Regarding patient:

3rd of August 2013.

To whom it may concern

This 40 year old previously healthy male from Eritrea, who is studying geology in Iceland, was admitted to our emergency room on the 27th of Ocober 2009 for difficulties with his breathing, including stridor at inhalation. A CT scan showed a 3 x 3 cm large and dense tumor in the distal part of his trachea, almost totally blocking the major airways. It was decided to take him for acute surgery, both to get tissue from the tumor for diagnosis and at the same time excise the tumor to relieve the obstruction.

The operation was performed in general anasthesia at Landspitali National on the 29th of October. The tumor was dense and easily bleeding, and therefore difficult to resect. During the operation a perforation of the right main bronchus occurred, extending into both the azygos vein and a large branch of the right pulmonary artery. This resulted in a major intraoperative bleeding with severe shock. During the cardiac arrest that followed a heart and lung maschine was used to support the circulation and pulmonary function. The trachela and vascular injury was repaired through sternotomy and the tumor debulked, still leaving parts of it in site (posterior wall). Postoperatively multiorgan failure occurred, with both respiratory and renal failure. This resolved for the next weeks and after a week in the ICU, the patient was discharged to the cardiothoracic ward for further treatment. There he received treatment for pulmonary fungal infection. Gradually he did better and was discharged to his home in Reykjavik, 43 days after surgery.

PAD showed the tumor to be a low grade mucoepidermoid cancer, but they are extremely rare tumores and have much more favorable diagnosis than the more common adeno- or squamous cell carcinomas. The Karolinska hospital in Stockholm was consulted and it was decided to further treat the tumor with local radiation therapy. This treatment, however, had to be delayed because shortly after discharge he was diagnosed with pulmonary infiltrates that turned out to be miliary tuberculosis. He started succcessfully TBC treatment in January 2010 that was continuded until July. He started radiotherapy with good result and satisfactory tumor response. He could get back to work and visited his home country. In early 2012 his symptoms worsened again with recurrent stridor. Bronchoscopy and CT showed major recurrence of the tumor, especially on the right side. There were no signs of distant metastases. Further radiotherapy was not deemed an option.

At this stage the Karolinska was contacted again and we got contact with Professor Macciarini at the ENT department in Huddinge. After detailed investigations and ethical approval it was decided to resect the tumor and use a stem cell seeded plastic trachea as a substitute. This operation was performed at Huddinge on the 9th of June 2011. Postoperatively he was admitted for several weeks in the ICU in Huddinge but 1 month after surgery he was transferred to Iceland for further treatment. He was admitted for one month in our hospital but was then transferred to a rehabilitation unit for a couple of months. In general he did well and gradually he could get back to work and finish his MSc studies.

For the last 2 years, since the operations, has has been sent several times to Stockholm for controls of the transplant. This has been scheduled every 3-5 months (Jan Juto). Stents have been placed into the graft and both main bronchi to reinforce the distal anastomosis. Some of these stents have been removed and new ones placed in stead. The last control was in April 2013 and then the left stent was removed but the right one, for some reasons, left in place.

For over a year 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 the stents. After intermittant treatment with Cycklocapron and bronchosopic controls in Stockholm these problems have got better. In stead, recurrent infections, mainly in his right lung, have been the main concern for the last 8 months. In December 2012 he was diagnosed with a rather large abscess in his right lower lobe that gradually responded to iv. antibiotics. Since then he 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. Still, earlier this summer, his trachea cultures were negative. Our infectious doctors have decided his treatment together with doctors from our pulmonology department.

Since early June has been more in our hospital than at home. He is not septic but his problems with copious sputum and hemoptysis are worrisom and reduce his quality of life significantly.

His symptoms stem very likely from his right lung. When he lies on his left side more sputum is coughed up. Chest X-rays have also shown pneumonia on the right side with a clear left lung. With multiple investigations we have shown that his right lung is non-functioning. This due to an early postoperative thrombus of a Vascutec graft to the right pulm. artery and multiple distal embolies to the right lung. His right lung therefore seems to contribute minimally to his respiration.

The question now is if it is not best to remove his right lung?

There are, however, several technical challenges that have to be dealt with.

- There are certainly a lot of adherances after 2 previous surgeries and extensive radiotherapy (70 Greys). His superior vena cava is almost obstructed. But he has not been thoracotomised before and previous surgeries were done through sternotomy.
- The question is how it is best to close the stump (some special stapling device?). First the remaining stent has to be removed, but for some reasons this stent was not removed at the last control in Huddinge (April 2013).
- The graft is covered with omentum. Since the transplant there has been a small hole on the distal left anastomosis, with air surrounding parts of the new trachea. This „hole" has to be checked and eventually closed if pneumonectomy is performed.
- There has also been problems, as seen on multiple CTscans and bronchosopies, with the upper part of the right distal anastomosis to the upper right lobe bronchus. The air around the trachea could therefore also stem from this part of the transplant together with the hole on the left anastomosis.
- In summary it is clear that there is a risk of empyema after pneumonectomy. Latissmus flaps or other muscle flaps could be used to cover both the stump and mediastinum. But here your team at KS have more experience.

We would be very happy to get help from you with this challenging patient. His problems are not easy to deal with but is a very strong character and has won all the small battles so far.

We would suggest that is transferred to Stockholm as soon as possible (his infection is relatively quit at the moment) for further work-up and decision on treatment.

We look forward to hear from you.

If further information is needed please do not hesitate to contact us.

Sincerely

Professor Tomas Gudbjartsson, MD, PhD
Department of Cardiothoracic Surgery
Landspitali University Hospital
Reykjavik, Iceland
Mobile: +354 8255016
Email: tomasgud@landspitali.is

Appendix 12

SVAR RÖNTGEN/ISOTOP

FRÅN
Karolinska Universitetssjukhuset
Röntgenavd, Huddinge
Röntgen C146
141 86 Stockholm
Tel 08-585 808 50

B: 11002-521-SV1
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F: 11002-521-SV1
R: 201301898233
L: 4729272701

TILL
Karolinska Universitetssjukhuset
Öron-,näs- och halskliniken, Huddinge
Avdelning B82
141 86 STOCKHOLM
Tel: 08-585 877 91 Fax: 08-585 873 25

Prioritet: Normal
Patienten bör undersökas på: Röntgen
Patienten kallas från: Vårdavd.

Remissdatum: 2013-10-21 08:30
Remittent: Gert Henriksson
Till sektion: Datortomografen
Önskad undersökning: CT thorax/esofagus
Frågeställning: Kartläggning av esofagusfistel.
Anamnes, status: Patient som opererades 2011 pga trachealcancer. Nu uppredade luftvägsinfektioner hö lunga + misstanke på esofagusfistel. Nu CT inför ställning till esofagusåtgärd och evt lungop hö sida. Thoraxkirurgerna solna och gastrokirurgerna Huddinge är med i diskussionerna. Pat kommer från Island idag, kommer att vara på B 82 from ca 14.00. Kerstin Cederlund är vidtalad i fallet.

Kreatinin: 60 2013-09-17

SVAR

Undersökning påbörjad: 2013-10-22 14:53 avslutad: 2013-10-22 15:22 Rek. C-koder:

Undersökningskod: .

Utlåtande:

DT thorax med iv och senarex po kontrast given i höger sidoläge
Jämförelse med föreg DT utförd 130917.
Det finns 2 till synes nytillkomna fistlar där
1) den ena kommunicerar mellan ventrala esofagus och transplantatets vänstra bens distala-dorsala kant, och
2) den andra är belägen 1 cm cranialt därom och förbinder esofagus med mediastinum och övergår där i en cirkumferent utbredd luftspalt omkring transplantatet i "carina" höjd.
Förutom dessa patologiska förbindelser finns (liksom tidigare) luftfyllda fistlar som utgår från patientens egen vänstra huvudbronk och kommunicerar med oregelbundet utbredda luftspatier i mediastinum.
Kontrasten som intagits per os rinner över i båda transplantatbenen, in i de mediastinala lufthålorna samt ut i de från bronkträdet avstängda bronkerna i höger överlob samt ner i den kända kaviteten i höger underlob.
Sedan 130917 har det skett en progress av de nu tämligen rikligt utbredda bronkiolitförändringarna i vänster underlob och lingula, dessa lungdelar var tidigare ganska normala.
Utbredda subcutana kollateraler lateralt på höger sida av bålen.

-Fistlar mellan esofagus och luftvägar samt mediastinum. Progress av luftspalter utanför tracheatransplantatet som nu till största delen torde sitta löst. Bronkiolit/sekretstaganation i vänster lungas periferi.

Cederlund, Kerstin
17:44 2013-10-22 Signering 1 Preliminärt svar: Cederlund, Kerstin

Framställd
13-10-23 08:50

			SVAR RÖNTGEN/ISOTOP	
FRÅN	Karolinska Universitetssjukhuset Röntgenavd, Huddinge Röntgen C146 141 86 Stockholm Tel 08-585 808 50	B: 11002-521-SV1 S: 11002-521-SV1 F: 11002-521-SV1 R: 201301898233 L: 4729272701		
TILL	Karolinska Universitetssjukhuset Öron-,näs- och halskliniken, Huddinge Avdelning B82 141 86 STOCKHOLM Tel: 08-585 877 91 Fax: 08-585 873 25		Prioritet: Normal Patienten bör undersökas på: Röntgen Patienten kallas från: Vårdavd.	

08:50 2013-10-23 Signering 2 Slutgiltigt svar: Wallier, Egon

---slut---

Framställd
13-10-23 08:50

Karolinska Universitetssjukhuset 11002303K72
MK-division1, Huddinge
Medicin- och kirurgavdelning K72
141 86 STOCKHOLM
tel: 08-585 802 72 / 08-585 823 68 fax:08-585 824 20

Appendix 13

* 2013-12-10 16:39 Mats Lindblad, Läk H - ÖAK kir avd K72 (signerad)
Rättelse: 2014-01-15 16:40 Mats Lindblad, Läk

OPERATIONSBERÄTTELSE

Operationsdatum 2013-12-10

Preop. bedömn. Patient med komplicerad sjukhistoria. Sammanfattningsvis patient ursprungligen från Eritrea och boende på Island. Har utvecklat en mukoepidermoid tumör i trakea som recidiverar. Op 2011 med resektion av trakea och ersättning med stamcellsklätt kompositgraft. Mycket komplicerad op med besvärliga blödningar som slutat med avstängs arteria pulmonalis dextra samt vena cava superior. Överstått det hela tämligen väl men har nu utvecklat esofagobronkiell fistel och tecken till att trakealgraftet lossnat både proximalt och distalt. Enligt tidigare diskussion är patienten inte aktuell för ny transplantation av trakea och inte heller aktuell för hjärt-lung/lung-transplantation. Ej aktuell för ECMO-behandling. Dock inga tecken till recidiv. Nu har vi beslutat att försöka palliera honom med intentionen att resecera esofagus och ersätta med koloninterponat subkutant.

Operations- åtgärdskod
JCD20 Transsektion av esofagus proximalt och distalt
JCD03 Esofagusbypass med interposition av kolon
JCA08 Endoskopiskt avlägsnande av främmande kropp i esofagus
JCF12 Endoskopisk inläggning av stent i esofagus
JDB00 Gastrostomi
JDH63 Pylorusplastik
UJD02 Gastroskopi

Operatör Lars Lundell (läk) /1gm2/

Operatör 2 Magnus Nilsson (läk) /1zbg/

/Mats Lindblad

Assistent Koshi Kumagai (läk) /78n4/

/Ioannis Rouvelas och Jon Tsai.

Sammanfattning Vi har tagit bort två stycken esofagusstent och sett att kompositgraftet i trakea eroderat främre väggen av esofagus. Avstår därför från stripping. Staplar därför av esofagus proximalt och distalt

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efter att ha lagt två nya esofagusstentar över fisteln. Vi lägger sedan ett koloninterponat subkutant mellan esofagusstumpen på halsen och ventrikeln. Kolostomi handsydd. Gastrostomi anlagd.

Operationstid: 5,5 h.
Blödning: 150 mL.

Operationsförlopp

Inleder med gastroskopi där vi tar bort patientens två stycken esofagusstentar. Går sedan ner och inspekterar, ser då att på cirka 25 cm nivå och hela vägen ner till carina har patientens trakeala kompositgraft eroderat in i esofagus framvägg. Det läckager en hel del luft, framför allt distalt i från. Bilder tagna. Med hänsyn till denna bild anser vi det allt för äventyrligt att strippa ut esofagus enligt plan då kompositgraftet sannolikt skulle disloceras fullständigt. Beslutar därför tillsammans med professor Juto och professor Lundell att anlägga två nya esofagusstent för att försöka täcka fisteln och sedan stapla av esofagus och lämna denna in situ och därefter göra en bypass.

Går därför vidare med övre medellinjesnitt och in i fri bukhåla. Identifierar den stjälkade omentdelen som går upp retrosternalt och denna skonas. Rikligt med luftläckage in i GI-kanalen varför vi snabbt öppnar upp ett hål i ventrikelns framsida och tömmer på luft. Härefter dissekerar vi rent i hiatus och friar upp esofagus som delas med Endo Gia Tri-stapler. Fripreparerar sedan vänster kolon stjälkad på arteria colica sinistra. Rikligt med välcirkulerad kolon. Syr en sida-till-sida kolokoloanastomos, handsydd. Går samtidigt in på halsen längst till vänster i sternocleido. Påtagligt fibrotiskt här men vi kan så småningom ta oss in medialt om jugularis och carotis till prevertebrala fascian. Identifierar esofagus och fripreparerar denna. Går nu ner via gastroskopi och anlägger två stycken esofagusstentar 8 cm långa för att täcka fistelhålen. Staplar sedan av esofagus på halsen strax ovanför det övre stentet. Fripreparerar en subkutan ficka längs med vänster bröstkorg och vi för här upp vänster kolon subkutant ända-till-ända på halsen. Lägger ner ett Exudrain 14. Försluter platysma med enstaka Vicryl, clips i huden. I buken sys en kologastrisk anastomos ända-till-sida. Anlägger distalt om detta en gastrostomi som sys upp mot främre bukväggen. Försluter sedan fascian med fortlöpande PDS där man sparar den övre biten för passage av kolon. Clips i huden.

Peroperativa ordinationer

V-sond lagd via näsan ner i koloninterponatet och får ligga kvar. Nexium i v 40 mg x 2.

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Postoperativ planering Tas till IVA för delayed extubation.

----------------------- slut utskrift -------------------------

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	Karolinska Universitetslaboratoriet	S: 11001-412-308	
	Klin Pat/Cyt lab	F: 11001-412-308	
	171 76 Stockholm	R: 1026-0496993-5	
	Tfn	L: OHS29-14	

Appendix 14

TILL
Karolinska Universitetssjukhuset
Thoraxkliniken, Solna
N14 Thorax-IVA
171 76 Stockholm

Regnr
OHS29-14

Patienten avliden	2014-01-30 10:18	Remittent:	Thomas Fux
Ankomstid lab:	2014-02-03		Tfn:

Diagnos/fråga: uttag av syntetiskt tracheatransplantat inkl nativ proximal/distal anastomosvävnad

Klinisk epikris: Bäste kollega,
Ytterligt komplext fall, 40årig man, tidigare trakealresecerad (2011) med syntetiskt tracheatransplantat, multipla komplikationer, anastomosinsufficiens trachea/nativt kvarvarande nativ luftväg både proximalt samt distal som lett till subtotal protesavlossning. 2013-12-10 mkt komplicerad esogfagusrekonstruktion med koloninterponat pga uppkomst av tracheaesofagel fistel som delvis kvarstått postoperativt. Betydande postoperativa luftvägsproblem med långvarig IVAvård, besvären aggraveras, stentdislocering, efter bronchoskopi 28.1 raskt försämrad, koldioxiretention, omöjlig att ventilera > multiorgansvikt, forts terapi bedöms utsiktslös varför terpiavbrott beslutas och patient avlider 2014-01-30.
Viktigt att uttagt transplant inkl anastomosområden fotograferas, delvis nedfryses samt läggs i NaCl för senare analys. Ut nås per direkttelefon 08 517 706 59

SVAR

PRELIMINÄRT UTLÅTANDE
2014-02-07 OHS29/2014
2011-442098

YTTRE BESIKTNING: Kroppen efter en 40-årig (utan svenska personnummer) med normal kroppsbyggnad och normalt hull. Hudfärg ua. Inga ödem. Sedvanliga likfenomen. På bröstkorgen och bukens övre medellinje ses ett 40 cm långt operationsärr. 3 cm på nedre delen av halsen påvisas en öppning av kolointerponat med normal färg, inga tecken på något tumörsuspekt eller på någon övertygande abscess. Mellan sjätte och sjunde revbenet på vänster bröstvägg finns en katerter i anslutning till vänster pleurahåla. Under höger arcus ses en öppningen för gastromi. På trigonum femoris ses en genom huden införd intravenös kateter.

INRE BESIKTNING:

KARDIOVASKULÄRA ORGAN: Pericardytorna är fastlödda av svårlösbara adherenser, ingen vätska står i hjätsäcken. Hjärtat är lätt allmänt förstorat, vikt 420 gram. I hjärtöronen ses inga tromber. Foramen ovale är slutet. Kamrarna är inte dilaterade och väggarna är ej

Framställd
2014-02-10 12:08

uppenbart förtjockade. Endokardiet har ordinärt utseende. Myocardium ua. Vänster kammarens vägg har normal tjocklek liksom den högra kammarens vägg; vid snittning ses inga tecken till genomgångna infarkter. Klaffar och ostier är ordinära. Koronarkärlen är gracila och företer inga arteriomatösa plaques. Aortan är fri från arteriomatösa plaques. Makroskopiskt ses ingen tydlig struktur av höger lungartär, p.g.a. kraftig fibros och adherens i hilus av höger lunga. Inga embolier i vänster aa pulmonales. Övriga större kroppsartärer ua. Vv cavae och v portae inspekteras ua.

RESPIRATORISKA ORGAN: Höger lunga och bröstväggen har kraftiga adherenser. Vänster pleurahåla innehåller ingen ökad vätskemängd, men fokala adherenser. På vänster pleura föreligger ojämn ytan med fokal fibros, fibrinbeläggning och en transbröstväggskateter. Larynx uppvisar ordinär slemhinna. Inget slem. Cirka 7 cm nedre delen av halsen föreligger esofagobronkiell fistel. I trakeaområde påvisas ett Y-typ transplantat med tunn och vitaktig komponent på ytan. Omkring transplantat föreligger purulent vätska och nekrotisk vävnad. Fokalt ses inga nativa strukturer av trakea och esofagus. Anastomosränderna mot höger och vänster trakea är urkopplade och av proximala anastomsranden är 90% lossnat med sparsamt adherenslik material mellan graftet och distala randen av huvudtrakea. Lungorna tillsammans med resten vävnad från trakea och esofagus väger färskt 2500 gram. Efter formalfixering väger den vänstra lungan 1400 gram och den högra lungan 870 gram. På snittytorna ses multipla, vitaktiga, solida förändringar i vänstra lungparenkymet med fokal anslutning till fibrinbeläggningen på pleuraytan. I höger lung påvisas uttalad fibros med några olikstora solida förändringar, inget tydligt ordinärt lungparenkym.

