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Challenges in the Development of Autologous Cell Therapy Products

By YAEL PORAT*, DANNY BELKIN, ADINA BELLELI, JACOV ELKAYAM, DAPHNA SHIMONI, SVETLANA POROZOV, INA SAREL, ISSAR ASH, and VALENTIN FULGA

In recent years, cell therapy has been suggested as a promising approach for repair and regeneration of damaged tissues. VesCell™, a blood-derived autologous cell therapy product consisting of *ex vivo* enriched angiogenic cell precursors (ACPs) was developed by TheraVitae for the treatment of severe heart diseases.

A non-mobilized, blood-derived cell population consisting of low density cells, termed synergetic cell population (SCP), was isolated and cultured in the presence of serum-free medium (X-Vivo 15, Lonza, Walkersville, MD, USA) supplemented with growth factors and autologous serum to yield VesCell. Significant cell numbers ($>50x10^6$) exhibiting morphological, immunocytochemical, and functional characteristics of the angiogenic cell lineage were obtained from blood samples. The ACPs expressed the hematopoietic stem cell (HSC) markers CD34, CD133 and CD117, as well as specific angiogenic markers such as vascular endothelial growth factor receptor 2 (VEGFR2) (receptor 2 [R2] is also known as kinase domain region [KDR]), CD144, and CD31. In addition, the ACPs demonstrated acetylated low density lipoprotein (AcLDL) labeled with 3,3´-dioctadecyloxacarbocyanine perchlorate uptake, formed tube-like structures *in vitro*, and secreted cytokines such as interleukin-8 (IL-8), VEGF and angiogenin.

The development of VesCell posed significant challenges in the generation of a safe and effective product, ready-to-use by physicians. These challenges were related to general aspects of cellular product development and characterization, initiation of methodologies, and procedures used in the manufacturing, packaging and shipping processes.

The development work described herein resulted in an efficient, tightly-controlled manufacturing process in which final product safety, quality and stability of 35 hours at 2–12°C enabled the product's clinical evaluation in patients suffering from end-stage cardiovascular diseases.

Introduction

Despite significant improvement in treatments for severe heart diseases, numerous cardiac patients are not responsive to medical and invasive treatment. Thus, there is a crucial need to develop novel, more effective therapies. The discovery that stem cells may promote regeneration of ischemic tissue has led to a large number of studies evaluating the use of stem/progenitor cell therapy as a potentially effective treatment for severe myocardial diseases.[1]

Previous clinical studies in which over 700 patients were treated with autologous adult stem/progenitor cells harvested mainly from the bone marrow (BM) have demonstrated improved myocardial function with very few adverse effects, suggesting that administration of autologous stem/progenitor cells is feasible, safe, and potentially of therapeutic benefit.[2-22]

BM-derived stem/progenitor cells can be obtained by aspiration directly

from the bone marrow, or from the peripheral blood following cytokine pretreatment that mobilizes the cells (mobilized peripheral blood). BM aspiration entails pain and discomfort and requires the use of anesthesia. The alternative technology of obtaining cells from mobilized peripheral blood might result in increased blood viscosity, metabolic demand, and platelet counts.[23] To circumvent the risks and discomfort associated with both technologies, we opted to use cells harvested from

Yael Porat, Ph.D., *Chief Technical Officer and Vice President R&D; Danny Belkin, Ph.D., Regulatory Manager; Adina Belleli, Ph.D., QC Manager; Jacov Elkayam, B.Sc., Director of Production and Therapeutic Cellular Products Manufacturing Program, Southeast Asia; Daphna Shimoni, B.Sc., Scientist; Svetlana Porozov, Ph.D., Senior Scientist; Ina Sarel, Ph.D., Director of Neuroscience Program; Issar Ash, M.Sc., Senior Director of Compliance and Quality Assurance; Valentin Fulga, M.D.; TheraVitae Ltd.* ****Dr. Porat is the corresponding author:*** *TheraVitae Ltd, 7 Pinhas Sapir St., Science Park, Ness-Ziona, Israel, Phone: 972(8)9409170, Fax: 972(8)9409167, Email: yaelp@theravitae.com.*

non-mobilized peripheral blood as the raw material for the manufacture of VesCell.

We have recently reported[24] the isolation of a multipotent progenitor SCP from non-mobilized blood and the generation of an enriched population of ACPs from the SCP. Similar to endothelial progenitor cells (EPCs), ACPs simultaneously express Ulex-lectin and uptake of AcLDL. Furthermore, the ACPs express the HSC markers CD34, CD133, and CD117, as well as the angiogenic cell markers VEGFR2, Tie-2, CD144, von Willebrand factor (vWF), and CD31.

