Cell Therapy for Vascular Diseases in Israel

23

Yael Porat, Michael Belkin, Shlomo Bulvik, Michael Frogel, Offer Galili, and Mark Niven

In Israel, as elsewhere in the world, people with vascular disease suffer severe complications, including critical limb ischemia, which can result in gangrene and amputation. This chapter describes the step-wise progression in translational medical research as a scientific breakthrough progresses from a laboratory invention to the start of a clinical trial. The novel idea is a method whereby a sub-population of non-mobilized peripheral blood cells can be turned, within a day, into a cellular therapeutic product code-named BGC101, composed of endothelial progenitor cells (EPCs) and Stem/progenitor cell (SPCs). In addition, the benefits of collaboration between an Israeli biotechnology company and an Israeli medical center in overcoming the hurdles of bringing the idea to fruition will be described.

In order to achieve the goal of bringing innovative therapy to the market, one has to undertake complex tasks involved in translating the idea from a concept to a novel technology with the purpose of prevention, diagnosis or treatment of diseases.

The inventor has to surmount many hurdles before initiating a translational research project:

1. About 80 % of inventions are made by employees of universities, hospitals, government agencies etc. These inventions, by law, belong to the employer. In order to foster innovation, most of these institutions have regulations by

Y. Porat (✉)
BioGenCell, Ltd., Netanya, Israel

Hematology Stem Cell Research Center, Sanz Medical Center, Laniado Hospital, Divrey Haim 16, Netanya 42150, Israel
e-mail: yael.porat@biogencell.net; yporat@laniado.org.il

M. Belkin
BioGenCell, Ltd., Netanya, Israel

Eye Research Institute, Sheba Medical Center, Tel-Aviv University, Tel-Aviv, Israel
e-mail: belkin@netvision.net.il

S. Bulvik
Department of Hematology, Sanz Medical Center, Laniado Hospital, 16 Divrey Haim Street, Netanya 42150, Israel
e-mail: sbulvik@laniado.org.il

M. Frogel
BioGenCell, Ltd., Netanya, Israel
Cohen's Children's Medical Center, New Hyde Park, NY, USA

NY Pediatric Disaster Coalition, Department of Pediatrics, Maimonides Infant and Children's Hospital of Brooklyn, 4802 Tenth Avenue, Brooklyn, NY 11219, USA
e-mail: mikefrogel@gmail.com; michael.frogel@biogencell.net

O. Galili
Hillel Yaffe Medical Center, Hadera, Israel
e-mail: offerg@hy.health.gov.il; galili.offer@gmaio.com

M. Niven
Department of Endocrinology and Diabetes, Sanz Medical Center, Laniado Hospital, Kiryat Sanz, Netanya 42150, Israel
e-mail: mniven@laniado.org.il

A. DARDIK (ed.), *Vascular Surgery*, DOI 10.1007/978-3-319-33745-6_23

which they share the monetary rewards of a successful invention with its originator. Therefore the inventor has to face the bureaucracy of the institution's office, usually termed the "Technical Transfer Office", dedicated to evaluating, patenting and commercializing innovations.
2. The intellectual property of the new idea has to be protected by applying for patents that protect the invention and guarantee the inventor's freedom to operate. Furthermore, without patent protection the chances of anyone financing the project are practically nil.
3. A translational issue unique to cellular therapy is the problem that pharma companies are not accustomed to dealing with the special requirements for marketing of products in the new era of personalized regenerative medicine.

This onerous process can be facilitated by collaboration between academic medical centers and biotechnology companies whereby the product and clinical application development are managed together by multi-disciplinary teams. The outcome is not only faster but also provides academic validation to the technology. At the same time the academic medical center enhances its reputation by serving as an integral part in the vision and development of cutting edge technologies that will constitute the next generation of medical practice.

We describe here one example of an Israeli biotechnology company's pathway to develop an innovative technology platform utilized for the generation of SPC cell-based regenerative therapy to treat vascular diseases and its successful collaboration with an academic medical center.

