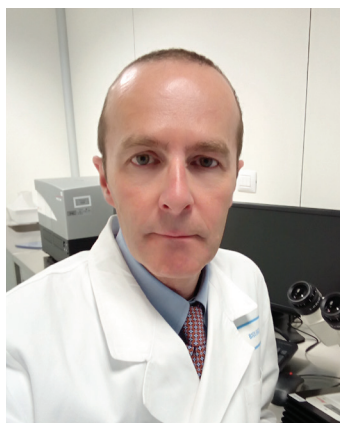


INTERVIEW

Advances in the use of cord blood as a potential source of MSCs



Giuseppe Astori is the head of the Advanced Cellular Therapy Laboratory (LTCA) at the Hematology of the Vicenza Hospital (Italy). His team focuses on the development of cellular therapies for the treatment of steroid-resistant graft-versus-host disease by using cord blood-derived mesenchymal stromal cells and for the immunotherapy of tumor relapse and viral reactivations after allogeneic hematopoietic stem cell transplantation. LTCA has an accredited laboratory at controlled contamination for the manipulation of transplants as for Directive 2004/23/EU and a GMP facility. Another area of interest of his group is the production and characterization of platelet derivatives. He has been nominated International Society of Cellular Therapy (ISCT) representative in the JACIE board and has been member of the European Leadership Committee of the ISCT (2014-2017). Before joining the Vicenza Hospital he worked at Cardiocentro Ticino, Switzerland where he was appointed Qualified Person of the GMP facility.

Q What are the features that make mesenchymal stem/stromal cells (MSCs) potential candidates for cell-based therapies?

MSCs possess interesting features that have facilitated their clinical use. The former MSCs to be brought into the clinic were isolated from bone marrow (BM) for the abundance of MSC clones in that tissue and their relative ease of isolation and *ex vivo* expansion. MSCs are interesting for their immunomodulatory capabilities that favored their clinical use to treat acute graft-versus-host disease (GvHD) in patients after allogeneic hematopoietic stem cell transplantation refractory to conventional therapies. Moreover, their ability to differentiate into mesodermal tissues such as bone, cartilage and fat has paved the way for their use in regenerative medicine.

Q You are working on developing cord blood as a suitable source of MSCs for clinical use. What are the advantages of cord blood-derived MSCs (CB-MSCs) compared to those derived from other sources?

As mentioned, MSCs were formerly isolated from BM. Several years ago the International Society for Cellular Therapy (ISCT) proposed the minimal criteria for defining MSCs, such as adhesion to plastic, the presence or absence of some surface markers and the ability to differentiate in three mesodermal lineages.

Nowadays, scientific evidence suggests that it is necessary to integrate these minimal criteria, as it has emerged that MSCs isolated from different tissues possess different functional and phenotypic characteristics. By way of example, MSCs derived from BM have a greater tendency *in vivo* to favor the formation of a hematopoietic niche.

We can say that MSCs can be distinguished depending on their origin in perinatal (e.g., those derived from umbilical cord, cord blood or placenta) or adult (derived from BM or adipose tissue among others). The phenotype of these cells is very similar; however, if you extend the analysis by including hundreds of antigens as we did and if you narrow the clustering criteria it is possible to highlight some distinctly expressed markers between perinatal and adult MSCs.

We are now studying the biological meaning of these findings. Returning to the CB-MSCs, our data allow us to say that these cells are endowed with an excellent capacity to inactivate *in vitro* T-lymphocyte proliferation. Another unique feature of CB-MSCs is their low immunogenicity, which makes them suitable for allogeneic cell therapies.

Q What *in vitro* parameters are useful to better define the quality of cord blood-derived MSCs prior to clinical application?

The data we have collected allow us to make two observations. Each batch of MSCs we isolate (irrespective of the tissue source) possess different immunomodulatory efficacy characteristics *in vitro* that could reflect their efficacy *in vivo*. That is, the immunomodulatory capacity of these cells varies from lot to lot and should be quantified in a specific immunomodulation assay.

We then realized that MSCs (no matter what source from which they are isolated) are not entirely ‘unknown’ to the host immune system. In fact, MSCs are now considered ‘immuno-evasive’ rather than ‘immunoprivileged’, meaning that they do not completely escape the attack of donor lymphocytes.

In collaboration with the colleagues at the Cell Factory “Franco Calori” in Milan directed by Rosaria Giordano and Lorenza Lazzari with whom we share this passion for CB-MSCs (they were among the first to isolate and characterize these cells), we have developed a cytotoxicity assay that allows us to make a choice of lot.

The transplant team of our hematology group (directed by Dr Marco Ruggeri) has utilized these cells in the clinic to treat our patients suffering for refractory acute and chronic GvHD. Cells are infused according to “hospital exemption”. We hold the authorization for infusion while the cells are produced under cGMP in Milan.

Prior to infusing the cells, we run this specific cytotoxicity assay; we mix the patient’s lymphocytes with CB-MSCs collected from the lots available for infusion and we select the one that displays lower lysis in the cytotoxicity assay. This is carried out in an effort to increase MSC permanence in the host and thus improve the efficacy of the treatment.

Our message is that it is now time to go beyond the concept of a ‘universal MSC’ and the type and lot of MSC should be chosen depending on the application required and their potency characteristics. This is true both for regenerative medicine and hematological applications.

Q How close are we to determining the ideal cell culture supplement for the expansion of MSCs?