In vitro functional tests show that ACPs migrate in response to chemoattractants and form tube-like structures on extracellular matrix test of angiogenesis. In addition to inducing angiogenesis, ACPs secrete several growth factors and cytokines such as IL-8, VEGF, and angiogenin which facilitate tissue survival and regeneration, as well as the mobilization of additional progenitor cells involved in the healing process of the ischemic area.[25-27] ACPs (rather than EPCs) are used to describe these cells as they induce angiogenesis by a variety of means and are not limited to differentiating into endothelial cells.

VesCell, a cell therapy product consisting of HSC/progenitor cells and ACP lineage cells is used to treat patients suffering from end-stage, no-option heart disease. It was aimed at promoting formation of blood vessels and rescue of myocardial tissue, thus improving cardiac function and ameliorating symptoms.

The development of a cell-based product required a comprehensive design and implementation process incorporating scientific, manufacturing, regulatory, and clinical inputs. Ultimately, overcoming these challenges resulted in an efficient manufacturing process carried out under tightly-controlled conditions including in-process control (IPC) and release testing, as well as stringent batch labeling and tracking procedures.

Following establishment of the manufacturing facility and process, VesCell was used in preclinical and clinical studies evaluating its safety and efficacy for the treatment of severe angina pectoris in patients suffering from end-stage heart disease not amenable to other therapies (manuscript in preparation). This article illustrates the challenges and shares the experience gained by our team in the development stages of this cell therapy product.

Experimental

Cell Culture

Low-density SCPs were isolated from individual adult blood samples as described previously.[24] Blood specimens of 450 ml were obtained from the Israeli Blood Bank, whereas patient batches were processed from blood specimens of 250 ml each. Blood was collected from the patients after obtaining their informed consent and flown to TheraVitae's manufacturing laboratory in Israel. For generating ACPs during development, SCPs were cultured at a concentration of 1.5–3.0x10^6 cells/ml in endothelial basal medium (EBM) (BulletKit, Lonza) or in "Medium I" composed of X-Vivo 15 serum-free medium supplemented with 10% autologous human serum, 1–10 ng/ml VEGF (R&D Systems, Minneapolis, MN, USA) and 5 IU/ml heparin (Kamada, Beit-Kama, Israel). Cells were counted and assessed for viability using the trypan blue dye-exclusion method.

Safety Tests

VesCell batches were examined by a panel of microbial tests to ensure that no contamination had occurred during the manufacturing process. These tests were all validated. Each batch was tested for: bacterial and fungal sterility according to current USP chapter <71>; presence of endotoxin according to current USP chapter <85>; Limulus amebocyte lysate (LAL) gel-clot method; rapid quantitative Gram stain test; and mycoplasma test according to the recommendations of USP NF (2007) chapter <1046>, Japanese Pharmacopoeia Fourteenth Edition chapter <9>, and Draft FDA guidance Cell Therapy IND-August 2003.[28-30]

Flow Cytometry

The percentage and total number of ACPs, the lineage-specific cells, as well as $CD34^+$ hematopoetic stem cell markers[31-33] were determined using flow cytometry (fluorescence-activated cell-sorting [FACS]) analysis. Harvested cells were tested for the expression of CD31, a molecule expressed by progenitor as well as mature angiogenic and endothelial cells[34-36] and uptake of AcLDL, a molecule which is rapidly internalized by endothelial and angiogenic cells, widely used for their characterization. ACPs were defined as cells exhibiting high levels of CD31 ($CD31^{Bright}$) concomitantly with uptake of AcLDL. Briefly, harvested cells were incubated in the dark, on ice, for 30 minutes with specific fluorochrome-conjugated CD34-APC (BD Biosciences, San Jose, CA, USA), CD31-PE (e.Bioscience, San Diego, CA, USA), or with isotype-matched non-specific controls.

In the case of CD31, the results represent the percentage of cells with bright intensity ($CD31^{Bright}$). Cell staining was considered bright if staining intensity was at least 50 times higher than the intensity of the corresponding isotype control staining. For AcLDL uptake, cells were incubated in the presence of 0.8 µg/ml AcLDL (Alexa Fluor488 AcLDL or DiO-AcLDL, Biomedical Technologies, Inc., Stoughton, MA, USA) for 15 minutes at 37°C after which they were washed and stained with PE-conjugated CD31.

Exclusion of dead cells was performed using 7-amino-actinomycin D ([7-AAD], BD Biosciences). In order to assure result reliability, cell suspension triplicates of 500,000 cells each were stained, assessed by FACSCalibur (BD Biosciences) and analyzed by CellQuest Pro software (BD Biosciences). For each replicate, at least 30,000 cells were acquired.

The percentage of each marker was determined in each test tube and the mean and percentage coefficient of variance (%CV) was calculated for each marker. The results were expressed as mean ± standard error (SE) of the percentage of stained cells. The number of stained cells was calculated by multiplying the number of harvested cells by the staining percentages obtained using the FACS.

