A risk assessment study of SARS-CoV-2 propagation in the manufacturing of cellular products

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The potential infection of cellular therapies by SARS-CoV-2 present high risks, as the target patients for these treatments are often immunocompromised or have chronic diseases associated with a higher risk of serious illness and death by COVID-19. The multicellular tropism of this virus presents challenges for the manufacturing of cell therapies, whereby the material could potentially become infected at the source or during cell processing. In this review we assess the risk of a SARS-CoV-2 propagation in cell types used to date in cellular therapies. Altogether, the risk of SARS-CoV-2 contamination of cellular products remains low. This risk should be evaluated on an individual basis, considering ACE2 and TMPRSS2 expression, existing literature regarding the susceptibility to infection, and single cell RNA sequencing data of COVID-19 patients. This analysis should ideally be performed for both the cells being manufactured and the cells used to produce the vector to ensure patient safety.

Plain language summary: Cell therapies are medicines based on the utilization of different cell types that are manufactured in special facilities. SARS-CoV-2, the virus that causes COVID-19, can infect a wide range of cell types. Patients requiring a cell therapy may be at higher risk of severe COVID-19 due to their underlying medical conditions. In this context, it is of importance to evaluate the risk of a SARS-CoV-2 contamination during the production of cell therapies to avoid possible infections. In this review, the authors assess the risk of an infection for cells being used as therapies to date and propose a systematic way to evaluate this risk.

Tweetable abstract: In this review the authors explore the multicellular tropism of SARS-CoV-2 and the corresponding challenges for the manufacturing of cell therapies, where the material could potentially get infected at the source or during cell processing.

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first identified in December 2019, with subsequent rapid spread across the globe. The virus causes COVID-19, a respiratory disease that affects persons of all ages but is particularly deadly in the elderly and in patients with pre-existing conditions [1,2]. Importantly, patients who may require treatment with cell therapies are generally less fit and present a higher risk of serious illness and death due to COVID-19 [3]. For example, the adjusted risk ratio (aRR) of death from COVID-19 is 1.39 for patients who are immunosuppressed, 1.28 for patients with cardiovascular disease and 1.31 for patients with chronic lung disease of [3]. SARS-CoV-2 uses the human protein ACE2 as entry receptor through its receptor-binding domain in its spike protein [4,5]. Glycan-recognizing C-type lectins, CD209 and CD209L, which have a broad virus tropism have also been suggested as entry receptors for SARS-CoV-2 [5,6], as well as AXL, NRP1 and TIM1 [7–10]. Once internalized, the virus needs to be proteolytically activated by human proteases through a priming of its spike protein. TMPRSS2 plays a major role in this process, but other proteases such as lysosomal...
cathepsins may be involved in its absence [4,11]. These molecules are expressed across a variety of organs (Figure 1). ACE2, for example, is highly expressed in respiratory and intestinal epithelium [12] and in cardiac pericytes [13]. Additionally, there is direct evidence that exposure to SARS-CoV-2 produces multiorgan invasion, pointing to a multiorgan tropism of this virus [14].

This broad tropism presents potential challenges for the manufacturing of cell therapies, whereby the material could conceivably become infected at the source (incoming or starting cell material) or during cell processing (final product). Cellular therapies are advanced therapeutic medicinal products (ATMPs) that are produced under current good manufacturing practices (cGMP). In USA, cGMP regulations are defined in the US Code of Federal Regulations (CFR) and in the European Union in the EudraLex Volume 4 and include, among others, strict aseptic methodology during the processing, packaging, or holding of drug products, extensive documentation, sterility testing and the path for product approval. Some ATMPs may also be manufactured under Good Tissue Practice (GTP). GTP requirements are less comprehensive than GMP requirements and are aimed primarily to avoid product contamination with transmissible agents.

Since these products contain human cells, the risk of the cells being infected by a human pathogen must be taken into consideration. Extensive rules and guidelines are in place to test the products for release. These tests include checking the cell donors for the presence of infectious disease, including the human immunodeficiency virus and hepatitis B and C viruses as defined in the CFR title 21 section 1271.75 [16]. In January 2021, the US FDA posted updated recommendations on COVID-19 regarding Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) and blood establishments [17]. At the time of this update, the FDA indicated that there had been no reported cases of transmission of COVID-19 via HCT/Ps or blood products.

However unlikely, ATMPs manufacturers must prevent or mitigate potential risks on the quality and/or safety of their products. There are two main channels which could lead to the contamination of ATMPs with SARS-CoV-2. First, the donor of the cells or tissue may be infected at the time of the donation. The FDA does not currently recommend molecular testing to screen asymptomatic cell and tissue donors [17]. Nevertheless, it was recommended to take into consideration if the donor was in close contact with a COVID-19 patient, had a diagnosis of COVID-19 or a positive SARS-CoV-2 test 28 days prior to donation. The FDA also considers that vaccinated individuals should still be considered as donors and that they would not need to be screened. Second, the cells could potentially get infected during product manufacturing despite the adherence to cGMP regulations.
This risk would mainly depend on the susceptibility of the target cells and vector producing cells to SARS-CoV-2 infection and propagation.

The FDA recommends that ATMP manufacturers perform a risk assessment regarding the potential transmission of SARS-CoV-2 by their products and describe possible mitigation strategies [17]. The risk assessment should consider such factors as cellular and tissue source, manufacturing processes, product testing, and number of patients to potentially be treated with the ATMP. In this review we assess the risk of SARS-CoV-2 infection for the cell types used to date in cellular therapies with approval designation (PRIME Designation, RMAT Designation, Breakthrough Designation, Fast Track Designation and SAKIGAKE Designation) in Europe, the USA and Japan [18].

**Blood-derived products**

Different blood products can be used as starting material to produce cell therapies (i.e., whole blood, peripheral blood mononuclear cells, bone marrow mononuclear cell and cord blood). Broadly speaking, blood cells express low levels of ACE2 and TMPRSS2 [15]. However, it is important to consider that cellular types present in this kind of products may be susceptible to SARS-CoV-2.