The process was started based on a patented method for generating specific populations of therapeutic SPC cells using immune system dendritic cells (DCs) for guiding specific differentiation of the cells in vitro towards pro-angiogenic cells. In order to pursue the project with all its complexities, a company, BioGenCell, was established. Cooperation was then established with Laniado Medical Center, Netanya, Israel, an academic affiliate of the Technion School of Medicine. Laniado Hospital serves as the only medical center for a growing population of 400,000 people in the city of Netanya and surrounding suburbs. Laniado Hospital was founded by The Grand Rabbi of Klausenberg, a survivor of Auschwitz, who lost his wife and 11 children in the Holocaust. As a direct result of this, he included in the medical center's charter a request for participation in innovative medical research projects whose ultimate purpose is the improvement in patient care and outcomes.

The collaboration with BioGenCell enabled the growing Medical Center to support this innovative regenerative medicine research from its inception. BioGenCell conducted the research, providing proof-of-concept for the patented technology, completion of the preclinical phase and the establishment of a stem cell research laboratory and GMP compliant clean room manufacturing facility that produces BGC101 for clinical trials. The company was able to maintain control of the intellectual property while working toward the common goal of developing an innovative technology platform with the promise of treating vascular diseases, reversing disability, and improving the quality of life. The team worked together on developing the clinical trial as one of the first studies in which Laniado is the lead hospital, as well as, obtaining regulatory approval from the Ministry of Health in Israel to begin human trials. Clinicians, researchers, academicians and company personnel will provide the needed input and skills for conducting this first in human trial directly at the Medical Center. Once the initial safety and efficacy trial in 30 patients is completed at Laniado, trials will be expanded to the United States and the European Union. This rapid trajectory has been made possible by the combined efforts of the multidisciplinary joint team approach that benefits the company, the medical center and most importantly the patients.

Patients suffering from cardiovascular diseases were chosen as the first target of the collaborative effort.

Etiology of Vascular Diseases

Degenerative vascular diseases are the major cause of morbidity and mortality worldwide. Cardiovascular Diseases (CVD) including

coronary ischemic heart disease (IHD), cerebrovascular disease (Stroke) and Peripheral Artery Disease (PAD) are the primary cause of death worldwide (in the US 31 % with over 750,000 deaths/year) [1–3]. The estimated yearly cost of CVD in the US exceeds $320 billion with projected costs of $918 billion in 2030. By then 43.9 % of the US population will have some form of CVD [4]. A dramatically increased risk for the development of vascular diseases is related to diabetes that currently affects 10 % of the US population, and by 2020 will affect more than 20 million people in the USA alone [4].

Critical Limb Ischemia

BioGenCell has chosen peripheral artery disease (PAD), which affects more than 12 million people in the US, and its most serious form, critical limb ischemia (CLI), as its initial vascular disease target. PAD is characterized by partial or total blockage of blood supply to a limb, usually the leg. As the disease progresses it reaches the stage of intermittent claudication, followed by the development of critical limb ischemia (CLI).

CLI is defined as a severe obstruction of the vasculature, manifested by poor physical functioning due to leg or foot pain, and/or non-healing ulcers with tissue loss [5]. It is estimated that by 2020 CLI will affect one million patients in the USA. About 30 % of CLI patients are defined as 'No Option' and require amputation. CLI has an increased risk of comorbidities mainly due to cardiovascular ischemic events with a 1 year mortality, after the development of CLI, of 25 % [6].

Current best practice treatments of CLI include treatment of ulcers and gangrene, revascularization and/or medication therapy, depending on the location of the obstruction to flow. Revascularization options are either endovascular or bypass surgery. However, in many cases the disease occurs in capillaries and small vessels, too numerous and small to revascularize by currently available procedures. Therefore patients are treated with medications aimed at improving blood flow (such as the phosphodiesterase inhibitor cilostazol), reducing blood viscosity (antiaggregants and anticoagulants), and reducing pain (including several levels of analgesics). In addition, due to the ulcers and subsequent gangrene and recurrent infections, these patients often need antibiotic therapy.