Analysis of Interleukin-8 Secretion

VesCell secretion of the chemokine CXCL8/IL-8, an anti-apoptotic cytokine involved in angiogenesis and known to induce mobilization of progenitor cells from the bone marrow[25,27,37,38] was tested using quantitative sandwich ELISA. IL-8 levels were determined in culture media samples collected on the harvesting day using a commercial detection kit (DouSet DY208E, R&D Systems, Inc.) according to the manufacturer's instructions. Triplicates of standard curve consisting of seven dilutions and tested samples (each at four dilutions) were analyzed. The intensity of the color proportional to the IL-8 amount was measured using a Multiskan EX microplate reader with Ascent Software (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

Tube Formation Assay

Tube formation was assessed using an *in vitro* angiogenesis assay (Cultrex basement membrane extract without phenol red, R&D Systems, Inc.). Briefly, harvested ACPs (0.1–0.4x10^6 cells/ml) were cultured overnight in a 96-well microplate using M199 medium (Sigma-Aldrich, Inc., St. Louis, Missouri, USA) containing 10% autologous human serum, 10 ng/ml VEGF, 10 ng/ml basic fibroblast growth factor (bFGF), 5 IU/ml heparin, and 25 µg/ml endothelial cell growth supplement ([ECGS] Biomedical Technologies, Inc., Stoughton, MA, USA) on basement membrane extract gel. Tube formation was assessed visually using an inverted light microscope (ECLIPSE TS-100, Nikon Corp., Japan). Angiogenic pattern and vascular tube formation were scored (as previously described).[24]

Results and Discussion

Strategy for the Development of a Safe Product

Critical decisions during product development were choosing to avoid the use of antibiotics and animal-derived materials such as bovine brain extract and fetal bovine/calf serum in the manufacturing process. Furthermore, a strong emphasis was placed on utilizing only materials with a history of clinical use in humans. For example, it was decided to use X-Vivo 15, a culture medium which is listed by the FDA in a product master file and which had already been used in several clinical trials.

In three preliminary experiments, the efficacy of Medium I containing X-Vivo 15 as the basal serum-free medium, supplemented with autologous serum, growth factors and heparin was compared with the EBM BulletKit medium, used as a positive control. At that time, EBM BulletKit (containing EBM-2, fetal bovine serum, BFGF, epidermal growth factor [EGF], VEGF, insulin-like growth factor [IGF], heparin, bovine brain extract, and antibiotics, [Clonetics, Lonza]) was considered the 'gold standard' medium for the generation of endothelial cell progenitors. Results calculated, based on the percentage of cells expressing CD34 and CD133 showed that Medium I was at least as good as the EBM BulletKit or even more effective in supporting VesCell production. Viability of cells grown in EBM BulletKit was 93.6% ± 4.0% and that of cells grown in Medium I was 89.1% ± 8.9%. The num-

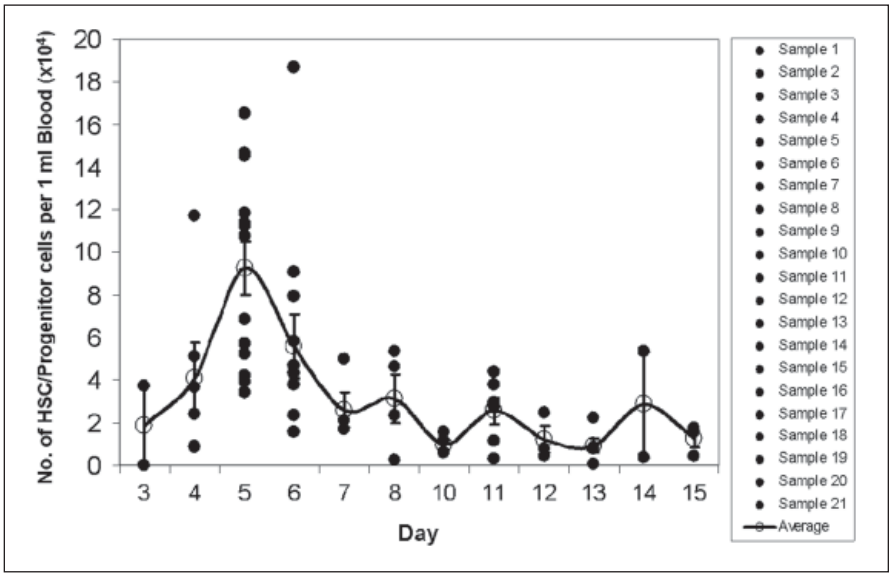

Figure 1. Results from kinetics experiments of 21 development batches, evaluating the number of HSC/progenitor cell number per 1 ml of blood at different time points. The largest number of these cells was achieved on day 5 of the manufacturing process.