**B cells**

Single-cell RNA sequencing (scRNASeq) of COVID-19 patients has shown no evidence of B cell infection with SARS-CoV-2 [19]. Moreover, B cells express only low levels of ACE2 and no TMPRSS2 [15]. B cells are thus most likely not susceptible to SARS-CoV-2 infection; however, we could not find any in vitro studies to corroborate this affirmation. In contrast, B cell function is affected in patients who have recovered from COVID-19 [20], which may affect the functionality of cell therapies manufactured from such donors. Of importance, CD19 is also downregulated in such patients, which may have implications for the isolation of B cells through methods that relay on CD19 expression [20].

**Erythroid cells**

CD34neg early erythroid progenitors express high levels of ACE2 and TMPRSS2. This expression is downregulated during the course of erythroid maturation. Most interestingly, CD34neg erythroid progenitors are targeted by SARS-CoV-2 in vitro, leading to their expansion [21,22]. These observations explain the anemia observed in COVID-19 patients, which plays a major role on the hypoxia suffered by these patients [21,22]. It is unclear whether mature erythrocytes can be infected, but most likely they could not support virus replication [22].

**Hematopoietic stem & progenitor cells**

CD34+ hematopoietic stem and progenitor cells (HSPCs) are one of the main starting materials for cellular therapies [23]. They express low levels of ACE2 and no TMPRSS2 [24]. In addition, direct evidence shows that they cannot be infected by SARS-CoV-2 [21]. Of interest, CD34+ cell exposure to the SARS-CoV-2 Spike (S) protein alters their growth in vitro [24]. Cell therapies using HSPCs have been approved under the commercial names of Libmeldy®, Skysoma®, Strimvelis®, Zolgensma® and Zynteglo®, for the treatment of metachromatic leukodystrophy, early cerebral adrenoleukodystrophy, adenosine deaminase severe combined immune deficiency and beta thalassemia, respectively. The presence of a gene modification manufacturing step in all these therapies should be considered in the risk assessment (see vector-producing cell lines section below). All these therapies are autologous, and thus would not have a risk of SARS-CoV-2 expansion to multiple patients.

**Monocytes, macrophages & dendritic cells**

The coronaviruses Middle East respiratory syndrome coronavirus (MERS-CoV) and SARS-CoV-1 are both known to infect macrophages [25,26]. Similarly, SARS-CoV-2 can infect both monocytes and macrophages, but this infection is abortive, meaning, SARS-CoV-2 virus does not replicate in these cells [27]. Infection and viral protein production without virus replication were also observed in monocyte-derived dendritic cells [28]. scRNAseq of bronchoalveolar lavage fluid from intubated COVID-19 patients detected SARS-CoV-2 sequences in monocyte-derived alveolar macrophages and migratory dendritic cells, although neither ACE2 nor TMPRSS2 could be detected in either cell type [15,19]. These data put into question the validity of an infection risk assessment exclusively based on the expression of the two markers mentioned above. A possible explanation to this observation could be that macrophages become infected in vivo after phagocytosis of infected cells in the alveoli [19]. These
observations may also be explained by direct infection through NRP1 and AXL in dendritic cells and monocytes, or CD209 on monocytes [15]. Finally, SARS-CoV-2 could also infect these cells by means of antibody-dependent enhancement [29]. Provenge® is an approved dendritic cell-based therapy against hormone refractory prostate cancer. The manufacturing of this therapy does not involve the transduction of the cells and is autologous, minimizing the risks associated with a SARS-CoV-2 infection to one patient.

Natural killer cells
Natural killer (NK) cells do not express ACE2 nor TMPRSS2 [15]. scRNAseq showed no evidence of infected NK cells in the lungs of COVID-19 patients [19]. Unfortunately, to the best of our knowledge, no in vitro studies have been performed to date regarding the susceptibility of infection of these cells. However, data show that NK cells in COVID-19 patients present a dysfunctional status, with a decrease of numbers of circulating cells that might derive from virus-induced apoptosis [30]. In this context, testing of the donors would be recommended to avoid the manufacturing of dysfunctional NK products.

Platelets & megakaryocytes
Platelets and megakaryocytes do not express ACE2 [31]. However, platelets can present SARS-CoV-2, as demonstrated by samples of circulating platelets of COVID-19 patients [32]. The origin of these cells may be infected megakaryocytes that differentiate into platelets, as megakaryocytes have been shown to be infected in samples from COVID-19 autopsies [32]. Other molecules like NPR1 have been proposed as a possible point of entry for SARS-CoV-2 in megakaryocytes [31]. What is more, patients with COVID-19 show a platelet hyperactivation [33]. This does not seem to be mediated by the virus itself or their proteins, but rather by the expression of different factors by the infected cells [33]. Aurix™ is an autologous platelet-based therapy for the treatment of chronic non-healing wounds. Since platelet activity may be influenced by the presence of COVID-19, a test of the donors would be recommended.

T cells
T cells show no signs of ACE2 nor TMPRSS2 expression [15]. In a study, scRNAseq of COVID-19 patients showed no evidence of T cell infection [19]. Taking these two facts into account, the likelihood that T cells could be infected by SARS-CoV-2 is relatively limited, however, no studies directly testing the susceptibility of this cell type were found. Therapies using T cells as a starting cell material have been approved under the commercial names of Abecma®, Breyanzi®, Kymriah®, Tecartus®, Yescarta® and Zalmoxis® for treatment of relapsed or refractory multiple myeloma, relapsed or refractory large B-cell lymphoma, acute lymphoblastic leukemia, chronic lymphoid leukemia and diffuse large B-cell lymphoma, mantle cell lymphoma, non-Hodgkin lymphoma and follicular lymphoma. All these therapies include a gene modification step, which should be considered during the SARS-CoV-2 propagation risk assessment. The immune compromised state of these patients presents an additional risk for their health with relation to a SARS-CoV-2 infection.

