, therapeutic approaches based on stem cells offers a glowy wish for patients suffering from HIV. Since *'Berlin patient'*, HSCs become in focus as a treatment approach for HIV. This is a patient that became functionally cured from HIV after he received HSC transplant with donor cells defective for the chemokine receptor type 5 (CCR5), mandatory for the virous invasion of cells.[21]

HIV is a tough and challenging infections known since 30 years ago. Not only it weakens the patient but also it integrates its genome in that of the host to guide the host cell functions. It targets immune cells so breaks down the defense mechanism of the body.It also adapt to the host microenvironment. Finding a single curative therapy is inapplicable with the exception of "*Berlin patient*". Even this approach isn't easily realized as it is hard to find matching donor homozygous for defective CCR5 needed for virus entry inside cells.[22]

Gupta and collaborates announced '*London patient'* who in the same way underwent HSC transplant using cells defective for *CCR5*. However, Allogenic HSCs can't be transplanted in HIV carriers without limitations such as Strong immune reaction as well as graft versus-host disease (GvHD).[23]

MSCs are contradicting to HSCs as they are less immunogenic and also they have a characteristic power of immunosuppression. This renders these type of cells an appealing approach to cure HIV-infected persond. Zhang and colleagues[22] conducted an interesting pilot study in 2013 to evaluate the safety as well as the

competence of MSCs isolated from the umbilical cord (UC-MSCs) and injected in NIR group of patients. UC-MSC based therapeutic approach under the registration number NCT01213186 showed tolerance by all patients and no remarkable side effects. In brief, administration of UC-MSC caused a marked increase in CD4 subset of T-cells titre and a decrease in proinflammatory cytokines titre. The exact pathway by which MSC of UC caused a decline in the overreaction of the immune system in NIRs wasn't stated yet. Infusion of allogenic adipose derived MSCs regarding safety and effectiveness was tested by a phase I/II clinical trial (NCT02290041) in patients affected by HIV and expressing strange immunologic and virologic reactions to HAART.[24]

HAART reached a great success in impeding HIV multiplication and ameliorating results clinically. However, HAART showed shortage in getting rid of reservoirs of HIV that remain latent and this is why its failed to help curing HIV infected patients. Therefore, there is a raising need to develop new approach for reactivation of pool of latent HIV and this may allow to subsequently clear them. A late in vitro study that uses cell lines infected with latent HIV proved a strategic role for MSCs and their secretome in reactivating latency of HIV. This is mediated by PI3K and NFkB signaling mechanisms. More specific studies are mandatory to discover the effectiveness of MSCs in reactivating HIV in microenvironments of reservoirs *in vivo*.[24] Depletion of CD4+ T cells is a main characteristic of HIV infection, leading to eventually to a clinical, significant immunodeficiency. MSCs have the ability to improve host immune reconstitution by decreasing the activity of subsets of T cells, CD8+ and this may help in restoring an efficient CD4+ T cell subset. Recipients of transplant of MSCs showed a promising enhancement of the count of naïve as well as memory CD4+ subset of T cells and in cytokine formation as a response for HIV antigen.[21]

2. MSCs for hepatitis B virus (HBV) treatment

Getting infected by Hepatitis B virus (HBV) is a chronic, dangerous and life menacing status. This attacks nearly 260 million persons and this is greater than 3 % all over the world. Due to liver cell failure or hepatocellular carcinoma, it causes a huge number of deaths. Patients suffering from chronic HBV may be exacerbated by an acute on top of chronic state of liver failure. Because no treatments are available, mortality rates are unexpected.[25] As a restoration for cirrhosis, nucleos(t)ide analogues are the treatment of choice and this may help in declining mortality rates as they inhibit division of virus but can't eradicate it. Another line of treatment is Interferon-α with a power of getting rid of the virus in only few candidates but with very limited clinical application owing to its adverse effects. The last line of choice is Artificial Liver Support System (ALSS) therapy which is used as adjunct prior to liver transplant approach in hepatic patients. Liver transplantation is the best and most effective and permanently efficient approach especially for unresponsive patients. Unfortunately, the course of HBV is very progressive and get worse before a donor is found.[26]

A recent modality to manage HBV-ACLF is MSCs. MSCs have a number of advantages that encourage clinicians to use them. First of all, they home damaged, cirrhotic liver and they are able to differentiate into functionally active hepatocytes. If used before allogenic liver transplantation, they exert an antiinflamatory as well as immunosuppressive effect favouring the field for transplantation. Self BMMSCs are safe and resist HBV infection but aren't the best source for stem cell based therapeutic intervention because their isolation is too invasive and distressing to the patients. The effect of treating HBV induced liver failure by transfusion of a single injection of ex-vivo expanded self BMMSCs was assessed in a study guided by Peng and colleagues. Laboratory results as liver function tests improved greatly but this improvement was of short duration but long term follow up wasn't promising. An explanation for the long term improvement decline of auto BMMSCs is that stem cells isolated from hepatic patients have slow proliferation rate and this delays the possible intervention.[27]

Clinical trials.gov has registered four studies of MSCs based approaches for HBV treatment. They used allogenic MSCs and not autologous stem cells. The first one is a phase II trial (NCT01322906) was conducted by Lin and collaborates on patients suffering from HBV liver failure. Results of this study showed that infused allogeneic BM-MSC were safe and have no side effects. In addition, MELD scores and liver function tests were better. This is owing to their immunomodulatory effects.[27,28]