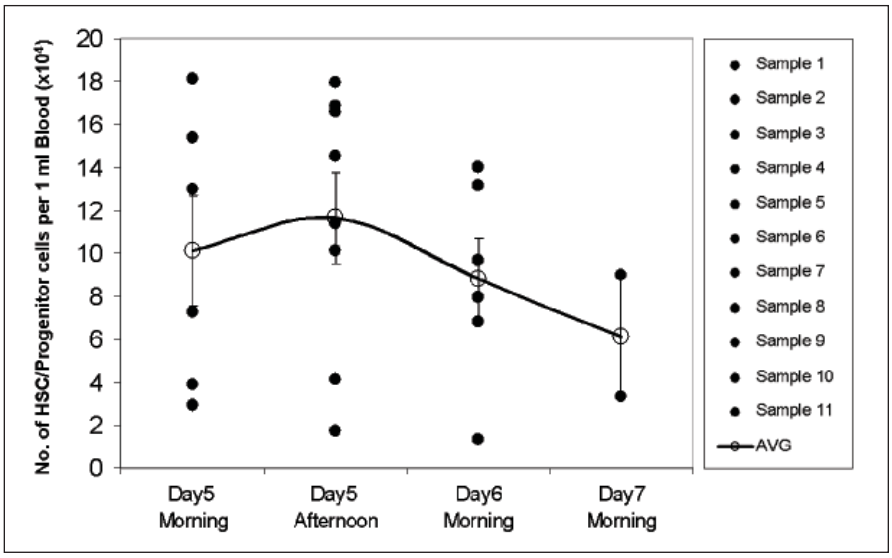

Figure 2. Results from kinetics experiments of 11 patient batches, evaluating the number of HSC/progenitor cells at different time points. The largest number of cells was achieved on day 5 of the manufacturing process.

ber of HSC/progenitor cells per 1 ml blood was $4.5x10^4 \pm 4.0x10^4$ for cells grown in EBM BulletKit, and $14.1x10^4 \pm 13.9x10^4$ for cells grown in Medium I (Table 1). These results supported the incorporation of X-Vivo 15 into the manufacturing process.

Efforts for increasing the safety of the cellular product were also directed towards the culture dish coating step. Human fibronectin (HFN), a commercial protein purified from human plasma, is widely used as a factor in the propagation of cells *in vitro,* but it might be unsafe. We hypothesized that donor's autologous plasma may supply the amount of fibronectin and other extra cellular signals required for cell propagation. Results from experiments comparing coating culture dishes with HFN or donor's autologous plasma in ten development batches showed that both substances were similarly effective in supporting VesCell production. Viability of cells grown in flasks coated with HFN was 95.6% ± 0.7%, and that of cells grown in flasks coated with autologous plasma was 95.2% ± 0.8% (p=0.351). Specific cell numbers per 1 ml of blood were calculated based on the percentage of cells expressing CD34, CD133, CD117, KDR and Tie-2. The average cell number obtained from cells cultured in flasks coated with HFN was $12.0x10^4 \pm 1.9x10^4$ and from cells cultured in flasks coated with autologous plasma was $10.5x10^4 \pm 1.5x10^4$ (p=0.215) (Table 2). It was thus decided to use autologous plasma as the coating material of the culture dishes.

Growth Kinetics

The establishment of the cell manufacturing process included kinetics experiments designed to evaluate the