DIGESTIONSORGAN: Subkutant, mellan 2 cm:s nedre del av halsen och ventrikeln ses ett koloninterponent som innefattar vitaktigt skummigt innehållet. I den delen av kolon ses makroskopiskt ordinär slemhinna, utan ischemisk förändring. Den proximala delen av esofagus är stängd i cirka 3 cm:s nivå i nedre delen av halsen. Mellan 6 cm proximalt och 6 cm distalt esofagus föreligger en 9 cm lång stent. Omkring stenten och längs distal del av esofagusen till gastroesofagus junktion påvisas i purulent vätsk, fibrotiserade vävnad och abscessbildning. På peritonealytorna finns fokala adherenser mellan tarm och enstaka adherenser ut mot gallblåsebädden. En gastrostomi påvisas med normalt utseende i ventrikelskropp. Ventrikel och duodenum uppklippes ua. Slemhinnan uppvisar inga ulcerationer eller tumörer. Jejunum och ileum palperas ua. En kolokoloanastomos ligger 50 cm från valvula ileocaecalis med ordinär utseende. Kvarvarande delar av kolon och rektum uppklippes och uppvisar blek, ordinär slemhinna. Pankreas ua. Levern är slappt i konsistensen med slät yta

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171 76 Stockholm

Regnr
OHS29-14

och skarp främre kant. Levern väger 1600 gram. På snittytorna ses ordinärt leverparenkym. Inga härdar ses. Gallblåsan är ordinär utan konkrement. Ductus hepticus och ducts choledochus ua.

UROGENITALA ORGAN: Njurarna är normalstora med sammanlagd vikt 320 gram. På snittytorna är barkskiktet normaltjockt, bark/märgteckningen är tydlig i båda njurarna. Njurbäcken och uretärer bilateralt ua. Urinblåsan är ordinär. Prostatan har normal storlek med mjuk konsistens

ENDOKRINA ORGAN: Thyreodea ua. Binjurar ua.

LYMFATISKA OCH HEMATOPOETISKA ORGAN: Inga patologiska lymfkörtlar påträffas. Mjälten har normalt konsistens, väger 360 gram.

CENTRALA NERVSYSTEMET:
Hjärnhinnor ua. Lillhjärna och hjärnstam uppvisar normala ytor. Vikt 1439 gram. Inga tecken till ökat intrakraniellt tryck. På snittytorna ses makroskopiskt normal vävnad, inga avgränsbara härdar eller blödningar. Basala hjärnkärl ua.

ÖVRIGT: Färskt undersökning på områden av mediastinum, respiratoriska ogran och esofagus förrättas vid visning av Dr. Thomas Fux.
I mediastinum påvisas kraftig inflammation, abscessbildning, esofagobronkiell fistel, fibros och inläggade stent. Retroperitoneum ua. Kalotten och kotpelare är ordinära.
DIAGNOS
Prel. PAD:
Kraftigt inflammation, abscessbildning i mediastinum.
Esofagobronkiell fistel.
Pericardit.
Misstanke på lunginflammation.
Status efter syntetiskt tracheatransplantat.
Status efter esofagusbypass med kolointerponat.
Status efter inläggning av stent i esofagus.

BIOBANKSINFORMATION

Naining Wang 2014-02-10
---slut---

Framställd
2014-02-10 12:08

Stockholms läns landsting

KOPIA

Karolinska(Huddinge) Öron, näsa, hals - Brev och intyg

Informed Consent Mr.

I, Mr. born on the 1973 (Personal ID in Iceland:), and resident in Reykjavik, Iceland, patient from the Department of ENT of the Karolinska Hospital in Huddinge declare voluntarily that:

I have been extensively informed by Prof. Paolo Macchiarini about the possibility of a complete resection of my primary malignant tracheal tumor and its reconstruction with a synthetic polymer-based and completely biocompatible tracheal scaffold reseeded *ex vivo* with autologous mesenchymal stem cells and *in vivo* with upper respiratory cells. I understand that I have currently shortness of breath and was found to have a primary carcinoma (a mucoepidermoid carcinoma) of the trachea, judged inoperable with traditional airway surgery. The tracheal tumor extends across the right tracheobronchial angle over a length of 5 cm, confirmed by CT and PET scans. The PET-CT shows no distant spread.

I have read as well the protocol of the transplant procedure, written in English, and understand that this represents the only chance of survival I have. A trachea of corresponding length and size will be custom-made at the University College in London by Prof. Seifalian and that Prof. Macchiarini will take the scaffold to Stockholm. I would then undergo a redo median sternotomy (re-opening of the chest) to take of the tracheal tumor using classic surgical airway principles. Before surgery (on June 7th), 200 to 300 mL of bone marrow would be aspirated from my left or right iliac crest and processed by Prof. LeBlanc K so that undifferentiated mesenchymal stem cells would be used for the reseeding process (48-72 hrs) using the bioreactor described in the Protocol.

At the time of transplantation, islands of respiratory cells will be taken from the right and left nose, and used to resurface the internal layer of the graft to promote re-epithelialization. Once the primary tracheal tumor has been resected, the tracheal scaffold will be reseeded with the above mentioned cells (respiratory cells on the internal) within the native tracheal bed of my body. Once the reseeding has been made, the tracheal graft will be anastomosed proximally and distally to recreate a trachea and tracheobronchial bifurcation, and wrapped with the *omentus major* (vascularised fat from the big stomach curve) to provide vascularisation and protect against radiation therapy (usual manoeuvres in lung transplantation). To boost the regeneration process, a perioperative treatment with local injection of transforming growth factor-B1 (50µg) (transforms mesenchymal stem cells into chondrocytes), granulocyte-coloning stimulating factor (10 mg/kg) (recruits progenitor endothelial cells) and erythropoietin (10.000UI) (reduces apoptosis) will be given for 2 weeks only. These drugs will be given at "regenerative" doses and have no side-effects. I have also been informed that tissue engineered graft will not require any immunosuppression at any time, and especially its side-effects.

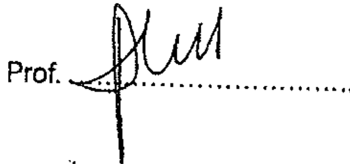
KOPIA

Anaesthesia will be general and through selective orotracheal tube, using arterial monitoring lines, urinary bladder catheter, epidural analgesia and cardiopulmonary by-pass stand-by. Complications from this transplant could be postoperative bleeding, left recurrent nerve palsy, respiratory infections, anastomotic complications, wound infections, respiratory insufficiency and requirement of mechanical ventilation.

I have been informed clearly about every single details, and my questions and doubts have been clarified without any restrictions. It is therefore that I liberally take the decision to authorize the above mentioned procedure with the understanding that I could retract this consent at any time. As proof of willingness, I sign this document.

Huddinge, 26 the June 2011

Doctor Signature

Prof. 

Signature of the patient

Mr

Appendix 16a

Articles

Tracheobronchial transplantation with a stem-cell-seeded bioartificial nanocomposite: a proof-of-concept study

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Summary

Background Tracheal tumours can be surgically resected but most are an inoperable size at the time of diagnosis; therefore, new therapeutic options are needed. We report the clinical transplantation of the tracheobronchial airway with a stem-cell-seeded bioartificial nanocomposite.

Methods A 36-year-old male patient, previously treated with debulking surgery and radiation therapy, presented with recurrent primary cancer of the distal trachea and main bronchi. After complete tumour resection, the airway was replaced with a tailored bioartificial nanocomposite previously seeded with autologous bone-marrow mononuclear cells via a bioreactor for 36 h. Postoperative granulocyte colony-stimulating factor filgrastim (10 μg/kg) and epoetin beta (40 000 UI) were given over 14 days. We undertook flow cytometry, scanning electron microscopy, confocal microscopy epigenetics, multiplex, miRNA, and gene expression analyses.

Findings We noted an extracellular matrix-like coating and proliferating cells including a CD105+ subpopulation in the scaffold after the reseeding and bioreactor process. There were no major complications, and the patient was asymptomatic and tumour free 5 months after transplantation. The bioartificial nanocomposite has patent anastomoses, lined with a vascularised neomucosa, and was partly covered by nearly healthy epithelium. Postoperatively, we detected a mobilisation of peripheral cells displaying increased mesenchymal stromal cell phenotype, and upregulation of epoetin receptors, antiapoptotic genes, and miR-34 and miR-449 biomarkers. These findings, together with increased levels of regenerative-associated plasma factors, strongly suggest stem-cell homing and cell-mediated wound repair, extracellular matrix remodelling, and neovascularisation of the graft.

Interpretation Tailor-made bioartificial scaffolds can be used to replace complex airway defects. The bioreactor reseeding process and pharmacological-induced site-specific and graft-specific regeneration and tissue protection are key factors for successful clinical outcome.

Funding European Commission, Knut and Alice Wallenberg Foundation, Swedish Research Council, StratRegen, Vinnova Foundation, Radiumhemmet, Clinigene EU Network of Excellence, Swedish Cancer Society, Centre for Biosciences (The Live Cell imaging Unit), and UCL Business.

Lancet 2011; 378: 1997–2004

Published Online
November 24, 2011
DOI:10.1016/S0140-6736(11)61715-7

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Introduction

Primary tracheal cancers are rare neoplastic lesions characterised by a high mortality rate. The gold standard treatment for these lesions is surgical resection with primary reconstruction.[1] However, epidemiological studies have shown that, because of difficulties in the definitive diagnosis, most patients with primary malignant tracheal cancers present with local inoperable disease (exceeding 6 cm or >50% of the total tracheal length) and are, therefore, treated with palliative measures. For these patients, prognosis is poor with a reported 5-year survival rate of about 5%.[2] Moreover, because safe reconstruction of the trachea is not possible, even in patients with operable tumours, the proportion of complete tumour resection is less than 60%.[3] This outcome would be greatly improved if a trachea substitute with similar anatomical, physiological, and biomechanical properties of the native trachea were available.

In 2008, we reported the first fully tissue-engineered tracheal transplantation with a non-immunogenic decellularised human donor trachea reseeded with bone-marrow-derived mesenchymal stem cells (MSCs) and respiratory cells.[4] However, this approach is limited by the shortage of donor organs of an appropriate size and has other disadvantages (webappendix p 9). As a result, an alternative, tailor-made synthetic tracheal scaffold is an urgent clinical need. We report the clinical transplantation of the tracheobronchial airway in a patient with recurrent primary trachea cancer, with use of a tailor-made artificial scaffold reseeded ex vivo with mononuclear cells (MNCs)[5] and a growth factor-induced endogenous stem cells mobilisation.

Methods

The recipient

Webappendix pp 2–9 provides a detailed description of the methods. A 36-year-old man presented in May, 2011,

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See Online for webappendix

See Online for webvideo

at the Karolinska University Hospital (Huddinge, Sweden) with stridor, cough, and respiratory difficulties. The patient, previously treated elsewhere with tumoral debulking surgery and postoperative regional radiation (70 Gy),[6] presented with a recurrence of a primary tracheal mucoepidermoid carcinoma affecting the distal trachea and both main bronchi (figure 1A). The patient underwent an extensive staging,[1] including ^{18}F-fluorodeoxyglucose (^{18}F-FDG) PET scan, multistage biopsies of the respiratory mucosa proximal and distal to the macroscopic tumour burden, and bone marrow biopsy and aspiration. Results showed no local or distant lymphatic or systemic metastasis, and normal stromal cells. The tumour extended from 5 cm above the right tracheobronchial angle into the first 1·3 cm of the right main bronchus, leaving the origins of the upper and intermedius take-offs tumour free, and the first 1·5 cm of the origin of the left main bronchus (webvideo 1). On the basis of surgical standards,[3] this extension was deemed beyond resectability; therefore a transplant procedure, with an artificial biomaterial, was offered to the patient.

We obtained written informed consent from the patient, and the transplant procedure was approved by the local scientific ethics committee.

Pretransplant preparation

We manufactured a tailor-made trachea from the preoperative chest CT and three-dimensional volume rendered images of the patient with a nanocomposite polymer (POSS-PCU; polyhedral oligomericsilsesquioxane [POSS] covalently bonded to poly-[carbonate-urea] urethane [PCU]) processed by an extrusion-phase-inversion method.[7] On the basis of our previous experience with the physical and mechanical properties of human trachea (webappendix p 11)[8] and the patient's preoperative CT scan (figure 1B), we developed a POSS-PCU nanocomposite polymeric airway of appropiate size and morphology, reproducing the exact dimensions of the patient's tracheobronchial structure (webappendix p 12). A Y-shaped three-dimensional glass mandrel was fabricated, and U-shaped rings of POSS-PCU, analogous to the cartilaginous rings of tracheobronchial tissue, were manufactured with casted methodologies and placed around the mandrel. The entire mould was then placed in the POSS-PCU solution to form a coagulated porous scaffold.

A bioreactor to accommodate precisely the maturation requirements of the Y-shaped synthetic windpipe construct used in the transplantation was developed (Hugo Sachs Elektronik-Harvard Apparatus GmbH, March-Hugstetten, Germany; figure 1C, D). The design was based on a sterilisable rotating-construct bioreactor, previously validated but with novel elements to drive a recirculating fluid flow within and around the developing graft,[5] enabling consistent and uniform delivery of cells, nutrients, gases, and hydrodynamic shear forces within the bioreactor. This process is accomplished without external fluid pumps and packaged within the bioreactor assembly so that ease of handling and simplicity of use in good manufacturing practice and clinical environments is not compromised.

Autologous MNCs were obtained 2 days before transplantation from a bone marrow aspirate through density gradient separation. Analyses of white blood cells, mononuclear cells, CD34+ cells, viability, colony-forming unit-fibroblast, flow cytometric characterisation, and sterility were done. To obtain the synthetic bioengineered tracheobronchial construct, cells were resuspended in low-glucose Dulbecco's modified Eagle's medium (Invitrogen, Stockholm, Sweden) and seeded onto the synthetic graft by incubation of the construct in the bioreactor at 37°C for 36 h before transplantation.

Immediately before transplantation, a second bone marrow harvest was done, and MNCs were separated and transferred to the operating theatre. Immediately before implantation, the airway construct was transported to the operating theatre, 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). We assessed sections of the graft, surplus to clinical need, by scanning electron microscopy, fluorescence, and bright field light microscopy and confocal live cell imaging (webappendix pp 14–15; webvideo 2).

Transplantation

Under general anaesthesia and orotracheal intubation, a redo sternotomy was done and the tumour-burden area dissected and mobilised, according to the principles of tracheal surgery.[1–3] Because of the previous postsurgical and radiation-induced scar tissue formation, the tumour had to be resected along with the right intrapericardial pulmonary artery, and subsequently revascularised with a Dacron 8F graft (Gelsoft, Vascutek, Terumo, Ann Arbor, MI, USA). The graft was interposed between the retroaortic and the extrapleural origin of the pulmonary artery, clamping both vena cavae for 26 min and without use of cardiopulmonary bypass. Tumour resection included the postlateral and mediastinal aspect of the distal truncus of the superior vena cava via lateral clampage and direct suture. The resected trachea included its distal intrathoracic 6 cm, the entire right main bronchus, and first 2 cm of the left main bronchus. All tumour margins were negative on frozen section, and a complete mediastinal lymph-node dissection was done. The airway was then reconstructed by implantation of the reseeded nanocomposite end-to-end, first to the right and left main bronchi and then to the proximal trachea, with standard techniques.[3] Finally, the omentum major was wrapped around the construct and the median

sternotomy and laparotomy closed in a standard manner. From our past experience with carinal surgery when cough reflex, mucous clearance, and patient's full mobilisation are suboptimal in the early postoperative course, a temporary tracheotomy above the implanted graft was made at the end of the procedure.[9]

Regenerative boosting therapy

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.

Follow-up assessment

Control endoscopies were done postoperatively daily for the first 7 days, for inspection of the graft and anastomoses. Bronchoscopies were then done once a week during admission to hospital, and once a month thereafter. Postoperative biopsies of the bioartificial graft were assessed by immunohistochemistry, haematoxylin-eosin stain, periodic acid-Schiff stain, and Masson's trichrome stain. Micro-RNA assessment in serum and analyses of soluble factors in plasma (multiplex cytokine assay and ELISA) were done every second day for 2 weeks after transplantation.

Peripheral blood mononuclear cells (PBMCs) were isolated by gradient centrifugation, with Lymphoprep (Nyegaard, Oslo, Norway) and washed twice with phosphate-buffered saline (Gibco, Grand Island, NY, USA). We assessed cell count and viability assays by Türk and trypan blue dye exclusion or by Nucleocounter NC-100 (ChemoMetec A/S, Allerød, Denmark). We analysed gene expression, chromatin immunoprecipitation, and analyses of PBMC subsets and phenotyping 2 days before surgery, and for 2 weeks postoperatively.

Statistical analysis

We undertook data analysis, preparation of graphs, and statistical comparisons with Prism software (Graphpad Prism version 5.0a) and three-dimensional surface modelling with Microsoft Excel 2010.

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

The patient was awake 24 h after transplantation. The immediate postoperative course was characterised by a right upper lobe pneumonia (day 2); *Candida albicans* (>10 000 CFU/mL) and *Stenotrophomonas maltophilia* (>10 000 CFU/mL) were isolated and treated with broad

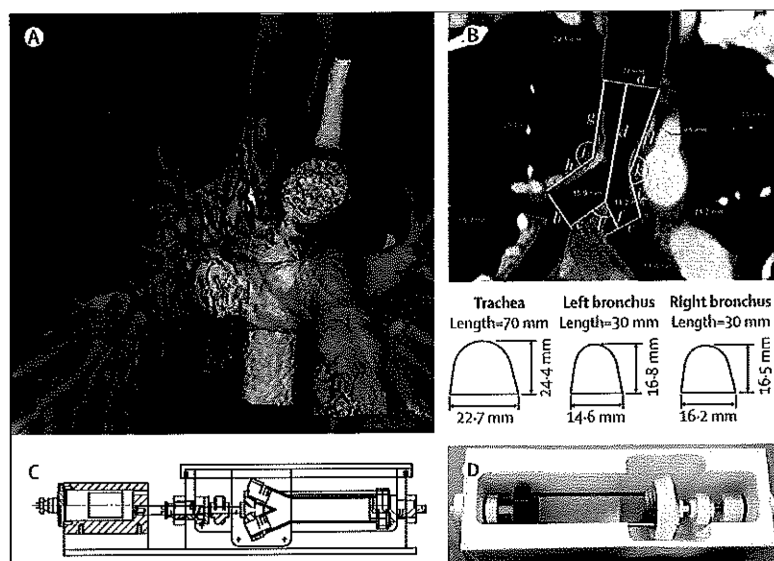

Figure 1: **CT scan and three-dimensional volume rendered (VR) images**
(A) Preoperative VR frontal plane image. Extension of tumour is shown in green. Tracheal and bronchial tree air space is shown in bright blue. The image shows the relation between tumour and right main pulmonary artery. (B, top) Measurement image. An angulated 2 mm thick CT image is used for measurements for individualised scaffold production. The image was optimised for concurrent visualisation of the tumour involvement of trachea inclusive of carina and proximal bronchi aligned parallel to tracheal longitudinal axis. Yellow lines mark proximal trachea (a) and distal right (b) and left (c) bronchi free from tumour involvement, and measurement of the transverse lumen diameters. Sagittal diameter was calculated from angulated transverse image perpendicular to the central lumen line (not shown). (d) Trachea free from tumour (carina); (e) right bronchus free from tumour (carina); (f) left bronchus free from tumour (carina); (g) trachea free from tumour, angle of trachea and right main bronchus; (h) angle of trachea and right main bronchus, right main bronchus free from tumour; (i) 1+2 corresponding left side measurements; (j) angle of trachea and right bronchus; (k) angle of trachea and left bronchus; (l) carinal angle. (B, bottom) Cross section of mandrel for (a) trachea, (b) left bronchus, and (c) right bronchus. (C) Frontal cutaway view of the improved bioreactor. (D) Macroscopic view showing bioreactor without the lid.

spectrum antibiotics and intensive physiotherapy. Other than this complication, the patient improved gradually and was weaned from the mechanical ventilation on day 5 postoperatively.

