The Ministry of Health and Social Development of the
Russian Federation
Ministry of Education and Science of the
Russian Federation

February 5, 2012

Dr. Paolo Macchiarini

Tracheal Transplantation
Clinical Trial Protocol
in "Molecular and Cellular Biology, Biotechnology, Regenerative Medicine"
as part of the Russian Government Grant Federation for governmental support of scientific
research conducted under the supervision of leading scientists at Russian institutions of
higher educational training according to contract "19" October 2011 N2 11.034.31.0065
between the Ministry of Education and Science of the Russian Federation and the State
Budgetary Educational Institution of Higher Professional Education "Kuban State Medical
University," the Ministry of Health and Social Development of the Russian Federation and
the leading scientist Paolo Macchiarini performing scientific research for the time period
October 19, 2011 to December 31, 2013
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1.0 Clinical doctors - researchers

1.1 Leading researcher: Dr. Paolo Macchiarini, Karolinska Institutet, Stockholm, Sweden.

1.2 Clinical research coordinator: Professor Vladimir Porkhanov A., chief physician GBUZ "Krasnodar Regional Clinical Hospital No. 1, n.a. S.V. Ochapovsky Department of Health Care Krasnodar Krai.

1.3 Other researchers: Ph.D. Polyakov IS, Ph.D. IA Pashkov, Gilewicz IV, Fedorenko TV.

1.4 Research centers / clinics:
Clinics: State budget institution of Higher Professional Education "Kuban State Medical University" Ministry of Health and Social Development Federation Krasnodar Regional Clinical Hospital No. 1, n.a. S.V. Ochapovsky Department of Health Care Krasnodar Krai.


2.0 Monitorering system
This protocol is designed as a request for permission to transplant trachea as an intraoperative solution for obstructive tracheal tumors and other conditions requiring replacement of the native trachea (table 1). The procedure involves the use of bioengineered synthetic scaffold seeded with autologous mononuclear cells, which is considered to be the only treatment option in some patients. Lead researcher, Dr. Paolo Macchiarini, will oversee the process with the assistance of a team of doctors and researchers who, together with the funding organization, will be responsible for monitoring of the patients before, during and after the procedure. These data are recorded in accordance to the requirements adopted for individual registration cards, which can be individually reviewed by an independent data monitoring committee or similar committee.
3.0 Introduction / brief overview

Tracheal transplantation is the only therapeutic alternative when endoscopic and other examinations shows that localization and extension of the obstruction (approximately 6 cm or more than 50% of the total length of the airway) make it impossible to perform surgery to remove the abnormal segment with adequate remaining length of healthy airway (table. 1). In the interim, patients can get temporary relief by endotracheal curettage and / or by inserting a T-tube into the trachea to maintain an open airway, but without surgical transplantation the disease will usually lead to death of the patient.

<table>
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<tr>
<th><strong>Type of Disease</strong></th>
<th><strong>Rationale</strong></th>
<th><strong>Contraindications to Transplantation</strong></th>
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<tbody>
<tr>
<td>Primary malignant tracheal tumors (benign and malignant)</td>
<td>Extension of the affected area beyond the limits for standard trachea resectability *</td>
<td>The presence of systemic metastases and mediastinal lymph nodes (malignant tumors); Conventional functional and psychological contraindications</td>
</tr>
<tr>
<td>Tracheal and esophageal fistula</td>
<td>Tracheo-esophageal defect exceeding the limits for standard trachea resectability</td>
<td>Malignant neoplasm</td>
</tr>
<tr>
<td>Tracheal stenosis</td>
<td>Extension of the affected area beyond the limits for standard resectability</td>
<td>Conventional functional and psychological contraindications</td>
</tr>
<tr>
<td>Tracheobronchial malacia (primary or secondary)</td>
<td>Extension of the defect area beyond the limits for standard trachea resectability treatment (luminal or stenting dilatation)</td>
<td>Conventional functional and psychological contraindications</td>
</tr>
</tbody>
</table>

*(6 cm of the entire length of the respiratory tract)*

The proposed protocol involves replacement of the trachea of terminal patients by transplantation of bioengineered synthetic skeleton, seeded with autologous mononuclear cells.
3.1 Special training / experience

In addition to surgical methods for trachea transplantation the protocol requires the knowledge and the experience of preparation of autologous cells, as well as the cell seeding procedure of bioengineered synthetic scaffolds. Operation and appropriate training will be carried out by the lead researcher; cell preparation is monitored by a specialist from Karolinska Institutet, Stockholm, Sweden.

4.0 Approval of the clinical trial protocol

The protocol and the informed consent were approved by the Ethics Committee of the Kuban State Medical University at the meeting on December 21, 2011 (protocol no. 8). The new version of the protocol, the study booklet, voluntary informed consent and the patient registration card were approved by the Ethics Committee of the Kuban State Medical University at the meeting of February 15, 2012 (protocol no. 9) and by the local Ethics Committee at GBUZ "Krasnodar Regional Clinical Hospital No. 1, n.a. S.V. Ochapovsky Department of Health Care Krasnodar Krai" University on January 24, 2012 (protocol no. 45).

5.0 Brief description of previous research in humans

Clinical success of similar operations with transplantation of artificial tracheobronchial airway, performed at Karolinska University Hospital in Stockholm, Sweden, on June 6, 2011 and November 17, 2011, have shown that tracheobronchial transplantation using bioengineered nanocomposite scaffold and autologous mononuclear cells can offer the only chance for the recovery in incurable patients.¹

Previous studies have confirmed the ability of mononuclear cells to stimulate the migration of peripheral blood stem cells into the different tracheal layers and make them differentiate

into respiratory epithelium and cartilage cells.\textsuperscript{2} In carrying out this transplantation procedure, we can not only fully remove the affected airway, but also give the patient an optimistic chance for cure and normal quality of life.

\textbf{Fig. 1:} Protocol for tracheal transplantation procedure, the first bioscaffold for transplant of artificial bioengineered trachea nanocomposite (PET bioscaffold).

\textbf{Description}

Preoperative procedures:
- Computed tomography of the patient 3 weeks prior to transplantation
- Patient data is delivered to the companies Nanofiber Solutions (Columbus, Ohio) and Harvard Apparatus (and other manufacturers) for fabrication of individual nanocomposite and bioreactor.
- Admission of the patient to the hospital (3/4 days before surgery), bone marrow collection and processing
- Allocation mononuclear cells
- Dynamic seeding of bioscaffold inside the InBreath bioreactor at 1.5 rev / min for 48/72 hours.