The second research was done by Xu et al. (NCT01724398) to assess the safety and utility of a new approach combining UC-MSC and plasma exchange (PE) for HBV-ACLF patients. Safety of this combination was proved.[29] This was in accordance with an earlier study by Li et al. However; combination hasn't improved short term prognosis compared to single dose administration. The two other trials (NCT03209986; NCT03109236) aim to apply stem cell based therapy on patients suffering from liver cirrhosis.[30]

Stem cells isolated from adipose tissue are recently suggested as a new source to propagate hepatic like cells. The study of Wang et al. proved that AD-MSCs were able to differentiate into hepatocyte-like cells that are functionally active. Surprisingly, it has been proven that AD- MSCs and hepatocytes like cells differentiated from ADMSCs were sheltered from being infected by HBV *in vitro*.[31] Concluded from that, ADMSCs are the most ideal autologous source for MSCs to be chosen to treat patients of chronic hepatitis B. As usual, further long-term surveying of liability AD-MSC transplant to get infected by HBV *in vivo* is needed and also in randomized clinical trials.[31]

Although preliminary results are promising, they are not conclusive. More detailed explanation and further studies to test therapeutic potential of MSCs and their mechanism in regenerating live.[32]

3. Respiratory Viral Infections

1. Influenza Virus (IV)

Bird species are attacked by the Avian influenza virus (AIV). Many subtypes of AIV are set by scientists such as H5N1, H1N1, H7N9, and H9N2. Crossing species barriers has become a nightmare as these viruses are capable of infecting other species as mammals.[33] Clinical symptoms of avian influenza infection in humans depends on the specific virus that caused the infection. They varied along a wide scale from sore throat, fever and muscle aches fatal attack of pneumonia followed by deteriorating systemic inflammatory response that may lead to life threatening conditions as acute respiratory distress ARDS or moreover acute lung injury ALI. To date, the available drug as are different antiviral drugs.[34] They have no positive effect neither on the signs nor on the tissue injury caused by the virus associated inflammation. Antiviral drugs can only alleviate symptoms and shorten their duration. Clinicians found that antiinflamatory drugs are capable of attenuating lung injury, so, started to include them in treatment protocols.[35]

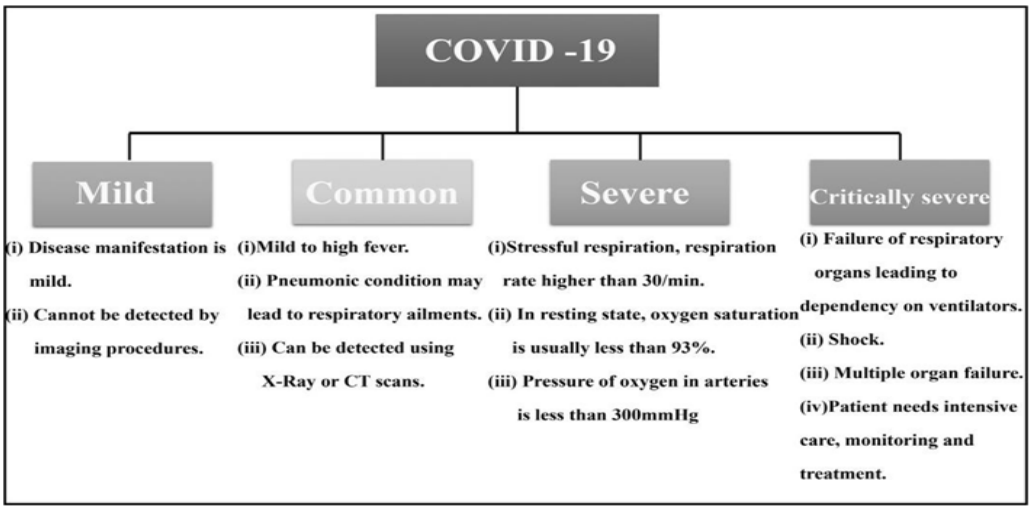
Owing to their immunomodifying, anti-inflammatory and repair characteristics, MSCs are now on the fire to enter regimens of Avian Flu remedies. Li et al. conducted a study using MSCs to manage ALI caused by H9N2 virus. Mice lungs showed promising improvement. Unfortunately, Prophylaxis using MSCs has no positive impact on alleviating the acute state of influenza in recent studies.[36]

In addition, there is a conflicting observations of MSCs-mediated tissue regeneration. Further studies both in vitro and in vivo are needed to reveal MSC power of regeneration after Avian viruses infection.