Understanding the best supplement for growing MSCs is a matter of debate in the scientific community. Historically, MSCs have been expanded in D-MEM or Alpha MEM supplemented with fetal bovine serum (FBS).

FBS is harvested from fetuses in slaughterhouses and is used as a source of cytokines and growth factors for the cells. Regulatory agencies recommend reducing or completely eliminating animal derivatives in cell cultures that will be used to treat humans. This is to reduce the risk of transmission of zoonoses especially after the incidences of Bovine Spongiform Encephalopathy or avoid xenogeneic reactions in the host. In addition, the availability of FBS is fluctuating and depends on the request of calves on the market.

For these reasons, human platelet lysates (PL) – produced by breaking human platelets by physical or physiological methods – are used as cell supplements rather than FBS. Platelets are rich in growth factors, in particular stored on ‘alpha granules’. Once broken, these factors are released into the culture medium and made available to the cells.

PL is now produced by commercial providers or directly by academic blood centers and whilst its introduction solved the issues presented with using FBS, it has opened created new problems: their biological safety (PL are collected from donors), standardization and suitability for clinical use. In fact, we still do not know what key factors to use as quality markers for these products. Furthermore, not all MSCs ‘love’ PL. For example, the MSCs we are most familiar with (those isolated from cord blood) must still be isolated in FBS. Only when the MSC clones have adhered to the plastic, can they be further expanded in presence of PL. This data is further evidence of how perinatal MSCs differ from those isolated from adult sources.

The scientific community within industry and academia are working hard on resolving these issues around PL. My personal feeling is that we need a close interaction with the regulatory agencies in order to define

who is allowed to produce and release PL. Another key consideration is availability: will be possible to meet supply demands of PL if FBS is abandoned in the future?

In any case, it is important to remember that the safety of an Advanced Therapy Medicinal Product (ATMP) is the responsibility of whoever releases it (the Qualified Person) and as a consequence in the production process the ancillary material that offers the highest safety standard (PL among them) is preferable.

Q How far are we from using cord blood-derived MSCs in clinical trials? What are the main barriers?

CB-MS*C* isolation has advantages and disadvantages compared to other sources. Their benefits include non-invasive collection (the cord blood if not banked is discarded), the minor ethical concerns, the possibility to access all the samples that public banks do not store for volume or cellular criteria and again, CB-MS*C* are 'naïve' cells possessing peculiar characteristics compared to other sources.

The critical disadvantage is the difficulty of isolation. The CB unit has to arrive at the laboratory within a few hours from collection, so it is necessary to put in place a functional network across the supply chain: who collects, who banks and who processes the unit. We receive samples daily, thanks to the excellent collaboration with our blood center directed by Dr Alberta Alghisi.

Another critical point is that there are low numbers of mesenchymal clones in the CB which reduces the overall isolation efficiency. In addition, not all the MSC colonies that we isolate from CB are identical. The group of Milano has described (and we have confirmed) the existence of at least two distinct mesenchymal cell populations: one mainly short-living and less proliferative, the other long-living, with higher growth rate and significantly longer telomere constituting the ideal MSC subset to be expanded and used *in vivo*.

It is also necessary to adopt special cultivation arrangements and the experience of our biologist is the main factor contributing to the successful isolation rates we achieve. However, the ease of accessibility to samples to be used in research or in the clinic is a tremendous advantage that we would not have using adult sources. In addition, the fact that blood comes from a blood bank guarantees its traceability with respect to the anonymity of the donor.

Q There have been reports of tumorigenicity as a matter of concern with CB-MS*C* transplantation. What weight do you give to these findings and what do you think needs to be done to address this?

The risk of creating genetic instability is possible and is inherent to any cell manipulation. The more we manipulate the cells, the more the risk. However, thousands of MSC infusions from several sources have

been performed in clinical trials to date that have evidenced the safety of the procedure.

The strict legislation on the production of ATMP requires the manufacturer to demonstrate the safety of the product released.

Regarding MSCs the scientific community agrees that it is best practice to expand the cells no more than five passages *in vitro*. This reduces the risk of eliciting genomic instability and the appearance of cellular aging phenomena that could reduce cell efficacy. For the product release, the latter can be monitored by measuring the length of the telomeres. The eventual appearance of genetic abnormalities in the MSC expanded population can be monitored by using the classic karyotype along with the Comparative Genomic Hybridization array. Cell tumorigenicity can be monitored for example by cell growth in soft agar.

Q Could you tell us about the aspects of your research you are most optimistic about in terms of their clinical application?

I believe that the immunomodulatory capacity of these cells can be immediately applied not only in the hematological context but in all autoimmune diseases. There are hundreds of clinical trials now with MSCs. However, I think it is essential that people recognise that it is an MSC from adult or perinatal sources are the same. The choice of MSC should depend primarily on the question of ‘what do I want to obtain from my cells’. In the past, this aspect has not been explored adequately because we did not have enough laboratory data available on MSC biology. So ‘the song remains the same’: from the bench to the clinic and back again.

Something similar happened in regenerative medicine with the use of bone marrow autologous mononucleated cells for the treatment of acute myocardial infarction. A huge amount of clinical data have been produced with completely discordant results that in the end have cast doubt on the effectiveness of the therapy itself.

To help move these cells into clinical applications, we need to develop *in vitro* and *in vivo* models that will enable prediction of efficacy of our MSCs in the clinic. To me this is a truly exciting challenge.

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