Intraoperative procedures:
Preparation of graft: an injection of granulocyte colony stimulating factor G-CSF, erythropoietin, transforming growth factor beta-3, insulin, dexamethasone and parathyroid hormone.
Transportation of the transplant to the operating room

Transplantation

Postoperative procedures:
The patient receives the drugs (systemic application): recombinant G-CSF factor analogues and synthetic analogs of erythropoietin every second day for two weeks after transplantation.
Transplant. Blood sampling is performed daily.

To date, two tracheal transplantations with bioengineered synthetic scaffolds have been successfully performed by Dr. Macchiarini together with colleagues from Karolinska Institutet in Stockholm, Sweden. In the first operation (June 2011) a nanocomposite bioengineered synthetic scaffold made of POSS-PCU (polyhedral oligomeric silsesquioxane) was used, while in the second operation (November 2011) a nanocomposite bioengineered synthetic scaffold made of PET (polyethylene terephthalate) was used. In both cases luminal ingrowth with healthy cells of respiratory epithelium was observed.

Fig. 2 shows the bronchoscopy results with respiratory epithelial cells after the operation in November 2011 with a bioengineered synthetic PET-nanocomposite bioscaffold was used, which is the same type of bioscaffold which is proposed in this protocol. The bronchoscopy and the pattern of stained cells show the presence of a normal mucosa in the bioscaffold at one week after transplantation.

**Fig. 2:** A. Bronchoscopy of the PET-nanocomposite trachea one week after implantation (November 2011), showing presence of a normal mucosa on the bioscaffold. B. Ciliated respiratory epithelial

Tracheal transplantation research plan

Confidential

Version A
cells (white arrow) obtained from a brush biopsy from the center of the transplanted trachea one week after implantation. The fact that this biopsy was done almost immediately after transplantation, allows us to assume that the cells derive from the differentiated stem cells, and not from the spread of normal epithelial cells from the proximal or distal end of the transplant.

6.0 Rationale for not cancelling

In the previous research it has not been any negative effects or complications which would cause rejection of the proposed research plan (protocol).

7.0 The procedure for obtaining voluntary informed consent

Patient information is presented by informed consent (attached to the protocol). The form of informed consent for reference is issued to patients before the procedures and is necessary for inclusion in the study protocol. The patient is given the opportunity to familiarize themselves with the content of the informed consent in a separate room or bring it home to familiarize themselves with it, get answers to their questions and sign with the date and time of signing. All preparing of the patient for transplantation provided by the protocol starts after the agreement has been signed. Researchers register the date and time of its completion in the patient card.

8.0 General study plan

The tracheal transplant procedure will be performed sequentially, as shown in Fig. 1

"Protocol for tracheal transplantation procedure, transplantation for the first time applied for the with artificial bioengineered trachea nanocomposite (PET bioscaffold)".

Preoperative assessment will include the following procedures:

- Tomography of the neck and chest including a three-dimensional reconstruction of the respiratory tract.
- Rigid / Flexible fiberbronchoscopy.
- Evaluation of cardiac function (scanning with thallium during exercise).
- Assessment of respiratory function (spirometry).
- Analysis of blood, including blood coagulation factors.
- Evaluation of liver and kidney function.
- Immunogenic assess of peripheral blood sample for the determination of the phenotype HLA and serologic infections (HIV, syphilis, EBV, etc.).
• Evaluation of the patients hematopoietic stem cells baseline levels; approximately 30 ml of peripheral blood will be taken at admission for assessment basic level of hematopoietic stem cells, and a portion of this sample is frozen for further analyzes.
• Evaluation of endogenous baseline erythropoietin levels in the peripheral blood. In case of successful preoperative evaluation bone marrow samples will be selected for approximately 1-4 weeks prior to surgery.

**Harvesting of mononuclear cells from bone marrow and seeding of the bioscaffold**

[After 48 or 72 hours before transplantation depending on the necessary shape of bioscaffold (tubular or bifurcated shape)].

• The procedure will be performed under general anesthesia.
• Around 250-300 ml of bone marrow (BM) will be aspirated. BM will be passed to the Department of Hematology or other department to isolate mononuclear cells (MNCs).
• Cell medium DMEM (Dulbecco Modified Eagle´s Medium) * + autologous serum (10%) + antibiotics.
• Peripheral blood sample (50 ml) will be aspirated with heparin (operating) and transferred to the laboratory for cell culture in compliance with good manufacturing practices. These MNCs will be isolated and frozen in liquid nitrogen.
• Bronchoscopy and bronchoalveolar lavage (BAL). It is necessary to keep the BAL (take away the supernatant, add PBS, pelleted by cell centrifugation and freeze).
• Bioreactor will be sterilized beforehand (locally with using plasma sterilization according to manufacturer).
• Synthetic bioscaffold will be sterilized with alcohol (ethanol) or by gamma-irradiation, and then incubated in cell medium in 2 hours before adding cells.
• Necessary materials: stitching and forceps, sterilized scissors
• Incubator.
• Seeding and growing the cells on a synthetic bioscaffold (scanning electron microscopy (SAM), microscopic studies of living cells, histology).

* - environment and their manufacturers may differ.

48 hours before transplantation:
The patients receive treatment to stimulate mobilization of cells by intravenous injection of recombinant granulocyte colony stimulating factor (Filgrastim, 10 μg/kg, not more than 30 million IU), and erythropoietin (EPO alpha or beta, not more than 40000 IU) \(^3\)\(^4\)\(^5\).
The patient will receive full and accurate information through the informed consent form, orally and in writing, about the risks of the therapeutic procedure.

Cell preparation procedure
Bone marrow separation and further manipulation
72/48 hours before transplantation (depending on the desired shape of the bioscaffold: tubular or bifurcated) bone marrow samples (BM) will be selected by bilateral repeated aspiration from the iliac crest, general volume 250-300 ml. This procedure will be carried out under general anesthesia and lasts about 20 minutes.

Explanted aspirate will be transferred to the hematology unit (or to another department) to isolate mononuclear cells (MNCs). MNCs are obtained by Ficoll gradient separation, at density 1.077 g/ml. After separation the cells are washed three times with saline (with the addition of 5% human albumin) to remove the residual ficoll and left in a solution consisting solely of components approved for clinical use. The whole procedure will be carried out in a closed system (Sepax 3 Biosafe America Inc, Houston,

Texas) to ensure sterility and a complete automatic process⁶. Isolated MNCs are transferred into a bag with 600 ml medium (Dulbecco’s Modified Eagle Medium [DMEM 10% human albumin) and transported from operation at temperature 4°C to a laboratory working under the principles of GMP for filling synthetic bioscaffold (sterilized by ethanol or gamma radiation). After incubation (DMEM) for 2 hours the bioscaffold will be secured in the bioreactor. MNCs (medium DMEM) are seeded on the surface of the graft. Then corresponding medium and growth factors is added (10μg/cm² of recombinant human transforming growth factor β-3 (R & Systems, Minneapolis, Minnesota, United States), 10 nmol/l recombinant parathyroid hormone-related peptide (PeproTech), 100 nmol/l dexamethasone and 10 μg/ml insulin (Sigma-Aldrich, Dorset, United Kingdom). The bioreactor is placed into an incubator running with an initial rate of 1 cycle per minute for 18 hours, then the speed is gradually increased to 1.5 cycles per minute. After 48/72 hours the chamber is placed in a sterile container and carefully transferred to the operating room.