2. COVID-19 (The invisible enemy)

By the end of 2019, a new emerging virus is causing an epidemy crossing the continents. Covid-19 (*coronavirus infectious disease)* is menacing the world and not hesitating. Starting in China, in Yuhan state, it spreads to nearly cover us all. The main pathogenic agent is SARS-CoV-2, subtype of *severe acute respiratory syndrome coronavirus 2*. Its proteome and genome mimics its counterpart family members, SARS-CoV that attacked China in 2003 and Middle East Respiratory Syndrome (MERS) that attacked Saudi Arabia in 2012. Scientists all over the world are in a hurry to control or better prevent this pandemic.[38]

Unfortunately, we lack powerful drugs with antiviral activity to treat infections and vaccines to protect from infections. This is why patients are treated by only alleviating their symptoms such as reducing fever. Medicine role in this pandemic is limited to symptomatic treatment with a special care towards supporting vital organs and preserving their functions especially with severely affected candidates. Across continents, scientists eyes are turned towards finding an efficient therapeutic regimen that can be applied to COVID-19 patients especially the end stage ones.[39]



Autopsies from cadavers of Covid-19 patients revealed that although the virus itself was only found in the tissues of respiratory system and was undetectable in spleen, bone marrow and lynph nodes, the major pathological changes were found in the lungs and immune organs. Added to this, vasculitis affected small systemic blood vessels. This was explained by the exaggerated overreaction of immune system, cytokine storm, in response to viral load. This cause a damaging effect causing the pathogenesis seen in lungs, immune organs as well as small systemic blood vessels. Regarding the previous results, trying to modulate or regulate immune response to this virus may have a better impact clinically and accelerate patients recovery as well as increasing survival rate.[40]

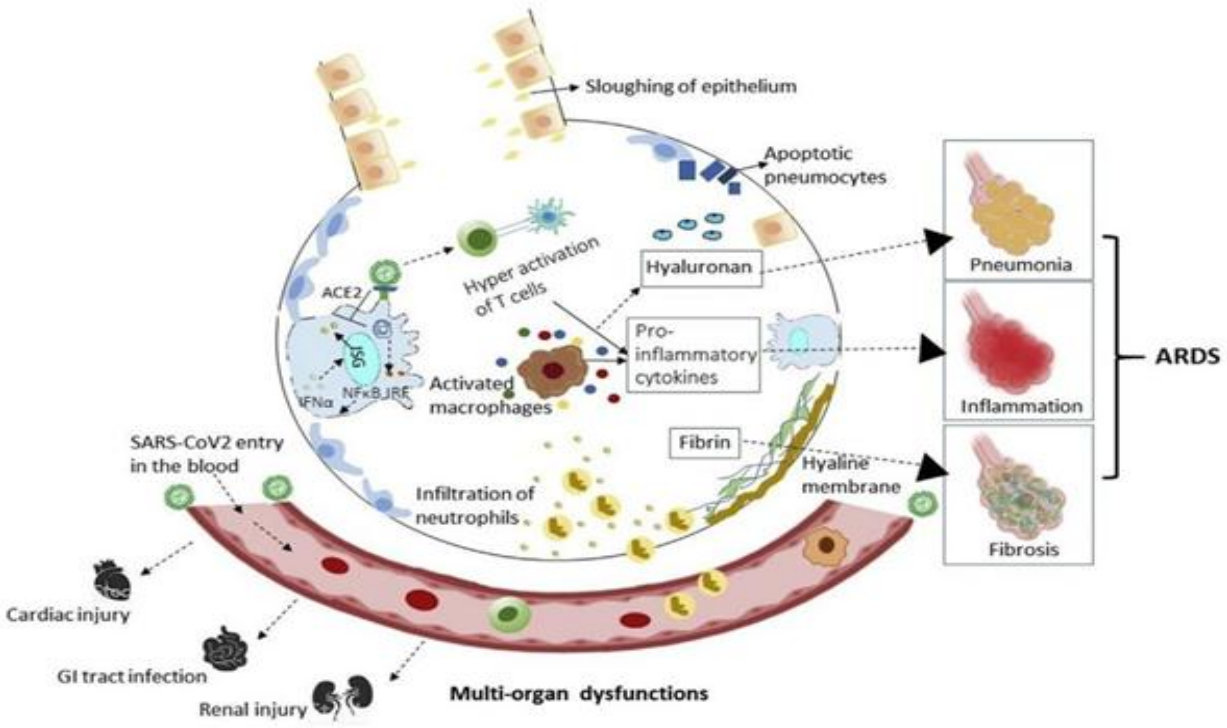


Figure. 4: After infection with Covid-19, MQ and T-cells are activated by organism attachment to ACE2 receptors favouring its endocytosis. This turns on NFkB-IRF pathway and stimulates IFN with antiviral activity. SARS-COV2 escapes this clearance trial and in response to this, a storm of cytokines is secreted from hyperinflamed neutrophils called to the site as well as MQ secreting huge amount of proinflamatory mediators causing fibrosis. Excessive inflammation causes hypersecretion of hyaluronan that enhance fluid absorption leading to pneumonia and ARDS. When virus entry to blood is favoured, other organs as heart, kidney, GIT are attacked by virus translocation causing failure in multiple organs. ***[Aditi M. et al, J.Biomed 2020]***

It was proved in a study conducted by Huang and collaborates that the viral load in COVID-19 causes a state of hyperinflamation and uncontrolled reaction of immune cells.