1 week after surgery, the bronchoscopy (webvideo 3, figure 2A) showed a normal and patent airway bleeding from its inner layer at the contact with the scope; the obtained biopsy samples showed the presence of necrotic connective tissue associated with fungi contamination and neoformed vessels (figure 2B). The temporary tracheotomy cannula was removed 18 days later. The patient was then transferred to a normal ward and discharged to the referral hospital 1 month after surgery. The biopsy sample 2 months after transplantation showed large granulation areas with initial signs of epithelialisation and more organised vessel formations, and no bacterial or fungi contamination (figure 2B). The patient was discharged from the referring hospital to start rehabilitation and later resumed his university studies. 5 months after transplantation, the patient is asymptomatic, breathes normally, is tumour free, and has an almost normal airway (figure 2C) and improved lung function compared with preoperatively (table, webappendix p 16).

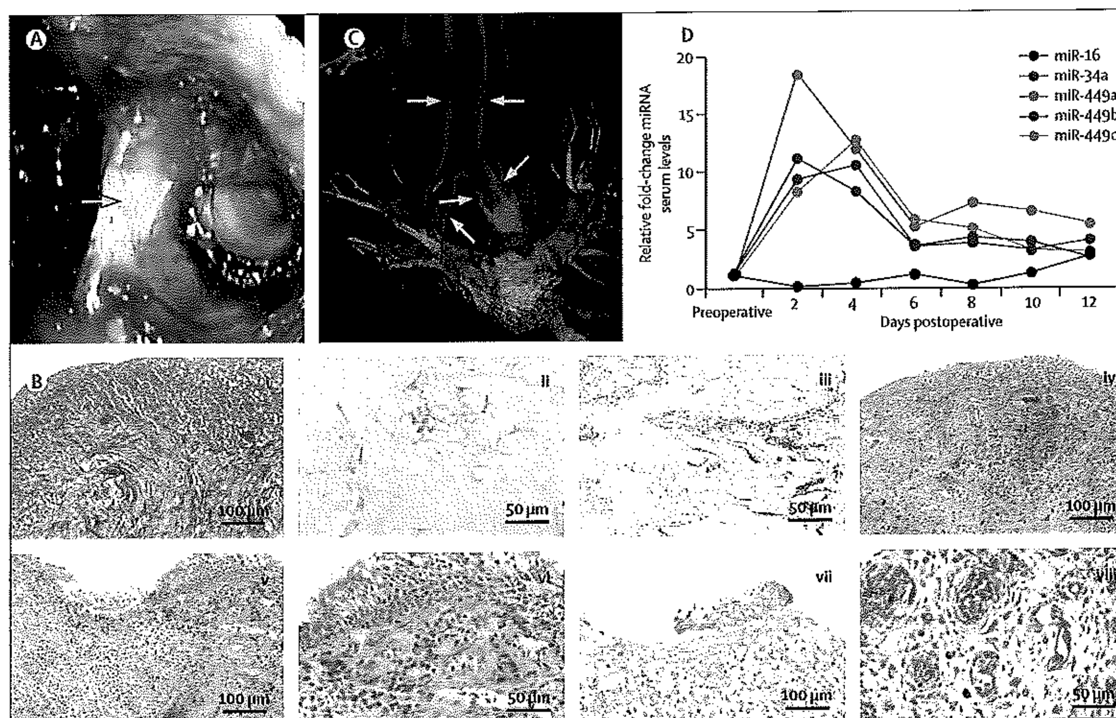

Figure 2: **Postoperative follow-up**

(A) Bronchoscopy image showing the transplanted bioengineered construct integrated with surrounding tissues. (B) Histological evaluation (Masson) of the first postoperatively obtained biopsy sample (at day 7) from the distal part of the graft showed necrotic connective tissue (i) with fungi contamination (periodic acid-Schiff stain; ii) but developed vascular structure (CD146 with Bond Polymer Refine Detection brown colour; NGFR with Bond Polymer Refine Red Detection red colour [without stained structures]; immunolabelling was done on Leica -Bond-Max automated immunostainer; iii). By contrast, the follow-up biopsy at 2 months (haematoxylin-eosin stain) showed ulceration with granulation tissue (iv) and still inflammation, but also respiratory epithelium with mucus secreting cells (haematoxylin-eosin stain; v and vi). Additionally, P63-DAB/CK17 staining showing detached metaplastic squamous epithelium (red; vii) and Mib1-DAB/Vimentin staining showing proliferating (brown) endothelial structures in capillaries. (C) Postoperative volume rendered (VR) image. Air in airways is shown in bright blue. Note that the VR technique displays only the factual air and not the scaffold material. Yellow arrows show borders for scaffold insertion. (D) Serum levels of miR-16, miR-34, miR-449b, and miR-449c were measured by quantitative PCR in serum samples gained at the indicated timepoints before and after surgery.

	May, 2011 (before surgery)	October, 2011 (4 months after surgery)
FVC (L)	3·80	2·63
FEV_1 (L)	1·52	1·95
FEV_1:FVC (%)	40·02%	74·37%

FVC=forced vital capacity. FEV_1=forced expiratory volume in 1 s.

Table: **Lung function tests**

Analyses by micro-RNA expression have shown that serum levels of miR-34 and miR-449 members are potential biomarkers for promotion of terminal differentiation of airway epithelium.[10] Compared with preoperative levels, we noted upregulation 2 days after transplantation, which gradually decreased (figure 2D). By contrast, serum levels of miR-16—a ubiquitous miRNA frequently used for normalisation of serum miRNA levels[11]—remained unchanged.

The autologous bone marrow MNCs were seeded on the synthetic graft and incubated in the bioreactor (webappendix p 14), resulting in a bioengineered tracheobronchial construct suitable for transplantation (webappendix p 17). Scanning electron microscopy of cells incubated in the bioreactor and confocal microscopy of live cells statically exposed to the biomaterial identified cells of different morphologies inside the scaffold, including long processes, filopodia, and lamellipodia (webappendix pp 16–17), whose formation requires anchorage to the scaffold. Although a shortage in material prevented us from undertaking a thorough analysis of cell division, some of the observed cells seemed to be newly divided (webappendix p 17) and cells exposed to the bioreactor aggregated in dense clusters, suggesting clonal expansion. Staining with the CD105 marker showed a subpopulation of cells of mesenchymal lineage (webappendix p 17). Flow cytometric phenotyping of the bioreactor medium showed a selective reduction of MSCs and haemopoietic stem cells (HSCs) after the reseeding process, with a particular decrease in CD90 high and CD59 dim cells (webappendix p 17), suggesting their preferential attachment and engraftment to the scaffold.

Monitoring of patient cell counts and plasma markers showed formation of typical acute phase reactants,

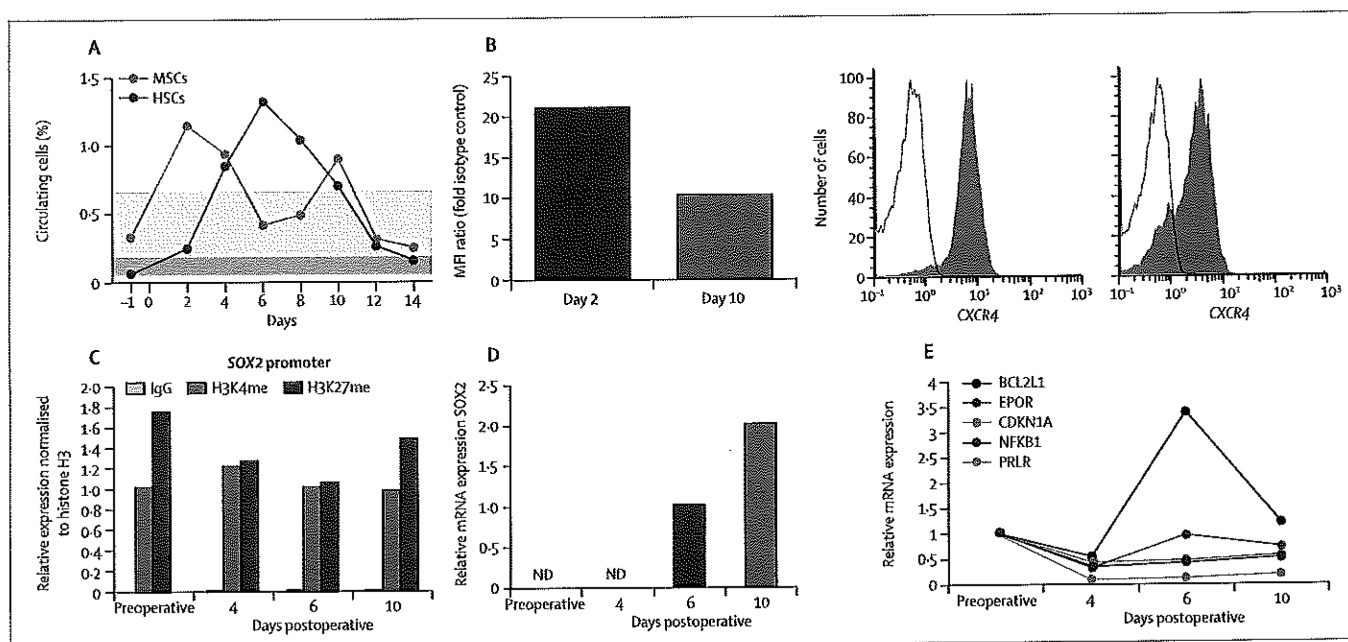

Figure 3: **Analysis of peripheral blood mononuclear cells after surgery (flow cytometry, gene expression, and epigenetic regulation)**
(A) Dynamics of circulating MSCs and haemopoietic stem cells (HSCs) as analysed by high-resolution flow cytometry. MSCs in circulation reached a peak level 2 days after surgery and subsequently decreased, and thereafter reached a secondary peak at day 10. The detection ranges from four healthy donors matched for age and sex (male donors aged from 32–39 years, randomly selected at the Karolinska Institutet, Stockholm, Sweden) are shown in light grey. The percentage of circulating HSCs increased and reached to a plateau at day 6 but then rapidly decreased to normal levels at day 14. Reference ranges assessed from four donors are shown in dark grey. (B) Expression of CXCR4 on circulating MSCs on days 2 and 10 as acquired by flow cytometry. Left panel: comparison of mean fluorescence intensity (MFI) ratios on days 2 and 10. Right and centre panels: red lines indicate isotype controls and green histograms indicate MSCs. (C) Quantitative PCR results after chromatin immunoprecipitation procedure using antibodies against histone H3 (for normalisation), trimethylated lysine 4 (H3K4me3), and trimethylated lysine 27 (H3K27me3) on histone 3 at the promoter regions of the gene SOX2. (D) Expression of the SOX2 gene relative to GAPDH, analysed by quantitative RT-PCR. (E) Fold change of genes with antiapoptotic function, relative to the preoperative condition, analysed by quantitative RT-PCR array. ND=not detectable.

wounding, tissue remodelling, and regenerative factors after surgery (webappendix pp 18–19). Webappendix p 19 shows results of a heat map analysis with the exact kinetics and numerical values for individual factors. Findings from a flow cytometric analysis of the peripheral blood showed an increase in HSCs at day 6, with a particular increase of the CD90+ subpopulation (webappendix pp 20–21). We noted an increased amount of circulating MSCs at day 2, to 15-fold higher levels than the age and sex matched healthy donor range (figure 3A), possibly as a result of the boosting therapy before surgery; this increase was followed by a three-fold decrease at day 4 and 6, to show a secondary two-fold increase at day 10. In line with these findings, the relative gene expression of homing-associated factor SDF-1 receptor CXCR4 was two-fold higher in MSCs at day 2 than at day 10 (figure 3B).

We then undertook a molecular analysis of the stem-cell phenotype. Chromatin immunoprecipitation showed that the *SOX2* gene, encoding a progenitor-associated transcription factor, showed stable concentrations of the H3K4me3 activity mark throughout the postoperative analysis. However, the H3K27me3 repressive mark was decreased in samples retrieved 4 and 6 days after surgery, showing an increased activity and decreased repression at these timepoints (figure 3C). Accordingly, this increased total activity preceded an induction of SOX2 gene expression at day 6 and a further increase at day 10 (figure 3D). Another progenitor-associated gene, GNL3, also displayed an increasing ratio of active and repressive marks H3K4me3 and H3K27me3 at day 6, correlating with increased gene expression (data not shown). These results on chromatin state and gene expression lend support to the flow cytometric analysis indicating a second wave of circulating MSCs (figure 3A, webappendix p 20).

We investigated the expression in PBMCs of genes involved in the JAK/STAT pathway—a major signalling transduction pathway activated by epoetin beta. Most genes showed a consistent trend of downregulation at day 4 postoperatively, compared with the preoperative condition, with subsequent progressive recovery of the expression levels at days 6 and 10 (webappendix p 22). Analysis of antiapoptotic genes showed a peak in the expression of BCL2L1 and EPOR at day 6, suggesting increased antiapoptotic activity in PBMCs at that time (figure 3E).

Discussion

Findings from this proof-of-concept case study show the feasibility of tracheal transplantation with an artificial nanocomposite reseeded with autologous stromal cells.

Panel: Research in context

Systematic review

We searched Medline and PubMed without date or language restrictions for articles with the following terms: "trachea", "tracheal replacement", "tracheal cancer", "tracheal surgery", "transplantation", "stem cells", "cell mobilization", "tissue engineering", "scaffold", and "synthetic material". We identified several relevant articles showing challenges and outcome of tracheal reconstruction and replacement, published between 1997 and 2011.[3,4,9,10,20,21,23–25] However, we could not locate any report showing the successful transplantation of a cell-seeded synthetic scaffold-based tracheal transplantation in human beings. Moreover, we identified no publication showing detailed insights into active stem-cell mobilisation in tracheal transplanted patients.

Interpretation

Our study is the first to describe a successful transplantation of a synthetic-based stem-cell seeded scaffold in a patient. Our data were collected from preoperative and postoperative in-vivo measurements and in-vitro studies. We applied complementary methods to confirm findings. Even though we now describe only one case of tissue-engineered tracheal transplantation, the magnitude of data and the validation of a specific mechanism suggest solid evidence. Our specific findings are verified and discussed extensively in relation to earlier findings from independent investigators. Moreover, our data provide novel insights into cellular pathways.

We have also shown the possibility of stem-cell mobilisation and the dynamic patterns and profile of mononuclear cells in peripheral blood circulation. Despite much progress in the clinical translation of tissue engineered organs and complex tissues,[12,13] no safe and suitable solution has been identified to successfully replace the trachea.[14,15] With a human decellularised tracheal matrix, repopulated with in-vitro expanded and differentiated autologous chondrocytes of MSC origin and autologous epithelial cells via a novel bioreactor system, the first-in-man completely tissue-engineered trachea replacement was successfully done.[5] This strategy, improved by intraoperative graft seeding with autologous cells (bone marrow MSCs and respiratory cells) and conditioning with differentiation and tissue-protective factors,[16] was subsequently successfully used in patients with both benign and malign airway diseases. However, this approach has limitations—eg, a long period for the decellularisation process (15–20 days), the need for different patient-specific sizes, the risks for altering long-term natural matrix mechanical properties, bacterial contamination during the in-vitro natural graft manipulation, and, most importantly, the absolute requirement of obtaining a donor organ.

The primary tumour of our patient involved the last 5 cm of the distal trachea along with the tracheobronchial bifurcation, which represents an absolute contraindication to any surgical resection. Because the patient had a tumour recurrence with severe stridor, despite 70 Gy of radiation therapy, and the waiting time for a donation would have been unpredictable, we decided to attempt a curative surgery by replacement of the resected airway with an artificial POSS-PCU-based nanocomposite combined with a novel pharmacological boosting strategy. This decision was based on the fact that the POSS-PCU is biocompatible, non-toxic, non-biodegradable, inert, and has negligible immunoreactivity.[17] Additionally, it displays mechanical properties, in-vivo chemical stability, and nanostructural features,[18,19] which approach the ideal for a bioengineered tracheal implant. And, it is patient specific, since it could be designed to replace not only the trachea but also the bronchi, or a combination of both. Lastly, it can be produced in a rapid and clinically appropriate timeframe.

Previous attempts to replace the airways of patients with tracheal cancer with synthetic materials have been unsuccessful because of graft's limited cell seeding, infection, migration, stenosis, necrosis, and ultimately death of the patients.[4] These drawbacks are clearly related to the fact that the trachea is not located in a mesenchymal environment, but is in direct contact with the breathing air, making infection and contamination more likely to occur. Thus we used a bioreactor environment to reseed the bioartifical scaffold with autologous mononuclear cells. Results showed that 36 h of dynamic incubation of the mononuclear cells in the bioreactor was sufficient for them to adhere to the biomaterial. 2 days after seeding, the cells exposed to the bioreactor formed dense clusters, whereas those statically incubated were more evenly distributed (webappendix p 17), indicating that the bioreactor might help nested cells to proliferate. Antibody labelling and morphological analysis showed the presence of proliferating CD105+ cells with mesenchymal but not haemopoietic phenotype within the graft. Although an extracellular matrix (ECM)-like structure was observed on the scaffold (webappendix p 17) after the reseeding and bioreactor process, whether this structure is related to the autologous serum or the production of ECM by engrafted cells is unclear.