Isolated MNCs will be checked for the following indicators:

- The number of mononuclear cells (MNCs): the minimum amount of 2x10⁶ cells/ml.
- Cell viability by fluorescence microscopy analysis (7-AAD): range 94-98%.
- Evaluation of mesenchymal progenitor cells CFU-F.
- Evaluation of hematopoietic progenitor cells CD34+.

Altogether 15x10⁶ cells are placed in three separate cryo-vials and frozen in DMSO, in accordance with the standard procedure for quality control analysis. The sterile graft is then re-inoculated with

cells in the operating room immediately before implantation (see Transplantation airway, page 14).

**Artificial nanocomposite airway transplantation**

The company Nanofiber Solutions (Doctor Jed Johnson, Columbus, Ohio) developed the graft trachea and tracheobronchial airways which is made of polyethylene terephthalate (PET). PET has been used successfully for more than 10 years for production of components to surgical implants and medical devices, ranging from non-absorbable suture thread including vascular transplants and orthopedic implants. Proposed polymer for the manufacture of a tracheal transplant is the biologically non-absorbable polyethylene terephthalate, that has been transformed into nanofibers (intermediate diameter of 350 nm), embedded in a semicircular spacer made of the material Dacron and forms a nanocomposite being completely biocompatible having the nanofiber structure of a natural trachea (Fig. 3 and 4).

![Transformed nanofibers](image)

**Fig. 3.** Transformed nanofibers with a diameter in the range 300-400 nm.
Recent published studies have shown that three-dimensional bioscaffold of PET is fully biocompatible with human hematopoietic cells and may even encourage the expansion of cells CD34+\(^7\). Additional data biocompatibility can be found in the Appendix at the end of the procedure description.

Extensive research in vitro and long-term in vivo PET research, in vivo on toxicity and biocompatibility have been conducted previously\(^7,8,9,10,11,12,13,14\). Furthermore,


biocompatible nanocomposite has been successfully used for transplantation, which was done in Sweden, at Karolinska University Hospital in November 2011. In the November study mononuclear cells were isolated from bone marrow aspirates and plated on bioscaffold via plasma sterilized bioreactor, which is described below. The results showed improved survival rate of cells (increased number of cells, improved orientation and the accumulation of extracellular matrix) in the PET bioscaffold compared to the POSS bioscaffold (Fig. 5 and 6).

Fig.5: Structure of PET-nanocomposite after seeding with autologous progenitor cells

Fig. 6: Bioscaffold of PET gives a higher cell acceptability compared to bioscaffold of POSS - PCU.

We offer manufacturing of synthetic bioengineered trachea transplant for individual patients based on the results of recent computed tomography and endoscopic studies; the graft will be made of polymeric nanofibers transformed (Nanofiber Solutions®, Columbus, Ohio, United States), having mechanical and structural properties that mimic the natural respiratory tract (fig. 4). The company Nanofiber Solutions will manufacture the cartilage rings of the trachea with mechanical properties similar to those of a natural trachea with its resistance of mechanical collapse. The cartilaginous rings are sandwiched between the nanofibers and placed at regular intervals in a special form, exactly reproduced after the shape of the patient’s trachea, and then transformed nanofibers will be used to cover each ring inside and outside, respectively.
The company Nanofiber Solutions does not see any problems in terms of manufacturing the device. As previously described, the inert nature of the polymer, combined with biomimetic topography transformed graft nanofibers provides the necessary surface properties for improved engraftment of cells, including mononuclear and epithelial cells specific to the trachea.

**Bioreactor InBreath**

This protocol includes the design of the bioreactor design previously used by our group during the first successful human implantation of a bioengineered trachea. The device, known by the market name InBreath 3D Organ Bioreactor (Harvard Bioscience, Holliston, Massachusetts), is placed inside the incubator for cell culture and consists of a single chamber of polysulfone, where the artificial organs are placed, as well as motor and remote control. Information about the materials used for the construction of the bioreactor can be found in Appendix 2.

The bioreactor InBreath chamber is easily separated from the motor block and can be subjected to plasma sterilization. The motor provides constant rotation of the cellular basis inside the chamber, thereby providing controlled effect of the hydrodynamic forces on the growing tracheal transplant. Protective chassis fully covers the brushless electric motor, protecting it from the corrosive action of moisture generated inside the incubator (fig. 7). Remote controller placed outside of the incubator, giving the possibility to adjust the speed rotation, without affecting the incubator.
According to this protocol, the synthetic bioscaffold will be specially manufactured for a patient using the synthetic material (PET). Both internal and external surfaces of the bioscaffold will be seeded with autologous undifferentiated mononuclear cells in the bioreactor.

**Seeding cells on bioscaffold**

1. Isolated MNCs are placed in a bag containing 300 ml DMEM (added with 10% human albumin) at temperature 4°C for transporting from the operation room to a laboratory working under the principles of GMP (Good Manufacturing Practice).
2. Bioscaffold (sterilized by ethanol or gamma radiation), bioreactor (plasma-sterilized) and surgical instruments (autoclaved) will be delivered in to a sterile room for cell culturing.
3. All persons who perform the manipulation of cells, bioreactor and bioscaffold must comply with Good Manufacturing Practices, including having sterile gloves, special protective clothing, etc.
4. The bioreactor is opened in a fume hood under sterile conditions and placed onto a sterile surface.
5. After that, the researcher must use a new pair of sterile gloves.
6. The bioscaffold will be removed from the primary sterile packaging and secured within the bioreactor at the respective fixtures.

7. After the bioscaffold has been docked within the bioreactor, MNCs (+ DMEM) will be seeded on the surface of bioscaffold.

8. 250 ml volume of medium (with the addition of autologous plasma and human albumin) will be added to the bioreactor chamber.