Proinflamatory mediators that are secreted caused an irreversible damage to the lungs that caused death of patients due to fibrosis and ARDS.ICU patients expressed higher levels of these mediators if compared to nonICU patients. Scientists concluded that a successful treatment of COVID-19 must be based on preventing lung damage by inhibiting immune cels hyperfunction so that the over secretion of cytokines is aborted. This must be accompanied by repairing any lung damage as a consequence of the virus either the damage was in structure or in function.[41]

Scientists eyes are turned on therapeutic regimens based on stem cells to control COVID-19 infection due to their miraculous properties of immune regulation and inhibition of inflammation added to their basic homing characteristics.[42]

MSCs based approaches not only make use of cells but also utilizes stem cells products either conditioning media or vesicles secreted extracellularly as exosomes. Cells other than MSCs are also in clinical researches.[42]

In Beijing Hospital, China a study registered as (ChiCTR2000029990) and conducted by Leng group,[44] intravenous injection of MSCs succeeded in improving respiratory signs as fever , breath shortness as well as ameliorating functions of lungs. The seven patients included in the trial felt better clinically in two to four days. Accordingly, C-reactive protein was downregulated and proinflamtory cytokines were declined in patients treated with MSCs. This sharp decline was accompanied by a remarkable increase in IL-10, a major anti-inflamatory mediator. So it can be concluded that MSCs are safe to be injected intravenously with excellent results in shifting cytokine storm and enhancing damaged lung repair. These action are exerted through ameliorating lung microenvironment. Optimistic results presented in this pilot study encouraged scientists and gave hope to researchers to achieve more trials on using MSCs to treat COVID-19 affected candidates,[44]

Clinical trials.gov announce on the fifth of March 2020 the starting point for five Chinese new clinical trials using MSCs as an approach to treat COVID-19 patients. In two of these trials, single Intravenous injection of UC-MSCs was the method of application. While in (NCT04252118) Phase I clinical trial, three doses of MSCs were given with a concentration of 3×10^7 each. From these trials, safety of MSCs terapy was concluded.

In the same way, a trial numbered (NCT04288102) phase I/II study assessed the safety and efficiency three intravenous injections of MSCs with a concentration of 4 x 10^7 each at days 0, 3 and 6.[42] (NCT04276987) trial is evaluating a new MSCs product in terms of safety and efficacy, inhaled aerosol of exosomes derived from allogenic ADMSCs in ICU Pneumonic patients due to COVID-19.[40] Scientists stated that using Exosomes derived from MSCs instead of using MSCs is superior in advantages. From these advantages is that exosomes are able efficient and succeded in migrating easily to the affected site due to their nano-scaled size. Also, they escape from being entangled in small vessels. Also the infused dose of exosomes don't decline as their cellular counterpart after infusion. This ensures that a sufficient 'dose' will reach the damaged target.[40]

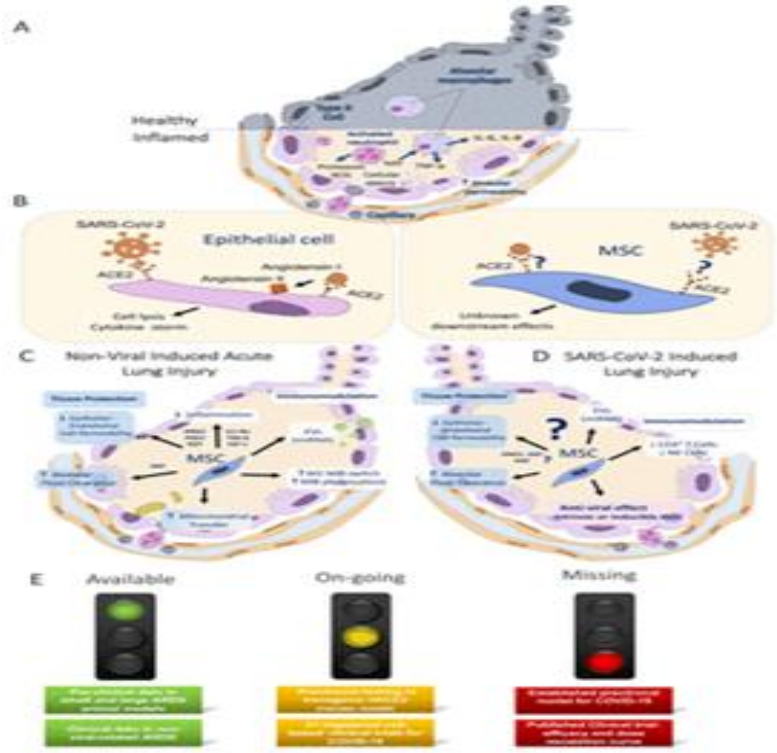


Figure 5: The therapeutic power of MSCs to repair lung damage is mainly mediated by secreted factors. They have immune regulating as well as antiinflamatory functions.

Benefits of using MSCs to control Viral infection

MSCs have different impact on T-cell response facing virual pathogen

Nowadays, the immune modulatory and antiinflamatory MSCs effect is out of discussion. Stimulation of different substes of T-cells as well as their functional activity can be controlled to a great extent by MSCs. MSCs can orchestrate all immune cells as T cells, B cells, natural killer cells, dendritic cells, monocyte/macrophages and neutrophils. T cells have a wide range of functions. They can cause deleterious autoimmune conditions and inflammatory disorders. They mediate GVHD and cause rejection of transplanted organs.Subsets of T-cells include T helper cells either *Th1* or *Th2* as well as *T cytotoxic* cells. Another subset is *Treg* cells, *T regulatory*, that are able of shutting down immune response and controlling inflammation that may lead to tissue injury. An imbalance between the number or function of *Treg* and *effector T* cells is responsible for many immune disorders.[45]

MSCs paracrine functions and their secreted molecules play a role in modulating T cell response either ditrectly by activating or suppressing them ot indirectly by acting on APC(Antigen Presenting Cells). Researches have proved that allotransplant patients receiving MSC gained beneficial effects. This may help also in controlling autoimmune disorders.[45]

However, some researches found that giving MSCs may not be beneficial but worsen and aggravate tissue injury mediated by T cell response. So, the main modulatory effect of MSCs is mediated through their dual action on Treg cells either stimulatory or inhibitory effect.

