Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Transfusion and Apheresis Science 61 (2022) 103490

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Editorial: Time to explore the missing links: the cross-talk between COVID infection and cancer cells; lingering questions on the longitudinal efficacy of the front line immunotherapies against unpredictable "Omicron ghosts" and insights from CAR-T cell therapy pointing toward better clinical outcomes

In this issue of What's is Happening, as a follow-up to my previous joint theme on "*Platelet and extracellular vesicles in COVID-19 infection and its vaccines*", I have invited professor Hadi Goubran, *Saskatoon Cancer Centre and College of Medicine, University of Saskatchewan, Saskatoon, Canada* to lead another concise synthesis of what we know on the interactions of SARS-CoV-2 viral infections and its virion on cancer cells and the disease progression or regression.

While there are a few studies on coronavirus convalescent plasma [CCP] in cancer patients, who suffered a higher toll from the infection, the intended use of CCP was to assess its impact on the control of the viral infection rather than cancer progression. Still, insights from CAR-T cell therapies offer some promising outcomes in cancer treatments. However, many challenges, including improving responses in blood cancers and efficacy in solid tumours still remain to be resolved. Nevertheless, work is in progress to develop a delivery method that enhances treatment by adding CAR T cells and specializing signalling proteins to a hydrogel that is injected next to a tumour.

During the early Coronavirus pandemic, in addition to the deployment of two doses of vaccines directed at the mRNA of coronavirus spike proteins which has provided some promising outcomes, several observational and evidence-based studies supported the usefulness of CCP containing a considerable amount of polyclonal CoV-2 neutralising antibodies, for preventative and therapeutic applications. In fact the use of high titre CCP coupled with timely deployment of vaccines against the original strain of CoV-2 infection are well-documented mode of passive immunity and have been approved in accordance with recommendations of leading institutions [EU, FDA, UK, and WHO]. However, enhancing the longitudinal safety/efficacy and tolerability of these front line measures of the immunotherapies, against the heavily mutated Omicron subvariants was found to be problematic. This is potentially due to the enormous individual variability in the levels and poor affinity or functionality of the circulating neutralising antibodies. It is therefore important to further investigative the avidity and specificity of the circulating neutralizing antibodies. Hence, going back to basic, exploring insights on the clinical outcomes of the deployment of CCP and vaccines against mutated CoV-2 subvariants are of current interest to many readers.

In a recent study to better understand the potential therapeutic safety/efficacy of pooled CCP, in an animal validation study, 12 adult rhesus macaques were inoculated with SARS-CoV-2 by intra-tracheal and intranasal routes. One day later, eight animals were infused with pooled human CCP with a high titer of neutralizing antibodies (RVPN NT50 value of 3003), while 4-control animals received normal human plasma. The animals were monitored for 7 days and those treated with CCP had only marginal detectable levels of antiviral antibodies after infusion in comparison to the control animals. The overall therapeutic benefits were inferior to results obtained earlier with monoclonal antibodies in this animal model.

Interestingly, in a separate study, when the readily available hyperimmune intravenous immunoglobulin (hIVIG) to SARS-CoV-2, derived from recovered donors for use as a specific passive immunotherapy was compared with placebo it was observed that the hIVIG group did not have a significantly greater odds of a more favourable outcome at day 7. Furthermore the infusion reactions were more common in the hIVIG group being twice as high as in the placebo groups. Clearly more innovative approaches to the quality, safety, and tolerability of active principles of products derived from CCP are required.

In this context, I have invited one of my regular contributors, professor Tor Hervig and other colleagues from Norway to provide an update on the Nordic preparedness on the use of "Convalescent plasma in the treatment of Covid-19, in line with the principle lessons learned from the past from von Behring, Kitasato and Ehrlich": [i] The treatment should start early; [ii] The severity of the condition and the antibody dose should match [iii]; There must be a minimal dose of antibodies. Preparedness for these principles is essential for the next generation of the pandemic.