9. The following ingredients are added to the medium (see Appendix 3 "List of biological agents and characterization TGF β-3"): 10 mcg/ml of recombinant human transforming growth factor β-3 (R & D Systems, Minneapolis, Minnesota, United States), 10 nmol/l of recombinant parathyroid hormone-related peptide (PeproTech), 100 nmol/l dexamethasone, 10 µg/ml of insulin (Sigma-Aldrich, Dorset, United Kingdom). *(Note: hereinafter products from other manufacturers may be used than the products from companies which have been used in previous transplants referred to).*

10. Thereafter, the bioreactor will be placed in an incubator and the chamber closed (containing the bioscaffold, MNCs + 250 ml of medium).

11. NOTE: a bifurcate tracheobronchial bioscaffold does not perfectly fit to the shape of the chamber and this can lead to dynamic tension in the bioreactor. This tension (shear) will be transmitted through the external connection to the electric motor and can lead to termination of the chamber rotations. In that case, the bioreactor must manually be monitored every 20 minutes. In the case of a low speed rate due to influence of dynamic tension, it is possible to balance the chamber with an object to ensure continuous communication between these two components. In the event of a significant dynamic tension (shift), it is necessary to understand additional action to secure the bioscaffold. As a rule, such an event is adverse for tubular bioscaffolds.

12. The bioreactor is started with an initial speed of 1 rpm for 18 hours and then the rate will gradually be increased to 1.5 rpm.
13. After 24 hours, 50ml of the above medium is added into the chamber to a total volume of 300 ml.

14. After 48/72 hours, the chamber will be placed in a sterile container and gently moved to the operating room.

**Transplantation of the airway**

The patient will be subject to general anesthesia and intubated with an endotracheal tube. Any existing t-tube will be removed. The patient will also be subject to bone marrow aspiration, according to the procedure for preparing cells (see above). Median sternotomy will be carried out in supine position. After resection of the damaged airway segment the airway graft will be introduced (conditioning) with growth factors, including 10 ng/ml recombinant human transforming growth factor β-3 (R & D Systems, Minneapolis, Minnesota, United States), 10 nmol/l recombinant parathyroid hormone-related peptide (PeproTech), 100 nmol/l dexamethasone, 10 μg/ml insulin ( Sigma-Aldrich, Dorset, United Kingdom, G-CSF (of 1 μg/kg) and erythropoietin (40000 IU), to stimulate the mobilization of peripheral hematopoietic cells. The graft will be adjusted in size, and then proximally and distally anastomosed to correct the defect of the respiratory tract by using non-absorbable sutures, such as Cardionyl 3/0 (Peters Surgical). The graft will then be covered by major omental flap wrapping (vascularized adipose tissue is separated from large stomach bend and the right gastro-omental artery and diaphragmatically or substernal transferred to the mediastinum) to provide long-term protection of the graft anastomoses, and indirect stimulate neovascularization of the graft.

**Sterility tests**

The risk of bacterial/fungal medium contamination will be assessed be microscopic examination prior to the transplantation. The quality of all components and medium are controlled by the manufacturers, and remain sealed until the cell seeding procedure.
Subsequent quality assessment of the medium is carried out by completion of the bioengineering process (Table 2).

**Table 2 - Tests and acceptance criteria**

<table>
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<th>Анализ</th>
<th>Критерий приемлемости / Метод</th>
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</thead>
<tbody>
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<td>Стерильно / Критерии стерильности (Бпр. Ф. 2.6.1)</td>
</tr>
<tr>
<td>Эндотоксины</td>
<td>&lt;0,5 эндотоксиновых единиц/мл / Лал-тест</td>
</tr>
<tr>
<td>Микоплазма</td>
<td>Микоплазма не обнаружена / Культивация</td>
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</table>

*British Pharmacopoeia Volume IV. Appendix XVI a. Test for Sterility*

A sample of the culture medium with the culture of MNCs will be added to the vial cultivation for growing blood cells shortly before the incubation procedure. The appearance of any foreign cultures in these samples within the next 48-72 hours will be deemed to be a significant event and may result in termination of the entire procedure. During the incubation period in the bioreactor, 24 hours after opening of the bioreactor, fresh medium is added to the growing blood cells and samples of fluid from the bioreactor are incubated to check for contamination. On the day of surgical implantation, as mentioned above, the neotracea is evaluated for cell growth and coating of the bioscaffold surface; at this point, samples from the medium from the bioreactor are sent for STAT analysis and Gram-staining, as well as being stored in vials for cultivating blood cells. If Gram staining gives a negative result, the graft will be considered as microbiologically sterile and ready for implantation into the patient’s body. A small piece of the graft will be selected in the operating room prior to implantation for analysis of cellular culture. It will be placed in a tube for a standard smear and subjected to the standard analysis for [...] fluid from the wound. All samples will be analyzed a total of 14 days to evaluate the availability microbacteriological and fungal infections. Certificates of analysis will be included in the reports.
**Postoperative procedures**

To stimulate the process of regeneration in the postoperative period, the patient will receive pharmacological agents with following systemic injections:

a) Recombinant analogues of G-CSF (Filgrastim, 10mcg/kg/day, no more than 30 mcg/kg/day)

b) Synthetic analogues of erythropoietin (EPO alpha or beta, max 40000 IU).

Both of these factors will be administered in adequate concentrations (in reduced doses not associated with any side effects) for stimulating mobilization and transformation of progenitor stem\(^3,4,5,15,16\) and bone marrow cells […] day automatic controlled plasma erythropoietin level and calculation of whole blood (including leukocyte blood count). Levels above 50000-60000 cells in the blood will be considered a manifestation of toxicity and as a result will be reduced dose or discontinued. The treatment is carried out every other day for two weeks after transplantation.

**Follow-up**

Follow-up will include:

- Endoscopy (flexible or rigid bronchoscopy) of the transplanted respiratory system every day or every other day (when clinically indicated) during the first week and at least once before discharge from the hospital.
  
  Bronchoscopy is performed on a monthly basis for the first six months and then every 6 months for the first 5 years. Samples from the respiratory airway mucosa shall be collected and stored for quality analysis.

- Counting of blood cells, (including leukocyte blood count) every day during the first two weeks.


- Evaluation and calculation of mobilized progenitor cells according to the graph below:

- Assessment of immunogenicity. After 3, 7 and 30 days after transplantation blood samples will be taken for analysis of HLA by OIA antibodies. Subsequent immunogenic studies will also be performed on 3, 6 and 12 months after transplantation.
- Computer tomography of the neck and chest with three-dimensional reconstruction of the transplanted airway will be done during the first, third, and sixth months during follow-up, and then every 6 months within the first 5 years.
- Subsequent cancer surveillance in children will be performed throughout the patient’s life and include standard examinations.
9.0 Expected risks

The positive effect of this operation is supposed to be greater than the risk since this procedure may be the only possible chance of cure for some patients. Training will be held in appropriate cell laboratory in compliance with good manufacturing practices. In addition, numerous sterility tests will be carried out on all cells and materials before tracheal transplantation. If the sterility of cells and materials will be called into question, which seems unlikely, the whole procedure will immediately be terminated.