Many in-vitro studies dealed with MSCs dual function, one of them showed that culturing T cytotoxic cells specific for EBV or CMV with MSCs didn't negatively affect their cytotoxic function. Instead they continue to proliferate and secrete IFN-γ to kill the virus.[45]

In controversy, Sundin and colleagues stated that T cell proliferation in response to CMV antigen was inhibited if MSCs are present. In a study conducted by Khoury et al , He showed that MSCs downregulated the proliferation capacity and declined the release of IFN-γ from T cells

specific for influenza and CMV. Coculture of T cells against H1N1 influenza virus and UC-MSCs causes suppression of their cytotoxicity power *in vitro*. This is why, it is mandatory for workers of the medical field to be aware of double face impact of MSCs on cells of the immune system facing viral infection.[47]

Many researchers tried to test MSCs dual effect on immune cells. Clinically, two patients who were trated by MSCs for acute GvHD maintain effector T against CMV cells as well as IFN- γ secretion. A number of clinical studies suggested that children given MSCs did not become more liable to viral infections in comparaison to control.[47]

The issue to be discussed is the reactivation of latent viruses in patients receiving SCs. Patients receiving HSCs and tested positive for VZV are at great risk of viral reactivation due to immune suppression mediated by stem cells. However, contradicting results concerning viral reactivation in vitro and in vivo have emerged. Results are conflicting between *in vitro* and clinical studies. T cells specific for HAdV was not affected by coculture with MSCs *in vitro*, while children treated with MSCs suffered from low survival rate due to HAdV infection.[48]

Results are conflicting and not conclusive due to shortage of studies addressed to clarify this issue. More studies must be directed towards exploring differential impact of MSCs on immunecells response while using MSCs based clinical approaches.[48]

Studying Viral pathogenesis aided by MSCs

When studying the pathogenicity of a virus, its life cycle clarification is mandatory. Stem cells are not just helping in virus treatment but also in revealing the way the virus enters our body and the way it causes a disease. Till now, The life cycle of HBV in its early steps isn't clear because of the absence of in vitro study for its way of infectivity. Scientists have developed hepatoma cell lines to study these period of early infectivity in lab, but unfortunately, they are no more suitable for researches. They tried to shift to using primary hepatocytes extracted from human liver. This was the ideal as they are the site of virus entry and replication but this was unapplicable and many obstacles were faced such as the scarce resources and culturing difficulties. This is why human BM-MSCs have offered an genius cell source for the preclinical researches of HBV. Many animal researches showed the ability of Human BMMSCs to differentiate into actively functioning cells that mimic hepatocytes. Hepatocytes like cells derived from BM-MSCs allows mimicking the whole life cycle of HBV in liver in terms of infection and replication as it was obtained from primary hepatocytes cultures. They achieve a supportive viral proliferation without losing native viral characteristics in a way far superior to primary hepatocytes.[49]

Kaposi sarcoma virus causes a sets of malignancies in the human body of HIV. How the virus was able to cause these malignancies isn't clear yet. However a study conducted by Jones et al on MSCs neighbouring the nephrons gave a model and allowed studying KSHV pathogenic mechanisms.[50]

Safety of Clinical use of MSCs

Side effects of using auto-MSCs are very few. Patients in a meta analysisonly reported transient fever. Concerning MSCs derived products,they are multiple and differ according to stem cells type and source as well as ways of production and collections. All of the above make it difficult to define their safety for clinical use. Reported complications were emboli formation or thrombosis and some reported death.

When dealing with alloMSCs, the virus reservoirs that may be sheltering latent viruses in MSCs must be taken into consideration. This is why PCR and screening tests for these viruses must be made as a routine survey before transplanting allo-MSCs. This includes AdV, CMV and EBV. MSCs are vulnerable to getinfected by one of the mentioned viruses after being administered asvreported by many evidences *in vitro*.

1. Hepatitis-B Virus

In fact, MSCs may act as a HBV reservoir. MSCs may take the role of HBV reservoir outside the liver. They can implant HBV genome in a mouse heart tin a model of myocardial infection after their administration.[51,52]

2. Herpes viruses/Parvovirus

Herpes virus family has many members. All of which can easily infect MSCs making them defective in function and proliferation power. Studies by Smirnov et al. showed MSCs infection by CMV downregulated surface markers important for their differentiation capability. Also, CMV attack of MSCs inhibits their immunomodulatory effect. Other members of the Herpes family also can attack MSCs and become latent and sheltered. They become reactivated after infusion in receipients.[53,54,55]

FM-MSCs, *Fetal membrane derived Mesenchymal Stromal Cells*, are emerging as a new source of stem cells and must be screened as they are a potential reservoir for hepes virus family infection. This explaind the urgent need for sensitive methods to test for viral DNA presence in MSCs both in donors and recipients. This may ensuring the safety and efficacy of MSCs- approaches.[54]

