WHITE PAPER

GMP-Compliant Human Bone Marrow for Cell Therapy Applications

Authors
Dominic Clarke, PhD, Global Head of Cell Therapy
Brad Taylor, PhD, Global Marketing Director

CELL THERAPY STARTING MATERIALS

Developing cell therapies involves the use of stem cells, immune cells, and various other cell types derived from human donors. While these cells have been used for years to support medical research, the use of living cells as the basis of a therapeutic drug necessitates an additional level of screening, qualification, and source material characterization.

Regulatory guidelines for safety and efficacy validation of cell therapy starting materials for clinical trial and commercial use require GMP compliance (Giancola, 2012). Therefore, all successful cell therapies ultimately need to transition to using GMP-compliant starting materials for their product development process.

Cell therapy starting material suppliers familiar with regulatory guidelines concerning advanced medicinal products can give developers an advantage in understanding and implementing regulatory agency requirements. Establishing a solid partnership with GMP-compliant suppliers means that they will already be able to anticipate company sourcing needs for various projects when it comes time to begin product scale-up.

HUMAN BONE MARROW THERAPEUTIC APPLICATIONS

Bone marrow aspirate is richly populated by stem and progenitor cells capable of regenerating and differentiating into many different cell types (Fig. 1) (Jaime-Perez, 2016; Holton, 2016). Hematopoietic stem cells (HSC) originate in the fluid portion of the bone marrow and can differentiate into red blood cells (erythrocytes), white blood cells (leukocytes), and platelets. These cells are collected from the bone marrow via aspiration. Mesenchymal stem cells (MSC), also termed mesenchymal stromal cells, are cultured from freshly collected adult human bone marrow. MSC can differentiate into cartilage cells (chondrocytes), bone cells (osteoblasts), and fat cells (adipocytes).

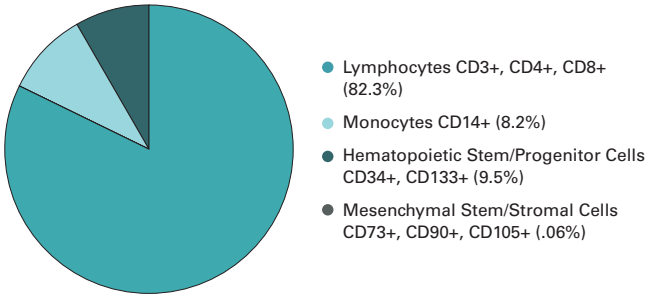

Figure 1. Bone Marrow Aspirate Mononuclear Cell Composition
Average percentages of bone marrow mononuclear cell (BMMC) populations present in healthy donor bone marrow aspirate.

Injecting patients with bone marrow-derived cells is the oldest form of human stem cell therapy. Bone marrow transplantation was first used successfully to treat leukemia back in the late 1960s. Today, researchers know a great deal more about stem cell physiology than they did in those early years, and as a result, stem cell therapy is a major area of investigation (See Table 1).

Stem cell transplants commonly use cells mobilized from the bone marrow and then collected from peripheral blood. However, proponents of sourcing stem cells directly from bone marrow point out that evidence shows this reduces the incidence of Graft vs. Host Disease (GvHD), a common and sometimes dangerous side effect (Alousi, 2019). Both methods have their advantages and disadvantages, and the jury is still out on whether one source is inherently better than the other for transplants. Scientists do know, however, that the bone marrow niche microenvironment is different from that of peripheral blood in terms of growth factors and cell subpopulations.