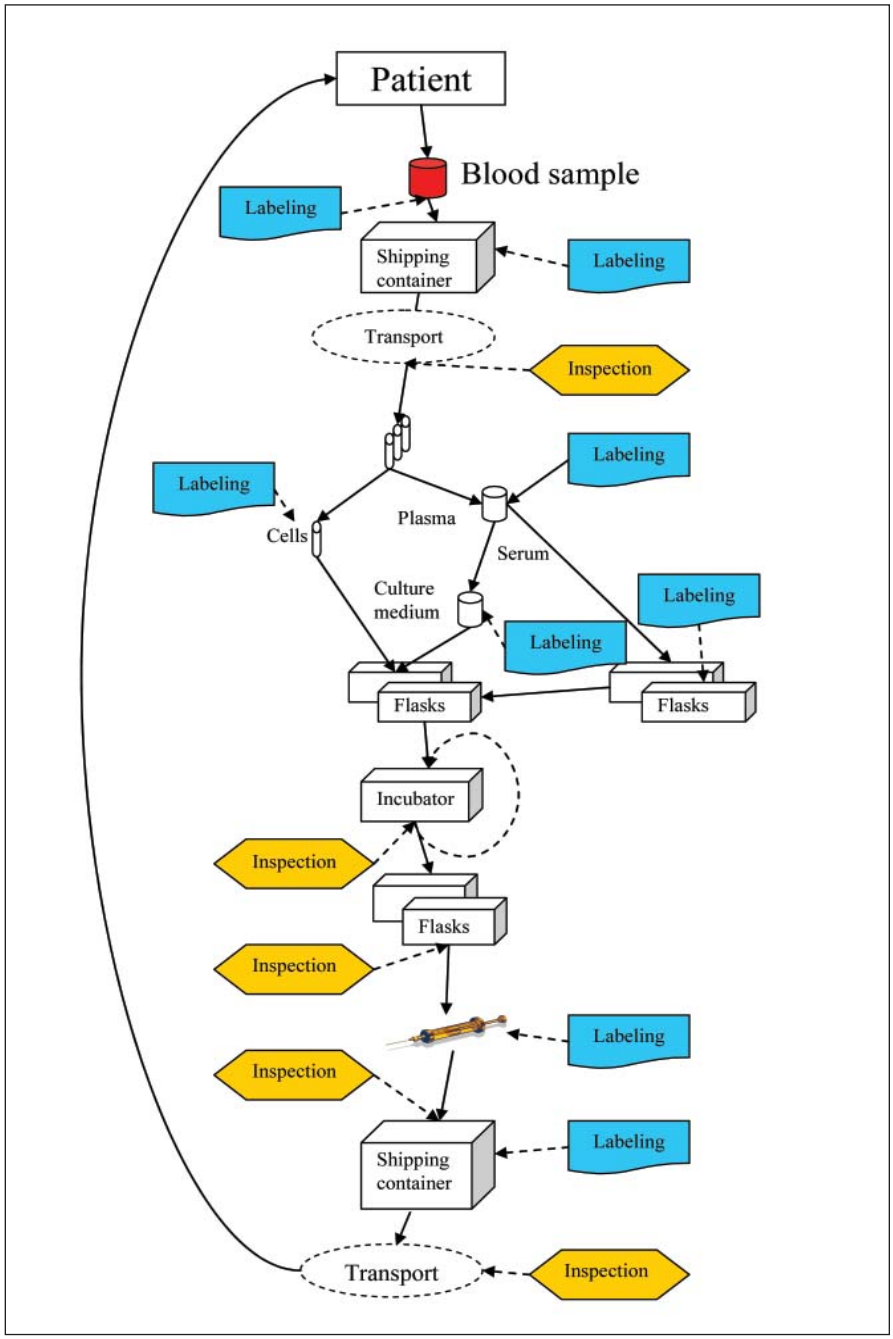

Figure 3. Schematic flowchart illustrating the batch tracking system employed at TheraVitae. This system includes label inspection and batch identity verification at critical points along the manufacturing process.

Table 1. Results from experiments comparing EBM BulletKit medium and Medium I containing X-Vivo 15 serum, growth factors, and heparin in the generation of research VesCell batches. The results indicate that the Medium I is more effective in supporting the production of VesCell.

	N	EBM Bullet Kit Average ± SE	Medium I Average ± SE	Ratio Medium I / EBM	T test p Value
% Viability	3	93.6 ± 4.0	89.1 ± 8.9	95.1	0.469
No. ($x10^4$) HSC/Progenitor Cells/ml Blood	3	4.5 ± 4.0	14.1 ± 13.9	312.0	0.437

best time point for harvesting of ACPs. Analysis of 21 development batches examining the attainable number of HSC/progenitor lineage-specific cells expressing CD34, CD133 or KDR per 1 ml of blood on days 3–15 indicated that the highest absolute number is obtained in the early days of culture with a clear peak on day 5 (Figure 1).

Equivalence of Products Manufactured from the Blood of Healthy Donors and Severe Heart Disease Patients

Development batches were produced using blood from healthy donors, which could potentially differ from blood samples taken from the generally older population of severely ill cardiac patients who are routinely treated with multiple drugs. In order to ensure the adequacy of the manufacturing process for patient samples, manufacturing process equivalence using blood samples from 11 cardiac patients was carried out. VesCell batches produced from these patients fully complied with quality control (QC) specifications for sterility, viability, and cellular markers. Results from patient batches, similar to results from the development batches, indicated that harvesting of cells on day 5 of the culture is preferable (Figure 2). The positive results seen in these trial runs reaffirmed the consistency and reliability of the cell manufacturing process and allowed the initiation of VesCell preclinical and clinical usage.

From R&D to Manufacturing

Additional efforts were addressed at the adaptation of the technology developed during research and development stages to a GMP environment. This included preparation and implementation of standard operating procedures (SOPs), establishment of IPC and product release criteria, along with transfer of the manufacturing process from a lab to a cleanroom facility (see below). Manufacturing is performed according to established manufacturing instructions which stipulate all steps of each process in great detail. Every step is documented, signed and dated. The completed manufacturing instructions are reviewed and authorized by the quality assurance (QA) department. All other documentation associated with batch processing such as QC test results, deviation reports, and environmental monitoring are reviewed by QA prior to batch release.

Ten VesCell batches produced during the validation step fully complied with QC specifications for sterility, viability and cellular markers. An average viability of 95.6% ± 0.7% and $18.1x10^4$ ± $3.8x10^4$ HSC/progenitor cells per 1 ml of blood were obtained.