Non-blood derived products
Aortic endothelial cells
A study using immunofluorescence detected both ACE2 and SARS-CoV-2 in the vascular endothelial cells of multiple organs of COVID-19 patients, although aortic endothelial cells were not examined [14]. Additionally, the presence of the virus in vascular endothelial cells was confirmed in a study using material from autopsies from COVID-19 patients, without including aortic tissue [34]. In vitro studies demonstrated that SARS-CoV-2 can infect and replicate in human blood vessel organoids [35]. Finally, in vitro studies also showed that the presence of SARS-CoV-2 nucleocapsid proteins activated and damaged aortic endothelial cells, offering a possible explanation for the endothelial injury observed in some COVID-19 patients [36].

Cardiosphere-derived cells
Cardiosphere-derived cells (CDCs) are a progenitor cell population found in the heart [37]. Co-expression of ACE2 and the spike protein of SARS-CoV-2 has been observed in the heart of COVID-19 patients [14]. Moreover, the Human Protein Atlas shows that all the tested cell types present in the heart express ACE2 and low levels of TMPRSS2 [15]. In vitro infectivity was also demonstrated in cardiomyocytes derived from induced Pluripotent Stem Cells (iPSCs) [38], cardiosphere-derived stromal cells [39] and in a cardiosphere model [40]. Moreover, infected cells
can develop into a hyper-inflammatory phenotype that may explain the cardiac complications observed in COVID-19 patients [39]. Finally, patients with cardiovascular disease have a COVID-19 mortality aRR of 1.28, highlighting the importance of preventing SARS-CoV-2 infection in patients who need cardiac-derived cell therapies [3].

**Human embryonic stem cells**

Human embryonic stem cells (hESCs) express low levels of both ACE2 and TMPRSS2 [41]. No information could be found regarding the susceptibility of hESCs to SARS-CoV-2 infection, but the fact that iPSCs are refractory to the infection may point to a similar phenotype for hESCs [42]. Importantly, cells differentiated from hESCs can become susceptible to the infection, especially if the daughter cells express high levels of ACE2 and TMPRSS2 [43–45].

**Induced pluripotent stem cells**

iPSCs express low levels of both ACE2 and TMPRSS2 [41] and are refractory to SARS-CoV-2 infection [42]. Nonetheless, it should be considered that iPSCs may present different expression profiles and differentiation capacities depending on their tissue of origin and donor characteristics which could theoretically influence SARS-CoV-2 susceptibility [46,47]. As in the case of hESC, more differentiated cells derived from iPSCs can become susceptible to the infection, especially if the daughter cells express high levels of ACE2 and TMPRSS2. As an example, derived lung, neural and cardiac cells are permissive for SARS-CoV-2 infection *in vitro* [48,49].

**Mesenchymal stem & progenitor cells**

Mesenchymal stem cells (MSCs) do not express ACE2 nor TMPRSS2, and thus would not be expected to be targets for SARS-CoV-2 infection [50]. In addition, there are published reports that MSCs are resistant to SARS-CoV-2 infection *in vitro* [50,51]. These data are especially encouraging due to the potential use of MSCs as treatment for COVID-19 [52,53]. Alofisel®, Stemiac® and Temcell®, all MSC-based products, have gained market authorization for the treatment of, among others, chronic obstructive pulmonary disease, graft-versus-host disease, Type I diabetes, and myocardial infarction: all diseases with an increased risk of death by COVID-19 [3]. These are all allogenic therapies, increasing the importance of a proper risk assessment.

**Oligodendrocyte progenitor cells**

Oligodendrocyte progenitor cells (OPCs) express both ACE2 and TMPRSS2 [54], making them potential targets for SARS-CoV-2. A study using BrainSpheres (organoids derived from iPSCs containing neurons, astrocytes, and oligodendrocytes) demonstrated that SARS-CoV-2 can infect and replicate in this system, but a definitive statement of which cell types were infected was not made [55]. Recently a report showed that OPCs overexpress genes related to COVID-19 pathogenesis which suggests that they may be susceptible to infection [56].

**Discogenic cells**

Discogenic cells are progenitor cells originating from adult human intervertebral disc tissue, specifically from the nucleus pulposus tissue, and have a notochordal origin [57]. These cells exhibit a multipotency for mesenchymal lineage differentiation [58] which could suggest a similar phenotype to MSCs, namely no ACE2 and TMPRSS2 expression and no susceptibility to coronavirus infection [50,51]. However, no literature could be found regarding these topics for this cell type.

**Retinal cells**

An *in vitro* model based on hESC-derived eye organoids demonstrated that retinal pigment epithelial (RPE) cells express ACE2 and TMPRSS2 and are targeted by SARS-CoV-2 [59]. Moreover, iPSC-derived retinal organoids express ACE2 and TMPRSS2 and a SARS-CoV-2 spike protein pseudovirus can enter these cells [60]. These findings are consistent with the fact that COVID-19 patients present retinal manifestations of the disease [61]. No cell therapies using retinal cells have been approved so far, but Luxturna®, an AVV based gene therapy, has been approved for the treatment of Leber congenital amaurosis.

**Vector producing cell lines**

Viral vectors (often based on retroviruses and lentiviruses) are used to transduce cells to express specific proteins in ATMPs. Commonly, human embryonic kidney-derived cell lines (HEK293 and HEK 293T cells) are used for viral vector production [62,63], but these cells express only low levels of ACE2 [15]. These lines have been shown to be
susceptible to SARS-CoV-2 infection, but only with a limited viral replication capacity [64]. Interestingly, HEK293 and 293T cells transfected with ACE2 and TMPRSS2 can be easily infected by SARS-CoV-2 [11, 65].