Disease Area	Recent Sample Publication(s)	Stem Cell Type
Cancer	Friedrichs et al., 2010 Lancet: Oncology	Bone marrow-derived HSC
Ischemic heart disease	Farag et al., 2011 European Cardiology Review	Bone marrow-derived HSC
Myocardial infarction	Murrow et al., 2011. Journal of Nuclear Cardiology	Bone marrow-derived HSC
Sickle cell disease	Aslam et al., 2018. Bone Marrow Transplantation	Bone marrow-derived HSC
Liver cirrhosis	Eom et al., 2015. World Journal of Gastroenterology	Bone marrow-derived HSC
Neurological disease	Zakerinia et al., 2018. International Journal of Organ Transplantation Medicine	Bone marrow-derived HSC
Bone regeneration	Yasuhara et al., 2010. Artificial Organs	Bone marrow-derived HSC
Disease Area	**Recent Sample Publication(s)**	**Stem Cell Type**
Severe acute pancreatitis	Zhao et al., 2016. Stem Cells International	Bone marrow-derived MSC
Ischemic heart disease	Farag et al., 2011. European Cardiology Review	Bone marrow-derived MSC
Pain management	Centeno et al., 2018. Advanced Procedures for Pain Management	Bone marrow-derived MSC
Type II diabetes	Bhansali et al., 2014. Cell Transplantation	Bone marrow-derived MSC
Reperfusion injury	Li, et al., 2015. Journal of Cellular and Molecular Medicine	Bone marrow-derived MSC
Cardiomyopathy	Hare, et al., 2012. Journal of the American Medical Association	Bone marrow-derived MSC
Multiple sclerosis	Uccelli et al., 2012. Trials	Bone marrow-derived MSC
Liver cirrhosis	Eom et al., 2015. World Journal of Gastroenterology	Bone marrow-derived MSC
Glaucoma	Mead B, and Tomarev S, 2017. Stem Cells and Translational Medicine	Bone marrow-derived MSC
Crohn's disease	Barnhoorn et al., 2019. Journal of Crohn's and Colitis	Bone marrow-derived MSC
Cartilage repair	Fellows, et al., 2016. Frontiers in Genetics	Bone marrow-derived MSC
Osteoarthritis	Shadmanfar et al., 2018. Cytotherapy	Bone marrow-derived MSC
Rheumatoid arthritis	Shadmanfar et al., 2018. Cytotherapy	Bone marrow-derived MSC
Pulmonary fibrosis	Zhou et al., 2014. Experimental and Therapeutic Medicine	Bone marrow-derived MSC
ALS	Nabavi et al., 2019. Cell Journal	Bone marrow-derived MSC

Table 1. Overview of Current Research and Clinical Applications for Human Bone Marrow-Derived Stem Cells

These differences have the potential to impact stem cell proliferative capacity and plasticity (Morrison, 2015; Szade, 2018).

HSC cell and gene therapies are being developed to fight blood diseases such as cancer, sickle cell anemia, and hemophilia (Chang, 2017). White blood cells, most notably T cells, form the basis of cancer immunotherapies, and more recently, autoimmune diseases (Fernandez, 2018). In the last 3 years, T cell-based treatments for leukemia, lymphoma, and prostate cancer made headlines upon their approval by the U.S. FDA (NCI, 2017). This success has significantly bolstered the number of cancer immunotherapies entering clinical trial. Dendritic cells, B cells, and NK cells are also generating significant interest for their potential as therapeutics for the treatment of cancer, hemophilia and other protein deficiency disorders, autoimmune diseases, and infectious diseases (Seattle Children's Research Institute, 2019).

HSC derived directly from bone marrow are currently being evaluated in a number of clinical trials as the basis for advanced medicinal products. One of the primary applications under investigation is the treatment of sickle cell disease. In one clinical trial, patients are being treated with bone marrow-derived CD34+ HSC genetically engineered to correct the malformation of red blood cells that is signature to the disease. The study is still in its pilot stage (Williams, 2019).

Additional studies are focused on using bone marrow-derived HSC to improve tolerance to organ transplantation in patients suffering from kidney failure (Ildstad, 2019; Ildstad, 2019). These studies are headed to Phase 2 clinical trials.

Bone marrow-derived MSC are the subject of an extraordinary amount of innovative medical research due to their therapeutic potential across a wide scope of applications. (Rizvanov, 2016). These applications range from treatments for osteoarthritis and rheumatoid

arthritis, to treatments for diabetic neuropathy, multiple sclerosis, pulmonary disease, and tissue and organ repair (clinicaltrials.gov, 2019). Recent research has shown that MSC derived from bone marrow are phenotypically and functionally distinct from both primary MSC cultures (Barra, 2014) and induced pluripotent stem cell-derived MSC (Xu, 2019). This implies that bone marrow-derived MSC are more biologically relevant than those obtained by other means.