Recently, several attempts were made to shorten the interval between two doses of vaccine deployment and then increase the number of booster doses to three and even 4 to increase and enhance the durability and effectiveness of antiviral antibodies. Moreover, attention is focused on targeting more specific vaccines to enhance the therapeutic benefits of vaccination programs. In parallel, attempts were made to optimize and even improve the safety, efficacy, durability, and reduce toxicity just by lowering the dose required by using self-amplifying RNA rather than the mRNA approach used in existing COVID – 19 vaccines. The main difference is the dose needed for use. Self-amplifying RNA (saRNA) is just a type of messenger RNA (mRNA) that has all the same structural components, but it encodes four extra proteins that make up a replicase enzyme. This enzyme is able to make copies of the RNA once it gets into a cell. With mRNA, the amount of RNA that is really delivered is in the vaccine. As soon as it gets into a cell it starts making protein, but it also starts getting degraded. With saRNA, once it gets into a cell it starts

https://doi.org/10.1016/j.transci.2022.103490

Available online 14 June 2022

making protein, but it also makes copies of itself so then you get many thousands more copies of RNA than you get with the mRNA. It's also getting degraded, but since its replicating, a longer duration of protein expression is expected.

Moreover, with mRNA, protein expression is for three to five days following injection, and with the saRNA, a much longer time span, expected to usually be about 30–60 days.

The duration of immunity is also important. The mRNA vaccines work really well early on, and then we start to see the antibody levels tailing off; and the T-cell responses becoming important in controlling COVID infections but with the saRNA, and sustained exposure to the antigen over a longer period, a longer duration of antibody response expected.

The dose-ranging studies that were done specifically for COVID indicate that the side effects of the vaccine are directly correlated with the dose of RNA. For example, Moderna tried doses of 50, 100, and 250 micrograms in their Phase I clinical trial, and the data from that trial make it obvious that the higher the dose of mRNA, the more side effects and hence the more severe side effects. Moderna is currently using a dose of 100 micrograms for their COVID vaccine and the Pfizer/BioNTech mRNA vaccine is a 30-microgram dose. AstraZeneca in their Phase III trial was using 5 micrograms, much lower than the other mRNA COVID vaccines, which helped reducing the side effects and the cost of vaccination.

Another important point to take into consideration is delaying the second and now the booster doses. A recently published study of changing the Pfizer/BioNTech's BNT162b COVID-19 vaccine from the standard three- to six-week interval to an eight- 16-week interval significantly increased SARS-CoV-2 neutralization titre to the alpha, beta, and delta variants of SARS-CoV-2. Moreover, with mRNA, protein expression for three to five days following injection, and with the saRNA, a much longer time span, is usually about 30–60 days is expected.

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Clearly, much remains to be done to enhance the durability and longitudinal efficacy of these practical front-line measures against the rather unpredictable emerging heavily mutated fast-spreading CoV-2 Omicron subvariants that are showing different patterns of inhibition by neutralising antibodies derived from CCP and the existing vaccines.

In this context, some investigators are trying to extract the active principle of CCP, at the early peak stage to enhance the safety/efficacy of the enriched final product. In fact, some investigators, including this author, are exploring the production of neutralizing antibody hyperconcentrates, either using online apheresis technology or selective pools of CCP or selective pools of vaccinated individual volunteers that more closely reflect the nature of circulating variants in real-time. No wonder an enormous amount of CCP has already been collected internationally by several blood banks in view of there potential benefit as an alternative to vaccines but such products still require validation through animal studies before clinical trials. Other investigators are opting to inhibit or removing potential toxic elements from CCP, such as the IL6 cytokine, activated complement, or by modifying anti-inflammatory antithrombotic potential.