The human cervical adenocarcinoma cell line HeLa has also been used for viral vector packaging [62]. HeLa cells do not express ACE2 [66] and are not naturally infected by the SARS-CoV-2 [67]. However, HeLa cells transfected with an ACE2 plasmid (transiently expressing ACE2) are capable of being infected [68].

The Vero line is a monkey kidney epithelial cell line which expresses ACE2 [69] and can be infected by the SARS-CoV-2 virus [60–71]. Although our literature search found no evidence that Vero cells are used for GMP production of gene therapy viral vectors, they have been used in other manufacturing processes such as in vaccine production [72].

Discussion

The COVID-19 pandemic caused by the SARS-CoV-2 virus has forced many industries to adapt their way of working, having also had a great impact on the ATMP field [73]. As shown in this article, different cell types present highly distinct susceptibilities to SARS-CoV-2 infection. These range from cells which cannot be infected by the virus (i.e., MSCs), to cells completely receptive to virus infection (i.e., endothelial cells), including cells which can be infected but show no virus replication (i.e., dendritic cells). A summary of the susceptibility to SARS-CoV-2 infection of the cellular types discussed in this review is shown in Table 1. A summary of the therapies with full approval in Japan, the EU and the USA and their cellular material is shown in Table 2.

An ATMP could potentially become infected at the source (incoming or starting cell material) or during cell processing (final product). This was the case during the Zika virus outbreak, which could be transmitted through blood products and semen [74, 75]. This fact led the FDA to recommend persons diagnosed with Zika virus infection in the previous 6 months or that visited a Zika high-prevalence area to be ineligible as cell and tissue donors [16]. To date, there have not yet been cases of transmission of COVID-19 via HCT/Ps or blood products reported in the literature. The FDA also states that it is not aware of any case of transmission via these products [17]. Additionally, coronaviruses are not known to be transmitted by HCT/P transplants [17]. However, it is the responsibility of the manufacturers to prevent this risk. It may be beneficial from a risk management perspective to test cell donors for SARS-CoV-2 if they have been in close contact with a known positive, have had symptoms, have had a positive SARS-CoV-2 test, or have developed COVID-19 in the last 28 days, as recommended by the FDA [17]. The risk of SARS-CoV-2 transmission from an ATMP into a patient would be highest for allogeneic cell lines/banks of epithelial or endothelial cells that express ACE2 and TMPRSS2. Such cell banks could be used for multiple (patient non-specific) doses of final product, so safety measures and potentially donor screening should be implemented.

During cell processing an ATMP could potentially become contaminated in two ways. First, the product may be contaminated due to direct contact with an operator suffering COVID-19. Considering the strict sterility and hygiene standards held by the industry, a direct contamination of the cell product from an operator is highly unlikely. However, testing, mask mandates and vaccination are still beneficial to prevent virus spread between operators and would minimize the risk of contamination of the product. A second way a product could potentially be contaminated would be through cell culture materials of human origin used during the manufacturing process, which could have been contaminated at the source. Currently, the FDA does not recommend testing incoming materials for SARS-CoV-2 [17]. The risk would be unlikely for products such as albumin USP, which has alcohol fractionation and is pasteurized (60°C for 10 h) to inactivate viruses (e.g., see CSL Behring Albuminar-5 factsheet). The risk could, however, be higher for pooled human serum, which does not use these types of processes. For pooled serum, donors are screened and tested following FDA guidelines, which do not currently include testing for SARS-CoV-2 [17]. A close collaboration with suppliers of biological materials of human origin to make sure they are taking all adequate measures to avoid contamination of such products will be necessary. It is important to remark that the current cleaning procedures used in cleanrooms for ATMP production rely on high-level disinfectants, based on isopropanol, bleach, hydrogen peroxide and phenols. These disinfectants are adequate for the destruction of coronaviruses, further minimizing the risk of a product contamination [76].

Although a seemingly good strategy to avoid a SARS-CoV-2 infection of an ATMP would be to test the operators, donors, and the cell product all the way through the production of the drug product, several challenges make this approach very difficult to implement. First, the availability of the material for testing is limited, with many processes relying on precious material where every cell counts toward the success of the therapy. Second, there is a financial consideration, with serial SARS-CoV-2 testing adding to the financial burden of producing ATMPs. Finally, the willingness of the operators and donors to get tested may also suppose a limitation to this broad testing strategy.
Table 1. Summary of the risk assessment of SARS-CoV-2 infection for all the cell types used to date in cellular therapies with approval designation (PRIME Designation, RMAT Designation, Breakthrough Designation, Fast Track Designation and SAKIGAKE Designation) in Europe, the USA and Japan.