Clinical batches are processed in a cleanroom. This cleanroom facility was designed to comply with cGMP and ISO guidelines[39-41] and consists of two ISO Class 7 (Class 10,000) cleanrooms and an ISO Class 8 (Class 100,000) entrance room. All critical operations related to batch processing procedures are carried out inside ISO Class 5 (Class 100) biological safety cabinets located in the ISO Class 7 cleanrooms. The cleanrooms also house incubators, centrifuges, refrigerators and microscopes. Each cleanroom's pressure differentials are monitored and recorded. Routine environmental microbial monitoring is performed during each batch processing.

Batch Tracking and Labeling

In manufacturing of autologous cellular products, batch tracking, ensuring that each patient is treated by his personal product, is crucial. To address this issue, a stringent process control and batch labeling system was designed and implemented, allowing the segregation and tracking of different patient batches. Under the tracking scheme (Figure 3), batch tracking begins at the time of blood collection and continues beyond the administration of the final product to the patient until all QC data have been collected and recorded. Throughout the cell manufacturing process, each critical step is performed and recorded by two employees assuring—beyond any doubt—the identity of the processed batch. Upon process termination, a certificate of analysis (COA) detailing release testing results is issued and sent to the medical center. In addition, a batch release (or rejection) statement ratified by QA personnel is issued, authorizing (or prohibiting) the administration of VesCell to the patient.

Packaging, Shipping and Stability

Both the raw material (blood) and the product (VesCell) are transported between the medical centers and the manufacturing facility. The 5,000 mile distance between Israel and Bangkok (approximately 20 hours total transport time) raised concerns about blood and cellular product stability during the long transportation period. A shipping procedure guaranteeing a defined temperature range (2–12˚C) was evaluated and implemented. According to this procedure, the blood sample and syringes are packed and placed in a temperature-controlled insulated cooling box which maintains its temperature at a constant range (Figure 4). Each insulated cooling box contains the blood bag or syringes, gel packs, and a data logger monitoring and recording the temperature during shipment.

Table 2. Results from experiments comparing culture dish coating with fibronectin or autologous plasma in the manufacturing of VesCell batches. The results indicate that the autologous plasma is similarly effective in supporting the production of VesCell.

	N	Fibronectin Average ± SE	Plasma Average ± SE	Ratio Plasma / Fibronectin	T test p Value
% Viability	18	95.6 ± 0.7	95.2 ± 0.8	99.5	0.351
No. ($x10^4$) HSC/Progenitor Cells/ml Blood	18	12.0 ± 1.9	10.5 ± 1.5	87.8	0.215

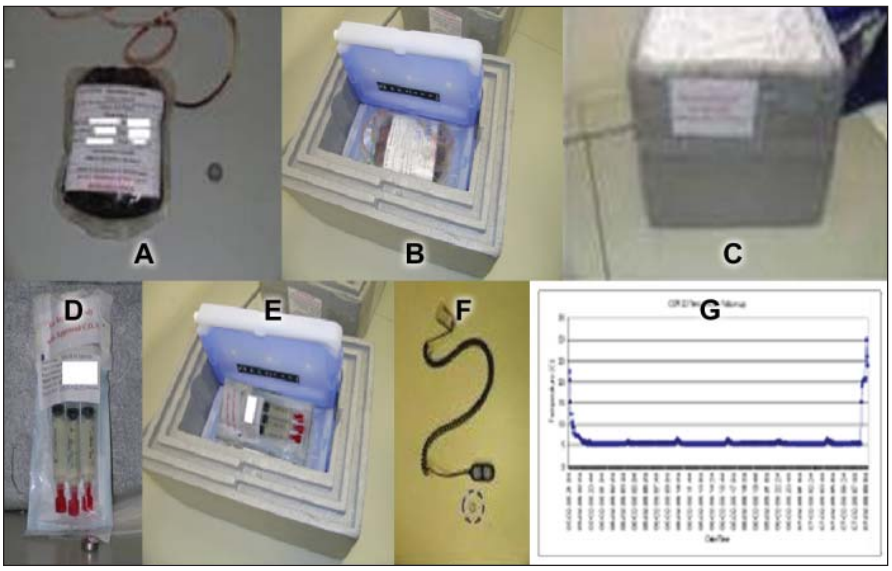

Figure 4. Schematic illustration of shipment conditions. A-C: Blood sample packaging for shipment; TheraVitae labels are placed on blood collection bags; data logger is attached to the blood bag; the bag is placed in a labeled cooling box for shipment to the manufacturing facility. D-E: Packaging is performed in a similar fashion for the transportation of the final product. F: The data logger (circled) is activated in order to monitor the temperatures during shipping; a computer interface cable is used to collect the temperature readings upon arrival at destination. G: Example of resulting shipment temperature log showing stable temperature during shipment.