10.0 Adverse events

Possible complications of the transplant are postoperative bleeding, injury to the nervus recurrence, respiratory infections, anastomotic leak, wound infection, respiratory failure and the need for mechanical ventilation. All adverse events should be documented and communicated to the lead researcher, the sponsor and the Ethics Committees of the University and the Hospital. Address and phone number of the clinic for emergency GBUZ "Regional clinical hospital (N) 2 (l) S.V. Ochapovsky Department of Health Care Krasnodar Krai "at the address: 350086, Krasnodar, street. May Day, d. 167, Tel.: (861) 252-73-02, 260-35-11, Head of the Oncology Department, doctor of higher category, PhD Polyakov, Igor Stanislavovich

11.0 Introduction of amendments

The clinical trial plan cannot be changed without the written permission from the lead researcher, the funder, the Ethical Committee of the University and the Clinic. Amendments may require regulatory approval prior to their entry into force. All the amendments to the protocol, the patient’s informed consent map, must be approval by the Ethical Committee of the University and the Clinic.
12.0 Publication policy

The results of this research can be used for publication.

13.0 Individual registration card (separate application)
Appendix 1: Biocompatibility, acceptability / cell proliferation and mechanical characteristics of PET medical devices available on the market at present time.

**Bioscaffold material:** polyethylene terephthalate (PET) is not absorbable.

**Summary on biocompatibility:** polyethylene terephthalate has for more than a decade successfully been used in fabrication of numerous of surgical implants and medical devices, from intracardiac and vascular grafts to threads for surgical sutures. The company Nanofiber Solutions (Columbus, Ohio) has developed tracheal and tracheobronchial bioscaffolds imitating the nanofiber structure of the natural trachea (Fig. 8). These bioscaffolds are made of non-absorbable polyethylene terephthalate transformed into nanofibers with an mean diameter of 350 nm.

![Fig. 8. Electron micrograph scanning of purified human tracheal cells (A) and artificial PET trachea manufactured by the company Nanofiber Solutions (B).](image)

An artificial tracheobronchial graft using bioscaffold from PET-material has already been successfully transplanted at Karolinska University Hospital, Stockholm, Sweden, in November 2011. The clinical success of this operation indicates that the tracheobronchial graft of bioengineered nanocomposite and autologous mononuclear cells may be the only chance of cure for some patients.

We propose to use the same material for the bioscaffold and the same procedure for its production, which has been successfully used for the tracheal transplantation in November,
2011, using this Protocol. The polymer nanocomposite PET has carefully been studied on cell compatibility, and recent surgery, performed at Karolinska University Hospital, demonstrated its acceptability, ability to allow for proliferation of autologous mononuclear cells and early (7 days) re-epithelialization with respiratory epithelium.

**Conclusion:** information on the biocompatibility of implantable prostheses made of PET-material such as spinal cord, esophagus and cardiac valves is provided in the table below. All data confirm excellent biocompatibility when using the material for medical implants for vital organs. These data, and also the successful tracheal transplantation in a patient in November 2011, have shown excellent biocompatibility, allow concluding that the proposed bioscaffold material meets the requirements of biocompatibility and is safe for use.

**Data on biocompatibility of medical devices made from PET, at the present available on the market.**

<table>
<thead>
<tr>
<th>Тест на биосовместимость Per ISO 10993</th>
<th>Критерии приемлемости</th>
<th>Ссылка #1— спинальный имплантат Zimmer Dynesys (FDA 510(k) KO92234)</th>
<th>Ссылка #2— эндоскопический имплантат Enteryx™ для лечения гастрозофагеальной рефлюксной болезни (FDA PMA P020006)</th>
<th>Ссылка #3— сердечный чрескатетерный клапан Edwards Sapien™ (FDA PMA P010041)</th>
<th>Ссылка #4— Capentier-Edwards S.A.V.™ биопротез, модель 2650 (аортальный) (FDA PMA P10044)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Цитотоксичность</td>
<td>0, 1 или 2 (нетоксично)</td>
<td>СООТВЕТСТВУЕТ (Отсутствие признаков токсичности)</td>
<td>СООТВЕТСТВУЕТ (Отсутствие признаков токсичности)</td>
<td>СООТВЕТСТВУЕТ (Отсутствие признаков токсичности)</td>
<td>СООТВЕТСТВУЕТ (Отсутствие признаков токсичности)</td>
</tr>
</tbody>
</table>


18 Medical Technologies Enteric FDA Summary of Safety and Effectiveness, 2003, for PO20006: [http://www.fda.gov/ohrms/dockets/ac/03/briefing/3921/SSED.pdf](http://www.fda.gov/ohrms/dockets/ac/03/briefing/3921/SSED.pdf)