The most frequently reported airway complications after lung transplantation are necrosis, dehiscence, and stenosis, probably related to ischaemia of the transplanted bronchus during the immediate period after transplantation.[20–22] In this report, an avascularised, Y-shaped nanocomposite was implanted and the initial fungal infection had resolved within 4 months from transplantation; later the endoluminal surface was partly lined with respiratory mucosa, at which we noted nearly healthy epithelium and proliferating endothelium. This finding provides evidence that a bioengineered synthetic tracheobronchial nanocomposite can be recellularised in vivo with site-specific cells to become a living and functional scaffold completely integrated into the adjacent tissues. The measured levels of miR-34/449

micro-RNAs, which have been proposed as potential biomarkers of terminal differentiation of airway epithelium,[10] suggest the presence of postoperative airway epithelial differentiation in the patient.[11]

One of the key issues in a synthetic transplantation setting is the recruitment of repair cells that promote the integration and remodelling of the newly transplanted material. The cellular components contributing to regeneration can be recruited either from local tissue or from circulating progenitor cells. We observed HSC mobilisation together with increased amounts of circulating MSCs, which contrasts with previous findings[23] of no mobilisation of MSCs, when G-CSF was used as mobilising agent alone. Indeed, surgery-induced inflammation and chemokine and anaphylatoxin release at the implantation site could be the reason for the observed MSC mobilisation in our patient. We detected release of a large array of soluble mediators associated with wound healing, which could promote MSC mobilisation, as previously reported by other investigators.[24,25] Additionally, G-CSF induced neutrophil expansion, and release of proteases, which we also detected, could have promoted progenitor mobilisation.[26] Progenitor mobilisation presumably occurs by weakening their anchoring within the niche, either by degradation of the anchoring ECM components or their retention factor SDF-1a itself.[26] Bone marrow progenitor cell activation, recruitment, tissue repair, and local immune suppression at the surgery site could be enhanced by blood activation products, such as C3a and thrombin,[26–28] which were formed upon scaffold implantation. We detected a strong systemic release of growth factors and matrix metalloproteinases, which are probably produced by recruited progenitor and other immune cells. Furthermore, we recorded a substantial and very early increase of the epoetin-receptor expression with simultaneous upregulation of antiapoptotic genes, such as *BCL2L1*. During inflammation, trauma cytokines are released which upregulate epoetin receptors but inhibit tissue protection by downregulation of local epoetin production and antiapoptotic downstream pathways, favouring cell apoptosis. To avoid this early postoperative absence of local epoetin production, we administered regenerative (500 UI/Kg) epoetin doses, aiming to favour and trigger early local tissue protection and regeneration.

Taken together, these results provide evidence that a successful organ regeneration strategy has been accomplished (panel). The successful overall clinical outcome of this first-in-man bioengineered artificial tracheobronchial transplantation provides ongoing proof of the viability of this approach, in which a cell-seeded synthetic graft is fabricated to patient-specific anatomical requirements and incubated to maturity within the environment of a bioreactor. Additionally, in-depth cellular biochemistry analyses have provided new insight into the mechanisms by which the so-called pharmacological boosting factors contribute to cell mobilisation, differentiation, and ultrastructural organisation of the fully engrafted tracheobronchial construct.

Contributors

PJ was responsible for the bioreactor-based cell seeding; assisted the surgery and with collection of secondary data; and wrote corresponding methods, results, and interpretation sections. EA and TS undertook all flow cytometry characterisation of the cells, interpreted the results, and wrote corresponding methods. SB provided preclinical data for human tracheal biomechanics, and helped to write the report. KLB, BN, and GM designed, undertook, and assessed the multiplex analyses and wrote corresponding methods. KLB undertook the bone-marrow isolation. PB organised and supervised standards at the good manufacturing practice facility. BB did histological evaluation. CC and AS designed and developed the three-dimensional nanocomposite trachea, and wrote corresponding methods. OE and TG are responsible for the clinical follow-up of the patient and provided biopsy material and blood samples; TG also participated in the surgery and wrote corresponding methods. K-HG and JL assisted the surgery. SLG and SS did all cell imaging and wrote corresponding methods. OH and TL undertook and assessed all epigenetic analyses and wrote corresponding methods. JEJ and GH assisted in the preoperative and postoperative care. BL did all radiological imaging, interpretation, and three-dimensional reconstruction, and wrote corresponding methods. TL and CR undertook and assessed the miRNA studies, and wrote corresponding methods. VL and AIT undertook and analysed gene expression experiments, and wrote corresponding methods. EW isolated the mononuclear cells. PM was the primary investigator and leading author of the report, indicated how to built the three-dimensional nanocomposite, was leading surgeon and was responsible for the preoperative and postoperative course, and oversaw the review process. All authors provided primary data for modelling scenarios, and assisted with interpretation of results and report revision.

Conflicts of interest

We declare that we have no conflicts of interest.

Acknowledgments

The European Commission (FP7 EU Project: 280584-2BIOtracheaCP-FPFP7-NMP-2011-SMALL-5), the Knut and Alice Wallenberg Foundation, Swedish Research Council, and the StratRegen funded preclinical and postoperative in-vitro studies, gene expression, cell characterisation, and imaging. The Vinnova Foundation, Radiumhemmet, and Clinigene EU Network of Excellence funded multiplex and flow cytometric analyses. The Swedish Cancer Society supported the epigenetic studies. The Centre for Biosciences, the Knut and Alice Wallenberg Foundation, and the Swedish Research Council supported the Live Cell imaging Unit at the Department of Biosciences and Nutrition, Karolinska Institutet, used for live cell imaging. ERC-2007-Stg/208237-Luedde-Med3-Aachen funded the miRNA studies. The UCL Business supported the development of the three-dimensional nanocomposite scaffold. We thank all the health professionals of the Karolinska University Hospital in Huddinge, Stockholm, Sweden, without whom the transplantation could not have been done; Harvard Apparatus (Hollison, MA, USA) and Hugo Sachs Elektronik-Harvard Apparatus GmbH (March-Hugstetten, Germany) for supporting us with the bioreactor and the supervision of its use; Peter Güntner, for image analysis and measurements for scaffold production, and Anders Svensson, for three-dimensional volume image rendering, both from the Department of Radiology, Karolinska University Hospital Huddinge, Stockholm, Sweden; all the staff of the Landspitali University Hospital in Reykjavik, for the excellent support with the preoperative and postoperative care of the patient; Alessandra Bianco and Costantino Del Gaudio from Department of Science and Chemical Technologies, Intrauniversitary Consortium for Material Science Technology (INSTM), Research Unit Tor Vergata, Rome, Italy, for bi-dimensional and three-dimensional mathematical model for tracheal lateral area assessment; Iyadh Douagi from the Center of Hematology and Regenerative Medicine, Department of Medicine, Karolinska Insitutet for his assistance with flow cytometry; and Ulrika Felldin from ACTREM, Karolinska Institutet for her assistance with cell isolation.

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Airway Transplantation

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KEYWORDS

• Trachea replacement • Tissue engineering • Allogenic transplantation
• Immunosuppressive medication • Rejection

KEY POINTS

- Replacing long segments or the entire trachea in humans.
- Allotransplantation.
- Surgical challenges.
- Organ rejection.
- Tissue engineering.

A variety of benign or malignant disorders affecting the trachea can theoretically be treated by simple resection and subsequent end-to-end anastomosis of the remaining trachea.[1] This primary reconstruction is, so far, the only curative treatment in patients with tracheal diseases but, unfortunately, it is feasible only when the affected tracheal length does not exceed 6 cm in adults and about 30% of the entire length in children. Besides this technical restriction, local anatomy, previous treatments, and type of pathologic condition can further restrict the already few therapeutic options.

Longer segments cannot be treated surgically because it is impossible to perform safely a direct reconstruction of the airway that, under these circumstances, would ultimately fail because of the excessive tension at the anastomotic site. Benign diseases have been approached with various endoluminal solutions.[1,2] Because most of primary tracheal malignancies are diagnosed in an advanced local stage, only palliative options remain available, such as stenting, tumor debulking or radiotherapy.[1,3] Consequently, tracheal transplantation could be a valid alternative for many patients (Table 1). To this end, different replacement strategies have been investigated in experimental settings and some of them translated to the clinic. However, so far, none of them has turned into a routine clinical procedure. The requirements of an ideal tracheal substitute are multifaceted but crucial for a successful clinical application (Table 2).

TYPES OF TRANSPLANTATIONS

In 1963, Fonkalsrud and Sumida,[4] and, in 1971, Fonkalsrud and colleagues,[5] reported two initial clinical cases of tracheal replacement using the patients' own esophagus in congenital agenesis and long-segment stenosis. Initially, the patients recovered remarkably but then died within the first 6 weeks postoperatively. Both neotrachea required permanent stenting and did not provide normal

The authors declare no competing financial interests.
This work was supported by European Project FP7-NMP- 2011-SMALL-5, BIOtrachea; Bio- materials for Tracheal Replacement in Age-related Cancer via a Humanly Engineered Airway (No. 280584e2); ALF medicine (Stockholm County Council), Transplantation of Bioengineered Trachea in Humans (No. LS1101e0042); The Swedish Heart-Lung Foundation, Trachea Tissue Engineering; Doctor Dorka Stiftung (Hannover, Germany), bioengineering of tracheal tissue; and a megagrant of the Russian Ministry of Education and Science (agreement No. 11.G34.31.0065).
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Thorac Surg Clin 24 (2014) 97–106
http://dx.doi.org/10.1016/j.thorsurg.2013.09.005
1547-4127/14/$ – see front matter © 2014 Elsevier Inc. All rights reserved.

Table 1
Indications for potential tracheal transplantation and eligibility criteria

Indications	Benign Diseases	Malignant Diseases
—	• Trauma • Benign stenosis • Relapsing polychondritis • Osteochondroplastica • Amyloidosis • Tuberculosis	• Unresectable tumors • Postlaryngectomy recurrences or diseases
	Eligibility Criteria	
	• Extended (>60% total length) benign & malignant diseases • Already maximally pretreated • Age between 10 and 75 y • No absolute surgical contraindications • No regional and/or micrometastasis (bone-marrow biopsy proven) • Normal psychological or psychiatric habitus • Independent review board, ethics and national transplant clearance • Written informed consent	

function. No similar transplants have been made since. Instead, a variety of approaches have been attempted clinically that use either allotransplantation or tissue engineering (TE) approaches.

TRACHEAL ALLOTRANSPLANTATION

Fresh Cadaveric Trachea

In 1979, Rose and colleagues[6] described the first case of allogenic tracheal replacement using a fresh cadaveric tracheal graft in a 21-year-old male patient with extensive benign tracheal stenosis. In a two-stage procedure, the graft was initially implanted into the sternocleidomastoid muscle region to provide indirect vascularization and subsequently transferred to the orthotopic site. The patient was discharged 9 weeks after the transplantation without immunosuppressive medication and no signs for organ rejection or health status impairment. At that time, it was assumed that the tracheal immunogenicity was not relevant and graft failure was only provoked by graft ischemia and infection.[6] In contrast, Levashov and colleagues[7] transplanted on a donated trachea but with different findings. They used the omentopexy to obtain indirect blood supply of the cadaveric trachea and reported signs of organ rejection at day 10 postoperatively. The investigators affirmed the promising overall outcome 4 months after the transplantation but emphasized the essential need of adequate donor-recipient selection and modern immunosuppressive medication. Similar

Table 2
Requirements of ideal tracheal substitute

Scaffold for Tissue Replacement	Characteristics
General properties	• Nonimmunogenic • Nontoxic • Nontumorigenic • Allows for cell adhesion, migration, proliferation, and differentiation
Tracheal-specific properties	• Airtight and liquid-tight seals • Mechanical properties to react on both lateral and • Longitudinal forces • Support airway patency and respiratory function
Required mechanical properties based on native trachea	± 75° (right/left axial rotation at 0° maximum flexion) ± 75° (right/left axial rotation at 0° flexion) Flexion/extension 70°/60° and 40% of strain limit (flexion-extension bending) 40%:20% for tension/compression (axial/tension/compression) Lateral (right/left) bending: 48° and expected strain limit of 40%

findings were obtained by Delaere and colleagues[8] in a small animal model confirming the necessity of immunosuppressive medication for tracheal transplantation. In another in-depth evaluation of the antigenic profile of the human trachea, Shaari and colleagues[9] discovered the underlying mechanism of the immune competence of human trachea.

Despite the obvious significance of immunogenicity, most research was focused on graft revascularization. Klepetko and colleagues[10] demonstrated that the structural and functional properties of an allogenic graft could be maintained with heterotopic transplantation into the omentum. In a large animal study, Macchiarini and colleagues[11] described a harvesting technique to revascularize the entire trachea that, to be successful, required heavy immunosuppression to control rejection and perfect venous drainage. This experience has been applied by Duque and colleagues,[12] in Columbia, who have reported a series of 20 clinical laryngeal and tracheal transplants. An extension of this technique, a composite laryngotracheal (7 cm) graft transplant, was recently used in a 51-year-old woman already on immunosuppression.[13] Postoperatively, the patient continues to rely on a tracheotomy but has had the return of an oral and nasal airway, vocalization, smell, and taste.

The pros of this method are excellent vascularity and maintenance of functional integrity. The cons include life-long heavy immunosuppression and donor dependence.

In Situ Processed Fresh Cadaveric Trachea

Like Rose and colleagues,[6] Delaere and colleagues[14] investigated the interesting approach of using fresh cadaveric allografts. The harvested trachea was initially transplanted into the subcutaneous tissue of the recipient's forearm to induce neovascularization of the graft. Meanwhile, immunosuppressive medication was initiated and continued for the next 229 days. Within the observation period, the posterior wall of the heterotopic implanted graft became necrotic, and was removed and replaced by buccal mucosa of the recipient. After 4 months, the allograft was placed into the orthotopic position and a 4.5 cm defect of the patient's trachea replaced. At the time of orthotopic transplantation, the graft showed cartilage rings composed of viable cartilage tissue and epithelial lining consisting of squamous epithelium and respiratory epithelium.

The pro of this method is the need for immunosuppression is only early, not life-long. The cons are long-lasting and multiple procedures. So far, only short-segment replacement could be resected using standard techniques.

Cryopreserved, Irradiated, or Chemically Preserved Trachea

To avoid immunosuppressive medication, various attempts to reduce graft immunogenicity, such as cryopreservation,[15] irradiation, or detergent enzymatic treatment were investigated. Unfortunately, it was soon evident that the mechanical and structural macroscopic properties and cell adhesion and behavior would be damaged.[15–18] The long processing time was also a concern, especially for patients with malignancies. Regarding chemically fixated tracheae, Jacobs and colleagues[19] reported on 131 patients (100 adults and 31 children) who were treated with such processed grafts.[20] The technique was only applied in patients with benign tracheal disorders, except for one who had adenoid cystic carcinoma, with satisfactory results. However, because it requires an intact posterior tracheal wall, this technique is unfeasible for extended circumferential malignancies. The common denominator of these reconstructive methods is the need of permanent stenting. The pro of these methods is that there is no need for immunosuppressive medication. The cons are that they are donor dependent, have an extended processing time, are partly posterior wall dependent, involve stenting, and are nonvital.

Fresh or Cryopreserved Cadaveric Aorta

Some groups investigated the use of aortic grafts to replace the affected part of the windpipe. Carbognani and colleagues[21] introduced the technique of using a cryopreserved aortic allograft. Their study showed the technical feasibility of the method but also the imperative necessity of always placing a permanent stent into the graft to provide normal function and limit fibroblasts colonization. Wurtz and colleagues[22] reported satisfying clinical results without immunosuppressive medication by using either fresh or cryopreserved aortic homografts.[23] Nevertheless, the lack of cartilage-ring development and, therefore, related loss of structural integrity requires permanent stenting and other mechanical support.[22,24] The pro for these methods is that there is no need for immunosuppressive medication. The cons are donor dependence and permanent intraluminal stenting.

TE

Hermes Grillo,[1] a pioneer of airway surgery, stated that TE could become the most promising therapeutic concept for patients with

inoperable tracheal lesions. Experimental studies demonstrated the potential of TE for nearly every solid or tubular organ and each type of tissue. Initial clinical applications for a variety of these, such as heart valves, urine bladder, tubular structures, suggest the technologic feasibility and potential.[25–28] The concept of TE is based on four components: (1) scaffold or matrix, (2) cells, (3) bioreactor, and (4) various bioactive molecules.

The Scaffold

The scaffold is the basic component that provides the structural integrity of the graft. Characteristics must meet both general and organ requirements, including trachea-specific criteria. General requirements are that it is nonimmunogenic, nontoxic, and nontumorigenic, as well as allows for cell adhesion, migration, proliferation, and differentiation. Trachea-specific characteristics include airtight and liquid-tight seals at the start; mechanical properties that will react on both lateral and longitudinal forces, and support airway patency; and adequate respiratory function.

The biologic scaffold

Various biologic scaffolds have been investigated for their potential use but the ideal scaffold source depends on the target tissue. For the trachea in particular, three natural tissues have been tested in detail: the trachea,[29–31] porcine jejunum,[32] and the aorta.[33] The primary goal of TE is to provide a nonimmunogenic scaffold that can be transplanted without any subsequent need of immunosuppressive medication. Because the major histocompatibility complexes I and II are key for adverse immune reaction, it is imperative to remove them from the donor tissue. Decellularization, using various methods such as detergent and enzymatic solutions (ionic, nonionic, resolvent, chelating, alkaline, acidic, zwitterionic) and/or physical methods (perfusion, agitation, static), has demonstrated its efficiency in doing so. During the removal of the genomic DNA, the nanofiber architecture of the extracellular matrix (ECM) remained intact and the bioengineered ECM consisted of several proteins (laminin, elastin, collagen) of significant importance for cell homing, differentiation, migration, and proliferation. Each decellularization strategy has a different impact on the in vivo degradation process of the ECM, which essentially influences tissue remodeling stimulation, angiogenesis (neoformation of vessels), and cell homing.

To date, initial cases of clinical transplantation using biologic scaffolds processed by detergent enzymatic method have been reported with promising early outcome for both a decellularized trachea[27,34] and a porcine jejunum.[32,35] Macchiarini and colleagues performed a series that included nine patients suffering from various disorders and treated with a decellularized and reseeded trachea. Notably, they recognized unpredicted mechanical impairments within 12 months in about 30% of the patients (Macchiarini, personal communication, 2013). The pros of this method are that there is no need for immunosuppressive medication and preservation of the ECM. The cons are donor dependency, processing time, and biomechanical degeneration.

The artificial scaffold

Even though the initial clinical experience with biologic scaffolds seems promising, drawbacks do still exist, even though the biologic scaffolds do yet require a human donation. The decellularization protocol lasts approximately 2 to 3 weeks, which might not be practical for patients with malignant diseases that require an early treatment. In addition, the mechanical properties are a disadvantage, particularly for longer segments that require recurrent stenting because of the clinically observed instable biomechanics. Therefore, other alternatives are more desired.

As discussed previously, the assumption that the trachea is only a tubular structure to transport air from the larynx to the lungs is obsolete; other functions and characteristics have been elucidated. This complexity explains why synthetic grafts failed in serving as tracheal grafts.

> *Only a living substitute, therefore vascularized, can pretend to fulfill the anatomic mechanical and anti-infectious functions of the trachea.*
>
> —*Macchiarini*[36]

Artificial material that is not vascularized and is nonvital incorporates a low level of integrity, indicates a trend for migration and contamination, and is usually rather stiff and nonflexible. The absence of vascularization and immunocompetence must be solved to overcome all drawbacks of synthetic materials and their associated complications.[1]

Why artificial?

Synthetic scaffolds have unlimited availability, can be customized and manufactured to meet the patients needs, are inexpensive and fast to process, and can be sterilized without altering cell biology. Various biodegradable and nondegradable materials have been evaluated, including Marlex mesh (Chevron Phillips Chemical Company LP, TX, USA), polyethylene oxide/polypropylene oxide copolymer (Pluronic F-127, Invitrogen, Ltd,

Paisley, UK), polyester urethane, polyethylene glycol–based hydrogel, polyhydroxy acids, poly-ε-caprolactone, polypropylene mesh, poly (lactic–co-glycolic acid) polymer, gelatin sponge, and alginate gel.[1] To date only few clinical applications have been reported using synthetic based scaffolds.[37–39]

Various matters must be considered to turn synthetic materials into viable tissue. Aside from using cells to reseed the scaffold, pharmaceutical interventions are crucial to support the regeneration of the implanted graft. Moreover, the blood supply can be provided by indirect vascularization via surgical techniques, such as with the omentum major, latissimus dorsi flap, musculofascial flap, or other pedicles. The further development of these strategies will help to improve the outcome of synthetic-based trachea because of the enhanced integrity of the graft and ameliorated in situ regeneration of the transplant.