| Material                                         | ACE2 expression | TMPRSS2 expression | Infection susceptibility | Additional infection information                                                                 | Other considerations                                                                 | Refs.               |
|--------------------------------------------------|-----------------|--------------------|--------------------------|---------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|---------------------|
| Aortic endothelial cells                         | Yes             | No                 | Yes                      | Infection and replication in human blood vessel organoids                                          | Activated by SARS-CoV-2 nucleocapsid protein                                         | [14,35,36]         |
| B cells                                          | Low             | No                 | No                       | scRNASeq no evidence of B cell infection                                                          |                                                                                      | [15,19]            |
| Blood products (PBMC, whole blood, bone marrow)  | Expression in some cell types | No                   | Yes                      | Some small subsets of cells are susceptible                                                       | Susceptible cell types may not part of the final cell product                         | [15,19,21]         |
| Cardiac progenitor cells                         | Yes             | Low                | Yes                      |                                                                                                   | Infected cells can cause a hyper-inflammation                                         | [14,38-40]         |
| Dendritic cells                                  | Low             | Yes                | Yes                      |                                                                                                   | Permissive to infection and protein expression but not virus replication               | [28]                |
| Erythroid cells                                  | Yes (early erythroid progenitors) | Yes (early erythroid progenitors) | Yes                      | CD34- early erythroid progenitors susceptible to SARS-CoV-2 infection with viral replication       | Cause of anemia in COVID-19 patients                                                 | [21]                |
| HEK293T (embryonic kidney)                       | No              | No                 | Yes                      | Susceptible infection, low viral replication                                                      | Transfection with ACE2 and TMPRSS2 allows infection                                   | [15,64,65]         |
| HeLa (cervical cancer)                           | No              | No                 | No                       | Transfection with an ACE2 plasmid allows for infection                                            |                                                                                      | [15,66-68]         |
| Hematopoietic stem and progenitor cells          | Low             | No                 | No                       | Exposure to S protein alters proliferation/expansion                                               |                                                                                      | [21,24]            |
| Human embryonic stem cells                       | Low             | Low                | No information available |                                                                                                   |                                                                                      | [41]                |
| Induced pluripotent stem cells                   | Low             | Low                | No                       | Derived lung, neural and cardiac cells permissive to infection                                   |                                                                                      | [41,42,48,49]      |
| Mesenchymal stem cells                           | No              | No                 | No                       |                                                                                                   |                                                                                      | [50,51,53]         |
| Monocytes and macrophages                        | No              | No                 | Yes                      | Susceptible, but the infection is abortive                                                        |                                                                                      | [15,27]            |
| Natural killer cells                             | No              | No                 | No information available |                                                                                                   | No information available                                                               | [15]                |
| Oligodendrocyte progenitor cells                 | Yes             | Yes                | No information available | Overexpression of genes related to COVID-19                                                        |                                                                                      | [54-56]            |
| Progenitor cells from intervertebral disc tissue | No information available | No information available | No information available |                                                                                                   | No information available                                                               |                    |
| Retinal cells                                    | Yes             | Yes                | Yes                      | COVID-19 patients have retinal symptoms                                                            |                                                                                      | [59-61]            |
| T cells                                          | No              | No                 | No                       | scRNASeq no evidence of infection                                                                |                                                                                      | [15,19]            |
| Vero (Monkey kidney epithelial)                  | Yes             | No                 | Yes                      |                                                                                                   |                                                                                      | [69-71]            |

PBMC: Peripheral blood mononuclear cell; scRNASeq: Single cell RNA sequencing.
Table 2. Summary of therapeutic cell types with products with full approval in either Japan, the EU or the USA.

| Material                                      | Indications                                                                 | Commercial products                  |
|-----------------------------------------------|------------------------------------------------------------------------------|-------------------------------------|
| Blood products (PBMC, whole blood, bone marrow) | Treatment of wounds                                                         | Aurix™                               |
| Dendritic cells                               | Hormone refractory prostate cancer                                           | Provence™                            |
| Hematopoietic stem and progenitor cells       | Metachromatic leukodystrophy, early Cerebral Adrenoleukodystrophy, adenosine deaminase severe combined immune deficiency, beta thalassemia | Libmeldy®, Skysona®, Strimvelis®, Zolgensma®, Zytéglo® |
| Mesenchymal stem cells                        | Crohn’s disease, spinal cord injury, acute radiation injury, chronic obstructive pulmonary disease, graft-vs-host disease, Type I diabetes and myocardial infarction | Alofisel®, Stemrac®, Temcell®        |
| Retinal cells (as target cell)                | Leber congenital amaurosis                                                  | Luxturna®                            |
| T cells                                       | Relapsed or refractory multiple myeloma, relapsed or refractory large B-cell lymphoma, acute lymphoblastic leukemia, chronic lymphoid leukemia and diffuse large B-cell lymphoma, mantle cell lymphoma, non-Hodgkin lymphoma, follicular lymphoma | Abecma®, Breyanzi®, Kymriah®, Tecartus®, Yescarta®, Zalmoxis® |

PBMC: Peripheral blood mononuclear cell.

Cell type

ACE2 + TMPRSS2 expression

The human protein Atlas (mRNA expression)

literature research

In vitro SARS-CoV-2 susceptibility

Literature research

In vivo SARS-CoV-2 susceptibility

COVID-19 patients scRNAseq

Transduction necessary?

Yes

No

Risk assessment report

Figure 2. Strategy for the risk assessment of SARS-CoV-2 propagation in cell types used for the manufacturing of advanced therapies.

scRNAseq: Single-cell RNA sequencing.

Importantly, a risk assessment should ideally be based on experimental data and not only on annotations of ACE2 and TMPRSS2 expression from existing databases. While such information may be useful, some cell types, (i.e., monocytes and dendritic cells), do not show high expression of these markers but are still susceptible to SARS-CoV-2 infection [19,27]. This fact could be explained by the role of further surface molecules and proteases in SARS-CoV-2 cell infection [5–10], limiting the value of ACE2 and TMPRSS2 as standalone markers. What is more, when using databases that assess expression at the organ level, some organs which are highly damaged in
COVID-19 patients may appear to have a low expression of these markers (i.e., the lung, Figure 1). This may be an effect of the low resolution of this kind of data, for example, only epithelial cells may be susceptible to infection, but the lung is a heterogeneous tissue formed by several types of cells. Data with a cellular resolution is always to be preferred when performing a risk assessment for an ATMP.

**Conclusion**

Altogether, the risk of SARS-CoV-2 contamination of cellular products is low but not non-existent. The risk should be evaluated on an individual basis for each therapy being produced. The expression of ACE2 and TMPRSS2 should be investigated for the cell type being used. A literature review on *in vitro* experiments evaluating the susceptibility to virus infection and primary scRNASeq data of COVID-19 patients should complete this risk evaluation (Figure 2). This evaluation should be performed for both the cells being manufactured as well as the vector-producing cells.

