

COMMENTS to DOCKET No. FDA-2008-D0520

“Potency Tests for Cellular and Gene Therapy Products”

I applaud and commend the FDA/CBER for the Draft Guidance to Industry on “Potency Tests for Cellular and Gene Therapy Products”. This may be one of the only comments endorsing this Draft Guidance, because many, if not all, of the Standards Organizations and other organizations involved in cellular therapy, particularly those involved in bone marrow, mobilized peripheral blood and umbilical cord blood transplantation, will probably try and resist any deviation from their present course to be mandated to develop and perform new tests. Much of this will come from a lack of understanding the definition and meaning of potency and the procedures involved in developing and using a potency assay. Therefore, it would not surprise me if other comments try and fight these new recommendations “tooth and nail”, or try and impose their own meaning/definition of potency and their own ideas of how cell potency should be determined. However, I urge the FDA/CBER to stipulate and maintain the requirement for potency assays, for whatever cell and gene therapy procedure it might be, because to date, many of the tests that are employed are primitive and provide little, if any relevant clinical information that might benefit the patient. It is also of interest to note that this Draft Guidance comes on the heels of the European Medicines Agency (EMA) “Guideline on Potency Testing of Cell Based Immunotherapy Medicinal Products for the Treatment of Cancer” that came into effect on May 15, 2008. To the FDA’s credit, this Draft Guidance is more detailed and provides far superior guidelines for potency testing.

Before embarking on my comments, I should state that my company, HemoGenix[®], Inc, is not impartial and would certainly profit from the Draft Guidance being converted into a Guidance for Industry for Immediate Implementation. This is because earlier this year, HemoGenix commercialized the first and only stem cell potency assay, called HALO[®]-96 PQR. This assay was designed and developed specifically for the purpose of cord blood stem cell potency evaluation with the ability to ascertain acceptance/rejection criteria prior to transplantation. HALO[®]-96 PQR is also the only assay that incorporates a cord blood reference standard in order to measure cell potency.

On 8/15/2007 I submitted comments regarding Docket No.2007D-0025 Guidance for Industry: “Class II Special Controls Guidance Document: Cord Blood Processing System and Storage Container, For Immediate Implementation”. My comments criticized the use of the colony-forming cell (CFC) assay as a quality control assay for umbilical cord blood, especially since the assay is, with few exceptions, used only as a “growth – no growth assay” in order to satisfy the regulations and the documentation required. An alternative assay called HALO[®]-96 SPC-QC (Stem and Progenitor Cell-Quality Control) was also described. In my comments, I also requested that the FDA/CBER “seriously consider cell potency assays, such as HALO[®], that have the assay characteristics designed specifically for this purpose”. When those comments were written, we had considered HALO[®] SPC-QC as a type of potency assay. It was of course not a true potency assay, but the forerunner to the PQR. However, in writing those comments, the ideas of what a potency assay means and encompasses were set in motion and have culminated in HALO[®]-96 PQR (Potency, Quality Release). Regardless of what assay is used for a particular cell type and intended purpose, it is imperative that those developing and employing such an assay understand how to interpret the results and what the results imply.

The PQR assay was developed specifically for umbilical cord blood because organizations such as FACT-JACIE and NetCord-FACT mandate the use of “appropriate” and “validated” assays for cord blood and the AABB requires a “potency” assay for cellular therapy products. It is interesting that although these organizations state categorically what is required, it is not enforced. The published standards of these organizations are compiled and written by those who are supposed to know and understand the requirements. It has been an eye opener to realize that few, if any, who are supposed to be specialists in their field, have any idea what it means to validate an assay, let alone understand the meaning of “potency”. It is a pitiful situation. At the recent NMDP Council Meeting in Minneapolis, I was invited to be one of the presenters. During the introduction to our workshop, the definition of potency as stated in the Code of Federal Regulations (CFR) was provided. Virtually everybody in the room looked blank indicating a lack of understanding of the definition and its implications. Indeed, it has been quite surprising to learn that those who are supposed to be leaders in the field and to whom others look up to, are themselves ignorant, and in many ways complacent, of such fundamental knowledge.