Amputations have devastating psychological and diminished quality-of-life effects on patients [7]. They also can have a tremendous negative impact on their survival.

Amputations are also associated with significant expenses (e.g., hospitalization, surgery, constructing and fitting of prosthesis, rehabilitation, home health aides, construction and adaptations at the patients' homes, influence on family and productivity economics, long-term health care costs, etc.) [8]. The economic burden per CLI amputee exceeds $100,000 in the first year and an additional cost of $15,000 for each consecutive year for outpatient care (~85 % of the patients) or $70,000–100,000 for nursing home care (~15 % of the patients).

Based on all these statistics there is an obvious pressing need for new therapeutic modalities that promote regeneration of blood perfusion in the limbs, such as the use of SPC-based products.

Results and Challenges of SPC Cell-Based Therapy in CLI

The rationale for using SPC as a therapeutic modality to treat CLI is the assumption that their plasticity allows them to differentiate in vivo or in vitro in response to the environmental cues and, more specifically, to support tissue revascularization and resultant reperfusion. Tissue sources, including healthy donors and patient-derived cells, are currently under research and development or have already been tested in patients. A variety of allogeneic and autologous tissues have been suggested as sources for SPC and EPC cell treatment, such as bone marrow (BM), peripheral blood mobilized cells, and from various mesenchymal organs.

A meta-analysis by Benoit et al. summarized 45 clinical trials of open-label and randomized clinical trials (RCTs) with 1272 patients who received cell therapy. Safety analysis included evaluation of death, cancer, unregulated angiogenesis, and procedure-related adverse events (AE) such as bleeding. The overall AE rate was low (4.2%). Cell therapy patients did not have a higher mortality rate than control patients and demonstrated no increase in cancer incidence when analysed against standard population rates. Efficacy analyses included the clinical endpoints of amputation and death as well as functional and surrogate endpoints. Cell therapy patients had a significantly lower amputation rate than control patients (OR 0.36, $p=0.0004$). In addition, efficacy was demonstrated in a variety of functional and surrogate outcomes (such as ABI, TcPO2 and QoL) [9, 10].

These studies show that cell implantation is well tolerated. However, most of the reported AE stemmed from pre-procedural treatments connected to acquiring cells for the treatment [11–13]. For example, procedures for directly aspirating BM cells require the use of anaesthesia and entail pain and discomfort for these chronically ill patients. An alternative method for obtaining large amounts of BM cells is by extraction of mobilized BM cells from peripheral blood by aphaeresis. In the mobilization process, an inflammatory process is mimicked by the pretreatment of patients with high doses of granulocyte colony-stimulating factor (G-CSF; 1400 μg daily for 4–5 days). This has been reported to result in fever and chills, headache, muscular pain and bone pain, as well as, increased blood viscosity and platelet counts, which are problematic especially in patients suffering from microvascular diseases [11, 14, 15].

A further hurdle to commercialization of SPC technologies is lack of accessibility and high cost. For BM extraction there is a need for an operating theatre and team. This is both costly and accessible only in hospitals. Cell collection after mobilization requires aphaeresis utilizing specialized machines and dedicated teams. This process, similar to BM harvesting, is also costly and not available in every medical facility.

Beyond the State-of-the-Art

In order to overcome the above mentioned issues, BioGenCell applies a patient-oriented approach in the development of its novel technology for producing the therapeutic cell population (BGC101 enriched with EPCs) from a standard peripheral blood draw of 250 ml within a short culture period of 1 or 3 days. Most importantly, both the blood collection and the treatment can be performed in *any* outpatient setting.

Furthermore, this standardized and highly reproducible method enables development of a fully automated production process in a closed system device that will make the product much safer and accessible.

Since the number of EPCs and SPCs in the blood is relatively low, an ex vivo method for the enrichment and augmentation of specific cells was invented.