<table>
<thead>
<tr>
<th>Тест на биосовместимость</th>
<th>Критерии приемлемости</th>
<th>Ссылка #1 — спинномозговой имплантат Zimmer Dynesys (FDA 510(k) K092234) — Компонент спинного мозга</th>
<th>Ссылка #2 — эндоскопический имплантат Enteryx™ для лечения гастрозофагеальной рефлюксной болезни (FDA PMA P020006)</th>
<th>Ссылка #3 — сердечный резектационный клапан Edwards Sapien™ (FDA PMA P010041)</th>
<th>Ссылка #4 — Carpentier-Edwards™ S.A.V.™ биопротез, модель 2650 (аортный) (FDA PMA P10041)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Острая системная токсичность</td>
<td>Тестируемый имплантат должен продемонстрировать ≤ биологическую реакцию по сравнению с контрольной группой (мышь); &lt; 2 мышей могут иметь признаки токсичности; &lt; 3 мышей могут продемонстрировать потерю веса &gt; 2 г</td>
<td>СООТВЕТСТВУЕТ (не наблюдается признаков токсичности и потеря веса &gt; 2 г)</td>
<td>СООТВЕТСТВУЕТ (удовлетворяет критерию FSP, USA (USP))</td>
<td>СООТВЕТСТВУЕТ (Показано изменений в течение 72 часов)</td>
<td>СООТВЕТСТВУЕТ (негативно)</td>
</tr>
<tr>
<td>Тест на раздражение</td>
<td>Показатель основного первичного раздражителя (РПИ) Шкала от 0 — 0,4 (незначительный); не вызывает раздражения</td>
<td>СООТВЕТСТВУЕТ (не вызывает раздражения; PII: категория раздражения — 0)</td>
<td>СООТВЕТСТВУЕТ (удовлетворяет критерием USP)</td>
<td>СООТВЕТСТВУЕТ (не вызывает раздражения)</td>
<td>СООТВЕТСТВУЕТ (не вызывает раздражения)</td>
</tr>
<tr>
<td>Тест на мутагенность</td>
<td>Менее, чем двукратное увеличение числа ревертантных колоний на чашку по сравнению со средним числом колоний контрольной группы для каждого штамма</td>
<td>СООТВЕТСТВУЕТ (всемутагенный; менее, чем двукратное увеличение для каждого штамма)</td>
<td>СООТВЕТСТВУЕТ (негативные результаты)</td>
<td>СООТВЕТСТВУЕТ (негативные результаты)</td>
<td>Не проводился</td>
</tr>
<tr>
<td>Тест на биосовместимость</td>
<td>Критерии приемлемости</td>
<td>Ссылка #1 — спинной имплантат Zimmer Dynesys (FDA 510(k) K092234)</td>
<td>Ссылка #2 — эндоскопический имплантат Edwards Sapien™ для лечения гастроэзофагеальной рефлюксной болезни (FDA PMA P02006)</td>
<td>Ссылка #3 — сердечный клапан Edwards Sapien™ (FDA PMA P10041)</td>
<td>Ссылка #4 — Carpentier-Edwards™ S.A.V.™ биопротез, модель 2650 (аортный) (FDA PMA P10041)</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------------</td>
<td>-------------------------------------------------------------</td>
<td>-------------------------------------------------------------</td>
<td>-------------------------------------------------------------</td>
<td>-------------------------------------------------------------</td>
</tr>
<tr>
<td>Аллергическая проба</td>
<td>Класс &lt;1 или отсутствие кожной аллергической реакции, превышающей реакцию контрольной группы. С использованием модели Magnsson-Klingman, Класс 1, Класс аллергической реакции «Слабый аллерген»</td>
<td>СООТВЕТСТВУЕТ (реакция не превышает 0; аллергическая реакция 0%; классифицируется как Класс 1 (Слабый аллерген))</td>
<td>СООТВЕТСТВУЕТ (Класс 1: Слабая реакция, эквивалентна негативному контролю)</td>
<td>СООТВЕТСТВУЕТ (не вызывает аллергической реакции)</td>
<td>СООТВЕТСТВУЕТ (не вызывает аллергической реакции)</td>
</tr>
<tr>
<td>Тест на пирогенность</td>
<td>Температурная разница не превышает 0,5° от исходной температуры через 1-3 часа после введения</td>
<td>СООТВЕТСТВУЕТ (непирогенный; у животных не наблюдалось повышения температуры более чем на 3 градусов Цельсия)</td>
<td>Не проходила</td>
<td>СООТВЕТСТВУЕТ (не было зарегистрировано повышения температуры)</td>
<td>Не проводился</td>
</tr>
<tr>
<td>Тест на генотоксичность/мутации клеток мlekопитающих</td>
<td>Немутагенный, если изолированная культура тестируемого образца имеет частоту мутаций менее, чем двукратное увеличение для раствора контрольной группы.</td>
<td>СООТВЕТСТВУЕТ (экстрахи не провели мутагенности)</td>
<td>СООТВЕТСТВУЕТ (негативные результаты)</td>
<td>СООТВЕТСТВУЕТ (не проводился)</td>
<td>Не проводился</td>
</tr>
<tr>
<td>Анализ аберраций хромосом</td>
<td>Немутагенный, если значение $p &gt; 0.05$ для тестируемого</td>
<td>СООТВЕТСТВУЕТ (пемутагенный; нет)</td>
<td>СООТВЕТСТВУЕТ (негативные результаты)</td>
<td>СООТВЕТСТВУЕТ (недугатагенный)</td>
<td>СООТВЕТСТВУЕТ (не проведено)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>------------------------</td>
<td>-------------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>-------------------------------------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>Подкожная имплантация</td>
<td>Разница между средним показателем тестируемого образца и средним показателем контрольной группы равняется степени инкапсуляции; не вызывает раздражения, если разница равна нулю</td>
<td>СООТВЕТСТВУЕТ (удовлетворяет критерием USP)</td>
<td>Не проводилась</td>
<td>СООТВЕТСТВУЕТ (нетоксичный)</td>
<td>Не проводился</td>
</tr>
<tr>
<td>Подострая токсичность</td>
<td>Гемосовместимость</td>
<td>Не проводилась</td>
<td>Не проводилась</td>
<td>Не проводилась</td>
<td>СООТВЕТСТВУЕТ (нетоксичный)</td>
</tr>
<tr>
<td>Хроническая токсичность</td>
<td>Оценка внутримышечного имплантата у кролика; нетоксичный</td>
<td>Не проводилась</td>
<td>СООТВЕТСТВУЕТ (нетоксичный; слабая воспалительная реакция через 1 год)</td>
<td>СООТВЕТСТВУЕТ (нетоксичный после 90 дней)</td>
<td>СООТВЕТСТВУЕТ (нетоксичный после 50 дней)</td>
</tr>
<tr>
<td>Канцерогенность</td>
<td>Не проводилась</td>
<td>Не проводилась</td>
<td>СООТВЕТСТВУЕТ (нетоксичный)</td>
<td>Не проводилась</td>
<td>Не проводился</td>
</tr>
</tbody>
</table>

[^17]: Тест на биосовместимость Per ISO 10993.
### Engraftment and cell proliferation

PET polymer has been extensively analyzed for cellular compatibility and proved its ability to effectively support the implantation of cells, their dispersion and attachment for exogenous agents and preservation of the phenotype of cells, and was shown to have biomechanical dynamic ability to handle stress.\(^\text{21}\) In addition, bioscaffold, consisting of...
transformed nanofibers have demonstrated the ability to provide relevant micro-habitation for [...] and [...] differentiation of progenitor cells. 22

Fig. 9. PET bioscaffold of transformed nanofibers shows: (A) original microstructure fiber network (B) nano-pores on the surface of the fibers that contribute to cell attachment, (C) section fiber-demonstrates a lack of pores within it, (D) relatively dense flat bottom surface bioscaffold for slower cell migration cell in the spot with medium.

---


Mechanical characteristics of the bioscaffold

SPECIMEN  Electrospun bifurcated * scaffold produced by Nanofiber Solutions, Columbus, OH.

TEST  Uniaxial tensile test.

CONDITIONS  Universal testing machine Lloyd LRX
Load cell: 2500 N
Preload: 1 N
Speed of testing: 1 mm/s
Stainless steel custom-made grips fixed to the rigid rings of the scaffold.
In order to prevent slippage, grips were pre-glued to the sample.
Tests were carried out on both as-received scaffolds and scaffolds sterilized by immersion in ethanol overnight and then dried for 24 h.
For each specimen 5 measurements were performed.