Comparing active polyclonal antibodies versus passive vaccination immunization is now becoming a current topic of interest. In the UK some investigators are registering patients for polyclonal antibodies derived from the cultivation of B cells in a special technique using some specifically designated culture media for active immunisation (considering the hazard of autoimmune diseases) versus passive immunisation.

Meanwhile, SARS-CoV2 subvariants are still mutating in different parts of the world. The Pasteur Institute has recently published a preprint paper in medRxiv entitled "Culture and identification of a "Deltamicron" variant, in a three case cluster in southern France. According to WHO confirmation, this variant of concern had begun to appear in France, Denmark, Germany and the Netherlands. According to PCR results the scientist believe that the French strains were caused by a recombinant strain of Delta 21J/AY.4 and Omicron 21K/BA.1, which was named "Deltacron". In this SARS-CoV-2 recombinant, most of the spike gene was replaced in a Delta 21J/AY.4 matrix by an Omicron 21K/BA.1 sequence. It is now confirmed that the overall structure of the recombinant spike protein has been predicted according to the sequence of the recombinant virus. Compared to the Delta and Omicron variant strains, the main structural changes of this recombinant strain were located in the N-terminal domain which is attracted by lipid rafts and provides electronegative landing platforms for the spike in the initial interaction of the virus making it more electropositive to accelerate the binding of the virus to lipid rafts which may confer a selective kinetic advantage against virus competitors which may facilitate the interaction with the electronegative interface of the ACE2 cellular receptor. Overall, this structural analysis suggests an optimization of viral binding to the host cell membrane of the recombinant virus. The five typical key questions that need to be addressed are: [i] Is it easier to escape either vaccination-induced or natural immunity? [ii] Are currently used therapeutic drug combination still effective? [iii] Is it easier to replicate and spread? [iv] Does it cause more hospitalization? [v] Can the existing diagnostic reagents detect it?

Meanwhile, many new innovative strategies such as cellular and gene therapy are being explored in order to ensure future workflow and further mitigate patient safety. An Antibody-Drug Conjugate [ADC] is now considered to be a magic bullet for cancer treatment as it is designed utilising technology to bind complexes from the surface cells and to release the microtube disrupting agent that induces cell cycle arrest and apoptosis, an ADC directed to a T- cell checkpoint ligand. Furthermore, evidence is accumulating that SARS-CoV-2 vaccination induces immunological T cell memory enabling cross-recognization of variants from Alpha to Omicron. A new study suggests that a mRNA booster vaccine can induce neutralising immunity against 0 μm BA.1 and a second booster appears to significantly lowers the death rate. The Royal College of Physicians currently offers patients genetic tests to see if medical interventions are safe and effective.

COVID-19 is the seventh pandemic in the past 100 years and, clearly, we will face some others in the coming years. Understandably, viruses mutate frequently and some newly emerging subvariants are expected to threaten the predisposed unvaccinated and even insufficiently vaccinated individuals by bypassing vaccination-induced passive immunity against the original strain with devastating outcomes to our health care systems and beyond. In terms of preparedness for the next generation of vaccine development, Sonofi has begun construction of a Singapore vaccination plant facilitating a fully digitalised modular unit, which provides up to four vaccines simultaneously regardless of vaccine technology used. Other large-scale vaccine companies target fully industrialised manufacturing to scale up the autologous cellular immunotherapy that requires a fully closed system for processing and automation.

Looking back at the unpredictable nature of the emerged CoV-2 variants through the surveillance studies there are several logistical time consuming hurdles that might cause some delay in overcoming the management of CoV-2 variants respiratory infections. To state there have were more than 10 billion doses of vaccines administered globally for protecting people from COVID-19 variants prior to the emergence of the Omicron variant (B.1.1.529) across the globe. In the United States, both the average of new cases and daily deaths due to Omicron have already exceeded those during the delta variant's peak last September. The titer of neutralizing antibodies in vaccinated people's serum is significantly reduced against Omicron even in people who have completed two doses of vaccine. According to Reuters, COVID-19Tracher in Germany has reported 983 infections per 100,000 people over the last seven days, accounting for one in every six infections

reported worldwide each day. In the UK, one of the most heavily vaccinated countries, a similar of number is reported but the number with positive tests is dropping in line with the reducing severity of this infection, though the number of breakthrough infection cases due to Omicron has far exceeded that of the Delta subvariant.