SOURCING HUMAN BONE MARROW

Sourcing bone marrow for the research and development of this multitude of new therapies is challenging. Bone marrow donation is a surgical procedure that takes place in a hospital or certified donor center. Although an anesthetic is used, the procedure can still be painful, and many potential donors consider it to be intrusive. The result is that the availability of bone marrow from healthy donors is limited, and finding a reliable source of high-quality material is of primary concern to cell and gene therapy developers (Zhang, 2015).

The regenerative medicine industry is experiencing dramatic growth, both in terms of investment and in the number of cell and gene therapy candidates in clinical trial. The Alliance for Regenerative Medicine reports that the number of cell and gene therapies in Phase 1-3 clinical trials has increased from 631 in 2015 to over 1,028 at the end of 2018, with 2019 numbers expected to be even higher (Fig. 2) (McCormack, 2019). These numbers reflect a total of 59,575 enrolled patients. Investments in the industry are estimated to be at 13.8 billion worldwide, up 73% from just two years ago. There is already an unmet need for reliable sources of high-quality cell therapy starting material; sourcing GMP-compliant bone marrow material is even more problematic, as suppliers are few and far between. As the number of stem cell-driven clinical trials expand, this situation is creating a potential bottleneck for the cell therapy industry.

The process of recruiting and screening potential bone marrow donors takes significant time and resources (See Fig. 3). Qualifying donors must not only be willing to go through with the procedure, but also go through an intensive screening process. At HemaCare, all cellular products are collected at our FDA-registered collection center from informed, consented donors following IRB-approved protocols. Prospective donors must fill out a health-related questionnaire. Samples are drawn for a Comprehensive Metabolic Panel screening and then reviewed by a medical director along with other prescreening, physical exam, vitals, and CBC results. A physical exam must be performed by a qualified nurse or doctor. Additionally, all HemaCare bone marrow donors must undergo full infectious disease testing, including screening for HIV, Hepatitis B, and Hepatitis C prior to collection. If the donor is cleared, they are scheduled for a bone marrow donation. On the day of collection, the donor must again complete the health questionnaire and pass CBC qualification and vitals screening.

"Bone marrow is a critical starting material for a number of cell therapy manufacturing processes and there is an ongoing need for a more robust supply of high quality GMP bone marrow to enable the clinical and commercial manufacturing of these potentially life-changing cell therapy products."

- Head of Manufacturing, HCATS

Qualified back-up donors meeting requested criteria are also identified in case the original donor fails to pass screening or is unavailable upon the client's requested collection date. Cell therapy manufacturers who choose HemaCare as their supplier have access to an extensive and reliable pedigreed donor network, which increases the probability of a suitable donor being available when needed.

1,052 Clinical Trials Underway Worldwide by the end of Q3 2019			
Phase 1	**Phase 2**	**Phase 3**	
363	594	95	
Gene Therapy	**Gene Modified Therapy**	**Cell Therapy**	**Tissue Engineering**
Total: 370	Total: 418	Total: 218	Total: 46

Figure 2. Cell and Gene Therapy Clinical Trial Numbers Continue to Grow

As the regenerative medicine industry continues clinical trial and patient enrollment growth, the industry faces a bottleneck in the availability of GMP-compliant starting materials. (Alliance for Regenerative Medicine Q3 2019 Data Report)

Bone marrow aspiration requires technical expertise that can take years to perfect. Marrow volume differs among donors, as does the type of marrow cells present. Bone marrow volumes are affected by a donor's size, age, and lifestyle habits such as smoking (Hernigou, 2011; Beyth, 2015).

The nurse or doctor performing the aspiration needs to adjust their technique to match the anatomy of the donor. Anatomical landmarks along the iliac crest provide information as to where to puncture the medullary cavity to access the richest concentration of high-quality stem cells. The aspiration needle must be re-positioned multiple times during the procedure, all the while balancing the amount of local anesthesia that can be administered,

"Bone marrow aspiration requires a sharp eye and strong technical expertise, especially when higher volumes are required. Proper visual inspection and monitoring of collected material during the procedure can provide clues about the distribution of desired cellular components. With over 20 years of experience with these procedures, our staff knows how to expertly perform a bone marrow aspiration to maximize the collection of desired cellular components, even when larger aspirate volumes are required."