3. Human Immunodeficiency Virus

When dealing with MSCs, infection with HIV is of great importance. HIV infection of BMMSCs decreases their differentiation potential. Cotter et al. in his study showed that serum collected from an HIV patient was able to infect MSCs and thus reducing their multilineage differentiation power. Added to this, their paracrine function is altered. Also, MSCs may act as HIV reservoir

transmitting its genome to recipients. Cross infection between recipients and donors occur mediated by MSCs transplants.[56,57]

4. Respiratory system Viruses

In vitro infection of MSCs by subtypes of influenza virus has been studied and proved. Results showed a decline in cell proliferation and differentiation capability. MSCs are directed towards apoptosis and cell lysis. Also the immunoregulatory action of MSCs is altered. In accordance, Khatri et al. supported the fact that both H1N1 as well as H9N5 influenza replicate in primary MSCs of chicken so that a sharp decline of cytokine and chemokine production resulted. Invivo studies aren't conclusive yet.[38]

CONCLUSION

MSCs have many advantages making them the souece of choice for stem cell based therapy. This may be explained by the absence of ethical concerns as embryonic stem cells and no risk of teratoma formation as iPS. Plus, they exert an immunomodifying power and anti- inflammatory characteristics. It was previously proved that they have a regenerative capability and homing to damaged tissues. All of this make MSCs a new hope for human preclinical and clinical trials to treat patients facing viral infections.

Unfortunately, there are still some limitations that must be taken in consideration when talking about MSC-based therapies.

First, MSCs are heterogenous subpopulations and this may explain discrepancies in clinical trials. Also, not all MSCs subpopulations are neutral immunologically. Thus, advanced researches are needed to specify methods for isolating the "immune privileged" groups from the heterogeneous pool of MSCs to be help in clinical trials.

Using cell free MSCs products as exosomes may help avoiding these limitations. This concept emerged after discovering the paracrine function of MSCs.

There isn't enough data based on evidence concerning with |MSCs role in viral infections. This is why it is tooearly to conclude their safety and efficacy. Well designed clinical trials are from the major priorities in the field of stem cell research with short and long follow up.

MSC and virus play a double face game. Although MSCs are immunomodulator and can inhibit deleterious immune responses, they can also act as transmitters of virus genomes from donors to receipients. This is why careful screening of MSCs for virus reservoirs is mandatory before transplantation.

Approving MSCs and their products in COVID-19 treatment protocol is still under research. Safety of this developed approach must be first stated. The importance of MSCs intervention appears to be time dependant before irreversible damage to lungs occurred.

This is called *the window period for MSCs transplantation,* which is *the time when symptoms/signs are progressively getting worse.* Many factors are still under research including the exact dose as well as the route of administration.

Optimization of the microenvironment of NSCs may help one day in using them as the first line of treatment for viruses.

REFERENCES

1. Wang J, Chen Z, Sun M, Xu H, Gao Y and Liu J.: Characterization and therapeutic applications of mesenchymal stem cells for regenerative medicine. Tissue and cell, Elsevier Ltd, 2020; 101330.
2. Hass R, Kasper C, Böhm S and Jacobs R. Different populations and sources of human mesenchymal stem cells (MSC): a comparison of adult and neonatal tissue-derived MSC. Cell Commun Signal, 2011; 9.
3. Riham M. and Marwa M. Peripheral Blood Mononucleocytes: An Efficient and Reliable Source of Induced Pluripotent Stem Cells. Journal of Stem Cells, 2018; 13(4).
4. Volkman R and Offen D. Concise review: mesenchymal stem cells in neurodegenerative diseases. Stem cells, Vol. 35. Wiley-Blackwell, 2017; 1867–80.
5. Wilschut KJ, Ling VB and Bernstein HS. Concise review: stem cell therapy for muscular dystrophies. Stem Cells Transl Med, 2012; 1(11): 833–42.
6. Ra JC, Kang SK, Shin IS, Park HG, Joo SA, Kim JG.: Stem cell treatment for patients with autoimmune disease by systemic infusion of culture-expanded autologous adipose tissue derived mesenchymal stem cells. J Trans Med BioMed Central, 2011; 9: 1–11.
7. Phinney DG. Functional heterogeneity of mesenchymal stem cells: Implications for cell therapy. J Cell Biochem, 2012; 113: 2806–12.
8. Du, J., Li, H., Lian, J. et al. Stem cell therapy: a potential approach for treatment of influenza virus and coronavirus-induced acute lung injury. Stem Cell Res Ther, 2020; 11: 192.
9. K. Rajarshi : Combating COVID-19 with mesenchymal stem cell therapy Biotechnology Reports, 2020; 26: 00467.
10. Le Blanc K., "Mesenchymal stromal cells: tissue repair and immune modulation," Cytotherapy, 2006; 8(6): 559–561.
11. S. Aggarwal and M. F. Pittenger, "Human mesenchymal stem cells modulate allogeneic immune cell responses," *Blood*, 2005; 1815–1822.
12. Corcione, F. Benvenuto, E. Ferretti et al., "Human mesenchymal stem cells modulate B-cell functions," *Blood*, 367–372.