The pros of this method are that it is not donor dependent, there is no need for immunosuppressive medication, it is anatomically tailored, processing is fast and low-cost, and the nature of its mechanical properties. The cons are that the material is nonvital and there is a risk for contamination.

Composite scaffolds

Biologic and synthetic materials can also be combined into one scaffold to optimize the mechanical and bioactive properties of the graft. The coating of artificial scaffolds with various natural ligands and ECM-proteins may enhance cell adherence and proliferation while mechanical forces maintain. Research has been performed on collagen-coated poly-propylene scaffolds. In addition, various gels incorporating the cells for the internal and external surface were investigated.[40] The outcome of all these surface modification studies did not result in entirely convincing data; therefore, clinical transfer has not been realized.[41,42] In contrast, defects of the trachea smaller than 50 mm, have been successfully treated with the technology of composites using a Marlex mesh tube covered by collagen sponge.[43] Therefore, combinations of biologic and synthetic components may provide novel alternatives to TE in the future. The pros of this method are that it is not donor dependent, there is no need for immunosuppressive medication, it can be customized, and processing is fast, and cost-effective. The cons are that it is stent-dependent, nonvital, and there is a risk for contamination.

Scaffold-free

A recently developed technology of scaffold-free cell sheets is so far only applicable for very small defects but not for circumferential defects or longer gap reconstructions due to the lack of structural integrity.

The pros are that this method is not donor dependent and there is no need for immunosuppressive medication. The cons are that it is not feasible for long gap and circumferential reconstruction.

Cells

The cell type and source can differ and is highly dependent on the target tissue (**Fig. 1**). Considerations include stem versus differentiated cells, and allogenic versus autologous cells.

Aside from in vitro seeded cells, bone marrow-derived cells and resident stem or progenitor cells may contribute to tissue regeneration.[44] Go and colleagues,[45] and Jungebluth and colleagues,[38] demonstrated the necessity of seeding cells before tracheal transplantation to avoid graft collapse and bacterial contamination. Seguin and colleagues[44] provided similar evidence for the involvement of bone marrow–derived mesenchymal stem cells to tracheal regeneration. In addition, resident stem and progenitor cells, responsible for tissue regeneration and repair, have been detected in so-called stem cell niches along the airways, so far mainly in animals but similarities may exist in humans.[46,47]

Stem cells

Pluripotent stem cells The use of pluripotent cells, including embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs) is widely debated. In particular, ESCs are controversial because of significant ethical concerns. When Takahashi and Yamanaka[48] first described the technology of reprogramming mature cells into pluripotent stem cells, it was presumed that this cell type would soon be transferred to the clinic and be an ideal autologous alternative to ESCs. However, in experimental studies it has been elucidated that both iPSCs (depending on the reprogramming method) and ESCs seem to be prone to immune recognition and consequent rejection. In addition, the reprogramming for IPSCs is insufficient, which makes clinical use currently unrealistic. The pros of pluripotent cells are that they are not donor dependent (iPSCs) and there is no need for immunosuppressive medication (method dependent). The cons are ethical concerns, donor dependency (ESCs), and the need for immunosuppressive medication.

Multipotent stem cells Multipotent or adult stem cells, such as mesenchymal (MSCs), hematopoietic stem cells (HSCs), or amniotic fluid stem cells,

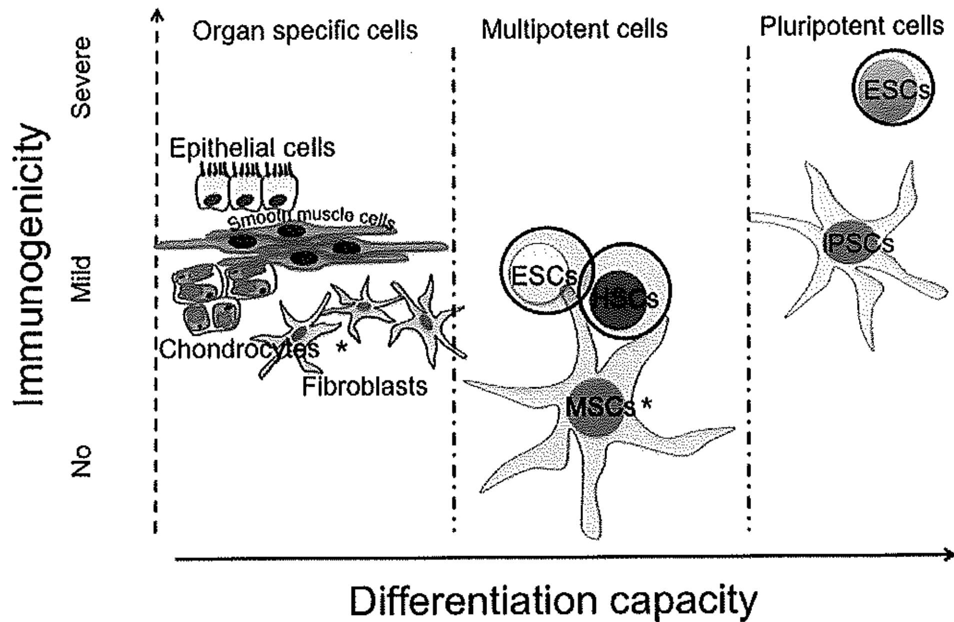

Fig. 1. Immunogenicity of various cell types potentially usable in TE. EPCs, endothelial progenitor cells; ESCs, embryonic stem cells; HSCs, hematopoietic stem cells; iPSCs, induced pluripotent stem cells; MSC, mesenchymal stromal cells. Already clinically applied (*asterisks*).

can differentiate into various cell types. These stem cells are usually obtained from the recipient (eg, bone marrow, peripheral blood, fat tissue) with essentially no ethical concerns and, therefore, can be applied without the use of immunosuppression. These so-called adult stem cells have already been used in many tracheal-related and nonrelated clinical settings.[27,49–51] Mononuclear cells (MNCs) have become clinically interesting (NCT01110252) because they include stem and progenitor cells, can be isolated from the bone marrow or the peripheral blood, and be actively mobilized to increase the yield of isolated cells.[37]

The pros of this method are there is no need for immunosuppressive medication, it is not donor dependent (depending on the cell type), multipotent differentiation, good availability, immunomodulatory capacity, and nearly no ethical issues. The con is that it is donor dependent.

Terminally differentiated cells

Epithelial, smooth muscle, and endothelial cells, and chondrocytes, have been clinically applied[14,27,32,35] for different tracheal replacement strategies. All these cells have low ethical implications, which make the clinical transfer simple.

The pros for this method are no need for immunosuppressive medication (depending on cell source), it is not donor dependent (if autologous), and isolation is simple. The cons are that it is donor dependent (if allogenic), immunosuppression (for allogenic) is needed, it is terminally differentiated, and the cell numbers are low.

Autologous versus allogenic cells

The advantage of using autologous cells is that it eliminates the patient's need for lifelong immunesuppressants. Regarding adult stem and progenitor cells, the available number and application field are high because of their self-renewal and differentiation capacity. In contrast, terminally differentiated cells have a limited source due to autologous cell or tissue donations making them inappropriate for some specific clinical scenarios. Allogenic cells have an unlimited availability but, aside from MSCs, require lifelong immunosuppressive medication.

The pros of allogenic cells are the unlimited cells numbers and the capacity for differentiation depending on the cell type. The cons are the need for immunosuppressive medication (except for MSCs). The pros of autologous cells are that they are not donor dependent and no immunosuppressive medication is needed. The cons are limited cell numbers and that they are inappropriate for malignant diseases (see Fig. 1).

Bioreactor

To provide the ideal conditions for TE of the airways, the natural environment should be ideally mimicked or used as a bioreactor. The external conditions influence all cellular parameters such as adhesion, engraftment, proliferation, differentiation, migration, and apoptosis. Asnaghi and colleagues[52] introduced a double-chamber rotating bioreactor for trachea engineering in a clinical setting with controlled and monitored conditions.

A more developed version of this bioreactor has been used for the clinical transplantation of natural decellularized scaffolds[27,34] and synthetic-based grafts.[37,38] Despite these initial promising clinical applications, there is the possibility of using the organism of the organ recipient as a biologic bioreactor with an single-[53,54] or multistage in vivo engineering strategy.[14]

In vitro pros are controlled and monitored conditions and customized devices. The cons are risk of contamination, lack of native characteristics, the need for additional substrates (eg, hormones, growth factors), cost, and labor demands.

In vivo pros are an ideal environment and it is cost-effective. The con is that there is no control of cell behavior (proliferation, migration, differentiation).

Pharmaceutical Intervention

Bioactive and signaling molecules, hormones, and other factors may be used to overcome the difficulties associated to engineered tissue transplantation, especially contamination and, even more significant, necrosis. Vascularization or, more precisely, neovascularization plays the most crucial role in this context and usually results in ischemia of the transplanted tissue. Direct and indirect vascularization can be achieved via surgical techniques as previously described.[11] However, additional strategies are necessary to provide sufficient angiogenesis within the transplanted construct. Therefore, other vascular growth promoting factors are required, such as vascular endothelial growth factor, fibroblast growth factor, and so forth. Aside from the bioactive factors that drive the neovascularization, further components such as endothelial cells are necessary to form the novel vessels. To this end, strategies that can mobilize progenitor and stem cells from their niches are of interest (Fig. 2), including granulocyte colony-stimulating factor (G-CSF), which was administrated in some early patients who underwent tracheal transplantation.[37] G-CSF can mobilize endothelial progenitor cells (EPCs) to support neoangiogenesis and MSCs can positively influence wound healing. MSCs, specifically CXCR-4–positive MSCs, can be attracted to the transplantation site and promote the regeneration of the implanted graft via their immunomodulatory capacity. The cell homing is initiated and driven by various blood activation products, chemokines, and/or growths factors and can be mediated through the SDF-1/CXCR4 pathway. Usually, an ischemic environment dominates the surgical wound due to initially poorly developed

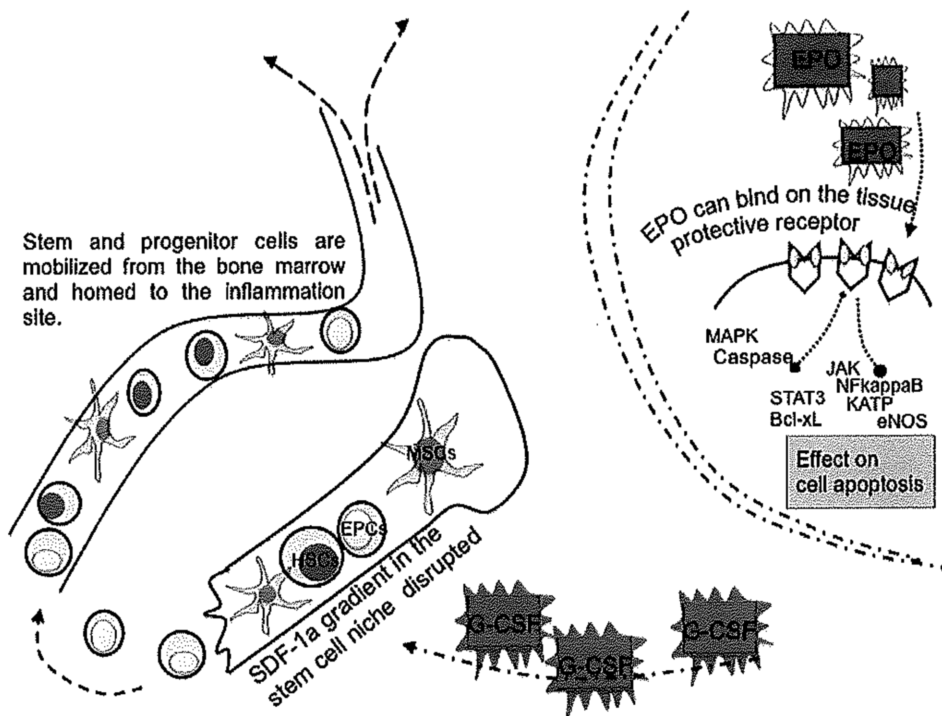

Fig. 2. Pharmaceutical intervention and bioactive molecules. EPCs, endothelial progenitor cells; EPO, erythropoietin; G-CSF, granulocyte colony-stimulating factor; HSCs, hematopoietic stem cells. Genes involved in apoptosis: Bcl-xL, B-cell lymphoma-extra large; eNOS, endothelial nitric oxide synthase; JAK, Janus kinase; KATP, ATP-sensitive K(+); MAPK, mitogen-activated protein kinases; MSC, mesenchymal stromal cells; NFkappaB, nuclear factor kappa-light-chain-enhancer of activated B cells; STAT3, signal transducer and activator of transcription 3.

neovascularization, which provokes increased cell death. Antiapoptotic strategies, therefore, may have beneficial impact on transplanted tissues. Erythropoietin (EPO) can certainly represent such a tool via its tissue protective function that has been described previously.[55,56] Aside from regulating the erythropoiesis, EPO influences the expression of several antiapoptotic genes, including Janus tyrosine kinase-2 (JAK-2) STAT5 (signal transducer and activator of transcription 5)-Bcl-2, phosphatidylinositol 3, protein kinase B, mitogen-activated protein kinase, and nuclear factor-κB. Using EPO may provide a protective effect for resident cells (within the wound environment) and to cells that are exposed to ischemic environment on the tracheal graft due to the lack of early vascularization.

Further strategies focus on the modification of the scaffold to improve cell–surface interaction and the material's biocompatibility and, therefore, the cell adhesion, proliferation, and differentiation. The pro of this method is support of the endogenous regenerative capacity. The cons are unknown risks and side-effects.

SUMMARY

Trachea transplantation remains a highly challenging procedure and, so far, no ultimate solution has been discovered. For many decades, physicians and researchers made immense efforts to overcome the hurdles of replacing a simple connection between the larynx and the lungs. It turned out to be much more difficult and, in particular, more complex than previously assumed. Various purely surgical techniques have been evaluated; however, because of technical challenges, they have not proven their clinical feasibility. In addition, conventional allogenic transplantation requires lifelong immunosuppressive medication and is associated with negative side effects. Recently, early clinical achievements in tissue-engineered trachea provide clinical evidence that this method might be the next promising therapeutic alternative in tracheal replacement (**Table 3**). Progress has been made in investigating underlying mechanisms and pathways of cell–surface interactions, cell migration, and differentiation; however, we are far from fully understanding the complexity of tracheal tissue regeneration. TE is

Table 3
Overview of clinical cases using TE tracheal grafts

	Decellularized Trachea Transplantation (2008–2011)	**Outcome**
Patients	9 male/female (3:6) (11–72 y)	4:5 (dead/alive)
Indications		
Benign diseases (n = 6)	4	All 4 alive, partly biodegradable stent-dependent
• Severe tracheomalacia or bronchomalacia	1	Alive, no need for stent support
• Long-segment congenital stenosis	1	Patient died of fulminant gastrointestinal bleeding
• Tracheoesophageal fistula		
Malignant diseases (n = 3)	3	All patients died of systemic tumor recurrence
• Primary tracheal carcinoma		
	Synthetic-based Trachea (2011–2013)	**Outcome**
Patients	Eight male/female, 3:5 (2–43 y)	2:6 (dead/alive)
Indications		
Benign diseases (n = 6)		1 (out of 6) patient died of unrelated causes
• Severe tracheomalacia or bronchomalacia	4	To date, all patients are alive (only the POSS/PCU scaffold requires stent treatment because of abnormal granulation tissue and fistula formation)
• Long-segment congenital stenosis	1	
• Congenital agenesis	1	
Malignant diseases (n = 2)	2	1 patient died of severe gastrointestinal bleeding
• Primary tracheal carcinoma		

a step in the right direction but only the future will elucidate the real impact of this technology on tracheal replacement.

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Appendix 17a

Från:
Till:

Datum:
Ärende:

Till: @karolinska.se
Från: "Bengus, Monica" <monica.bengus@roche.com>
Datum: 2014-09-19 12:58
Kopia: Reinhard Bucher <reinhard.bucher@roche.com>, "Schnetzler, Gabriel" <gabriel.schnetzler@roche.com>, Martin Bexon <martin.bexon@roche.com>, Margareta Olsson Birgersson <margareta.olsson_birgersson@roche.com>, Lena Ignatova <lena.ignatova@roche.com>
Ärende: Re: epoetin inquiry
(Se den bifogade filen: NeoRecormon SmPC recommendations (1) (2).docx)

Dear Dr.

I am International Medical Director at Roche taking care of the anemia compounds (as Neorecormon, Mircera).
Please see below our answers to the five questions below.

Please let us know if further clarification is needed.
Best regards,
Monica

Monica Bengus, MD
International Medical Director Mature Products
F. Hoffmann- La Roche Ltd
Global Medical Affairs
Grenzacherstr. 124
CH-4070 Basel, Switzerland
Phone: +41 61 688 0897

1. Is the term *"Regenerative boosting therapy"* in relation to NeoRecormon a known concept for Roche in the context of transplantation? What is Roche's opinion/experience/standpoint in using this type of *"supra-therapeutic"*-off label use?

The term *"Regenerative boosting therapy"* in the context of transplantation, is a concept not established and investigated by Roche.
There is no evidence for any clinical benefit to this and considerable reasons to be very concerned.
Roche is strongly recommending the use of Neorecormon in line with the approved indications and the information provided in the label.

2. Is there any scientific evidence by using this *"supra-therapeutic"* doses known by Roche regarding a *"regenerative"* effect as for example re-epithelialisation of synthetic implants?

There is no such scientific evidence.
There are some references that link EPO with wound healing and some that see it being used to condition stem cells that aid wound healing but there is nothing in human clinical experience that backs up the experimental evidence from assays of hormones and receptors and tissue culture experiments in the lab.
Bahlmann (Blood 2004; 1-3:921-926) has shown that administration of rhEPO increased the number of functionally active endothelial progenitor cells (EPCs) by differentiation in vitro in a dose-dependent manner, assessed in cell culture and by tube formation assay. The effect on EPC was already observed with an rhEPO dose of about 30UI/kg per week.

3. What is Roche´s opinion/recommendation regarding supra-therapeutic doses in pts on ECMO-support. Thromboembolic (TE) complications is a feared complication. 1 pts on ECMO support had 2 TE-complications, whereof as mentioned one developed a ECMO pump-thrombosis (otherwise extremely rare complication). Increased risk for TE-events? in previously non-anemic/non-epoetin-responder pts?