- Pia Gross, Executive Director of Operations, HemaCare

and the amount of material that can be collected without compromising the donor's comfort. The knowledge, experience, and physical conditioning necessary to performing high-volume aspiration is demanding; in fact, the literature suggests that mastery of these techniques has deteriorated in the last few years (Remberger 2015) and the regenerative medicine industry as a whole could benefit from increased training, in light of the rising demand for high-quality aspiration material. People willing to volunteer as bone marrow donors are relatively scarce, so establishing trust and keeping the donor as comfortable as possible is essential to encouraging repeat donations. Once the bone marrow collection is performed, the donated unit is packaged and made ready for transportation to the end-user for use in their project.

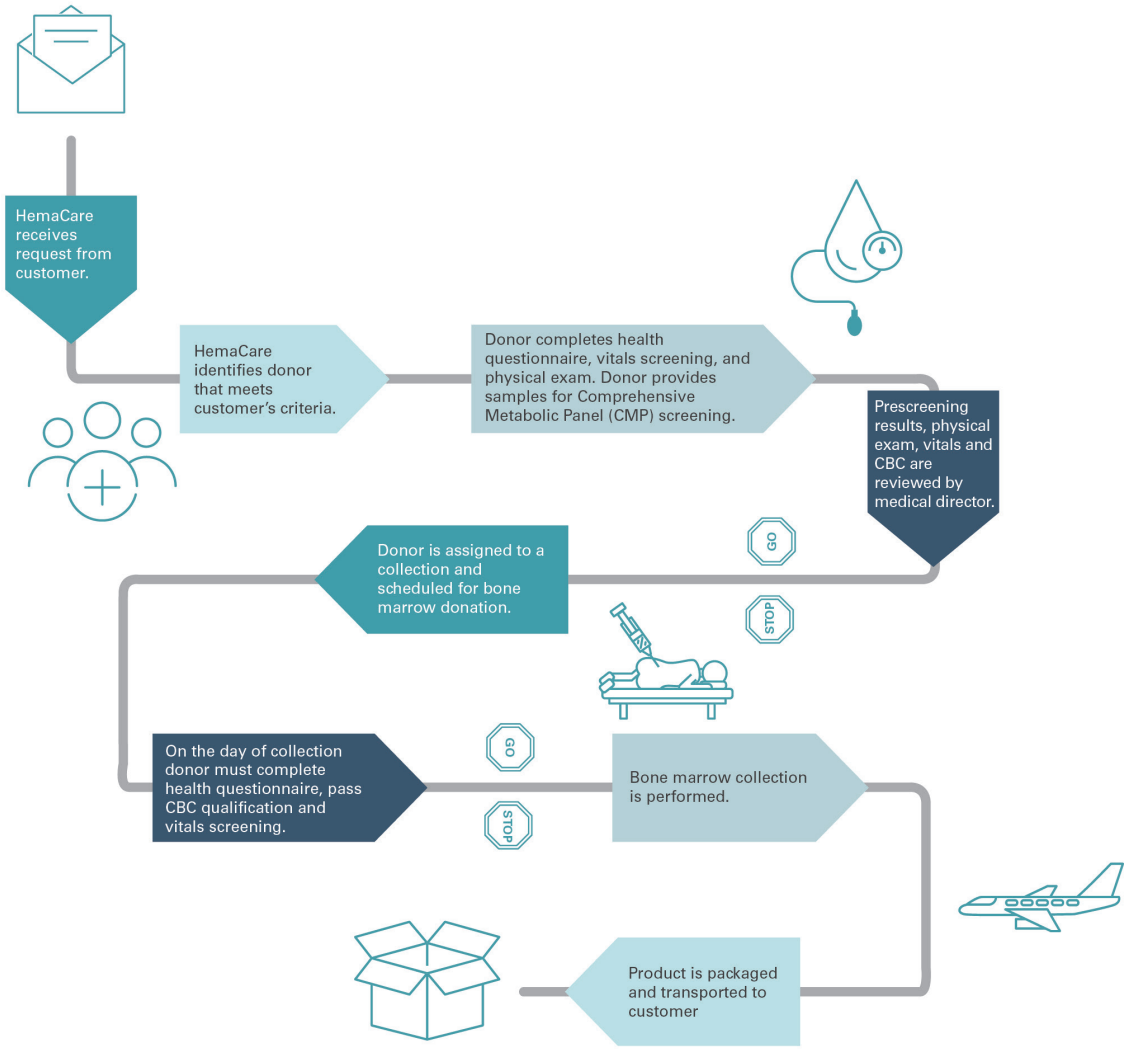

Figure 3. HemaCare Bone Marrow Collection Process

Collection of bone marrow aspirate from qualified donors is a complex process involving pre-screening for donor suitability to match the client's requested criteria, followed by the collection of demographic and medical history data, physical exam, and pertinent health screening.

It is essential that proper care is taken to safeguard the integrity of the unit throughout preparation and transport.

GMP-COMPLIANCE ENSURES CONSISTENT QUALITY

The extreme versatility of bone marrow-derived multipotent stem cells is highly prized for clinical applications. MSC derived from human bone marrow is currently the most common allogeneic cell product being tested in clinical trials (Jang, 2014; Rizvanov, 2016). As the number of stem cell therapies in clinical trial multiplies, sourcing sufficient GMP-compliant bone marrow is a consideration essential to commercial success (Wuchter, 2015).

Despite the acknowledgment of this fact, many companies hesitate to adopt GMP materials early in development due to their expense. The costs for a pharmaceutical company to enter the cell therapy space are already notoriously high. The regulatory burden for showing the safety and efficacy of a treatment relying on human cells and tissues that are responsive to their environment is onerous. Cell therapy developers need to carry out comprehensive cell characterization plus assure that their methods of development are robust enough to safeguard the integrity of a living product.