Although my comments refer primarily to umbilical cord blood (UCB), they can also apply to bone marrow and mobilized peripheral blood. Many in the field believe that total nucleated cell count (TNC), viability and the CD34 expression marker (as a measure of stem cell number) are the only parameters required to determine cell potency, quality and release of the UCB product for transplantation purposes. Indeed, the National Marrow Donor Program (NMDP) will probably respond to this Draft Guidance for Industry by arguing for TNC as one, if not the only measurement necessary. This is because the greater the number of cells transplanted, the greater the probability of engraftment. Of course, what is not taken into account is the reason why this might be the case. Few, if any, cord blood bankers and stem cell “transplanters” are also stem cell biologists. It has become very clear from the presentations I have given, including the one at the recent NMDP Council Meeting in Minneapolis, that few have even taken into account that a UCB unit of low volume and cell number might have a high proportion of primitive stem cells, while a UCB unit with a high cell number may consist primarily of mature stem cells on the verge of becoming lineage-specific cells and exhibiting such a low proliferation capability that the unit might not even demonstrate short-term engraftment.

Certainly, TNC is an important parameter, but according to the Draft Guidance criteria, measurement of TNC cannot be considered a potency assay. Similarly, although viability and CD34 number are important parameters, they cannot be used as a measure of potency. In addition, none of these measure the most important parameter, namely proliferation potential (please see below). For reasons described in detail in my comments to Docket No.2007D-0025, the CFC assay also does not meet the stipulated criteria for a potency assay because:

1. It was never designed as a potency assay or even a quality control assay. It is an investigative research tool.
2. It does not provide quantitative test results for release of the product.
3. It cannot be used to meet pre-defined acceptance and/or rejection criteria.
4. It cannot be calibrated or standardized and cannot be used with controls.
5. It lacks accuracy, sensitivity, reliability, reproducibility and robustness established through validation.
6. It cannot quantify the measure of strength (activity) of the cells.

Even though a potency assay may not exhibit all of the properties required in the Draft Guidance, the CFC assay should not even be considered as a potency assay on the grounds that to perform the assay, subjective, manual enumeration of colonies is required, a procedure that cannot be standardized (despite numerous attempts) and cannot be validated. Furthermore, any subjective assay should not even be considered where the safety of the patient is critical to the outcome.

As stated in the Draft Guidance and in the EMEA Guidelines, to measure potency, it is necessary to have a reference standard to which samples can be compared. When the potency of a compound is determined, a dose response is usually performed. Parallel displacement to the left or right of the reference standard provides the potency ratio of the sample and therefore the increase or decrease in activity compared to the reference standard. This certainly occurs if the sample being compared is of the same material as the reference standard. For example, if different erythropoietin preparations are examined in a dose-dependent manner against the erythropoietin reference standard, then the linear portions of the positive, sigmoidal dose response curves for each preparation should be parallel to that of the reference standard. The difference in potency or activity of each preparation would be represented by the ratio of displacement to the left or right of the reference standard. The parallel dose response curves are obtained because the material in all preparations is the same as that of the reference standard. If this were not the case, the dose response lines would not be parallel. The present Draft Guidance on Potency Testing seems to assume that cells would behave in a similar manner to that of compounds. This is not the case. Indeed, from our own studies, it is actually the exception rather than the rule and the FDA/CBER may want to consider the following evidence in preparing the final version of the Guidance.