Measured parameters:  Force at break (Fmax).
Elongation at break, defined as the percentage increase in length, before the break occurs, with respect to the initial length of the specimen.
путем погружения в этанол на одну ночь, а затем высушены в течение 24 часов. Для каждого изделия было выполнено по 5 измерений.
Измеренные параметры: Сила разрыва (F max)
Растяжение при разрыве, определяемое как процент увеличения длины перед моментом разрыва, по отношению к исходной длине изделия.

Ниже: Раздвоенный биокаркас из трансформированных нановолокон
Вверху слева: Типичные графики зависимости растяжения от силы и графики силы при разрыве для тестируемого биокаркаса до и после гамма-терилизации.

Анализ гамма-терилизованного биокаркаса показал отсутствие каких-либо изменений размера, цвета, прочности на разрыв, или структуры материала согласно сканирующей электронной микроскопии, как показано ниже:

<table>
<thead>
<tr>
<th>Размеры биокаркаса</th>
<th>Исходные</th>
<th>После гамма-терилизации</th>
</tr>
</thead>
<tbody>
<tr>
<td>Длина</td>
<td>6,1 см</td>
<td>6,1 см</td>
</tr>
<tr>
<td>Ширина</td>
<td>2,9 см</td>
<td>2,9 см</td>
</tr>
</tbody>
</table>
Вверху: СЭМ фотографии нановолоконного материала до стериллизации гамма-облучением.
Внизу: СЭМ фотография нановолокон после стериллизации гамма-облучением (28 kGy).

Данные измерений предела прочности при растяжении (n=5):

<table>
<thead>
<tr>
<th></th>
<th>Средняя (МПа)</th>
<th>Стандартное отклонение</th>
<th>Стандартная погрешность</th>
</tr>
</thead>
<tbody>
<tr>
<td>Контрольный образец</td>
<td>2,01</td>
<td>0,34</td>
<td>0,15</td>
</tr>
<tr>
<td>Образец после стериллизации гамма-облучением (28 kGy)</td>
<td>1,77</td>
<td>0,39</td>
<td>0,17</td>
</tr>
</tbody>
</table>
Приложение 2: Материалы биореактора (различные виды медицинских пластмасс и нержавеющая сталь)

Резюме: Медицинские материалы успешно применяются в целом ряде медицинских устройств, включая имплантаты, уже более двадцати лет. Используемый в данном клиническом исследовании биореактор помогает осуществить поддержку биокаркаса и обеспечить процесс выращивания клеток на его поверхности перед имплантацией. Биореактор изготовлен из основных из нескольких видов медицинских пластмасс, удовлетворяющих критериям Фарм. США (USP) Класса VI биосовместимости (Класс 6) (см. таблицы ниже). Основные материалы, покрывающие поверхность биореактора, полиоксиметилен (POM-C) и полиэтилен (PSU), уже использовались ранее для выращивания двух искусственных трансплантов трахеобронхиального дыхательного пути, операции по пересадке которых были успешно проведены в Госпитале Каролинского университета, Стокгольм, Швеция, в июне и ноябре 2011 года.

Заключение: Применение материалов Класса VI USP (медицинского класса/Класса 6) в сочетании с успешным клиническим исходом вышеупомянутых операций в июне и ноябре 2011 года указывает на то, что биореактор удовлетворяет критериям биосовместимости как аппарат для предоперационной обработки и процедуры переноса клеток на биокаркас в соответствии с протоколом для трахейного транспланта.

Материалы, использованные в конструкции биореактора:

<table>
<thead>
<tr>
<th>Компонент</th>
<th>Химический материал</th>
<th>Торговое название</th>
<th>Производитель</th>
<th>Удовлетворяет USP, Класс 6 (Медицинский материал)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Уплотнения, статические</td>
<td>EPDM резина</td>
<td>70 EPDM 291</td>
<td>Freudenberg Process Seals GmbH &amp; Co. KG, Германия</td>
<td>Да</td>
</tr>
<tr>
<td>Уплотнения, динамические</td>
<td>Фторкаучук (FKM)</td>
<td>FKM 80.445-01</td>
<td>Angst+Pfister AG, Швейцария</td>
<td></td>
</tr>
</tbody>
</table>
### Трансплантация трахеи

<table>
<thead>
<tr>
<th>Компонент</th>
<th>Химический материал</th>
<th>Торговое название</th>
<th>Производитель</th>
<th>Удовлетворяет USP, Класс 6 (Медицинский материал)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Зажимы</td>
<td>X10 CrNi 18-8 (нержавеющая сталь)</td>
<td>1.4310</td>
<td>AGILFedern, Германия</td>
<td>Нержавеющая сталь</td>
</tr>
<tr>
<td>Контейнер</td>
<td>Полисульфон (PSU)</td>
<td>TECASON™S</td>
<td>Ensinger Inc, США</td>
<td>Да</td>
</tr>
<tr>
<td>Крышка для контейнера с культурой</td>
<td>Полисульфон (PSU)</td>
<td>TECASON™S</td>
<td>Ensinger Inc, США</td>
<td>Да</td>
</tr>
<tr>
<td>Переносная крышка</td>
<td>Полисульфон (PSU)</td>
<td>TECASON™S</td>
<td>Ensinger Inc, США</td>
<td>Да</td>
</tr>
<tr>
<td>Переносная контейнер</td>
<td>Полиоксиметилен сополимер (POM-C)</td>
<td>Centrodal C</td>
<td>Centroplast Engineering Plastics GmbH, Германия</td>
<td>Да</td>
</tr>
<tr>
<td>Крышка переносного контейнера</td>
<td>Полипропилен (PP)</td>
<td>CentrolabHT™</td>
<td>Centroplast Engineering Plastics GmbH, Германия</td>
<td>Да</td>
</tr>
<tr>
<td>Вращающиеся детали</td>
<td>Полиоксиметилен сополимер (POM-C), Полиэфирфиркетон (PEEK)</td>
<td>Centrodal C, PEEK LSG</td>
<td>Centroplast Engineering Plastics GmbH, GermanySchmidt + Bartl GmbH</td>
<td>Да</td>
</tr>
<tr>
<td>Вал привода</td>
<td>X2 CrNiMo 18-15-3 (нержавеющая сталь)</td>
<td>1.4441 ESU</td>
<td>EZMetalstahlZieherei Mark GmbH, Германия</td>
<td>Не применимо</td>
</tr>
<tr>
<td>Оправка</td>
<td>Xb CrNiMoTi 17-12-2 (нержавеющая сталь)</td>
<td>1.4571</td>
<td>BSGStahlhandel, Германия</td>
<td>Не применимо</td>
</tr>
</tbody>
</table>