The repeated outbreaks of the new variants have given rise to thinking about pouring further resources into newer vaccine development that is tailored to the highly transmissible mutated variants. Moderna developed a new generation of COVID-19 vaccine, mrna-1273.529 (also known as mRNA-Omicron), as a booster shot that has undergone clinical trial and is used currently in the UK as the fourth booster vaccine and in children this is now undergoing clinical trials at one-third of the adult dose. However, early animal studies suggests that Omicron-specific boosters of Moderna offer no advantage over the third dose of current vaccines, which suggests that the development and improvement of vaccines need to continue as a high priority as the virus is still spreading globally and posing a new challenge on using human vaccination as the cornerstone to prevent and control the spread of newer, heavily mutated CoV-2 subvariants. The data on the Pfizer Inc. and BioNTech' Omicron-based booster vaccine from Israel indicate that the fourth dose of this new vaccine is effective against viral variants of the CoV-2. Moreover, recent studies showed that a fourth dose of Pfizer/BioNTech's BNT162b2 vaccine, broadly induces neutralizing antibodies and memory B-cell activity against all circulating variants of concern detected in individuals receiving the third booster. This is supported by the results of a non-human primate study in which macaques were challenged with high doses of the Omicron SARS-CoV-2 variant after homologous and heterologous prime-boost vaccination with Pfizer/BioNTech's BNT162b2 and Johnson & Johnson's COVID-19 vaccines.

A team of Microbiology of the University of Hong Kong, as the first research team in Asia, has successfully isolated and cultivated the Omicron mutant strain, and offered it to the Chinese Centre for Disease Control and Prevention, Sinovac biology, and Sinopharm for research and vaccine development. Consino Bio also announced their plan for developing a COVID-19 vaccine against the Omicron variant last year.

Another area of major interest is exploring the impact of T-cell therapy as the second pillar of passive immunotherapy. In fact, in Germany, some investigators used innovative technology to characterise antigen-reactive T-cells directly in combination with closed-system cell sorting to enrich and purify antigen-reactive T-cells. By characterising CoV-2 and pre-existing T-cell memory generated by "common cold" Coronavirus-specific T-cells from both healthy donors and COVID-19 patients and by using single-cell gene expression profiling, a better understanding of the induced immune reaction is possible. Also predicting the success of vaccination may become achievable. The experience gained can be applied to manufacturing newer bioproducts and safer therapy based on hyperimmune globulins against other emerging variants and viruses. This may be regarded as a helpful approach in countries with poorer infrastructures that are most in need of vaccines and alternative therapy.

Interestingly, some newer automated manufacturing units for chimeric antigen receptor (CAR) T-cell therapies, using allogeneic, off-the-shelf CD45RA-memory T-cells, obtained from a convalescent donor, for passive adoptive cell therapy CoV2 is currently in the development stage. In fact, German oncology scientists are working with Optima Pharma (OPTIMA packaging group GmbH, Schwäbisch Hall, Germany) to create an automated manufacturing unit for the decentralised production of CAR T-cell therapies.

Moreover, a dose-escalation clinical trial was conducted on patients in Spain to assess the safety and feasibility of the use of allogeneic memory T-cells obtained from convalescent donors. The result indicates a clinical improvement six days after infusion without serious adverse effects, with lymphocyte recovery two weeks after the procedure and donor microchimerism at least three weeks after infusion. The long-term effects of such an approach are under evaluation. However, no clinical trial was performed using SARS-CoV-2 specific memory T-cells, despite the procedure for obtaining these cells being feasible, easy to implement for small-scale manufacture, and is quick, cost-effective, involving minimal manipulation, and has no GMP requirements.