This initial study tested 56 frozen UCB samples for which the engraftment results were known. However, the engraftment results and other sample characteristics were not provided until the study of all 56 samples were completed. At this time, it was found that more than 50% of the sample vials had been stored at -80°C instead of in liquid nitrogen (LN2), even though the cord blood units were actually stored in LN2. This difference in storage temperature accounted for virtually all of the cord blood samples stored at -80°C to be incapable of proliferation as measured using HALO®. Of the 25 sample vials remaining that were stored in LN2, all were assayed using a minimum of a 3-point cell dose response to detect the mature *in vitro* multipotential stem cell (CFC-GEMM) and 23 of the 25 samples could be assayed for primitive *in vitro* stem cells, designated HPP-SP. (The 2 samples that could not be assayed using a 3-point cell dose response, did not have sufficient cells present). The CFC-GEMM (Colony-Forming Cell, Granulocyte- Erythroid, Macrophage, Megakaryocyte), as its name suggests, is responsible for producing lineage-specific hematopoietic cells. The High Proliferative Potential – Stem and Progenitor cell (HPP-SP) can produce both lymphopoietic and hematopoietic lineage cells and is therefore more primitive than the CFC-GEMM. The reason why these cell populations were chosen was because the goal of a cord blood (bone marrow or mobilized peripheral blood) transplantation procedure is to reconstitute the lympho-hematopoietic system. This can only be done when both primitive and mature stem cells are transplanted, thereby providing both short- and long-term engraftment and repopulation. In order to achieve any engraftment, the stem cells have to first home to the hematopoietic site (which is primarily the bone marrow). Engraftment is the process whereby the stem cells begin to proliferate and self-renew to reestablish the stem cell compartment. Similarly, to achieve repopulation, the stem cells have to first proliferate in order to start the differentiation process into the functional, mature blood cell elements. Since the aim

of the transplantation procedure is therefore to first establish stem cell proliferation, it is a stem cell proliferation assay that is required to measure cell potency. Without proliferation, no engraftment or repopulation will occur. For a potency assay that has to measure stem cell proliferation potential, it is necessary to understand that within the stem cell compartment, the cells exhibit different degrees of “stemness”, which in turn is directly related to their proliferation potential.

For lympho-hematopoiesis, proliferation capability only occurs up to the early precursor cells and then ceases under steady state conditions. The stem cells therefore have a greater proliferation potential than the lineage-specific progenitor cells. In fact, because the HPP-SP stem cell population is more primitive than the CFC-GEMM population, the former has a greater proliferation potential than the latter. The 3 hematopoietic progenitor cell populations, BFU-E, GM-CFC and Mk-CFC usually have similar proliferation potentials, but are significantly lower than the 2 stem cell populations. Finally, lymphopoietic cell populations (T-CFC and B-CFC) exhibit the lowest proliferation potential of all under steady-state conditions. If a cell dose response were performed for each of these 7 cell populations, it would be seen that proliferation potential, as measured by the slope of the linear regression would be different for each of the 3 groups, stem cells, hematopoietic lineage-specific cell populations and the lymphopoietic populations. The proliferation potential is provided by the slope of the cell dose response curve. The greater the slope, the greater the proliferation potential, the more primitive the cells.

It is the relationship between the slope of the cell dose response linear regression and the proliferation potential that is the key to understanding how stem cell potency can be determined the how acceptance / rejection criteria can be defined. All are interrelated.

HALO® is based on measuring the concentration of intracellular ATP (iATP). As cells proliferate, the iATP concentration changes proportionately. In fact, the iATP concentration is directly correlated not only with proliferation, but also with viability and cell number. Release of iATP after incubation acts as a limiting substrate for a luciferin/luciferase reaction to produce bioluminescence as light, which is detected in a plate luminometer. An external ATP standard calibrates the assay and high and low controls ensure that the assay is properly standardized. The PQR version of HALO® utilizes “Suspension Expansion Culture (SEC)” technology, because it is easier to use, results are obtained in only 5 days, it is twice as sensitive as the methyl cellulose version of HALO® and coefficients of variation are very low (mean, 5.5 %).

When a 3-point cell dose response was performed on the UCB samples from which sufficient cells were obtained, only 2 samples tested for CFC-GEMM and only 1 sample tested for HPP-SP exhibited statistical parallelism to the UCB reference standard. In contrast, the remaining UCB samples showed no parallelism to the reference standard for either stem cell population detected. Instead, the UCB samples demonstrated cell dose response linear regression lines with widely varying degrees of slope. For any cell dose used, e.g. 5,000 cells/well, a vertical line drawn through all sample dose response curves demonstrated that as the iATP increases, there is a concomitant increase in the slope of the linear regression. If the slope of the cell dose response linear regression was plotted against the iATP concentration, a direct correlation was obtained. It is this correlation that allows the measure of stem cell potency for a UCB sample to be determined. The ratio of the reference standard slope to that of the UCB sample provides the

potency ratio and therefore determines whether the UCB sample has a greater or lower potency than the reference standard.