Thromboembolic Events are considered as Important Identified Risk in the anemia in oncology indication. Clinical studies have shown a higher frequency of TEEs in cancer patients treated with epoetin beta compared with untreated controls or placebo. In a Roche meta-analysis of 12 controlled studies based on the individual data of 2301 studies in cancer patients, the reported rate of TEEs was 4% in the control group compared with 7% in the epoetin beta group (HR 1.62, 95% CI: 1.13 – 2.31). This is within the rate of TEEs as adverse reaction of 1% to 10% listed in the current EU SmPC.
Despite differences in definition of events described as TEE, an independent Cochrane Collaboration working group also observed a comparably increased rate in cancer patients treated with ESAs compared with control (RR: 1.67; 95% CI 1.35 – 2.06). The meta-analysis of Bennet et al. provided similar results.

-Bohlius J, Wilson J, Seidenfeld J, et al. Recombinant human erythropoietins and cancer patients: updated meta-analysis of 57 studies including 9353 patients. J Natl Cancer Inst 2006;98(10):708–14
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Please see attached an extract from the EU SmPC and the dose recommendations and discontinuation requirements depending on the Hb values.
In a placebo-controlled study using epoetin beta in 351 patients with head and neck cancer (ENHANCE), study drug was administered to maintain the haemoglobin levels of 14 g/dL in women and 15 g/dL in men. Locoregional progression free survival was significantly shorter in patients receiving epoetin beta (HR=1.62, p=0.0008). It should be mentioned that ENHANCE study used a double dose (60 000 IU) and is an extreme outlier for tumour progression.The results and interpretation of this study were confounded by imbalances between the treatment groups, especially with regard to tumor localization, smoking status and the heterogeneity of the study population.

-Henke M, et al Erythropoietin to treat head and neck cancer in patients with anaemia undergoing radiotherapy: randomised, double-blind, placebo-controlled trial The Lancet (2003) 362: 1255-1260

4. What is Roche´s opinion/recommendation regarding the use of this supra-therapeutic doses in malignant (tumor) pts - 1 pt had as mentioned a non- radically resected malignant tumor but still received the "*regenerative boosting*" regime. Increased risk for tumor growth/spread?

It is thought by some that EPO as a growth factor has the potential to stimulate tumour growth / spread. It is suggested that this is a dose sensitive effect. However, supra-therapeutic doses have not been investigated by Roche and enhanced tumour growth with supra-therapeutic doses can not be excluded. Roche strongly recommends to use the product according to the approved label, including maximal dose recommendation for anemia due to cancer chemotherapy.

5. Specific considerations from Roche´s point of view concerning the risk of increasing adverse/side effects of NeoRecormon when combining the substance with Neopogen in "*supratherapeutic*" doses

It has previously been suggested that the co-administration of two growth factors would increase the risk of stimulated tumour growth / spread. Certainly a role for both EPO and GCSF in angiogenesis has been described in both tumour and other settings. Nevertheless, Roche is strongly recommending the use of Neorecormon in line with the approved indications, dosage and the information provided in the label.

A clinical study Trial to Reduce Cardiovascular Events with Aranesp® Therapy (TREAT) was performed which indicated that in patients with diabetes, chronic kidney disease (CKD), and moderate anemia who were not undergoing dialysis, darbepoetin alfa did not reduce the risk of either of the two primary composite outcomes (either death or a CV event or death or a renal event) and was associated with an increased risk of stroke. Based upon the TREAT study results, the European Agency (EMA) requested a pooled post-hoc analysis

of all ESA clinical studies in chronic renal failure patients. A tendency towards increased risk estimates for all-cause mortality, cardiovascular and cerebrovascular events associated with higher cumulative epoetin doses independent of the diabetes or dialysis status was observed.

Please note that in light of this analysis, EMA is proposing the following wording to be added to the label: "*Adequate control of symptoms of anaemia compatible with haemoglobin concentration below 12g/dL should be achieved with the lowest effective ESA doses because there is evidence to suggest that cumulative high ESA doses may be associated with an increased risk of mortality, serious cardiovascular and cerebrovascular events. Caution should be exercised with escalation of ESA doses in chronic kidney disease patients with a poor haemoglobin response to ESA and alternative explanations for the poor response should be considered*"

Bilagor:

NeoRecormon SmPC recommendations (1) (2).docx

Appendix 17b

MedInfo

Dr.
Karolinska Universitetssjukhuset Solna
17176 Stockholm

September 18, 2014

Thank you for your request for information regarding the following topics:

- Is the term "Regenerative boosting therapy" in relation to Neupogen known concept for Amgen in the context of transplantation? What is Amgen´s opinion/experience/standpoint in using this type of "supra-therapeutic"-off label use?

- Is there any scientific evidence by using this "supra-therapeutic" doses known by Amgen regarding a "regenerative" effect as for example re-epithelialisation of synthetic implants?

- What is Amgen´s opinion/recommendation regarding supra-therapeutic doses in pts on ECMO-support.

- What is Amgens´s opinion/recommendation regarding the use of this "supra-therapeutic" doses in malignant (tumor) pts - 1 pt had as mentioned a non- radically resected malignant tumor but still received the "regenerative boosting" regime. Increased risk for tumor growth/spread?

- Specific considerations from Amgen´s point of view concerning the risk of increasing adverse/side effects of Neupogen when combining the substance with NeoRecormon in "supratherapeutic" doses?

You have been in contact with my colleague Dhru Patel, Pharm D, Medical information Oncology, Amgen Thousand Oaks, regarding your inquiries on Neupogen and "regenerative boosting therapy". Dhru has given you a verbal response to your inquiries, however, you also asked for a literature search based on your question "What complications can be expected when using Neupogen for these types of indications (off-label use)?".

Please find below his response to your inquiry.

"Upon review of the published literature, no data pertinent to this Neupogen and regenerative boosting therapy were identified (EMBASE, BIOSIS, MEDLINE, OVID, Google Scholar, Quosa searched). Therefore, Amgen does not cannot comment on or recommend the use with lack of the safety and efficacy of Neupogen in this population."

Neupogen (filgrastim) is approved for the following therapeutic indications:

Filgrastim is indicated for the reduction in the duration of neutropenia and the incidence of febrile neutropenia in patients treated with established cytotoxic chemotherapy for malignancy (with the exception of chronic myeloid leukaemia and myelodysplastic syndromes) and for the reduction in the duration of neutropenia in patients undergoing

In order to respond to you and to fulfill our obligations, we recorded information you chose to provide to us such as your name, contact information, personal health related information, your question and our answer in our business correspondence system. The recorded information is transferred to the United States, and stored securely by Amgen Inc. and trusted processors, because our documentation system is global and physically operated by Amgen Inc. in the United States. If you wish to access, correct or delete your information please contact Amgen`s privacy office at privacyoffice@amgen.com.

Medical Information Request MIR-216065

myeloablative therapy followed by bone marrow transplantation considered to be at increased risk of prolonged severe neutropenia.

The safety and efficacy of filgrastim are similar in adults and children receiving cytotoxic chemotherapy.

Filgrastim is indicated for the mobilisation of peripheral blood progenitor cells (PBPC).

In patients, children or adults, with severe congenital, cyclic, or idiopathic neutropenia with an absolute neutrophil count (ANC) of $\leq 0.5 \times 10^9/l$ and a history of severe or recurrent infections, long term administration of filgrastim is indicated to increase neutrophil counts and to reduce the incidence and duration of infection-related events.

Filgrastim is indicated for the treatment of persistent neutropenia (ANC less than or equal to $1.0 \times 10^9/l$) in patients with advanced HIV infection, in order to reduce the risk of bacterial infections when other options to manage neutropenia are inappropriate.

This information is intended for healthcare professionals and may contain information relating to medicinal products and/or indications that have not been approved by the European Commission or national medical agencies. It is supplied to you as a professional courtesy in response to your specific unsolicited request. Amgen recommends the use of its products only in accordance with the approved Summary of Product Characteristics (SmPC).

Please feel free to contact me if you have additional questions.

Sincerely

Pia Zygmunt Tamayo, M.Sc. Pharm., Ph.D.
Medical Information Manager
Amgen AB, Gustav III:s Boulevard 54
169 27 Solna, Sweden
Tel: +468 695 11 00

Medical Information Request MIR-216065

Appendix 17c

Från:
Till:

Datum:
Ärende:

Till: @karolinska.se
Från: Ali Ashraf <Ali.Ashraf@bio-techne.com>
Datum: 2014-09-10 15:57
Ärende: TGF Beta 3

Dear

Thank you for contacting Bio-Techne regarding your query.

As per our conversation earlier, unless otherwise expressly stated in writing By R&D, all products are for research use only, and not for human or animal therapeutic or diagnostic use.

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I hope this helps. If you have any other questions, please contact us.

Kind Regards

Ali Ashraf
Technical Sales Correspondent
Bio-Techne
19 Barton Lane, Abingdon Science Park
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Terms & Conditions Pricing and Payment Terms

1. Agreement. These are the Standard Terms and Conditions ("**Terms**") under which R&D Systems, Inc. ("**R&D**") sells its products and services ("**Products**"). A party purchasing Products ("**Purchaser**") from R&D or an R&D authorized agent will be subject to these Terms regardless of what method is used to submit its offer to purchase Products ("**Order**"). Some of R&D's Products are subject to intellectual property licenses, software licenses, or other contract terms that Purchaser will not find here ("**Supplementary Terms**"). If such Supplementary Terms exist for the Products being purchased, they will be found in a quotation, order acknowledgment, product insert, or written agreement that accompanies or is associated with the Products. If Purchaser is uncertain if any Supplementary Terms exist for Products, Purchaser should contact R&D's Customer Service. The Order, these Terms, and Supplementary Terms (if any) are sometimes collectively and individually referred to herein as the Agreement ("**Agreement**").

R&D's acceptance of any Order by Purchaser is expressly conditioned on Purchaser's agreement to these Terms. R&D will not be bound by, and specifically objects to, any term, condition, or other provision which is different from or in addition to the provisions of this Agreement (whether or not it would materially alter this Agreement) which is proffered by Purchaser in any order, receipt, acceptance, confirmation, correspondence, or otherwise, unless R&D specifically agrees to such provision in a written instrument signed by R&D.

2. Purchase and Sale. R&D shall sell to Purchaser, and Purchaser shall accept and pay for, all Products ordered by Purchaser pursuant to an Order that has been accepted by R&D. All Orders are subject to acceptance by R&D either in writing or by shipping Products. R&D may accept any Order in whole or in part.

3. Order Confirmation and Duplication. Written confirmation of a telephone, fax, e-mail, electronic, or Internet Order is not required; however, if confirmation of an Order is sent by Purchaser, it shall be prominently marked - "CONFIRMING ORDER, DO NOT DUPLICATE".

Duplicate shipments due to incorrectly marked confirming orders will be subjected to a returned products charge of 20% of the list price of the Products returned (irrespective of whether such Products were purchased at discounted prices) ("**Returned Products Charge**") plus shipping charges. R&D does not issue full credits for returned products since its products are perishable and R&D cannot be assured of the quality of returned products.

4. Delivery. All domestic and international shipments shall be Ex Works R&D's Minneapolis, MN facility, according to INCOTERMS® 2010 as issued by the International Chamber of Commerce. Shipments are made in a commercially reasonable manner as determined by R&D. R&D will select the carrier but will not be deemed thereby to assume any liability in connection with the shipment nor will the carrier be construed to be an agent of R&D. Purchaser shall pay or reimburse R&D for all transportation, freight, insurance, loading, packaging and handling charges, taxes, duties, fees, storage and all other charges applicable to the Products. All shipments are subject to availability. Shipment schedules are approximate and R&D will use commercially reasonable efforts to complete shipments as indicated. R&D shall not be liable for any damages or penalties for delay in delivery or for failure to give notice of delay for any reason. Claims for loss or damage of products in transit must be made to the carrier and not to R&D.

5. Title. Title to the Products will pass to Purchaser upon R&D's delivery of the products to the carrier, subject to the limitations provided in this Agreement.

6. Price. Purchaser shall pay the prices specified in the applicable Order or, if no price is specified in the Order, the price set forth in R&D's standard price list in effect on the date that R&D accepts the Order. All prices are subject to change without notice. Purchaser shall be responsible for all delivery and handling charges, taxes and other amounts payable to governmental authorities in connection with applicable transactions. These charges will be added to Purchaser's invoice.

7. Tax. Sales tax will be added when shipping to jurisdictions where R&D is responsible for collecting sales tax unless the receiving party has exempt information on file with R&D. Purchaser shall be solely responsible for any applicable sales, use or similar tax and agrees to indemnify R&D for any such tax if not properly paid by Purchaser. Purchaser is responsible to confirm that its account is set up properly and that any applicable exemption documents are provided. Purchaser should contact R&D to correct or update its account information and should note any updates to this effect on applicable orders.

8. Payment. Payment terms shall be net 30 days from the date of invoice and in the currency specified on the invoice. Purchaser will only be charged for products shipped. Products placed on back order will be charged when shipped. If Purchaser is late in making payment, R&D may, without affecting any other rights or remedies, suspend delivery, cancel Orders, reject future orders, and/or charge a late payment fee, from the due date until paid, at the rate of one and a half percent (1.5%) per month (18% per year) or such lesser amount as is the maximum rate of interest allowed by law. Purchaser shall pay any and all reasonable costs, including attorneys' fees, incurred by R&D in collecting any delinquent balance.

9. Inspection and Returns. Purchaser can return Products that are damaged or defective upon delivery, but Purchaser must contact R&D's Customer Service within 10 calendar days of the day Purchaser received the Products. A return authorization ("**Return Authorization**") must be obtained from R&D's Customer Service for

all returns. R&D will not accept returns, and no credit will be issued, without a Return Authorization. Custom Orders are ineligible for cancellation or return.

Upon receipt of Products that have a Return Authorization, a credit for the original purchase price less the Returned Products Charge (calculated as 20% of list price as described in Section 3 above) and the original shipping and handling charge ("**Partial Credit**") will be issued. To obtain a Partial Credit, products must be returned within sixty (60) calendar days of receiving a Return Authorization.

If an error by R&D results in shipment of incorrect Products, R&D will, at its sole option, either ship, at no charge, the correct Products per Purchaser's Order or credit Purchaser's account for the purchase price of the Products shipped in error plus shipping charges. If an error by Purchaser results in the shipment of incorrect Products and is reported to R&D within ten (10) calendar days, Purchaser may obtain a Return Authorization and return the incorrect Products for Partial Credit.

10. Transfer Prohibited. In the absence of an express written agreement to the contrary, all Products are sold by R&D for the exclusive use of the Purchaser and shall not to be resold, transferred, or conveyed, in whole or in part, to any other party.

11. Product Use and Restrictions. Unless otherwise expressly stated in writing By R&D, all products are for research use only, and not for human or animal therapeutic or diagnostic use. Products are to be used only in accordance with R&D's specifications, product inserts, online product descriptions, and published catalogs. Products are not submitted for regulatory review or validated for clinical, therapeutic or diagnostic use, safety and effectiveness, or any another other specific use or application unless expressly stated in writing by R&D. Purchaser is solely responsible for complying with all applicable laws, regulations and governmental policies when using R&D Products. Purchaser is solely responsible for obtaining all necessary approvals, permissions and/or licenses or intellectual property rights from applicable third parties as may be required for Purchaser's research and any other intended uses. It is solely Purchaser's responsibility to make sure the products are suitable for Purchaser's particular use.

As a material condition to R&D providing its Products to Purchaser, Purchaser agrees that it shall not, directly or indirectly, attempt to reverse engineer, disassemble, or otherwise perform any compositional, structural, functional or other analyses directed to learning the methodology, components, formulae, processes, make-up, or production of any Product or any portion thereof.

12. Export Controls and Related Regulations. Products and information that Purchaser receives from R&D are subject to United States export control laws and regulations. Purchaser may not, directly or indirectly, sell, export, re-export, transfer, divert, or otherwise dispose of any such Product or information (including products derived from or based on Products or information received from R&D) to any destination, entity, or person prohibited by United States laws or regulations.

Purchaser represents and warrants that it is not designated on, or associated with any party designated on, any of the United States government restricted parties lists, including without limitation, the United States Commerce Department Bureau of Industry and Security ("BIS") Denied Persons List; Entity List or Unverified List; the United States Treasury Department Office of Foreign Assets Control ("OFAC") Specially Designated Nationals and Blocked Persons List; or the United States State Department Directorate of Defense Trade Controls ("DDTC") Debarred Parties List. Purchaser shall comply with all applicable United States economic sanctions and export control laws and regulations, including without limitation, the regulations administered by OFAC, the Export Administration Regulations administered by BIS, and the International Traffic in Arms Regulations administered by DDTC.

R&D may terminate this Agreement and discontinue any ongoing supply to or business with Purchaser immediately, without notice and without liability, upon R&D becoming aware that Purchaser, or any party associated with Purchaser, is named on any restricted party list.

13. Limited Warranty. Unless a different written warranty is included with product inserts accompanying Products, R&D warrants each Product will meet its published specifications when used appropriately under normal conditions. The warranty shall last from the time delivery is made until the Product's expiration or "use by" date or its specified number of uses.

This Limited Warranty only covers issues caused by defects in material or workmanship during ordinary consumer use. The Limited Warranty does not cover issues caused by any other reason, including but not limited to issues due to normal wear and tear, acts of God, misuse, limitations of technology, custom manufacture in accordance with specifications Purchaser gave R&D, neglect or accident caused by Purchaser, contact with improperly used or unapproved chemicals or environments, or modifications of or to any part of the Product. R&D's sole and exclusive liability to Purchaser and Purchaser's sole and exclusive remedy for warranty claims hereunder shall be replacement of the non-conforming Product or refund of the purchase price.

The above warranties are exclusive, and R&D makes no other warranty or representation of any kind whatsoever, express or implied, including without limitation any implied warranties of Merchantability or Fitness for a particular purpose, of suitability, of non-infringement, or regarding results obtained through the use of any product, whether arising from a statute or otherwise in law or from a course of performance, dealing or usage of trade, all of which are expressly disclaimed. Our warranties extend only to the original Purchaser and cannot be transferred by the original Purchaser to any other party.

14. Limitations on Remedies. IN NO EVENT SHALL R&D BE LIABLE TO PURCHASER FOR ANY SPECIAL, INCIDENTAL, CONSEQUENTIAL, PUNITIVE, INDIRECT OR EXEMPLARY DAMAGES OF ANY KIND, OR LOST PROFITS OR REVENUE, HOWEVER CAUSED, WHETHER FOR BREACH OR REPUDIATION OF CONTRACT, TORT, BREACH OF WARRANTY, NEGLIGENCE, OR OTHERWISE, WHETHER OR NOT R&D WAS ADVISED OF THE POSSIBILITY OF SUCH LOSS OR DAMAGES.

NOTWITHSTANDING ANY OTHER PROVISIONS OF THESE TERMS AND/OR ANY OTHER AGREEMENT BETWEEN R&D AND PURCHASER FOR THE PURCHASE OF THE PRODUCTS, R&D'S TOTAL LIABILITY TO PURCHASER ARISING FROM OR IN RELATION TO THESE TERMS, AN AGREEMENT BETWEEN THE PARTIES OR THE PRODUCTS, WHETHER ARISING IN CONTRACT, TORT OR OTHERWISE SHALL BE LIMITED TO THE TOTAL AMOUNT PAID BY PURCHASER TO R&D FOR THE APPLICABLE PRODUCTS. IN NO EVENT WILL R&D BE LIABLE FOR THE COST OF PROCUREMENT OF SUBSTITUTE GOODS.

