Interventional
Orthopedics
Foundation

February 10, 2015

Thomas N. Tulenko, PhD
TNT Consulting
Retired Professor and Scientific Director, Stem Cell Centers:
Thomas Jefferson College of Medicine
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Via Email

Dear Dr. Tulenko:

Thanks for your abstract that was delivered via email on February 3, 2016 to the Interventional Orthopedics Foundation. Since we want a free flowing debate on this and other topics important to physicians who treat patients using orthobiolgics, we are happy to publish your opinions. At the same time, we think there is some confusion regarding our position and the statements you have made.

First, our position on placental or amniotic "stem cells" is not that these tissues may not have a place in regenerative medicine, but is focused more on the consumer fraud that is occurring in calling these tissues "stem cell therapy". While the definition of what is and is not a stem cell therapy remains a moving target, we can all agree that a physician who tells a patient that he or she is getting a stem cell based treatment must ensure that viable stem cells are being used. In the case of these tissues, when they are used as directed on the product labelling, we could find no cell population that under ideal culture continues survived to be able to form colonies, let alone stem cells. In your experience, you state, "*Unlike the freshly isolated stem cells from the amnion which are authentic, live regenerative stem cells, freshly thawed placental membrane products have relatively few live cells* (but more than in bone marrow concentrate)". You go on to state that *"…these living cells appear to have lost both their regenerative and proliferative capacity, the two defining criteria of stem cells."* **Hence you agree that the products being sold do not contain viable stem cells.** We hope you would agree that any physician claiming he or she is injecting viable stem cells by using an amniotic fluid product is misrepresenting what is being offered to the patient. At the IOF we believe this is unacceptable and unprofessional behavior. While your major point is that "the presence of living cells is largely irrelevant" with respect to these products, a patient being sold an amniotic "stem cell therapy" without actual stem cells would likely feel otherwise.

Regarding your assertion that amniotic tissue, once thawed contains more stem cells than bone marrow concentrate (even though these cells are nonfunctional), we disagree and can find no literature support for your position. First, as you have implied, the stem cells in amniotic fluid products currently being used clinically by physicians are damaged to the point where they no longer meet the minimal criteria of being a "stem cell[1]". Hence there seems little face validity to your position. However, let us also examine your claim based on the quantity of stem cells available in the different tissues (i.e. amniotic fluid vs. bone marrow). The published literature on bone marrow MSC expansion shows very low failure rates and the ability to get to significant stem cell numbers within the first few passages[2]. Using a similar culture method we have never observed a successful culture of cells from a frozen amniotic product. With fresh amniotic fluid, the culture is far more heterogeneous, containing many non-stem cell components believed to be of epithelial origin and only after extensive culturing does an MSC population emerge[3]. Hence, based on this information, there is *prima facie* evidence that the MSC content in amniotic fluid is quite different than that of bone marrow. The published experimental evidence that attempts to quantify these cell numbers also supports this contention. The absolute number of MSCs within bone marrow is significantly higher than those in amniotic fluid given the higher total nucleated cell count in BMA:

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Tissue Type	Nucleated Cell Count (Cells/mL)	Percentage of Stem Cells within Nucleated Cells	Total Stem Cells Available per mL of Tissue
Amniotic Fluid	1 - 100 thousand[4]	0.9% - 1.5%[5]	9 to 1,500
Bone Marrow	11 - 25 million[6]	0.001% - 0.01%[7]	110 to 2,500

Thus, we are unsure how you claim the opposite. Perhaps you are confusing the published literature which uses culture expansion to isolate MSCs from amniotic fluid or perhaps you are not adjusting the percentage of MSCs with respect to number of nucleated cells for the significantly greater number of nucleated cells in bone marrow? In addition, the above comparison does not include the much greater number of hematopoietic stem cells present in marrow in high concentration that would be applicable to orthopedic applications such as muscle repair. Either way, by your own admission, the cells present in amniotic fluid products at the time physicians use them are incapable of functioning normally, which is not an issue with a fresh bone marrow concentrate.

Your last point is that amniotic fluid contains important growth factors, cytokines, and other components and that you have observed that the products "work well". While about 6,000 patients have had their results reported after using bone marrow MSCs to treat orthopedic conditions since 1997, a PubMed search this morning reveals that not much has been published on the use of amniotic fluid to treat common orthopedic conditions. For knee osteoarthritis (OA), a single small case series was just published in late 2015 on 6 patients[8]. No human studies are listed under hip OA, ankle OA, shoulder OA, tendinitis, or rotator cuff tears. This of course does not rule out that there may be additional studies published in the future, but there is certainly no published peer reviewed evidence to support your statement of efficacy as of February, 2016.

With regard to growth factors, we agree that amniotic fluid likely has these as a component that could cause a positive clinical benefit in orthopedic conditions. However, having said that, we have serious concerns that amniotic fluid may not have a more clinically efficacious growth factor or cytokine profile than much less expensive platelet rich plasma (PRP). For example, in our study we observed that in-vitro platelet lysate (the growth factors extracted from PRP) had a better stimulatory effect on MSCs than did amniotic fluid. Hence, what we really need as an Interventional Orthopedics community is more head-to-head research comparing these two products: one very expensive (amniotic fluid) and the other relatively inexpensive (PRP). In addition, our studies showing that amniotic products hampered MSC metabolic rates when compared to platelet lysate are very concerning, as many physicians, based solely on blind faith and the hype of sales reps have begun combining the two products (PRP and MSCs from bone marrow concentrate) in hope of producing a better mousetrap.

In conclusion, as you concede, amniotic fluid has no viable stem cells. While we are agnostic to whether amniotic fluid products may play a clinical role in orthopedic diseases one day, we strongly oppose clinicians advertising to patients that they are injecting stem cells when using these products. They are not, and in our opinion any physician making this claim is committing consumer fraud.

Sincerely,

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