In the small number of cases where statistical parallelism to the reference standard was obtained, the interpretation is that the samples contain a greater or lower number of stem cells than the reference standard, but those stem cells in the samples are of the same degree of “stemness” as those in the reference standard.

For all the other samples that exhibited no parallelism to the reference standard, there are several implications. First, as described above, the steeper the slope, the greater the proliferation potential. Furthermore, the steeper the slope, the more primitive the stem cells in the sample and therefore the greater the stem cell potency. It follows therefore, that the greater the stem cell potency, the greater the probability and prediction of engraftment. In fact, for all of the tested samples, engraftment would have been predicted from the iATP concentration. When the iATP concentration calculated as iATP/kg body weight was plotted against the TNC/kg transplanted, a direct correlation was obtained.

Stem cell potency is therefore quite different to that of a compound in that only a few samples are going to demonstrate statistical parallelism with the reference standard. Ascertaining potency of a biological material such as the stem cells of cord blood, bone marrow or mobilized peripheral blood requires a different method of analysis and a different level of understanding. Although the principals of the potency assay may be similar, in that a dose response of the sample and comparison to a reference standard will be required, the potency of other biological materials is going to require different readouts, possibly different analyses and a different understanding of the biology of the cellular product involved. The Draft Guidance for Industry makes this very clear, since one assay will not fit all cellular applications.

From the foregoing discussion, I have the following comments:

1. As mentioned above, few understand the meaning of “potency”, potency assay” and “validation”. Since these are defined in the CFR, it might be a good idea to explain, in more simple terms, what these definitions mean with respect to cellular and gene therapy.
2. The Draft Guidance should be made more “biological” and “physiological” to tie in with the fact that to perform a cellular therapy, knowledge of the biology of the cellular product is required.
3. Potency cannot be measured without a reference standard. It is an absolute requirement. For UCB, this is probably going to be a problem because there are only a limited number of reference standards assays that can be obtained from a single unit of cord blood. Therefore, until a solution to this problem is found, each processing laboratory will probably have to incorporate their own reference standard (stated in the Draft Guidance). The reference standard used in the studies described here was a UCB unit that had been rejected for storage. The cells were processed, adjusted to 3 millions cells/well, rate frozen and stored in liquid nitrogen. It has been suggested that a reference standard using peripheral blood be used because a larger number of aliquots can be obtained. This may be true, but again one has to think about the biology of the system. The stem cell potency of a cord blood unit cannot be compared to that of a peripheral blood reference standard. One would be comparing apples with oranges.

4. The decision as to whether a cord blood unit can be used for transplantation purposes can be decided using an assay such as HALO®-96 PQR. At the present time, we have set the acceptance/rejection level at 0.04µM iATP. More studies will be conducted in the near future and it is to be expected that this level may change. However, the potency assay adopted for a particular tissue or cell preparation should be considered as a part of the decision-making process to go forward with a transplantation procedure. For hematopoietic tissues, the other parameters that should be included in the decision-making process would be TNC, viability and CD34. New assays may be developed that might be helpful in the process, but no single assay or parameter should be considered alone. We consider this to be too risky.
5. For a particular tissue, a consensus for a single potency assay should be employed. This assay should possess most, if not all, the qualities and characteristics required by the Guidance. The assay should be standardized and validated. By using an assay with an external standard, it allows results to be compared both within and between laboratories. In this way, results can be compared so that all laboratories using the same assay can be subjected to independent proficiency testing.

I hope that the information provides the FDA/CBER with some insights into what a cell potency assay for hematopoietic cellular therapy might involve. I believe that present procedures must be significantly improved in order to improve safety for the patient. We are a company on the outside looking in. In our discussions with many people who are working in the field “hands on”, we have been appalled at how lackadaisical some of the standards and procedures have been. Despite accreditation procedures, little attention has been paid to the importance of the practical procedures involved. This Draft Guidance can be an excellent example to improve these procedures.