15. Indemnification. Purchaser shall hold harmless, indemnify and defend (at R&D's request) R&D for any and all damages, liabilities, costs and expenses (including any costs of litigation, including but not limited to, attorneys' fees and any other costs and expenses), fines, or losses in connection with any threatened or actual claims, actions, demands, investigations, or suits, including, but not limited to, claims or suits by third parties,

arising out of any of the following: (a) Purchaser's negligent or willful acts, or those of its employees and/or agents, (b) such Products being repaired or altered by persons other than R&D (unless expressly authorized in writing by R&D), (c) in the event that Purchaser modifies, or combines with any non-R&D goods or products, any of the Products purchased from R&D, and such modification or combination results in the actual or alleged infringement of any intellectual property rights of any third party, (d) from Products produced by R&D according to Purchaser's specifications, (e) any violations of export control laws by Purchaser, or (f) Purchaser's breach of any provision in these Terms.

16. Confidentiality and Intellectual Property. "Confidential Information" means any of R&D's business information, specifications and all related writings, drawings, designs and similar works or any other information which is disclosed by R&D to Purchaser and labeled or marked as confidential, proprietary or its equivalent, or oral or visual information that is designated confidential, proprietary or its equivalent at the time of its disclosure. All Confidential Information shall be the exclusive property of R&D, and R&D retains all of its rights, title and interests. Purchaser agrees to use Confidential Information for the exclusive purpose of performing this Agreement. Purchaser shall not disclose or provide any Confidential Information to any third party and shall take all necessary measures to prevent any such disclosure by its employees, agents, contractors or consultants. Upon request of R&D, Purchaser shall return all Confidential Information to R&D.

Purchaser acknowledges that all intellectual property rights relating to Products, as between Purchaser and R&D, are solely and exclusively owned by R&D. R&D's sale of Products to Purchaser only grants Purchaser a limited, non-transferable right, for Purchaser to use the quantity of Products bought from R&D in accordance with this Agreement. The act of R&D selling Products to Purchaser, R&D does not grant Purchaser a license to R&D's intellectual property, or grant Purchaser the right to make or have made any Product or any portion thereof. The onus rests with the Purchaser to secure any required "freedom to operate" rights for other intended applications.

Any inventions (patentable or otherwise), discoveries, improvements, data, know-how or other results that are conceived, developed, discovered, reduced to practice, or generated by or for R&D, or jointly by R&D and Purchaser, will be and will remain R&D's sole and exclusive intellectual property, and Purchaser shall transfer and assign, and hereby does assign, all of its rights, title and interests in and to any such joint intellectual property to R&D and assist R&D, at R&D's request and expense, in securing and recording R&D's rights in such intellectual property.

17. Force Majeure. R&D shall not be liable for any failure to perform this Agreement when such failure is due to circumstances beyond its control. Circumstances beyond the control of R&D shall be deemed to include, but shall not be limited to, acts of God, governmental action, accidents, labor trouble, and inability to obtain materials, equipment or transportation.

18. Waiver. No oral statements, recommendations or assistance given by a representative and/or distributor of R&D to Purchaser or its representatives in connection with the use of the Products shall constitute a waiver by R&D of any of the provisions hereof, or affect R&D's liability herein. R&D's failure to exercise any rights under this Agreement is not a waiver of it rights to damages for Purchaser's breach of contract and is not a waiver of any subsequent breach.

19. Governing Law/Disputes. Purchaser hereby agrees that the only proper jurisdiction and venue for any dispute with R&D, or in any way relating to these Terms or to products purchased from R&D, is in the state and federal courts in the State of Minnesota. All disputes with R&D shall be governed by the laws of the State of Minnesota, without regard to provision on the conflict of laws. The United Nations Convention on Contracts for the International Sale of Goods shall not apply to this Agreement or any other written agreement with R&D unless expressly stated otherwise. Purchaser further agrees and consents to the exercise of personal jurisdiction in these courts in connection with any dispute involving R&D or its affiliates, employees, officers, directors, agents and providers. Any cause of action brought by Purchaser arising out of or relating to this Agreement or to Products purchased from R&D, must be brought within one year after such cause of action arose. Actions not commenced by Purchaser within one year are permanently barred. This paragraph survives expiration or termination of this Agreement.

20. Miscellaneous. All provisions set forth herein regarding warranty, confidential information, indemnification, liability and limits thereon, and any other provisions that survive by their terms will survive any termination or expiration of this Agreement and any other written instrument delivered in connection herewith pursuant to the terms of such sections. In the event that any provision of these Terms is held to be illegal, invalid or unenforceable under any present or future law, rule or regulation, such provision shall be deemed stricken from these Terms but such illegality, invalidity or unenforceability shall not invalidate any of the other provisions of these Terms. Purchaser may not assign, including by operation of law, its obligations hereunder without R&D's written consent.

Privacy Policy

The purpose of this Privacy Policy is to tell you what information we gather on this Website and how we use that information. By using this Website you accept the terms of this Privacy Policy, as well as the terms set forth in our Terms and Conditions.
This Privacy policy applies to the website owned and operated by [R&D Systems, Inc.][Techne Corporation] (referred to as "we," "us," the "Company" or similar terms), with a homepage located at [www.rndsystems.com] [www.techne-corp.com] (the "Website"). This Privacy Policy does not apply to any other other websites, and we make no representations as to the privacy practices of other websites.

What Information do We Collect?

In order to process and track orders, update your account, respond to your requests, operate our business, and maintain this Website and various features, we collect a variety of information about Website visitors. This section describes both the Non-Personal Information and Personal Information we collect.

Non-Personal Information.

Each time a computer visits this Website we may collect certain information from that computer. This information is automatically collected from the computer's web browser and may include information such as the following ("Non-Personal Information"):

- the type of web browser software the computer uses (for example, Netscape Navigator or Internet Explorer)
- the name of the domain from which the computer accessed the Internet
- the Internet address of the website from which the computer linked directly to the Website
- the date and time the computer accessed the Website

Non-Personal Information does not tell us who you are.

Also, we may place a text file called a cookie in the browser files of computers that visit this Website. Cookies are pieces of information issued by a website that, among other things, allow your computer to utilize all the features on this Website. If and when your computer accepts a cookie, the cookie may be stored on the computer's hard drive. US Government Information Bulletin (I-034) contains a good description of cookie technology at: http://www.ciac.org/ciac/bulletins/i-034.shtml You are always free to decline our cookies if your browser permits, but some parts of the Website may not work properly for you if you do so.

Personal Information.

In the course of opening or updating your account, placing an order, filling out a form on a page on this website, or taking any similar action, you may provide us with information such as your name, address, telephone number, purchase information and history, email address, credit card number, or similar information ("Personal Information"). ***The only way we get personal information is if you choose to give it to us.***

How do We Share and Use Information?

The ways we share, use, and protect information collected over this Website depends upon the type of information involved.

Non-Personal Information.

We may use non-personal information to help us make the Website more useful to you and for other business purposes. For example, we may tell business partners how many computers visited this Website or what pages were most popular. We may prepare reports and other materials using non-personal information. We may use Non-Personal Information for any other purpose and may share Non-Personal Information with third parties.

Personal Information.

If you submit personal information, you agree that we may keep a record of the personal information. For example, we may keep a record of your name, address, and credit card number in order to receive payment for your orders and in order to ship the orders. We also may use personal information to help us in our business, including but not limited to improving this Website and marketing and product development, or to contact you. You also agree that we may share personal information with other businesses that help us operate our business, such as credit card companies and shipping companies. If you complete the "Credit Reference" section you agree that we may contact the references and share your Personal Information with the references for the purpose of deciding whether to extend credit to you or to do business with you. Also, we may share personal information with other companies we do business with, such as business partners, affiliates, or successors
We may share Personal Information if required to comply with a law, regulation, court order, or other legal process. We may share Personal Information to protect our rights or property.

Email and Link Information.

Using the email address you provide us, we may send you email newsletters or similar documents from time to time. These emails may include technology that permits us to track if and when an email has been opened, as well as whether the recipient clicked on any particular link or advertisement contained in the email. This information is tracked and used for statistical analysis, to generate sales leads, and to help us operate our business. The information gathered from these emails may result in a call or email from our sales staff to provide further information on products that our emails have identified you as having an interest in.

Opt-In for Advertising and Other Communications.

You agree that we, or the third parties who help us operate this Website and conduct business, may contact you for advertising or any other purposes through any and all contact information you provide to us, such as email, telephone, fax, or mail.

California Residents only - Disclosures to Direct Marketing Entities.

This notice applies only to California residents and is made pursuant to California Civil Code § 1798.83. As noted above, we *may* disclose Personal Information about you, such as your email address, to third parties. If we disclose personal information about you to a third party and the third party will use that information for direct marketing purposes, you have the right to contact us at info@rndsystems.com to request that we notify you as to: (1) what categories of Personal Information we shared with the direct marketer; and (2) the name of the direct marketing businesses with whom we shared such information.

What Happens if the Privacy Policy Changes?

We may change this Privacy Policy at any time and for any reason. If we change this Privacy Policy we will post the change on this section of the Website.

What About Privacy on Other Sites?

This Website may contain links to other websites. We provide these links for your convenience, but we do not review, control, or monitor the privacy practices of websites operated by others. We are not responsible for the performance of these sites or for your business dealings with them. Your use of other websites is subject to the terms and conditions of those websites, including the privacy policies of those websites.

Are There Special Rules About Children's Privacy?

This Website is designed for an adult audience only, and anyone under 18 years old must not use this Website. We will not intentionally collect online any personal information (such as a child's name or e-mail address) from children under the age of 13. If you think that we have collected personal information from a child under the age of 13, please contact us as described below.

Security

Your information is stored on servers located in the United States. We and our agents who help us with hosting and security use a number of procedural and technical safeguards to protect your personal information against loss or theft as well as unauthorized access and disclosure, including use of encryption, "firewalls" and Secure Socket Layers. However, "perfect security" does not exist on the Internet.

User Conduct

You agree to follow the conduct rules listed below. If you fail to follow these conduct rules, R&D Systems may, in its sole discretion, terminate or suspend your account without refund, in addition to pursuing any other legal and equitable remedies including but not limited to money damages.

- You will not knowingly provide or post any false, misleading, or fraudulent information.
- You will not use this Site or any products purchased from R&D Systems for any illegal purpose.
- You will not hold yourself out as someone you are not or otherwise impersonate any living person.
- You will not interfere or tamper with the functioning of this Site, nor will you attempt to gain access to information or control of the Site not specifically granted to you.

- You will not use this Site to transmit any virus or similar destructive program or code.

Ownership and Use of Materials

The materials on this Site belong to R&D Systems or its third party licensors. There are some important rules about using and copying these materials. R&D Systems grants you a limited, revocable, license to download or print one copy of the materials on this Site, but only for the purpose of ordering products on the Site. When you download or print a copy of the materials on this Site, you must also include all copyright and other notices that are in the materials, including the copyright notice on the bottom of the page.
The materials on this Site are protected by United States and foreign copyright laws. If you use the materials in a way that is not clearly allowed by these Terms & Conditions and the license above, you are violating your contract with us and may be violating copyright, trademark, and other laws. In that case, R&D Systems automatically revokes your limited license to use the materials and you must immediately destroy any copies you have made. Title to the materials remains with R&D Systems or its third party licensors. All rights not expressly granted are reserved.

Site Disclaimer and Limitation of Liability

THE SITE IS PROVIDED ON AN "AS IS" AND "AS AVAILABLE" BASIS. TO THE FULLEST EXTENT PERMISSIBLE BY LAW, R&D SYSTEMS DISCLAIMS ALL IMPLIED WARRANTIES, INCLUDING BUT NOT LIMITED TO, IMPLIED WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE.
THE SITE DOES NOT GUARANTEE THE CONTINUOUS, UNINTERRUPTED OR SECURE ACCESS TO THE SITE. THE OPERATION OF THE SITE MAY BE INTERFERED WITH BY NUMEROUS FACTORS OUTSIDE THE CONTROL OF THE SITE OR R&D SYSTEMS.
UNDER NO CIRCUMSTANCES SHALL R&D SYSTEMS OR ANY OF ITS EMPLOYEES, DIRECTORS, OFFICERS, AGENTS OR SUPPLIERS BE LIABLE FOR ANY DIRECT OR INDIRECT LOSSES OR DAMAGES ARISING OUT OF OR IN CONNECTION WITH THE USE OR INABILITY TO USE THIS SITE.

Password and Account Security

If you have an account through the Site, you are responsible for (i) keeping your account password confidential and secured, (ii) restricting access to your computer and (iii) keeping the e-mail address associated with that account current. You accept full responsibility for all activities that occur within your Site account and/or using your password.

Links to Other Websites

This Site may contain links to websites operated by other parties. The linked websites are not under the control of R&D Systems, and R&D Systems is not responsible for the content available on any other websites linked from this Site. The appearance of the links does not imply R&D Systems' endorsement of the material on any other website and R&D Systems disclaims all liability with regard to your access to or use of such linked websites.

Advertising

This Site may include advertisements for products or services offered by other companies. R&D Systems may receive payment from these advertisers. Although R&D Systems believes you may find the information offered by advertisers to be helpful, R&D Systems does not endorse or guarantee any product or service offered by advertisers.

Geographic Scope

The Site is operated by R&D Systems from its offices in Minnesota, U.S.A. R&D Systems welcomes visitors from around the world. R&D Systems, however, makes no representation or warranty that the contents of the Site are appropriate or permitted by the laws and regulations of countries other than the U.S.A. If you choose to access the Site from other countries you do so at your own risk, and are responsible for compliance with applicable local laws.

Choice of Law/Disputes

You hereby agree that the only proper jurisdiction and venue for any dispute with R&D Systems, or in any way relating to your use of this Site or to products purchased from R&D Systems, is in the state and federal courts in the State of Minnesota, U.S.A. You further agree and consent to the exercise of personal jurisdiction in these courts in connection with any dispute involving R&D Systems or its affiliates, employees, officers, directors, agents and providers. Any claims relating to the information, services or products available on this Site will be governed by the laws of the State of Minnesota, excluding the application of its conflicts of law rules. You agree that any cause of action arising out of or relating to these Terms & Conditions or your use of the Site will be commenced by you within one (1) year after such cause of action arose. Actions not commenced by you within one (1) year are permanently barred. This paragraph survives expiration or termination of these Terms & Conditions.

Modification

R&D Systems reserves the right to make changes to the Site, including these Terms and Conditions, at any time without notice.

Trademark Information

The following is a listing of trademarks used by R&D Systems, Inc. This list is subject to change at any time. Registration applications for several marks are pending and may become registered marks in the near future. Any rights not expressly granted herein are reserved.

R&D Systems

- DuoSet®
- EvenCoat™
- ExactaChIP™
- Fluorokine®
- Quantikine IVD®
- MagCellect™
- MycoProbe®
- NorthernLights™
- Parameter™
- PlusCellect™
- Proteome Profiler™
- Proteome Purify™
- QuantiGlo®
- Quantikine®
- R&D Systems®

StemXVivo™
Surveyor™
Tools for Cell Biology Research™

Bio-Rad Laboratories, Inc.

Bio-Rad®
BioPlex®

BD Biosciences

PerCP™

ICI Americas

Tween®

GE Healthcare

Cy™

Luminex

Luminex®
Luminex 100™

Life Technologies

Alexa Fluor®
FluoroNissl™
Rhodamine Red™
SYBR®
Texas Red®

Qiagen

LiquiChip®

Trevigen

CardioTACS™
CometAssay™
Cultrex®
Cytonin™
DePsipher™
DermaTACS™
Flare™
FlowTACS™
MidaScan™
NeuroTACS™
OligoTag™
PathClear®
REC™
TACS™
TACS-Blue™
TACS-Blue Label™
TACS-Nuclease™
TACS-Red Label™
TACS-Sapphire™
TACS-XL®
TiterTACS™
TumorTACS™
VasoTACS™

Contact Information

If you have any questions or comments regarding the Site, the products offered, or R&D Systems, please contact us at:

R&D Systems
614 McKinley Place NE
Minneapolis, MN 55413
Tel: 1-800-343-7475
Tel: (612) 379-2956
Fax: (612) 379-6580
Email: info@RnDSystems.com

Search Technical Information:

Search

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Från:
Till:

Datum:
Ärende:

Till: @karolinska.se>
Från: Gaston Friedmann <Gaston.Friedmann@bio-techne.com>
Datum: 2014-09-25 12:42
Ärende: Biotechne RE: TGF Beta 3
(Se den bifogade filen: EXTEDOCU (1).doc)

Dear

Thank you for your patience.

In response to your enquiry, none of R&D Systems' protein or antibody products are produced according to the FDA regulation of Pharmaceutical cGMPs (21 CFR part 211). The FDA has never inspected the production of nor cleared any of R&D Systems' protein or antibody products. As stated in the data sheet it is >97% pure by SDS-PAGE silver staining. **The remaining impurities have not been characterised and may contain e.g. viral components, but also other unknown proteins with unforeseeable consequences for human applications.** Although we filter sterilize our proteins and take precautions to maintain sterility, we cannot guarantee this due to limitations of four filling and lyophilisation process.

The standard of our product is high enough for a **safe applications on cells in vitro, however applications in humans require highest safety standards and any applications on humans are against our licencing agreement.**

Further details of our policy are available in the attached document which applies to all our products and which we require to be signed by the end-user when purchasing products with extended documentation for ex-vivo applications.

I hope this information is useful.

Kind regards,

Gaston Friedmann

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EXTEDOCU (1).doc

Extended Documentation for Protein Products

R&D Systems produces proteins and antibodies for Research Use Only (RUO), not for *in vivo* use or for the production of therapeutic or other drugs, biologic products or devices. None of R&D Systems' protein or antibody products is produced according to the FDA regulation of Pharmaceutical cGMPs (21 CFR part 211). The FDA has never inspected the production of nor cleared any of R&D Systems' protein or antibody products.

R&D Systems has been working closely with investigators who would like to use our proteins for "*ex vivo*" cell therapy research. We strive to provide our customers with accurate information on the quality (purity) of our products and with some of the analytical characteristics of each product in order that our customers may determine whether our products are appropriate for their research.

R&D Systems provides for customer:

1. Lot-specific certificate of analysis*
2. Total amino acid digest analysis*
3. N-terminal amino acid analysis*
4. SDS PAGE analysis*
5. Protein concentration (as determined by A280 analysis or Coomassie assay)*
6. Endotoxin level (as determined by LAL assay)*
7. Results of microbial growth assay (using broth culture, Sabourand's dextrose and blood agar plates, following 72 hour incubation; reported at 72 hours, maintained for 7 days and checked once again before completion)
8. Bottling lot-specific bioassay results (ranges only)

* analyses performed on each bulk QC lot, not on individual bottlings of each QC lot

Production records and facilities are available for examination by appropriate personnel on-site at R&D Systems. Additional testing and documentation surrounding the product, as required by the customer, can be arranged at an additional cost.

