Stockholm September 24, 2014

Amendment to the Formal Appeal for an Investigation of Scientific Misconduct by Prof Macchiarini submitted to Karolinska Institutet on August 18, 2014.

To the Vice-Chancellor of Karolinska Institutet

RE: Prof Paolo Macchiarini, Department of Clinical Science, Intervention and Technology (CLINTEC) Karolinska Institutet and Departments of Ear, Nose, Throat, Karolinska University Hospital, Stockholm, Sweden.

Dear Professor Hamsten,

Since the submission of our Formal Appeal, submitted on August 18, 2014, new information concerning the three patients transplanted with synthetic tracheae has come to light which warrants your consideration. This new information was received from Landspitali, Reykjavik, Iceland (concerning Case 1) and from the biotech and pharmaceutical companies that have produced the substances which were administered experimentally to all three patients.

1. Lack of Verification of Malignancy, which was the Postulated Indication for Transplantation in Case 1 (transplanted Jun 9, 2011).

The reoccurrence of the malignant tumor in the trachea (mucoepidermoid cancer), which was postulated to be the indication for the transplantation of the synthetic trachea, ***cannot be verified*** in the patient's medical records from either Landspitali, Reykjavik or from Karolinska University Hospital pre-, peri-or postoperatively.

The patient underwent a complicated surgical procedure of the newly diagnosed tracheal tumor on Oct 29, 2009 at Landspitali, Reykjavik, Iceland. Histological analysis demonstrated a low differentiated mucoepidermoid cancer. Initially, postoperative radiation therapy was planned, but had to be postponed because of miliary tuberculosis (TB). Treatment for the TB was initiated in January and continued until July of 2010, after which radiation therapy was started. Early in the spring of 2011 the patient developed respiratory symptoms believed to be tumor reoccurrence. Clinical investigations were initiated on Iceland where biopsies of the trachea were performed on Feb 11, 2011, four months before the transplantation on Jun 9, 2011. These biopsies did not yield any signs of malignancy, but only inflammatory granuloma (Appendix 1). After assessment by Prof Macchiarini, the patient was transferred on May 24, 2011 to the Departments of Ear, Nose and Throat (ENT) at Karolinska University Hospital for further investigations and *"…planned major surgery in approximately two weeks."* (May 24, 2011, Admission notes, Departments of ENT, Appendix 2). The patient flew on a standard international flight to Stockholm to undergo these investigations and surgery and therefore the procedure has to be considered an elective procedure.

Computer tomography of the chest from May 24, 2011 (CT report, Appendix 3) could *not* verify if the changes in the patients' trachea were of malignant origin. A PET-(18-

FDG) examination performed on May 25, 2011 (PET report, Appendix 4) showed signs, which may be consistent with a tumor. However, 18F-FDG is not a cancer specific marker and false positive results may also be detected under various circumstances including active inflammation and granuloma, infection from for example tuberculo-lymphoadenopathy, active fibrotic injury, and radiation induced fibrosis or hyperplastic lymphadenopathy in the absence of malignancy (Appendix 5a, b, c). Two weeks before the planned transplantation two tracheal biopsies where performed at Karolinska University Hospital (May 26, 2011, Biopsy reports, Appendix 6a, b) as well as a bone marrow biopsy (May 27, 2011, Biopsy report, Appendix 6c). None of these biopsies verified the presence of any type of malignancy. ***Despite these negative histological findings surgery was performed June 9, 2011.***

In the surgical notes from the tracheal transplantation procedure it is stated:

Swedish:
”Så småningom kan sedan både höger huvudbronk och vänster huvudbronk fridissekeras. ***Man ser ingen makroskopisk överväxt av tumörvävnad,*** däremot en hel del fibrösa förändringar till följd av tidigare operationsingrepp samt strålning. Trachea delas av cirka 4-5 cm ovan carina och höger huvudbronk alldeles intill ovanlobsbronkens avgång. Vänster huvudbronk delas av cirka 1,5 cm från carina. ***Makroskopiskt ingen tumörväxt vilket även verifieras vid fryssnitt***.”
(Jun 9, 2011, Operation report, Appendix 7).

English translation:
“Eventually both the right and left main bronchus are exposed. One cannot see any macroscopic overgrowth of cancerous tissue, however a great deal of fibrotic changes subsequent to earlier surgical procedure and radiation. The trachea is divided about 4 to 5 cm above the carina and the right main bronchus proximal to the branch of the right upper love. The left main bronchus is divided about 1.5 cm from the carina. ***Macroscopically no tumor growth, which is also verified by frozen sectioning.”***

The biopsy (frozen sectioning) taken for intra-operative diagnosis demonstrated reactive lymphadenopathy without signs of malignancy (Jun 9, 2011, Pathology/Cytology report, Appendix 8). Despite this negative finding, Prof Macchiarini, who was leading the procedure, removed the native trachea and replaced it with the experimental synthetic trachea (Jun 9, 2011, Operation report, Appendix 7). There is no documentation in the Karolinska medical records of histological analysis performed post-operatively of the native trachea to verify the diagnosis of malignancy.