Since cell therapy companies realistically must back more than one candidate in their clinical pipeline, there is an ongoing logistical need to balance pipeline resourcing with the transition to GMP materials. GMP-compliant raw materials are more costly due to the supplier's prerequisite to carry out appropriate quality control assays, establish quality management systems oversight, and maintain extensive documentation.

Nevertheless, there is much to be said for early transition to GMP-compliant materials. Unlike other drug products, cell and gene therapies will always be subject to a certain level of starting material variability. GMP-compliance reduces risk by serving to standardize raw materials and processes, eliminating most external sources of variability, and leaving only those associated with the human donor.

Transitioning early saves time and monetary resources during more critical stages of development. It mitigates the risk of transmitting infectious disease or dealing with potential contamination, while assuring that materials are free of animal-derived components as required by regulatory agencies, and promoting consistent starting material quality throughout the development process.

CONCLUSION

The cell and gene therapy industry is showing an increasing awareness of the necessity of standardizing starting material production, processing, and validation (Clarke, 2019). Standardization will reduce product variability and mitigate the risk of failed or delayed manufacturing runs due to product quality issues.

Choosing GMP-compliant human bone marrow for research and clinical project needs ensures consistent, validated, and fully documented quality (HemaCare, 2019).

Identifying reliable suppliers for GMP-compliant raw materials during the early stages of product development can make the transition from preclinical to clinical development more seamless for multiple pipeline candidates (Nirenberg, 2019). HemaCare is invested in making GMP-compliant cell therapy starting materials more readily available while ensuring they meet the highest available validated quality standards. Starting material quality and consistency have a direct impact on the quality and efficacy of the final therapeutic product. The success of cellular therapeutics will greatly benefit from the early adoption of stringent quality management systems and consistent, high-quality materials.

REFERENCES:

1. Giancola R, et al. Cell therapy: cGMP facilities and manufacturing. Muscles Ligaments Tendons J. 2(3): 243–247. Oct; 2012.
2. Jaime-Perez J., et al. Flow cytometry data analysis of CD34þ/CD133þ stem cells in bone marrow and peripheral blood and T, B, and NK cells after hematopoietic grafting. Data in Brief 7: 1151–1155. 2016.
3. Holton J., et al. The Basic Science of Bone Marrow Aspirate Concentrate in Chondral Injuries. Orthop Rev (Pavia). 8(3): 6659. Sep 2016.
4. Alousi A., et al. Peripheral Blood versus Bone Marrow from Unrelated Donors: Bone Marrow Allografts Have Improved Long-Term Overall and Graft-versus-Host Disease-Free, Relapse-Free Survival. Biol. Blood Bone Marrow Transplant. 25(2):270-278. Feb 2019.
5. Morrison S. J., et al. The bone marrow niche for hematopoietic stem cells. Nature. 505(7483): 327–334. Jan 2014.
6. Szade K., et al. Where Hematopoietic Stem Cells Live: The Bone Marrow Niche. Antioxid Redox Signal. 29(2): 191–204. Jul 2018.
7. Chang K., et al. Long-Term Engraftment and Fetal Globin Induction upon BCL11A Gene Editing in Bone-Marrow-Derived CD34+ Hematopoietic Stem and Progenitor Cells. Molecular Therapy: Methods and Clinical Development. 4:137-148. Mar 2017.
8. Fernandez C. R. Taking CAR-T Cells Beyond Cancer: A New Therapy for Autoimmune Disease. Labiotech. Feb 2018.
9. With FDA Approval for Advanced Lymphoma, Second CAR T-Cell Therapy Moves to the Clinic. NIH National Cancer Institute. Oct 2017.
10. Researchers ready B cells for novel cell therapy. Seattle Children's Research Institute. May 2019.
11. Ildstad S. Induction of Donor Specific Tolerance in Recipients of Live Donor Kidney Allografts by Donor Stem Cell Infusion. University of Louisville. 2019.
12. Ildstad S. Delayed Tolerance in Recipients of Living Kidney Allografts by Donor FCRx Infusion. University of Louisville. 2019.

13. Rizvanov A. A. et al. Hematopoietic and Mesenchymal Stem Cells in Biomedical and Clinical Applications. Stem Cells Int. 2016: 3157365. Nov 2016.

14. Williams D. Pilot and Feasibility Study of Hematopoietic Stem Cell Gene Transfer for Sickle Cell Disease. Boston Children's Hospital. 2019.

15. Barra J. J., et al. Concise Review: Bone Marrow Derived Mesenchymal Stem Cells Change Phenotype Following In Vitro Culture: Implications for Basic Research and the Clinic. Stem Cells. Jan 2014.

16. Xu M., et al. Induced Pluripotent Stem Cell Derived Mesenchymal Stromal Cells Are Functionally and Genetically Different From Bone Marrow Derived Mesenchymal Stromal Cells. Feb 2019.

17. Zhang J. et al. The challenges and promises of allogeneic mesenchymal stem cells for use as a cell-based therapy. Stem Cell Research and Therapy. 6:234; 1-7. 2015.

18. McCormack K. By the numbers – a look at how the field of Regenerative Medicine is growing. Alliance for Regenerative Medicine. Jan 2019.

19. Hernigou P. Bone Marrow Aspiration Technique, Concentration Methods and Applications. Henri Mondor Hospital, University of Paris, France. Oct 2011.

20. Beyth S., et al. Cigarette Smoking Is Associated with a Lower Concentration of CD105+ Bone Marrow Progenitor Cells. Bone Marrow Research. 2015.

21. Remberger M., et al. Bone marrow aspiration technique has deteriorated in recent years. Bone Marrow Transplantation. 50: 1007–1009. 2015.

22. Jang J., et al. Development of GMP-grade mesenchymal stem cells derived from bone marrow of healthy donors. Cytotherapy. 16(4): S75. Apr 2014.

23. Wuchter P., et al. Standardization of Good Manufacturing Practice–compliant production of bone marrow–derived human mesenchymal stromal cells for immunotherapeutic applications. Cytotherapy. 17(2), 128–139, February 2015.

24. Clarke D, and Taylor B. Bridging the Gap: Laying the Foundation for Successful Advanced Therapies. European Biopharmaceutical Review. pp24-28. Apr 2019.

25. HemaCare Corporation. Understanding and Managing GMP and RUO Starting Materials.

26. Nirenberg U. Making an efficient & effective transition to GMP raw materials. Cell and Gene Therapy Insights. 5(2), 239-248. April 2019.

HemaCare Corporation
8500 Balboa Boulevard, Suite 130, Northridge, CA 91325 USA
(877) 310-0717 | info@hemacare.com | www.hemacare.com