The breakthrough innovation of BioGenCell is the utilization of autologous DCs with anti-inflammatory and angiogenic effects to mix in vitro with select SPCs in culture to create a final product for human use that specifically targets areas of ischemia and regenerates the microvasculature necessary for tissue repair (Fig. 23.1). At the time of the invention of BGC101 there were no published reports that utilized DCs to directly activate and differentiate SPC in vitro, outside of the immune system niche. In a set of experiments summarized by Porat et al. in Diabetes Metabolism Research and Reviews [16] selected immature plasmacytoid and myeloid DCs from 24 healthy and 2 diabetic donors were activated with anti-inflammatory and pro-angiogenic molecules to induce specific activation signals. Co-culturing of activated DCs with SPCs generated $83.7 \pm 7.4 \times 10^6$ BGC101 cells with 97% viability from 250 ml of blood. BGC101, comprising 52.4 ± 2.5% EPCs (expressing Ulex-lectin, AcLDL uptake, Tie2, vascular endothelial growth factor (VEGF) receptor 1 and 2, and CD31), 16.1 ± 1.9% SPCs (expressing CD34 and CD184), and residual B and T helper cells, demonstrated angiogenic, colony formation (stemness) potential and secretion of IL-8, IL-10, VEGF, and osteopontin.

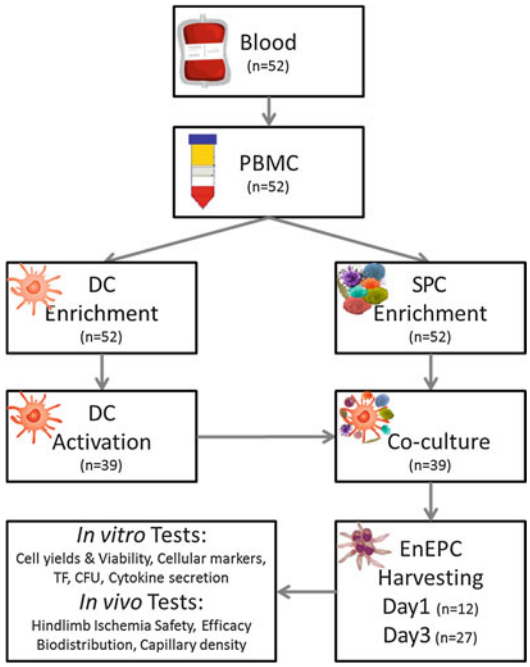

Fig. 23.1 Blood-derived SPC specifically activated by DCs [16]. Flow chart depicting the generation of potentially therapeutic enriched endothelial progenitor cells (EnEPCs). Non-mobilized blood-derived plasmacytoid and myeloid DCs activated with toleragenic and pro-angiogenic cytokines (such as IL-10, VEGF) are used in vitro to specifically direct the activity of SPCs which were enriched from the same blood sample and co-cultured for 1 or 3 days

BC101 is a typical biotechnology product that is defined in the US as 'Human Cells, Tissues and Cellular and Tissue-Based Products (HCT/Ps)' and is regulated by the FDA Center for Biologics Evaluation and Research (CBER). In EU it is defined as an advanced-therapy medicinal product (ATMP) and is regulated by the Committee for Medicinal Products for Human Use (CHMP) part of the European Medicines Agency (EMA) [17, 18].

In animal studies BGC101 cells administered to immunodeficient mice with limb ischemia (n = 40) yielded a high safety profile and significantly increased blood perfusion, capillary density, and leg function after 21 days. BGC101 cells tracked 21 days post-administration by hCD45 demonstrated homing and engraftment along the ischemic areas in the injured leg. Assessment of new blood vessel generation based on staining with hCD31 revealed enhanced vascularization that was restricted to the injected leg. Capillary density was significantly higher in the BGC101-treated mice than in the Vehicle Control treated group (105.2 ± 5.0 compared with 56.7 ± 2.7 capillaries/field; Figs. 23.2 and 23.3).