The surgical procedure was complicated by an injury to the right pulmonary artery with severe bleeding which was repaired by replacing it with a vascular prosthesis. Seven days post-operatively the patient was diagnosed with occlusion of the right pulmonary artery at the site of repair. This is a very uncommon complication since the blood flow through the pulmonary artery is significant and not prone to occlusion. The occlusion became chronic and a source of disability.

The patient was discharged from the Departments of ENT on July 8, 2011 and transferred back to Landspitali, Reykjavik, Iceland with the new experimental synthetic trachea along with an occluded right pulmonary artery and left-sided paralysis of the recurrent laryngeal nerve. An unfounded diagnosis is given in the Karolinska medical records with the wording “*malignant tumor in the windpipe*” (Jul 8, 2011, Discharge Summary, Appendix 9), which had not been histologically verified.

The patient recuperatated after surgery and could be discharged from the hospital. The development of granuloma formation in the transplant in the fall of 2011 lead to referral to the Departments of ENT, Karolinska University Hospital for evaluation and removal. The readmission notes from Nov 21, 2011 describe a patient suffering from a deteriorating general clinical status (Admission notes Nov 21, 2011, Appendix 10). Bronchoscopy at this time showed fistulation to the mediastinum and multiple stenting of the transplant is initiated five and half months after the transplantation (for detailed clinical outcome, bronchoscopy and biopsy findings see Tables 1-3 and Articles 1, 2, 4, 6 in the Formal Appeal).

The patient's status from the summer of 2012 is described in a letter from the Department of Cardiothoracic Surgery, Reykjavik, Iceland and reads as follows:

"For over a year[1] XX[2] has suffered bothering respiratory symptoms that have only got worse*. It started with* ***recurrent hemoptysis*** *that required admission to our hospital. It was thought that these bleedings were related to granulation tissue at the anastomotic sites but potentially also from the stents. After intermittent treatment with Cycklocapron and bronchoscopic controls in Stockholm these problems have got better.* ***Instead, recurrent infections, mainly in XX right lung, have been the main concern for the last 8 months****[3]. **In December 2012 XX was diagnosed with a rather large abscess** in XX right lower lobe that gradually responded to iv. antibiotics.* ***Since then XX has been admitted multiple times to our hospital for copious blood tinged sputum and pneumonia-like symptoms.*** *Streptomonas mult bacteria have been grown from his trachea, but have been resistant to treatment."…*

"Since early June[4] XX has been more in our hospital than at home. He is not septic but his problems with copious sputum and hemoptysis are worrisome and reduce his quality of life significantly."…

"With multiple investigations we have shown that XX lung is non-functioning. This is due to an ***early postoperative thrombus of a Vascutec graft to the right pulm artery and multiple distal emboli to the right lung. His right lung therefore seems to contribute minimally to his respiration."***

[1] Approx. summer 2012
[2] Refers to Case 1 who was transplanted on June 9, 2011. The patient died at Karolinska on Jan 30, 2014.
[3] "last 8 months" approximately December 2012.
[4] June 2013.
(Aug 3, 2013, Letter from the Department of Cardiothoracic Surgery, Reykjavik, Island Appendix 11).

The patient was more or less continuously hospitalized with a low quality of life since the beginning of June 2013 in Reykjavik on Island and finally and terminally hospitalized at Karolinska from Oct 21, 2013. CT-thorax Oct 22, 2013 shows "*Fistulation between the esophagus, the airways and the mediastinum. Progress of air filled gaps outside the tracheal transplant which probably now to the main part seems to be detached. Bronchiolitis and stagnation of secretion in the peripheral left lung."* (Oct 22, 2013, CT report thorax/esophagus, Appendix 12).

The tracheobronchial fistulation required multiple stenting of the esophageus and trachea as well as complicated esophagus surgery on Dec 10, 2013 (Dec 10, 2013, Operation report, Appendix 13). The patient died on Jan 30, 2014 due to therapy resistant respiratory insufficiency one year and eight months after transplantation subsequent to a physiologically dysfunctional tracheal transplant. The last eight

months of the patient's life, the patient was more or less hospitalized. The transplant showed before his death as well as at the time of autopsy no signs of established normal airway epithelialization and was nearly completely disconnected from its surroundings of necrotic tissue.
(Feb 3, 2014, Autopsy report, Appendix 14).
Conclusion

The transpired events raise the question if the patient ***de facto*** had a vital organ removed and replaced with an experimental implant in the absence of standard pre-, peri- or post-operative histological analysis. According to the medical records from both institutions the last positive histological analysis/sample was attained during the 1st operation in Oct 2009, ***1 year and 8 months before transplantation on June 9, 2011***. In the interim of the primary surgery and the tracheal transplantation, the patient had undergone high doses of radiation therapy to eradicate residual malignancy. Pre-transplant radiological examination did not substantiate a recurrent malignancy. Definite positive histological analysis should have been attained before extensive experimental surgery was embarked upon. The experimental procedure seems to have been performed without a registered ethical permission, since no ethical application seems to have been registered at the Regional Ethical Review Board. (See Formal Appeal filed to the President of Karolinska Institutet 2014-08-18, diary number 2-2184/2014).The patient signed an informed consent form ***17 days after the transplantation*** stating that he has been "*extensively informed"* that he has a malignant tracheal cancer and that his *"only chance for survival"* is a tracheal transplantation (June 26, 2011, Informed Consent Form, Appendix 15 and for further specific remarks concerning the Informed Consent Form, see the Formal Appeal on page 16-18).