**Future perspective**

As pandemics become more frequent due to factors such as overpopulation, globalization (e.g., easy travel), and climate change [77], stakeholders in the development and manufacturing of cell therapies will need to keep their processes and analytical methods up to date to comply with an ever-evolving regulatory environment. However, the tendency of the industry toward automation and the development of single-use closed systems will mitigate the risks of propagation of these pathogens during manufacturing. Moreover, technological advances in diagnostic methods tend to ever cheaper, faster, and reliable tests that will allow to screen cell and other human materials for use in cell therapy manufacturing without significantly increasing costs and time of production.

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**Executive summary**

- SARS-CoV-2 presents a multicellular tropism.
- Patients requiring cell therapies have a higher risk of serious illness and death due to COVID-19.
- The risk of SARS-CoV-2 propagation in manufacturing needs to be evaluated and minimized.
- Cell material could potentially be infected through the donor or could get contaminated during cell manufacturing.
- Each cell type presents different susceptibility to SARS-CoV-2 infection.
- Cell tropism can be evaluated through ACE2 and TMPRSS2 RNA expression, literature regarding *in vitro* infectability, and single-cell RNA sequencing data of COVID-19 patients.
- These types of analysis should be performed for both the therapeutic cells being manufactured and, in the case of gene therapies, the cells used to produce the vector.
- Similarly, each therapy and manufacturing process present its own specific risks.
- Strict sterility standards (personal and environmental monitoring) held by the manufacturers make a direct contamination from an operator highly unlikely.
- Cell culture materials of human origin could be contaminated at the source and may need to be tested.
- The risk should be evaluated on an individual basis for each therapy being produced, with the publication providing valuable information for this assessment.

**Author contributions**

All authors have contributed through bibliographic research, writing, and reviewing the manuscript.

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**References**

Papers of special note have been highlighted as: ● of interest

1. Modi C, Bohm V, Ferraro S, Stein G, Seljak U. Estimating COVID-19 mortality in Italy early in the COVID-19 pandemic. *Nat. Commun.* 12(1), 2729 (2021).
2. Rosenthal N, Cao Z, Gundrum J, Sianis J, Safo S. Risk factors associated with in-hospital mortality in a US national sample of patients with COVID-19. *JAMA Neurol. Open* 3(12), e2029058 (2020).

3. Kim L, Garg S, O'halloran A et al. Risk factors for Intensive Care Unit Admission and In-hospital Mortality Among Hospitalized Adults Identified through the US Coronavirus Disease 2019 (COVID-19)-Associated Hospitalization Surveillance Network (COVID-NET). *Clin. Infect. Dis.* 72(9), e206–e214 (2021).

- Shows that patients in need of cell therapies have a worse COVID-19 prognostic.

4. Shang J, Wan Y, Luo C et al. Cell entry mechanisms of SARS-CoV-2. *Proc. Natl Acad. Sci. USA* 117(21), 11727–11734 (2020).

- Demonstrates the cell entry mechanisms of SARS-CoV-2.

5. Shang J, Ye G, Shi K et al. Structural basis of receptor recognition by SARS-CoV-2. *Nature* 581(7807), 221–224 (2020).

6. Amraei R, Yin W, Napoleon MA et al. CD209/L-SIGN and CD209/DC-SIGN Act as Receptors for SARS-CoV-2. *ACS Cent. Sci.* 7(7), 1156–1165 (2021).

- Wang S, Qiu Z, Hou Y et al. AXL is a candidate receptor for SARS-CoV-2 that promotes infection of pulmonary and bronchial epithelial cells. *Cell Rex.* 31(2), 126–140 (2021).

- Cantuti-Castelvetri L, Ojha R, Pedro LD et al. Neuropilin-1 facilitates SARS-CoV-2 cell entry and infectivity. *Science* 370(6518), 856–860 (2020).

- Daly JL, Simonetti B, Klein K et al. Neuropilin-1 is a host factor for SARS-CoV-2 infection. *Science* 370(6518), 861–865 (2020).

- Mori Y, Fink C, Ichimura T et al. KIM-1/TIM-1 is a Receptor for SARS-CoV-2 in Lung and Kidney. *medRxiv* doi: 10.1101/2020.09.16.20190694 (2022).

- Hoffmann M, Kleine-Weber H, Schroeder S et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* 181(2), 271–280 e278 (2020).

- Zang R, Gomez Castro MF, McCune BT et al. TMPRSS2 and TMPRSS4 promote SARS-CoV-2 infection of human small intestinal enterocytes. *Sci. Immunol.* 5(47), eabc3582 (2020).

- Chen L, Li X, Chen M, Feng Y, Xiong C. The ACE2 expression in human heart indicates new potential mechanism of heart injury among patients infected with SARS-CoV-2. *Cardiovasc. Res.* 116(6), 1097–1100 (2020).

- Liu J, Li Y, Liu Q et al. SARS-CoV-2 cell tropism and multiorgan infection. *Cell Discov.* 7(1), 17 (2021).

- *Liu et al. (2021)* shows that SARS-CoV-2 has multiorgan and multicellular tropism proprieties.

15. Ponten F, Jirstrom K, Uhlen M. The Human Protein Atlas—a tool for pathology. *J. Pathol.* 216(4), 387–393 (2008).

16. Donor screening recommendations to reduce the risk of transmission of Zika virus by human cells, tissues, and cellular and tissue-based products. Food and Drug Administration (2018). www.fda.gov/media/145301/download

17. Manufacturing considerations for licensed and investigational cellular and gene therapy products during COVID-19 public health emergency. Food and Drug Administration (2021). www.fda.gov/media/96528/download

18. Alliance for Regenerative Medicine, Expedited Approval Designations. https://alliancerm.org/expedited-approval-designations/

19. Grant RA, Morales-Nebrada L, Markov NS et al. Circuits between infected macrophages and T cells in SARS-CoV-2 pneumonia. *Nature* 590(7847), 635–641 (2021).

20. Jing Y, Luo L, Chen Y et al. SARS-CoV-2 infection causes immunodeficiency in recovered patients by downregulating CD19 expression in B cells via enhancing B-cell metabolism. *Signal Transduct. Target Ther.* 6(1), 345 (2021).