Importantly, blood from diabetic patients yielded cells similar to those obtained from healthy donors (Table 23.1). Thus, in addition to its scientific merit this novel technology can facilitate the development of a series of standardized products as it only requires a blood volume of 250 ml that can be safely and easily acquired even from chronically ill patients.

Conclusion

For CLI patients, BGC101 will be used with the intention of rescuing ischemic limbs. This, we hope, will be achieved by promoting the formation and function of new blood vessels that will improve circulation, enhance tissue perfusion, alleviate signs and symptoms, delay or even prevent the need for amputations, and increase survival with improved quality of life. It is therefore our goal to bring BGC101 to market as soon as possible.

The cooperative efforts between BioGenCell and Laniado Medical Center have enabled the development of an efficient and timely plan that addresses safety and efficacy, as well as technical and regulatory issues, related to each step in the clinical treatment and manufacturing processes. Furthermore, this new therapy should be an accessible and affordable solution for patients, as well as healthcare providers.

In conclusion, the collaboration between a biotechnology company and medical center in Israel has overcome many of the significant hurdles in bringing a new concept from the bench to the bedside and has led to the rapid development of a novel technology for producing a therapeutic cellular product within a single day from a standard blood draw for the treatment of vascular diseases.

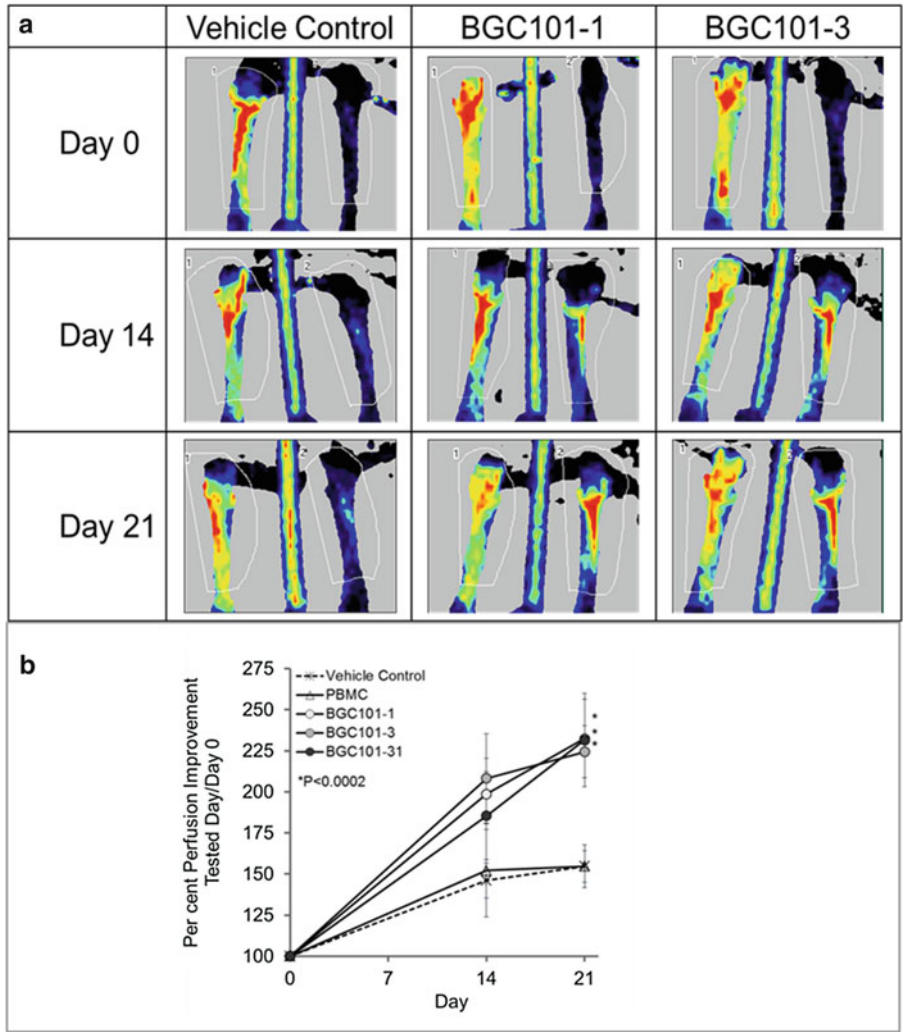

Fig. 23.2 In vivo efficacy of BGC101 in a mouse model of hind limb ischemia [16]. In vivo efficacy of BGC101 in a mouse model of hind limb ischemia, showing significant improvement in perfusion. Ligation and dissection of a femoral artery section led to ischemic damage, which was followed by intramuscular cell injections. (**a**) Representative laser Doppler scans following infliction of damage to the left leg (day 0), and repeated scans 14 and 21 days after treatment. During the experiment the per cent Perfusion was tested in the Vehicle Control treated group (n = 12), the peripheral blood mononuclear cell treated group (PBMC, 2.5×10^6 cells/mouse, n = 5), and in the BGC101 treated groups BGC101-1 (cells from 1-day culture, 2.5×10^6 cells/mouse, n = 10), BGC101-3 (cells from 3-day culture, 2.5×10^6 cells/mouse, n = 10), and BGC101-31 (cells from 3-day culture, 0.5×10^6 cells/mouse, n = 5) ($P < 0.0002$). (**b**) Percent perfusion improvement at each time point in each group versus that of day 0 compared with that of the Vehicle Control group at the same time point ($P < 0.0002$)

Successful implementation of this technology in clinical trials will place Laniado Medical Center and BioGenCell at the forefront of a new standard of care utilizing personalized regenerative medicine cell therapies. This novel technology could then be studied for other uses such as heart disease, ischemic stroke, pulmonary hypertension and even vascular dementia and several kinds of blindness.