Результаты тестов по биосовместимости для материалов PEEK и CentrolabHT™, проведенных производителями (выполнение критериев Класса 6 USP подразумевает тестирование материалов на острую системную токсичность, внутрикожную реакцию и приживание):

<table>
<thead>
<tr>
<th>Тест на биосовместимость Согласно ISO 10993</th>
<th>Критерии приемлемости</th>
<th>Материал PEEK</th>
<th>Материал CentrolabHT™</th>
</tr>
</thead>
<tbody>
<tr>
<td>Острая системная токсичность</td>
<td>Тестируемая группа должна продемонстрировать отсутствие признаков токсичности и потери веса &gt;2 г</td>
<td>УДОВЛЕТВОРЯЕТ (Отсутствие признаков токсичности и потери веса &gt;2 г)</td>
<td>УДОВЛЕТВОРЯЕТ (удовлетворяет требованиям USP)</td>
</tr>
<tr>
<td>Тест на биосовместимость согласно ISO 10993</td>
<td>Критерии приемлемости</td>
<td>Материал PEEK</td>
<td>Материал CentrolabHT ™</td>
</tr>
<tr>
<td>------------------------------------------</td>
<td>------------------------</td>
<td>--------------</td>
<td>------------------------</td>
</tr>
<tr>
<td></td>
<td>иметь признаки токсичности; &lt; 3 мышей теряют вес &gt; 2 г</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Внутрикожная реакция через 72 часа</td>
<td>Должны удовлетворять требованиям минимальной реактивности</td>
<td>УДОВЛЕТВОРЯЕТ (минимальная реактивность)</td>
<td>УДОВЛЕТВОРЯЕТ (минимальная реактивность)</td>
</tr>
<tr>
<td>Тест на приживление (7 дней)</td>
<td>Нет реакции</td>
<td>УДОВЛЕТВОРЯЕТ (нет реакции)</td>
<td>УДОВЛЕТВОРЯЕТ (нет реакции)</td>
</tr>
</tbody>
</table>
### Appendix 3: Characteristics of biological agents and factors TGF-β3

The following table presents the growth factors used preoperatively in the present protocol to accelerate tissue regeneration:

<table>
<thead>
<tr>
<th>Factor</th>
<th>Activity</th>
<th>Company</th>
<th>Distributor in Sweden</th>
<th>Quantity</th>
<th>Intra-operative dose</th>
<th>Pre- and post-operative dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recombinant human factor TGF-β3, CF*</td>
<td>TGF-β3</td>
<td>R&amp;D Systems</td>
<td>R&amp;D Systems Europe Ltd. <a href="mailto:info@RnDSystems.co.uk">info@RnDSystems.co.uk</a> <a href="http://www.RnDSystems.co.uk">www.RnDSystems.co.uk</a></td>
<td>10 μg</td>
<td>1 ampoule (5 μg)</td>
<td>1 ampoule (5 μg) preop.</td>
</tr>
<tr>
<td>Granulocyte colony-stimulating factor (G-CSF)</td>
<td>Filgastrim (Neupogen®)</td>
<td>AMGEN</td>
<td>Amgen Inc. One Amgen Center Drive Thousand Oaks California 91320-1799 USA</td>
<td>30 IU (0,6mg/ml)</td>
<td>1 ampoule (30 IU)</td>
<td>9 ampoules (30 IU)</td>
</tr>
<tr>
<td>NeoRecormon</td>
<td>Erythropoietin and recombinant Epoetin α</td>
<td>Janssen Biotech</td>
<td>Jansen Biotech, Inc. 800 Ridgeview Road Horsham, PA 19044</td>
<td>10000 IU or 40000 IU</td>
<td>4 ampoules à 10000 IU (in total 40000 IU)</td>
<td>9 ampoules à 40000 IU</td>
</tr>
<tr>
<td>Recombinant human factor PTHrP</td>
<td>rhPTH</td>
<td>PeproTech</td>
<td>PeproTech SE Klarabergsvidukten 70, 107 24 Stockholm, Sweden</td>
<td>50μg</td>
<td>0,5 ampoule preop.</td>
<td>0,5 ampoule preop.</td>
</tr>
<tr>
<td>Insulin</td>
<td></td>
<td>Sigma Aldrich</td>
<td>Sigma-Aldrich Technical Services PO Box 14508 St. Louis, MO 63178 USA</td>
<td>10ug/ml</td>
<td>5 μg/ml</td>
<td>5 μg/ml</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td></td>
<td>Sigma Aldrich</td>
<td>Sigma-Aldrich Technical Services PO Box 14508 St. Louis, MO 63178 USA</td>
<td>100 nmol/L</td>
<td>50nmol/L</td>
<td>50nmol/L</td>
</tr>
</tbody>
</table>
* TGF-β3: calculation of the necessary dose of TGF-β3 based on the size of the human trachea and as a rule, it is based on the average lateral area of the cartilage as shown below in figure 1B.

<table>
<thead>
<tr>
<th>Physical</th>
<th>Proximal</th>
<th>Medial</th>
<th>Distal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diameter (cm)</td>
<td>a</td>
<td>b</td>
<td>c</td>
</tr>
<tr>
<td></td>
<td>1.5±0.4</td>
<td>1.9±0.2</td>
<td>2.1±0.5</td>
</tr>
<tr>
<td>Lateral Area (mm²)</td>
<td>48.27±3.52</td>
<td>50.29±2.56</td>
<td>53.08±2.25</td>
</tr>
<tr>
<td>Mean lateral area</td>
<td>50.54±2.41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Mechanical</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Cartilaginous ring thickness</td>
<td>0.3±0.1</td>
<td></td>
</tr>
<tr>
<td>Cartilaginous ring width</td>
<td>0.12±0.02</td>
<td></td>
</tr>
<tr>
<td>Rupture force (N)</td>
<td>212±18</td>
<td></td>
</tr>
<tr>
<td>Tensile modulus (MPa)</td>
<td>2.0±0.0</td>
<td></td>
</tr>
</tbody>
</table>

Используя вышеприведенный пример определения средней латеральной площади, было подсчитано, что требуемая концентрация TGF-β3 составит 0,1 мкг/мм² (примерно 5 мкг на кольцо). Это дает в итоге, что необходимо подготовить раствор 10 мкг/мл TGF-β3 и вводить по 0,5 мл на каждое хрящевое кольцо.

Ведущий ученый [signature] П. Маккиарион