21. Huerga Encabo H, Grey W, Garcia-Albornoz M et al. Circuits between infected macrophages and T cells in SARS-CoV-2 pneumonia. *Semin. Immunopathol.* 39(5), 529–539 (2017).

22. Shahbaz S, Xu L, Osman M et al. Erythroid precursors and progenitors suppress adaptive immunity and get invaded by SARS-CoV-2. *Cell Stem Cell* 16(3), 428–436 (2021).

23. Morgan RA, Gray D, Lomova A, Kohn DB. Hematopoietic Stem Cell Gene Therapy: Progress and Lessons Learned. *Cell Stem Cell* 21(5), 574–590 (2017).

24. Ropa J, Cooper S, Capitano ML, Van’t Hof W, Broxmeyer HE. Human Hematopoietic Stem, Progenitor, and Immune Cells Respond Ex Vivo to SARS-CoV-2 Spike Protein. *Stem Cell Res. Rep.* 17(1), 253–265 (2021).

25. Channappanavar R, Perlman S. Pathogenic human coronavirus infections: causes and consequences of cytokine storm and immunopathology. *Semin. Immunopathol.* 39(5), 529–539 (2017).

26. Gu J, Gong E, Zhang B et al. Multiple organ infection and the pathogenesis of SARS. *J. Exp. Med.* 202(3), 415–424 (2005).

27. Boumaza A, Gay L, Mezouar S et al. Monocytes and macrophages, targets of SARS-CoV-2: the clue for Covid-19 immunoparalysis. *J. Infect. Dis.* doi: 10.1093/infdis/jiab044 (2021).

28. Yang D, Chu H, Hou Y et al. Attenuated Interferon and Proinflammatory Response in SARS-CoV-2-Infected Human Dendritic Cells Is Associated With Viral Antagonism of STAT1 Phosphorylation. *J. Infect. Dis.* 222(5), 734–745 (2020).

29. Lee WS, Wheatley AK, Kent SJ, Dekosky BJ. Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies. *Nat. Microbiol.* 5(10), 1185–1191 (2020).
30. Bi J. NK cell dysfunction in patients with COVID-19. *Cell Mol. Immunol.* 19(2), 127–129 (2022).
31. Shen S, Zhang J, Fang Y et al. SARS-CoV-2 interacts with platelets and megakaryocytes via ACE2-independent mechanism. *J. Hematol. Oncol.* 14(1), 72 (2021).
32. Zhu A, Real F, Capron C et al. Infection of lung megakaryocytes and platelets by SARS-CoV-2 anticipate fatal COVID-19. *Cell. Mol. Life Sci.* 79(7), 365 (2022).
33. Puhm F, Allaerts I, Lacasse E et al. Platelet activation by SARS-CoV-2 implicates the release of active tissue factor by infected cells. *Blood Adv.* 6(12), 3593–3605 (2022).
34. Bhatnagar J, Gary J, Reagan-Steiner S et al. Evidence of Severe Acute Respiratory Syndrome Coronavirus 2 Replication and Tropism in the Lungs, Airways, and Vascular Endothelium of Patients With Fatal Coronavirus Disease 2019: An Autopsy Case Series. *J. Infect. Dis.* 223(5), 752–764 (2021).
35. Monteil V, Kwon H, Prado P et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell* 181(4), 905–913 e907 (2020).
36. Qian Y, Lei T, Patel P et al. Direct activation of endothelial cells by SARS-CoV-2 nucleocapsid protein is blocked by Simvastatin. *bioRxiv* doi: 10.1101/2021.02.14.431174 (2021).
37. White AJ, Smith RR, Matsushita S et al. Human mesenchymal-derived stromal cells exposed to SARS-CoV-2 evolve into hyper-inflammatory/pro-fibrotic phenotype and produce infective viral particles depending on the levels of ACE2 receptor expression. *Cardioren. Res.* 117(6), 1557–1566 (2021).
38. Sharma A, Garcia G, Arumugawami V, Svensden CN. Human iPSC-Derived Cardiomyocytes are Susceptible to SARS-CoV-2 Infection. *bioRxiv* doi: 10.1101/2020.04.21.051912 (2020).
39. Amendola A, Garoffolo G, Songia P et al. Human cardiomyocyte-angiogenesis difference in iPSCs. *Cell Stem Res.* 10(1), 57–66 (2013).
40. Eriksen AZ, Moller R, Makovou B, Uhl SA, Tenoever BR, Blenkinsop TA. SARS-CoV-2 infects human adult donor eyes and hESC-derived ocular epithelium. *Cell Stem Cell* 28(7), 1205–1220 e1207 (2021).
41. Burrows CK, Banovich NE, Pavlovic BJ et al. Genetic variation, not cell type of origin, underlies the majority of identifiable regulatory differences in iPSCs. *PLoS Genet.* 12(1), e1005793 (2016).
42. Hu S, Zhao MT, Jahanbani F et al. Effects of cellular origin on differentiation of human induced pluripotent stem cell-derived endothelial cells. *JCI Insights* 1(8), e85558 (2016).
43. Huang J, Hum EJ, Aho KM et al. SARS-CoV-2 infection of pluripotent stem cell-derived human lung alveolar Type 2 cells elicits a rapid epithelial-intrinsic inflammatory response. *bioRxiv* doi: 10.1101/2020.05.30.175695 (2020).
44. Kase Y, Okano H. Expression of ACE2 and a viral virulence-regulating factor CCN family member 1 in human iPSC-derived neural organs susceptible to SARS-CoV-2 infection. *Stem Cell Transl. Med.* 10(4), 636–642 (2021).
45. Schafer R, Spohn G, Bechtel M et al. Human Mesenchymal Stromal Cells Are Resistant to SARS-CoV-2 Infection under Steady-State, Inflammatory Conditions and in the Presence of SARS-CoV-2-Infected Cells. *Stem Cell Reports* 16(3), 419–427 (2021).
46. Durand N, Mallea J, Zabair AC. Insights into the use of mesenchymal stem cells in COVID-19 mediated acute respiratory failure. *NPJ Regen. Med.* 5(1), 17 (2020).
47. Leng Z, Zhu R, Hou W et al. Transplantation of ACE2(-) mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. *Aging. Dis.* 11(2), 216–228 (2020).
48. Qi J, Zhou Y, Hua J et al. The scRNA-seq expression profiling of the receptor ACE2 and the cellular pro tease TMPRSS2 reveals human organs susceptible to SARS-CoV-2 infection. *Int. J. Environ. Res. Public Health* 18(1), 284 (2021).
49. Bullen CK, Hogberg HT, Bahadirli-Talbott A et al. Infectability of human BrainSphere neurons suggests neurotropism of SARS-CoV-2. *ALTEX* 37(4), 665–671 (2020).
50. Chamling X, Kallman A, Fang W et al. Single-cell transcriptomic reveals molecular diversity and developmental heterogeneity of human stem cell-derived oligodendrocyte lineage cells. *Nat. Commun.* 12(1), 652 (2021).
57. Silverman LI, Heaton W, Farhang N et al. Perspectives on the Treatment of Lumbar Disc Degeneration: The Value Proposition for a Cell-Based Therapy, Immunomodulatory Properties of Discogenic Cells and the Associated Clinical Evaluation Strategy. Front. Surg. 7, 554382 (2020).