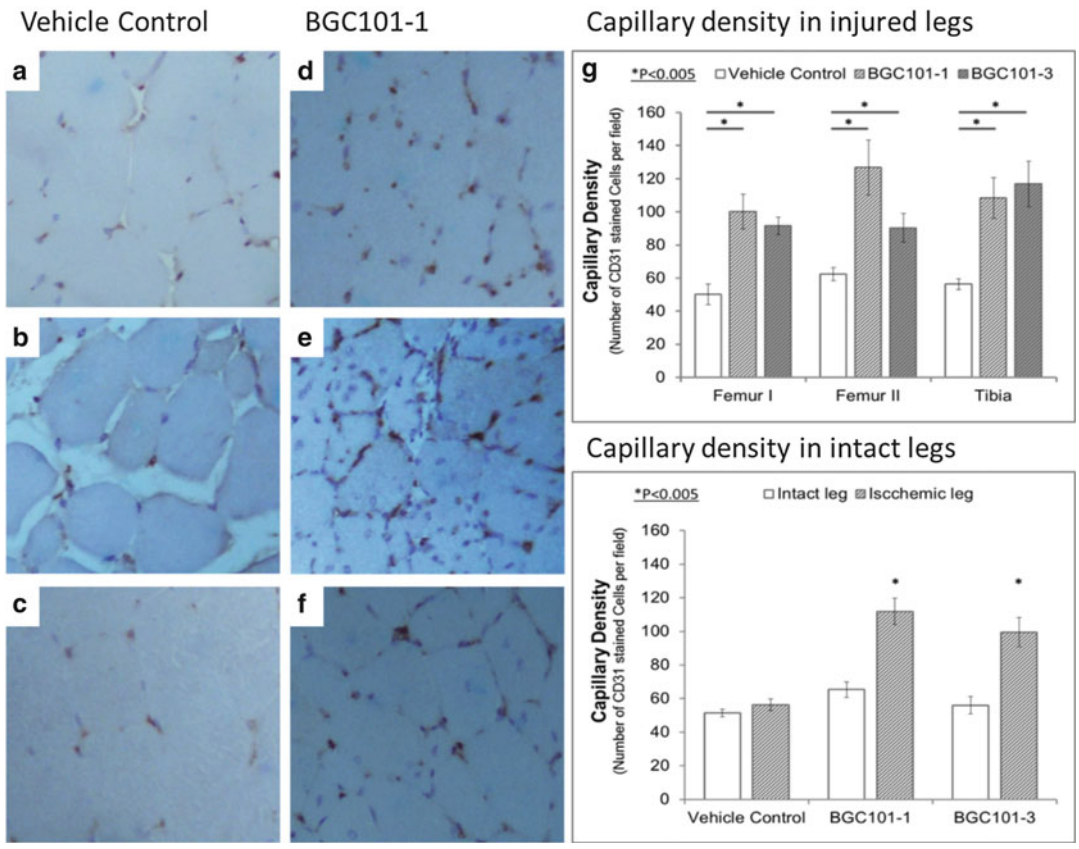

Fig. 23.3 Increased capillary density in BGC101 treated mice is restricted to the injured leg [16]. (**a**–**f**) are representative photomicrographs (×630) of tissue sections from ischemic and intact legs on day 21. New capillaries in tissue sections from femur at the site of damage (Femur I), femur at the implantation site (Femur II), and distally at the diaphysis of the tibia (Tibia), were stained with hCD31 (mouse cross-reactive) and H&E (**a**–**c**); (**d**–**f**) Analogous tissue sections from a BGC101-1 treated mouse. (**g**) Capillary density (average capillary count per field) in injured legs of mice treated with Vehicle Control, BGC101-1, and BGC101-3 ($P < 0.005$). (**h**) Capillary density in tissue sections from treated ischemic legs and from intact legs ($P < 0.005$)

Table 23.1 Per cent perfusion following treatment with EnEPCs originated from healthy and diabetic donors. Per cent Perfusion on Day 21 is presented in the Vehicle Control treated group (n = 12), the PBMC group (2.5×10^6 cells/mouse, n = 5, all from diabetic donor), and in the BGC101 treated groups BGC101-1 (cells from 1-day culture, 2.5×10^6 cells/mouse, n = 10, four from healthy donor and six from diabetic donor), BGC101-3 (cells from 3-day culture, 2.5×10^6 cells/mouse, n = 10, five from healthy donor and five from diabetic donor), and BGC101-31 (cells from 3-day culture, 0.5×10^6 cells/mouse, n = 5, all from diabetic donor) groups ($P < 0.0002$). Per cent Perfusion was calculated for all mice (All), as well as separately for mice that received cells from healthy (Healthy Donor) or diabetic (Diabetic Donor) donors

Group	Sub-group	%Perfusion Day 21	P Value Tested Group vs. Vehicle Control
Vehicle Control	All (n = 12)	35.1 ± 1.2	
PBMC	All (n = 5)	40.0 ± 4.4	$P = 0.167$
	Healthy donor (–)	–	
	Diabetic donor (n = 5)	40.0 ± 4.4	
BGC101-1	All (n = 10)	53.7 ± 2.7	$P < 0.0002$
	Healthy donor (n = 4)	56.8 ± 5.8	
	Diabetic donor (n = 6)	49.5 ± 2.1	
BGC101-3	All (n = 10)	47.7 ± 2.4	$P < 0.0002$
	Healthy donor (n = 5)	48.9 ± 4.1	
	Diabetic donor (n = 5)	46.4 ± 3.0	
BGC101-31	All (n = 5)	51.8 ± 4.0	$P < 0.0002$
	Healthy donor (–)	–	
	Diabetic donor (n = 5)	51.8 ± 4.0	