13. Maytawan T., Suradej H., and Arunee T.. Mesenchymal Stromal Cells and Viral Infection: Stem Cells International, 2015; 6: 1-8.
14. Krasnodembskaya A, Song Y, Fang X, Gupta N, Serikov V, Lee JW, Matthay MA. Antibacterial effect of human mesenchymal stem cells is mediated in part from secretion of the antimicrobial peptide LL-37. Stem Cells, 2010; 28(12): 2229-38.
15. Duffy M., Ritter T. and Ceredig R.: Mesenchymal stem cell effects on T-cell effector pathways. Stem Cell Res Ther, 2011; 2: 34.
16. Verena B., Daniel J. Weiss et al: International Society for Extracellular Vesicles and International Society for Cell and Gene Therapy statement on extracellular vesicles from mesenchymal stromal cells and other cells: considerations for potential therapeutic agents to suppress coronavirus disease-19 Cytotherapy, 2020; 22: 482-85.
17. Dander E., Lucchini G. and Vinci P.., "Mesenchymal stromal cells for the treatment of graft- versus-host disease: understanding the in vivo biological effect through patient immune monitoring," Leukemia, 2012: 26(7): 1681–84.
18. Spaggiari G., Capobianco A., Becchetti S., Mingari M., and Moretta L., "Mesenchymal stem cell-natural killer cell interactions: Eevidence that activated NK cells are capable of killing MSCs, whereas MSCs can inhibit IL-2-induced NK-cell proliferation," *Blood*, 2006: 107(4): 1484–90.
19. Meisel R., Zibert A., Laryea M., Göbel U., Däubener W. and Dilloo D., "Human bone marrow stromal cells inhibit allogeneic T-cell responses by indoleamine 2,3-dioxygenase-mediated tryptophan degradation," *Blood*, 2004; 103(12): 4619–4621.
20. Németh K., Leelahavanichkul A. and Yuen P. "Bone marrow stromal cells attenuate sepsis via prostaglandin E2-dependent reprogramming of host macrophages to increase their interleukin- 10 production," *Nature Medicine*, 2009; 15: 42–49.
21. Okoye and L. J. Picker, "CD4+ T-cell depletion in HIV infection: mechanisms of immunological failure," Immunological Reviews, 2013; (254): 54–64.
22. Zhang Z, Fu J, Xu X, Wang S, Xu R, Zhao M, Nie W, Wang X, Zhang J, Li T, Su L, Wang FS. Safety and immunological responses to human mesenchymal stem cell therapy in difficult- to-treat HIV-1-infected patients. AIDS, 2013; 27(8): 1283-93.
23. Allam, Ossama; Samarani, Suzanne; Ahmad, Ali Mesenchymal stem cell therapy in HIV- infected HAART-treated nonimmune responders restores immune competence, AIDS, 2013; 1349-1352.
24. Daniel R. Kuritzkes: Hematopoietic stem cell transplantation for HIV cure J Clin Invest, 2016; 126(2): 432–437.
25. Boyd A, Newsome P and Lu WY. The role of stem cells in liver injury and repair. Expert Rev Gastroenterol Hepatol, 2019; 13(7): 623–31.
26. Bing Zhu et al: A novel stem cell therapy for hepatitis B virus-related acute-on-chronic liver failure. Brazilian Journal of Medical and Biological Research, 2020; 53(11): 9728.
27. L. Peng, D.-Y. Xie, B.-L. Lin et al., "Autologous bone marrow mesenchymal stem cell transplantation in liver failure patients caused by hepatitis B: short-term and long-term outcomes," *Hepatology*, 2011; 820–828.
28. H. Aurich, M. Sgodda, P. Kaltwaßer et al., "Hepatocyte differentiation of mesenchymal stem cells from human adipose tissue in vitro promotes hepatic integration in vivo," *Gut*, 2009; 570–581.
29. Xu W., He H., Pan S., Chen Y., Zhang M., and Zhu S.: Combination Treatments of Plasma Exchange and Umbilical Cord-Derived Mesenchymal Stem Cell Transplantation for Patients with Hepatitis B Virus-Related Acute-on-Chronic Liver Failure: A Clinical Trial in China. 2019. Stem Cells International, 2019; 1-10. https://doi.org/10.1155/2019/4130757.
30. H. Li, L. Y. Chen, N. N. Zhang et al., "Characteristics, diagnosis and prognosis of acute-on- chronic liver failure in cirrhosis associated to hepatitis B," Scientific Reports, 2016.
31. Wang E., Kang SH, Kim MY, Lee JI, Baik SK. Mesenchymal stem cells to treat liver diseases. Annals of Translational Medicine, 2020; 8(8): 563.
32. Y. H. Li, Y. Xu, H. M. Wu, J. Yang, L. H. Yang, and W. YueMeng, "Umbilical cord-derived mesenchymal stem cell transplantation in hepatitis B virus related acute-on-chronic liver failure treated with plasma exchange and entecavir: a 24-month prospective study," Stem Cell Reviews and Reports, 2016; 645–653.
33. Y. P. Lin, M. Shaw, V. Gregory et al., "Avian-to-human transmission of H9N2 subtype influenza A viruses: relationship between H9N2 and H5N1 human isolates," Proceedings of the National Academy of Sciences of the United States of America, 2000; 9654– 9658.
34. Q. Liu, D.-Y. Liu, and Z.-Q. Yang, "Characteristics of human infection with avian influenza viruses and development of new antiviral agents," Acta Pharmacologica Sinica, 2013; 1257–1269.
35. T. T. Thanh, H. R. van Doorn, and M. D. de Jong, "Human H5N1 influenza: current insight into pathogenesis," International Journal of Biochemistry & Cell Biology, 2008; 2671–2674.
36. Li, Y., Xu, J., Shi, W. et al. Mesenchymal stromal cell treatment prevents H9N2 avian influenza virus-induced acute lung injury in mice. Stem Cell Res Ther, 2016; 159.
37. Wang, Ying & Wang, Feng & Zhao, Hongchang & Zhang, Xiaohe & Chen, Haiying & Zhang, Kaiyu. Human Adipose-Derived Mesenchymal Stem Cells Are Resistant to HBV Infection during Differentiation into Hepatocytes in Vitro. International journal of molecular sciences, 2014; 15: 6096-110.