Shipment and Stability of Blood Samples

Experiments evaluating the effect of shipment conditions were carried out on four blood samples from healthy Thai donors. Cell viability and the number of peripheral blood mononuclear cells (PBMCs) per 1 ml of blood were examined. Viability results of 97.4% ± 1.6% and PBMC yields of $1.5x10^6$ ± $0.5x10^6$ per 1 ml of blood obtained from these four batches were within the range of results acquired from 47 development batches obtained from the Israeli Blood Bank (viability of 99.2% ± 0.1% [p=0.79] and PBMC yield of $1.6x10^6$ ± $0.1x10^6$ per 1 ml of blood [p=0.34]). The conclusion from these experiments was that the blood is stable for up to 24 hours, and can be processed following air shipment from Thailand to Israel.

Shipment of Final Product

The limited shelf life of cellular products dictates that VesCell should be administered to the patient within a defined time period from the preparation of the final product (*i.e.*, from loading the cells into the syringes). Experiments performed on ten development batches were designed to test the stability of VesCell and to determine the product's shelf life. Harvested cells were packed in syringes and stored for 35 hours in an insulated cooling box. Cells were tested immediately after harvesting and following 35 hours of storage. The parameters examined were: a) viability; b) total number of cells; c) percentages of HSC/progenitor cells, assessed by the expression of the HSC marker CD34; d) percentages of ACPs, assessed by the expression of angiogenic lineage markers Tie-2, KDR and the concomitant expression of CD31 and uptake of AcLDL; and e) VesCell physiological activity assessed by tube formation assay and secretion of IL-8.

Results from these experiments (Table 3) show that the cells retained their features during the relatively long shelf life. Viability of 95.8% ± 1.0% and 96.1% ± 0.4% (p=0.749) and number of cells generated per 1 ml blood of $21.1x10^4$ ± $2.9x10^4$ and $16.6x10^4$ ± $2.5x10^4$ (p=0.249) were obtained at harvesting time and after storage, respectively. Percentages of cells expressing the HSC marker CD34 were 25.5% ± 3.5% at harvesting time and 22.4% ± 3.4% after storage (p=0.316). Percentages of ACPs co-expressing CD31 and uptake of AcLDL were 40.4% ± 6.5% at harvesting time and 41.8% ± 5.1% after storage (p=0.648). Likewise, similar levels of secreted IL-8 at time of harvesting and after storage were 44.8 ± 6.7 ng/10^6 cells/24h and 46.8 ± 5.6 ng/10^6 cells/24h (p=0.324) respectively, and comparable grades of tube formation, 3.7 ± 0.4 and 3.8 ± 0.4 (p=0.374) before and

Table 3. Results from experiments comparing VesCell characterization on before and after storage of 35 hours. The results indicate that VesCell was stable during preservation. (NS = not significant)

	N	Harvested Cells Average ± SE	35h Preserved Cells Average ± SE	% Recovery after 35h	T test p Value	
% Viability	11	95.8 ± 1.0	96.1 ± 0.4	100	0.749	NS
No. Cells ($x10^4$)/ml Blood	11	21.1 ± 2.9	16.6 ± 2.5	79	0.249	NS
% HSC	11	25.5 ± 3.5	22.4 ± 3.4	87	0.316	NS
% ACP	11	40.4 ± 6.5	41.8 ± 5.1	103	0.648	NS
IL-8 ng/10^6 Cells/ml/24h	9	44.8 ± 6.7	46.8 ± 5.6	105	0.324	NS
Tube Formation Grade	5	3.7 ± 0.4	3.8 ± 0.4	103	0.374	NS