The importance of robust and durable T-cell memory for CoV-2 for the management of the current pandemic is now confirmed by the outcomes of the first report of human passive adoptive cell therapy for COVID-19 using allogeneic, off-the-shelf CD45RA− memory T-cells obtained from a convalescent COVID-19 donor with no serious adverse effects as the most inflammatory parameters were stabilised post-infusion. Effective treatments are still needed to reduce the severity of symptoms, time of hospitalisation, and mortality of COVID-19 but the hypothesis that CoV-2 specific memory T-lymphocytes obtained from convalescent donors recovered from COVID-19 can be used as passive cell immunotherapy to treat pneumonia and lymphopenia in moderate/severe patients is great news showing the treatment efficacy progression in a biphasic way, initially viral and afterward inflammatory. It is interesting that the COVID-19-induced lymphopenia constitutes a therapeutic window that may facilitate donor engraftment and viral protection until recovery, and be used as passive cell immunotherapy to treat pneumonia and lymphopenia in moderate/severe patients.

A Spanish Regional Blood Transfusion Centre performed blood donor selection according to an approved protocol on patients $\leq$ 65 years old, during the disease and complete resolution of symptoms for at least 14 days before donation. The donor selection procedure for memory T-cells preservation was based on a high percentage of SARS-CoV-2 specific memory T-cells. A non-mobilised apheresis technology was used to obtain from the convalescent donor, CD45RA+ cells that were depleted by immunomagnetic separation using CliniMACS CD45RA Reagent from MiltenyiBiotec, following the manufacturer's instructions. CD45RA- cells were allocated for cryopreservation in doses adjusted to 100 kg of weight for three planned doses. Aliquots were cryopreserved in a double bag using autologous donor plasma with a final concentration of 5 % dimethyl sulfoxide (DMSO). CD45RA- aliquots were removed from liquid nitrogen storage and thawed in a dry defroster. Infusion of cryopreserved CD45RA− lymphocytes was performed 15 min after thawing. Cell count, viability, and microbiological studies were performed after each aliquot was thawed. The CD45RA-fraction viability and purity were analysed by flow cytometry (FCM).

In short, as we move forward of "preparedness", a lesson must be learned for the future. We must invest more in preparedness and not only have some checks and balances but also put more designated innovative research and development in place, as the current reductionist approach is too narrow. We also must act, well in advance and globally to tame the imported variants with all possible means, including improving vaccine supply and local manufacturing facilities and the appropriate, know-how for self-sufficiency and even learn how to coexist with some of the unpredictable emerging harmful mutated forms that are much expected to arrive.

Considering that passive immunity is a valid strategy as a treatment, especially in countries where vaccination is not reaching the desired numbers for an acquired high degree of immunity, more studies should be encouraged where antibody titre, plasma sources (vaccination or infection), and time of host infection and dosage (i.e. boosters with plasma from the same or different pools so as to assess provision with a varied antibody-antigen repertoire) are among the many points to be urgently considered. Another lingering unresolved question relates to the pathophysiology, causes, and long-term consequences of so-called "Long COVID" that still remain active areas of research. The current available data suggest that approximately 5–10 % of people who become infected with SARS-CoV-2 may develop persistent symptoms that last weeks or months after the infection occurred. Where is the power of immunotherapy to deal with the Long COVID issue! On a more positive side the future prospective replication of some proteins, as a new target for the COVID vaccine, would have huge prospects for a future vaccine which could activate T-cells to attack infected cells expressing the replication protein.

Life is full of" circles of dependence". In this section of TASCI, I am interested in the cycle of things that repeat themselves and how we should learn from them. I wish to take this opportunity to thank all my current colleagues and others who have helped me to produce this section of TASCI as real teamwork, because I could not do it without their enormous input and timely support.

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