58. Silverman LI, Dulatova G, Tandeski T et al. In vitro and in vivo evaluation of discogenic cells, an investigational cell therapy for disc degeneration. Spine J 20(1), 138–149 (2020).

59. Makovev B, Moeller R, Zebitz Eriksen A, Tenoever BR, Blenkinsop TA. SARS-CoV-2 Infection of Ocular Cells from Human Adult Donor Eyes and hESC-Derived Eye Organoids. SSRN doi: 10.2139/ssrn.3650574 3650574 (2020).

60. Ahmad Mulyadi Lai HI, Chou SJ, Chien Y et al. Expression of Endogenous Angiotensin-Converting Enzyme 2 in Human Induced Pluripotent Stem Cell-Derived Retinal Organoids. Int. J. Mol. Sci. 22(3), 1320 (2021).

61. Invernizzi A, Torre A, Parrulli S et al. Retinal findings in patients with COVID-19: results from the SERPICO-19 study. E Clinical Medicine 27, 100550 (2020).

62. Merten OW, Hebben M, Bovolenta C. Production of lentiviral vectors. Mol. Ther. Methods Clin. Dev. 3, 16017 (2016).

63. Van Der Loo, JC, Wright, JF. Progress and challenges in viral vector manufacturing. Hum. Mol. Genet. 25(R1), R42–52 (2016).

64. Harcourt J, Tamin A, Lu X et al. Severe Acute Respiratory Syndrome Coronavirus 2 from Patient with Coronavirus Disease, United States. Emerg. Infect. Dis. 26(6), 1266–1273 (2020).

65. Hu J, Gao Q, He C, Huang A, Tang N, Wang K. Development of cell-based pseudovirus entry assay to identify potential viral entry inhibitors and neutralizing antibodies against SARS-CoV-2. Genes. Dis. 7(4), 551–557 (2020).

66. Nie Y, Wang P, Shi X et al. Highly infectious SARS-CoV pseudotyped virus reveals the cell tropism and its correlation with receptor expression. Biochem. Biophys. Res. Commun. 321(4), 994–1000 (2004).

67. Chu H, Chan JF, Yuen TT et al. Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study. Lancet Microbe 1(1), e14–e23 (2020).

68. Zhou P, Yang XL, Wang XG et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579(7798), 270–273 (2020).

69. Ogando NS, Dalebout TJ, Zevenhoven-Dobbe JC et al. SARS-coronavirus-2 replication in Vero E6 cells: replication kinetics, rapid adaptation and cytopathology. J. Gen. Virol. 101(9), 925–940 (2020).

70. Case JB, Bailey AL, Kim AS, Chen RE, Diamond MS. Growth, detection, quantification, and inactivation of SARS-CoV-2. Virology 548, 39–48 (2020).

71. Yao P, Zhang Y, Sun Y et al. Isolation and Growth Characteristics of SARS-CoV-2 in Vero Cell. Viral. Sin. 35(3), 348–350 (2020).

72. Barrett PN, Mundt W, Kistner O, Howard MK. Vero cell platform in vaccine production: moving towards cell culture-based viral vaccines. Expert Rev. Vaccines 8(5), 607–618 (2009).

73. Qiu T, Wang Y, Liang S, Han R, Toumi M. The impact of COVID-19 on the cell and gene therapies industry: disruptions, opportunities, and future prospects. Drug Discov. Today 26(10), 2269–2281 (2021).

Qiu et al. (2021) summarizes some of the challenges that the cell and gene therapy industry is facing in face of the COVID-19 pandemic.

74. Musso D, Nhan T, Robin E et al. Potential for Zika virus transmission through blood transfusion demonstrated during an outbreak in French Polynesia, November 2013 to February 2014. Euro Surveill. 19(14), 20761 (2014).

75. Garcia-Bujalance S, Gutierrez-Arroyo A, De La Calle F et al. Persistence and infectivity of Zika virus in semen after returning from endemic areas: report of 5 cases. J. Clin. Virol. 96, 110–115 (2017).

76. United States Environmental Protection Agency. List N Tool: COVID-19 Disinfectants. https://cfpub.epa.gov/wizards/disinfectants/

77. Marani M, Katul GG, Pan WK, Parolari AJ. Intensity and frequency of extreme novel epidemics. Proc. Natl Acad. Sci. USA 118(35), e2105482118 (2021).