38. Du, J., Li, H., Lian, J. et al. Stem cell therapy: a potential approach for treatment of influenza virus and coronavirus-induced acute lung injury. Stem Cell Res Ther, 2020; 11: 192.
39. Golchin, T.Z. Farahany, Biological products: cellular therapy and FDA approved products, Stem Cell Rev, 2019; 166–175.
40. Muraca M., Augusto P.,Michela P., Massimo D., Umberto G., Lorenza L., Ornella P., Enrico L. and Giorgio Perilongoa, Mesenchymal stromal cells and their secreted extracellular vesicles as therapeutic tools for COVID-19 pneumonia? Journal of Controlled Release, 2020; 325: 135– 140.
41. Huang, X. Xiao, K., Hou, F., et al. Mesenchymal stem cells: current clinical progress in ARDS and COVID-19. Stem Cell Res Ther, 2020; 11: 305.
42. Aditi M. and Shalmoli B: Application of mesenchymal stem cell and secretome for combating mortality and morbidity in COVID-19 patients: A brief review J. Biomed, 2020.
43. Aleen S. and Fatima Sa.: Mesenchymal stem cells in the fight against viruses: Face to face with the invisible enemy . Current Research in Translational Medicine, 2020; 68: 105–110.
44. Leng Zikuan,Zhu Rongjia,Hou Wei, et al. Transplantation of ACE2- Mesenchymal Stem Cells Improves the Outcome of Patients with COVID-19 Pneumonia[J]. Aging and disease, 2020; 11(2): 216-228.
45. Weiss ARR and Dahlke MH Immunomodulation by Mesenchymal Stem Cells (MSCs): Mechanisms of Action of Living, Apoptotic, and Dead MSCs. Front. Immunol, 2019;10: 1191.
46. Sundin M, Törlén J, Thunberg S, Önfelt B, Ljungman P, Watz E, Mattsson J, Uhlin M. Individualization of Hematopoietic Stem Cell Transplantation Using Alpha/Beta T-Cell Depletion. Front Immunol, 2019; 10: 189. doi: 10.3389/fimmu.2019.00189. PMID: 30804948; PMCID: PMC6378311.
47. Khoury M, Cuenca J, Cruz FF, et al. Current Status of Cell-Based Therapies for Respiratory Virus Infections: Applicability to COVID-19. Eur Respir J, 2020.
48. Gao F, Chiu SM, Motan DA, Zhang Z, Chen L, Ji HL, Tse HF, Fu QL, Lian Q. Mesenchymal stem cells and immunomodulation: current status and future prospects. Cell Death Dis, 2016; 7(1): 2062.
49. Gaska JM, Ploss A. Study of viral pathogenesis in humanized mice. Current Opinion in Virology, 2015; 11: 14-20.
50. Sasirekha Ramani, Sue E Crawford, Sarah E Blutt, Mary K Estes, Human organoid cultures: transformative new tools for human virus studies, Current Opinion in Virology, 2018; (29): 79- 86.
51. Y. Wang, F. Wang, H. Zhao, X. Zhang, H. Chen, and K. Zhang, "Human adipose-derived mesenchymal stem cells are resistant to HBV infection during differentiation into hepatocytes in vitro," International Journal of Molecular Sciences, 2014; 6096–6110.
52. Y. Fellig, G. Almogy, E. Galun, and M. Ketzinel-Gilad, "A hepatocellular carcinoma cell line producing mature hepatitis B viral particles," Biochemical and Biophysical Research Communications, 2004; 269–274.
53. R. Meisel, K. Heseler, J. Nau et al., "Cytomegalovirus infection impairs immunosuppressive and antimicrobial effector functions of human multipotent mesenchymal stromal cells," Mediators of Inflammation, 2014.
54. S. Avanzi, V. Leoni, A. Rotola et al., "Susceptibility of human placenta derived mesenchymal stromal/stem cells to human herpesviruses infection," PLoS ONE, vol. 8, no. 8, Article ID e71412, 2013.
55. M. Sundin, A. Lindblom, C. Örvell et al., "Persistence of human parvovirus B19 in multipotent mesenchymal stromal cells expressing the erythrocyte P antigen: implications for transplantation," Biology of Blood and Marrow Transplantation, 2008; 1172– 1179.
56. K. Cheng, P. Rai, X. Lan et al., "Bone-derived mesenchymal stromal cells from HIV transgenic mice exhibit altered proliferation, differentiation capacity and paracrine functions along with impaired therapeutic potential in kidney injury," *Experimental Cell Research*, 2013; 2266–2274.
57. E. J. Cotter, N. Chew, W. G. Powderly, and P. P. Doran, "HIV type 1 alters mesenchymal stem cell differentiation potential and cell phenotype ex vivo," *AIDS Research and Human Retroviruses*, 2011; 187–199.