2. Administration of Growth Factors and Bone Marrow Stimulating Pharmaceuticals

All three patients received the following substances according to a protocol developed by Prof Macchiarini at the time of transplantation and for *"2 weeks after transplantation"* (table 1-2).

- **Recombinant Human Transforming Factor β3**
  (Manufacturer: R&D Systems, Minneapolis, MN, USA).

- **Filgrastim (Neupogen®) Granulocyte Colony Stimulating Factor (G-CSF)**
  (Manufacturer: Amgen Europe BV, Breda, Netherlands).

- **Epoetin beta (NeoRecormon®) synthetic analogue of Erythropoietin**
  (Manufacturer: Roche, Grenzach-Wyhlen, Germany).

The administration of these substances was described in the article by Prof Macchiarini, **Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study.** *Lancet* Vol. 378 Dec 10, 2011 (Appendix 16).

Under the section Pretransplant Preparation on p. 1998 it is stated: *"Immediately before implantation, the airway construct was transported to the operating theater, reseeded with the obtained MNCs, and conditioned with growth and regenerative factors - namely,* ***recombinant human transforming growth factor-β3*** *(R&D Systems, Minneapolis, MN, USA; 10 µg/cm2****) , granulocyte colony stimulating***

factor filgrastim *(G-CSF, Neupogen; Amgen Europe BV, Breda, Netherlands; 10 µg/kg), and* ***epoetin beta*** *(analogous synthetics of Erythropoietin Roche, Grenzach-Wyhlen, Germany; 40 000 UI)."*

Under the section Regenerative Boosting Therapy p. 1990 it is stated: *"To enhance the regenerative process, the patient was treated pharmacologically by subcutaneous injections of* ***G-CSF*** *(10 µg/kg)* ***and epoetin-alpha (40 000 UI), with a loading dose given the day before transplantation and every other day for 2 weeks during the postoperative period."***

Comments

There is no scientific support for the "Off-label" use of growth factors and bone marrow stimulating substances in "supra-therapeutic" doses in a so-called *"Regenerative Boosting Therapy"* in human beings. The three producers (Roche, Amgen and R & D Systems) deny any knowledge of the term *"Regenerative Boosting Therapy"* for their respective substances. All three producers have recommended against the use of their respective substances for this indication, since there is no scientific data to support their use in this context (Appendix 17a, b, c).

R & D Systems emphasize that their product (Recombinant Human Transforming Factor β3) ***is not approved for administration to either humans or animals because of the potential risk for viral transfer and is only approved for research in cell culture in a laboratory environment***.

Two of the three transplanted patients (Case 1 and 2) had a history of malignancy. At transplantation, Case 2 was not radically resected from his malignancy (Nov 17, 2011 Pathology/Cytology report, Appendix 18) but nonetheless the growth factors were administered according to the above-mentioned protocol. This is medically precarious considering that a pro-malignant effect of these growth factors cannot be ruled out.

All three transplanted patients who had received "supra-therapeutic" off label doses of Epoetin beta developed potentially fatal thrombo-embolic complications (Table 2).

- Case 1: Occlusion of the right pulmonary artery was diagnosed 7 days after start of treatment. Long term complication: chronically occluded right pulmonary artery (Jun 16, Jul 5, Nov 16 2011, CT reports, Appendix 19a, b, c).
- Case 2: Venous thrombosis in the left jugular, subclavian and axillary vein systems and a pulmonary embolus in the left lower lobe was diagnosed on the last day of treatment.
- Case 3: Pump-thrombosis on ECMO (extracorporeal membrane oxygenation)) support (extremely rare complication in modern ECMO circuits), massive hemolysis, acute tubular necrosis, and acute renal failure resulted in 7 weeks of hemodialysis and an arterial embolus in the right leg diagnosed 7 days after the start of treatment. Long term complication: chronic renal failure.

Conclusions

Growth factors and bone marrow stimulating pharmaceuticals, where one is not approved for administration to either humans or animals, have been administered in “supra-therapeutic doses”. Case 1 and 2 had a history of malignancy (although malignancy in Case 1 has not been histologically verified) and the necessary approval/registration from the Regional Ethical Review Board or the Swedish Medical Products Agency have not been filed (Table 2).

All three patients suffered from severe thrombo-embolic complications. It cannot ruled out that these complications were caused by the use of scientifically undocumented and by the manufacturer (Roche) not recommended supra-therapeutic doses of Epoetin beta (NeoRecormon®).

Sincerely,

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