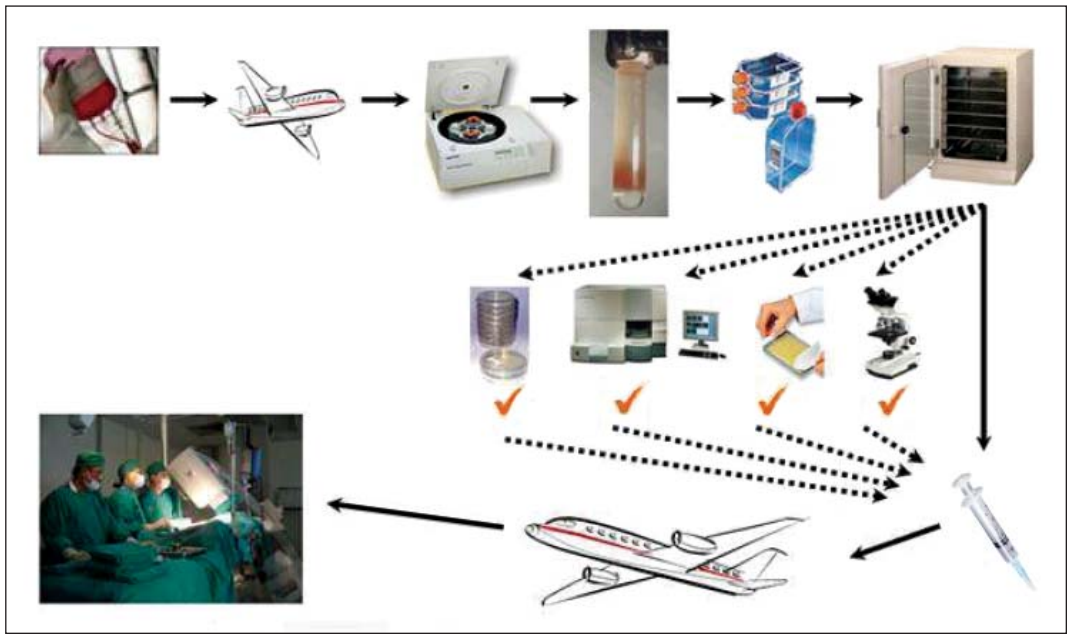

Figure 5. Schematic overview of the VesCell manufacturing process. Blood samples are transported to the manufacturing facility; cells are isolated by density gradients and cultured. Following incubation, cells are harvested, examined for viability, safety, identity, potency and purity. Pending passing these tests, the product is transferred to the hospital and administered to the patient.

after storage indicated the physiological activity was preserved. Development batches tested for sterility before and after 35 hours storage were all sterile and complied with tests release specifications. Thus, the shelf life of VesCell within syringes was determined to be 35 hours after packing.

In addition to determining VesCell's shelf life, its stability during administration was tested in order to assure that activities such as passage of cells through a catheter will not have a negative impact on product specifications. Experiments were done using ten development batches in which stored cells were examined before and following transfer through a standard injection catheter. High recovery of viability (98.7%) numbers per 1 ml of blood of HSC (79.8%) and ACPs (94.5%) indicated that cells administered to patients possess similar features to the harvested cells (Table 4).

The Manufacturing Process of VesCell

Blood samples of 250 ml are obtained from patients by routine venipuncture. The blood is flown to TheraVitae's manufacturing facility in Israel where it is processed. Low density cells are isolated using density gradients and cultured as described above to induce the generation of the ACPs. The final product, VesCell, is loaded into ready-to-use syringes. QC samples are tested for safety, viability, identity and potency, and pending passing release specifications, VesCell is released, flown to the hospital, and administered to the patient (Figure 5). The entire manufacturing process is carried out by two people—one of whom is the operator who carries out the majority of manual operations. The other is the supervisor (a more senior and experienced member of the staff) who is responsible for: a) receiving and identifying the blood sample; b) supervising the manufacturing process and solving any unexpected incidents that may impact the quality of the product; c) ensuring that all steps are carried out according to the SOPs and manufacturing instructions; d) reporting any deviations during the manufacturing process; and e) packaging the final product in a temperature-monitored, insulated cooling box.

Communication— Contact with Physicians

A key conclusion that was reached during process development and the clinical trial was the vital importance of maintaining a close dialogue with the treating physicians. As the cell manufacturing and administration processes are relatively complex on a logistical level, the physicians must always be kept informed of the timing of blood withdrawal and the arrival of the cell

Table 4. Results from experiments mimicking administration of the cells using catheter, testing the VesCell characterization before and after passage through a catheter. The results indicate that VesCell is stable during administration stages. (NS = not significant)

	N	Preserved Cells Average ± SE	Cells After Catheter Average ± SE	% Recovery	T test p Value	
% Viability	10	95.7 ± 0.6	94.5 ± 1.7	99	0.356	NS
No. ($x10^4$) HSC/ml Blood	10	8.0 ± 1.5	6.4 ± 1.5	80	0.058	NS
No. ($x10^4$) ACP/ml Blood	10	1.1 ± 0.4	1.1 ± 0.5	95	0.792	NS

product at the hospital. The administration of VesCell to a patient is allowed only if accompanying COA and batch release statement indicate that the results of all tests have passed the release criteria.

Summary

VesCell is an autologous stem/progenitor cellular product composed of non-mobilized, blood-derived cell populations of HSC/progenitor cells and ACP lineage cells.

VesCell development was carried out with the objective of generating a product which is: a) safe and effective; b) prepared from non-mobilized peripheral blood; c) stable, with a relatively long shelf-life; and d) ready-to-use and easily utilized by the physician. The development of VesCell required an in-depth understanding of the biological characteristics of the product and the establishment of a tightly controlled manufacturing process.

The resulting consistent and reliable manufacturing procedure of VesCell enabled its evaluation in preclinical and clinical studies designed to assess its safety and efficacy (manuscripts in preparation).

We hope that the experience gained during VesCell development will facilitate making the cell therapy available to an ever larger number of patients who are in need of novel, safe and effective treatment for hitherto incurable diseases.

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