References

1. CDC. Deaths final data for 2011. 2015. http://www.cdc.gov/nchs/data/nvsr/nvsr63/nvsr63_03.pdf. Accessed 27 July 2015.
2. Alwan, A. Global status report on non-communicable disease 2010, Geneva World Health Organization. 2011. http://www.who.int/nmh/publications/ncd_report_full_en.pdf.
3. Nichols M, Townsend N, Scarborough P, Rayner M. Cardiovascular disease in Europe 2014: epidemiological update. Eur Heart J. 2014;35:2950–9.
4. Mozaffarian D, Benjamin EJ, Go AS, et al. Heart disease and stroke statistics—2015 update: a report from the American Heart Association. Circulation. 2015;131:e29–322.
5. Lumsden AB, Davies MG, Peden EK. Medical and endovascular management of critical limb ischemia. J Endovasc Ther. 2009;16:II31–62.
6. Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FGR. Inter-society consensus for the management of peripheral arterial disease (TASC II). J Vasc Surg. 2007;45:S5–67.
7. Peacock JM, Keo HH, Duval S, Baumgartner I, Oldenburg NC, Jaff MR, Henry TD, Yu X, Hirsch AT. The incidence and health economic burden of ischemic amputation in Minnesota, 2005–2008. Prev Chronic Dis. 2011;8(6):141. http://www.cdc.gov/pcd/issues/2011/nov/11_0023.htm.
8. Alonso A, Garcia LA. The costs of critical limb ischemia. Endovasc Today. 2011;2011:32–6.
9. Benoit E, O'Donnell TF, Kitsios GD, Iafrati MD. Improved amputation-free survival in unreconstructable critical limb ischemia and its implications for clinical trial design and quality measurement. J Vasc Surg. 2012;55:781–9.
10. Benoit E, O'Donnell TF, Patel AN. Safety and efficacy of autologous cell therapy in critical limb ischemia: a systematic review. Cell Transplant. 2013;22:545–62.
11. Fadini GP, Agostini C, Avogaro A. Autologous stem cell therapy for peripheral arterial disease meta-analysis and systematic review of the literature. Atherosclerosis. 2010;209:10–7.
12. Fuh E, Brinton TJ. Bone marrow stem cells for the treatment of ischemic heart disease: a clinical trial review. J Cardiovasc Transl Res. 2009;2:202–18.
13. Gratwohl A, Baldomero H, Aljurf M, Pasquini MC, Bouzas LF, Yoshimi A, Szer J, Lipton J, Schwendener A, Gratwohl M, et al. Hematopoietic stem cell transplantation: a global perspective. JAMA. 2010;303:1617–24.
14. Losordo DW, Siscovick DS, Manolio TA, Udelson JE, Veereshwarayya V, Durgin M, Poh KK, Weinstein R, Kearney M, Chaudhry M, Burg A, Eaton L, Heyd L, Thorne T, Shturman L, Hoffmeister P, Story K, Zak V, Dowling D, Traverse JH, Olson RE, Flanagan J, Sodano D, Murayama T, Kawamoto A, Kusano KF, Wollins J, Welt F, Shah P, Soukas P, Asahara T, Henry TD. Intramyocardial transplantation of autologous

CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial. Circulation. 2007;115:3165–72.
15. Quirici N, Soligo D, Caneva L, Servida F, Bossolasco P, Deliliers GL. Differentiation and expansion of endothelial cells from human bone marrow CD133+ cells. Br J Haematol. 2001;115:186–94.
16. Porat Y, Assa-Kunik E, Belkin M, Krakovsky M, Lamensdorf I, Duvdevani R, Sivak G, Niven M, Bulvik S. A novel potential therapy for vascular diseases: blood-derived stem/progenitor cells specifically activated by dendritic cells. Diabetes Metab Res Rev. 2014;30:623–34.
17. U.S. Food and Drug Administration. 2011. Guidance for industry regulation of human cells, tissues, and cellular and tissue-based products (HCT/Ps) small entity compliance guide. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm062592.pdf. Accessed 21 Oct 2011.
18. European Medicines Agency. Advanced-therapy classification. 2015. http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/general/general_content_000296.jsp. Accessed 6 May 2015.