R&D Systems' quality focus includes:

1. Quality manual based on FDA QSR, 21 CFR 820
2. Documented processes and QA control of documentation
3. Personnel training programs
4. Raw material testing and vendor qualification
5. Equipment calibration schedules using CalMan calibration program
6. Facility maintenance, safety programs and pest control
7. Material review process for variance
8. QC data tracking

R&D Systems' QSR compliance weaknesses include:

1. Fermentor and purification columns are not dedicated to a specific product
2. Fermentor and purification column cleaning are not fully validated processes
3. Filling equipment and lyophilizer are not dedicated to a specific product
4. Heat sterilization and product bioburden loads are not fully validated processes
5. Product transfer is not validated for sterility
6. Filling and lyophilization areas are not classified and do not include HEPA filtration
7. Material and personnel flow control are not restricted
8. Design controls are not routinely followed during development of new RUO products

R&D Systems' products are sold for research use only. Resale as stand alone products, except by authorized distributors, is prohibited. If an R&D Systems' product is to be resold as a component of a purchaser's product, no license to use R&D Systems' name or trademark is granted, any label or printed material referring to R&D Systems must be removed and no reference to R&D Systems may be made. Nothing contained in this disclosure statement or any other statement of R&D Systems shall be construed as granting or conferring any rights by license or otherwise. R&D Systems may cease supplying any molecule for *ex vivo* research or any other use at any time without prior notice. For additional terms and conditions of sale, including limitations on warranty, see R&D Systems' current catalog or website and the attached certification, which is required to be executed at the time of purchase.

R&D Systems will seek to respond to appropriate requests for additional information.

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a bio-techne brand PRODUCT SEARCH SEARCH TIPS

Customer Service

Legal Information

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- Privacy Policy
- Trademark Information
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Terms & Conditions Pricing and Payment Terms

1. Agreement. These are the Standard Terms and Conditions ("**Terms**") under which R&D Systems, Inc. ("**R&D**") sells its products and services ("**Products**"). A party purchasing Products ("**Purchaser**") from R&D or an R&D authorized agent will be subject to these Terms regardless of what method is used to submit its offer to purchase Products ("**Order**"). Some of R&D's Products are subject to intellectual property licenses, software licenses, or other contract terms that Purchaser will not find here ("**Supplementary Terms**"). If such Supplementary Terms exist for the Products being purchased, they will be found in a quotation, order acknowledgment, product insert, or written agreement that accompanies or is associated with the Products. If Purchaser is uncertain if any Supplementary Terms exist for Products, Purchaser should contact R&D's Customer Service. The Order, these Terms, and Supplementary Terms (if any) are sometimes collectively and individually referred to herein as the Agreement ("**Agreement**").

R&D's acceptance of any Order by Purchaser is expressly conditioned on Purchaser's agreement to these Terms. R&D will not be bound by, and specifically objects to, any term, condition, or other provision which is different from or in addition to the provisions of this Agreement (whether or not it would materially alter this Agreement) which is proffered by Purchaser in any order, receipt, acceptance, confirmation, correspondence, or otherwise, unless R&D specifically agrees to such provision in a written instrument signed by R&D.

2. Purchase and Sale. R&D shall sell to Purchaser, and Purchaser shall accept and pay for, all Products ordered by Purchaser pursuant to an Order that has been accepted by R&D. All Orders are subject to acceptance by R&D either in writing or by shipping Products. R&D may accept any Order in whole or in part.

3. Order Confirmation and Duplication. Written confirmation of a telephone, fax, e-mail, electronic, or Internet Order is not required; however, if confirmation of an Order is sent by Purchaser, it shall be prominently marked – "CONFIRMING ORDER, DO NOT DUPLICATE".

Duplicate shipments due to incorrectly marked confirming orders will be subjected to a returned products charge of 20% of the list price of the Products returned (irrespective of whether such Products were purchased at discounted prices) ("**Returned Products Charge**") plus shipping charges. R&D does not issue full credits for returned products since its products are perishable and R&D cannot be assured of the quality of returned products.

4. Delivery. All domestic and international shipments shall be Ex Works R&D's Minneapolis, MN facility, according to INCOTERMS® 2010 as issued by the International Chamber of Commerce. Shipments are made in a commercially reasonable manner as determined by R&D. R&D will select the carrier but will not be deemed thereby to assume any liability in connection with the shipment nor will the carrier be construed to be an agent of R&D. Purchaser shall pay or reimburse R&D for all transportation, freight, insurance, loading, packaging and handling charges, taxes, duties, fees, storage and all other charges applicable to the Products. All shipments are subject to availability. Shipment schedules are approximate and R&D will use commercially reasonable efforts to complete shipments as indicated. R&D shall not be liable for any damages or penalties for delay in delivery or for failure to give notice of delay for any reason. Claims for loss or damage of products in transit must be made to the carrier and not to R&D.

5. Title. Title to the Products will pass to Purchaser upon R&D's delivery of the products to the carrier, subject to the limitations provided in this Agreement.

6. Price. Purchaser shall pay the prices specified in the applicable Order or, if no price is specified in the Order, the price set forth in R&D's standard price list in effect on the date that R&D accepts the Order. All prices are subject to change without notice. Purchaser shall be responsible for all delivery and handling charges, taxes and other amounts payable to governmental authorities in connection with applicable transactions. These charges will be added to Purchaser's invoice.

7. Tax. Sales tax will be added when shipping to jurisdictions where R&D is responsible for collecting sales tax unless the receiving party has exempt information on file with R&D. Purchaser shall be solely responsible for any applicable sales, use or similar tax and agrees to indemnify R&D for any such tax if not properly paid by Purchaser. Purchaser is responsible to confirm that its account is set up properly and that any applicable exemption documents are provided. Purchaser should contact R&D to correct or update its account information and should note any updates to this effect on applicable orders.

8. Payment. Payment terms shall be net 30 days from the date of invoice and in the currency specified on the invoice. Purchaser will only be charged for products shipped. Products placed on back order will be charged when shipped. If Purchaser is late in making payment, R&D may, without affecting any other rights or remedies, suspend delivery, cancel Orders, reject future orders, and/or charge a late payment fee, from the due date until paid, at the rate of one and a half percent (1.5%) per month (18% per year) or such lesser amount as is the maximum rate of interest allowed by law. Purchaser shall pay any and all reasonable costs, including attorneys' fees, incurred by R&D in collecting any delinquent balance.

9. Inspection and Returns. Purchaser can return Products that are damaged or defective upon delivery, but Purchaser must contact R&D's Customer Service within 10 calendar days of the day Purchaser received the Products. A return authorization ("**Return Authorization**") must be obtained from R&D's Customer Service for

all returns. R&D will not accept returns, and no credit will be issued, without a Return Authorization. Custom Orders are ineligible for cancellation or return.

Upon receipt of Products that have a Return Authorization, a credit for the original purchase price less the Returned Products Charge (calculated as 20% of list price as described in Section 3 above) and the original shipping and handling charge ("**Partial Credit**") will be issued. To obtain a Partial Credit, products must be returned within sixty (60) calendar days of receiving a Return Authorization.

If an error by R&D results in shipment of incorrect Products, R&D will, at its sole option, either ship, at no charge, the correct Products per Purchaser's Order or credit Purchaser's account for the purchase price of the Products shipped in error plus shipping charges. If an error by Purchaser results in the shipment of incorrect Products and is reported to R&D within ten (10) calendar days, Purchaser may obtain a Return Authorization and return the incorrect Products for Partial Credit.

10. Transfer Prohibited. In the absence of an express written agreement to the contrary, all Products are sold by R&D for the exclusive use of the Purchaser and shall not to be resold, transferred, or conveyed, in whole or in part, to any other party.

11. Product Use and Restrictions. Unless otherwise expressly stated in writing By R&D, all products are for research use only, and not for human or animal therapeutic or diagnostic use. Products are to be used only in accordance with R&D's specifications, product inserts, online product descriptions, and published catalogs. Products are not submitted for regulatory review or validated for clinical, therapeutic or diagnostic use, safety and effectiveness, or any another other specific use or application unless expressly stated in writing by R&D. Purchaser is solely responsible for complying with all applicable laws, regulations and governmental policies when using R&D Products. Purchaser is solely responsible for obtaining all necessary approvals, permissions and/or licenses or intellectual property rights from applicable third parties as may be required for Purchaser's research and any other intended uses. It is solely Purchaser's responsibility to make sure the products are suitable for Purchaser's particular use.

As a material condition to R&D providing its Products to Purchaser, Purchaser agrees that it shall not, directly or indirectly, attempt to reverse engineer, disassemble, or otherwise perform any compositional, structural, functional or other analyses directed to learning the methodology, components, formulae, processes, make-up, or production of any Product or any portion thereof.

12. Export Controls and Related Regulations. Products and information that Purchaser receives from R&D are subject to United States export control laws and regulations. Purchaser may not, directly or indirectly, sell, export, re-export, transfer, divert, or otherwise dispose of any such Product or information (including products derived from or based on Products or information received from R&D) to any destination, entity, or person prohibited by United States laws or regulations.

Purchaser represents and warrants that it is not designated on, or associated with any party designated on, any of the United States government restricted parties lists, including without limitation, the United States Commerce Department Bureau of Industry and Security ("BIS") Denied Persons List; Entity List or Unverified List; the United States Treasury Department Office of Foreign Assets Control ("OFAC") Specially Designated Nationals and Blocked Persons List; or the United States State Department Directorate of Defense Trade Controls ("DDTC") Debarred Parties List. Purchaser shall comply with all applicable United States economic sanctions and export control laws and regulations, including without limitation, the regulations administered by OFAC, the Export Administration Regulations administered by BIS, and the International Traffic in Arms Regulations administered by DDTC.

R&D may terminate this Agreement and discontinue any ongoing supply to or business with Purchaser immediately, without notice and without liability, upon R&D becoming aware that Purchaser, or any party associated with Purchaser, is named on any restricted party list.

13. Limited Warranty. Unless a different written warranty is included with product inserts accompanying Products, R&D warrants each Product will meet its published specifications when used appropriately under normal conditions. The warranty shall last from the time delivery is made until the Product's expiration or "use by" date or its specified number of uses.

This Limited Warranty only covers issues caused by defects in material or workmanship during ordinary consumer use. The Limited Warranty does not cover issues caused by any other reason, including but not limited to issues due to normal wear and tear, acts of God, misuse, limitations of technology, custom manufacture in accordance with specifications Purchaser gave R&D, neglect or accident caused by Purchaser, contact with improperly used or unapproved chemicals or environments, or modifications of or to any part of the Product. R&D's sole and exclusive liability to Purchaser and Purchaser's sole and exclusive remedy for warranty claims hereunder shall be replacement of the non-conforming Product or refund of the purchase price.

The above warranties are exclusive, and R&D makes no other warranty or representation of any kind whatsoever, express or implied, including without limitation any implied warranties of Merchantability or Fitness for a particular purpose, of suitability, of non-infringement, or regarding results obtained through the use of any product, whether arising from a statute or otherwise in law or from a course of performance, dealing or usage of trade, all of which are expressly disclaimed. Our warranties extend only to the original Purchaser and cannot be transferred by the original Purchaser to any other party.

14. Limitations on Remedies. IN NO EVENT SHALL R&D BE LIABLE TO PURCHASER FOR ANY SPECIAL, INCIDENTAL, CONSEQUENTIAL, PUNITIVE, INDIRECT OR EXEMPLARY DAMAGES OF ANY KIND, OR LOST PROFITS OR REVENUE, HOWEVER CAUSED, WHETHER FOR BREACH OR REPUDIATION OF CONTRACT, TORT, BREACH OF WARRANTY, NEGLIGENCE, OR OTHERWISE, WHETHER OR NOT R&D WAS ADVISED OF THE POSSIBILITY OF SUCH LOSS OR DAMAGES.

NOTWITHSTANDING ANY OTHER PROVISIONS OF THESE TERMS AND/OR ANY OTHER AGREEMENT BETWEEN R&D AND PURCHASER FOR THE PURCHASE OF THE PRODUCTS, R&D'S TOTAL LIABILITY TO PURCHASER ARISING FROM OR IN RELATION TO THESE TERMS, AN AGREEMENT BETWEEN THE PARTIES OR THE PRODUCTS, WHETHER ARISING IN CONTRACT, TORT OR OTHERWISE SHALL BE LIMITED TO THE TOTAL AMOUNT PAID BY PURCHASER TO R&D FOR THE APPLICABLE PRODUCTS. IN NO EVENT WILL R&D BE LIABLE FOR THE COST OF PROCUREMENT OF SUBSTITUTE GOODS.

15. Indemnification. Purchaser shall hold harmless, indemnify and defend (at R&D's request) R&D for any and all damages, liabilities, costs and expenses (including any costs of litigation, including but not limited to, attorneys' fees and any other costs and expenses), fines, or losses in connection with any threatened or actual claims, actions, demands, investigations, or suits, including, but not limited to, claims or suits by third parties,

arising out of any of the following: (a) Purchaser's negligent or willful acts, or those of its employees and/or agents, (b) such Products being repaired or altered by persons other than R&D (unless expressly authorized in writing by R&D), (c) in the event that Purchaser modifies, or combines with any non-R&D goods or products, any of the Products purchased from R&D, and such modification or combination results in the actual or alleged infringement of any intellectual property rights of any third party, (d) from Products produced by R&D according to Purchaser's specifications, (e) any violations of export control laws by Purchaser, or (f) Purchaser's breach of any provision in these Terms.

16. Confidentiality and Intellectual Property. "Confidential Information" means any of R&D's business information, specifications and all related writings, drawings, designs and similar works or any other information which is disclosed by R&D to Purchaser and labeled or marked as confidential, proprietary or its equivalent, or oral or visual information that is designated confidential, proprietary or its equivalent at the time of its disclosure. All Confidential Information shall be the exclusive property of R&D, and R&D retains all of its rights, title and interests. Purchaser agrees to use Confidential Information for the exclusive purpose of performing this Agreement. Purchaser shall not disclose or provide any Confidential Information to any third party and shall take all necessary measures to prevent any such disclosure by its employees, agents, contractors or consultants. Upon request of R&D, Purchaser shall return all Confidential Information to R&D.

Purchaser acknowledges that all intellectual property rights relating to Products, as between Purchaser and R&D, are solely and exclusively owned by R&D. R&D's sale of Products to Purchaser only grants Purchaser a limited, non-transferable right, for Purchaser to use the quantity of Products bought from R&D in accordance with this Agreement. The act of R&D selling Products to Purchaser, R&D does not grant Purchaser a license to R&D's intellectual property, or grant Purchaser the right to make or have made any Product or any portion thereof. The onus rests with the Purchaser to secure any required "freedom to operate" rights for other intended applications.

Any inventions (patentable or otherwise), discoveries, improvements, data, know-how or other results that are conceived, developed, discovered, reduced to practice, or generated by or for R&D, or jointly by R&D and Purchaser, will be and will remain R&D's sole and exclusive intellectual property, and Purchaser shall transfer and assign, and hereby does assign, all of its rights, title and interests in and to any such joint intellectual property to R&D and assist R&D, at R&D's request and expense, in securing and recording R&D's rights in such intellectual property.

17. Force Majeure. R&D shall not be liable for any failure to perform this Agreement when such failure is due to circumstances beyond its control. Circumstances beyond the control of R&D shall be deemed to include, but shall not be limited to, acts of God, governmental action, accidents, labor trouble, and inability to obtain materials, equipment or transportation.

18. Waiver. No oral statements, recommendations or assistance given by a representative and/or distributor of R&D to Purchaser or its representatives in connection with the use of the Products shall constitute a waiver by R&D of any of the provisions hereof, or affect R&D's liability herein. R&D's failure to exercise any rights under this Agreement is not a waiver of it rights to damages for Purchaser's breach of contract and is not a waiver of any subsequent breach.

19. Governing Law/Disputes. Purchaser hereby agrees that the only proper jurisdiction and venue for any dispute with R&D, or in any way relating to these Terms or to products purchased from R&D, is in the state and federal courts in the State of Minnesota. All disputes with R&D shall be governed by the laws of the State of Minnesota, without regard to provision on the conflict of laws. The United Nations Convention on Contracts for the International Sale of Goods shall not apply to this Agreement or any other written agreement with R&D unless expressly stated otherwise. Purchaser further agrees and consents to the exercise of personal jurisdiction in these courts in connection with any dispute involving R&D or its affiliates, employees, officers, directors, agents and providers. Any cause of action brought by Purchaser arising out of or relating to this Agreement or to Products purchased from R&D, must be brought within one year after such cause of action arose. Actions not commenced by Purchaser within one year are permanently barred. This paragraph survives expiration or termination of this Agreement.

20. Miscellaneous. All provisions set forth herein regarding warranty, confidential information, indemnification, liability and limits thereon, and any other provisions that survive by their terms will survive any termination or expiration of this Agreement and any other written instrument delivered in connection herewith pursuant to the terms of such sections. In the event that any provision of these Terms is held to be illegal, invalid or unenforceable under any present or future law, rule or regulation, such provision shall be deemed stricken from these Terms but such illegality, invalidity or unenforceability shall not invalidate any of the other provisions of these Terms. Purchaser may not assign, including by operation of law, its obligations hereunder without R&D's written consent.

Privacy Policy

The purpose of this Privacy Policy is to tell you what information we gather on this Website and how we use that information. By using this Website you accept the terms of this Privacy Policy, as well as the terms set forth in our Terms and Conditions.
This Privacy policy applies to the website owned and operated by [R&D Systems, Inc.][Techne Corporation] (referred to as "we," "us," the "Company" or similar terms), with a homepage located at [www.rndsystems.com] [www.techne-corp.com] (the "Website"). This Privacy Policy does not apply to any other other websites, and we make no representations as to the privacy practices of other websites.

What Information do We Collect?

In order to process and track orders, update your account, respond to your requests, operate our business, and maintain this Website and various features, we collect a variety of information about Website visitors. This section describes both the Non-Personal Information and Personal Information we collect.

Non-Personal Information.

Each time a computer visits this Website we may collect certain information from that computer. This information is automatically collected from the computer's web browser and may include information such as the following ("Non-Personal Information"):

- the type of web browser software the computer uses (for example, Netscape Navigator or Internet Explorer)
- the name of the domain from which the computer accessed the Internet
- the Internet address of the website from which the computer linked directly to the Website
- the date and time the computer accessed the Website

Non-Personal Information does not tell us who you are.

Appendix 18

FRÅN

STOCKHOLMS LÄNS LANDSTING
Karolinska Universitetssjukhuset
Karolinska Universitetslaboratoriet
Klin Pat/Cyt lab

Tfn

B: 11001-341-306
S: 11001-341-306
F: 11002-521-S03
R: 1026-8020499-1
L: K18251-11

TILL

Karolinska Universitetssjukhuset
Thoraxkliniken, Solna
N 13/23 Thorax
171 76 Stockholm

SVAR PATOLOGI/CYTOLOGI

Regnr
K18251-11

Provtagningstid:	2011-11-17 12:53	Remittent:	Lotta Orre
Ankomstid lab:	2011-11-17	SNABBSVAR:	Tfn: 77005

Preparatets natur: 1. Trachea (lång sutur i vä bronk, kort sutur i hö bronk2. Proximal trachealring (sutur i proximal ände

Frågeställning: Cancer? Radikalitet? Fryssnitt på resektionsränder

Anamnes: Adenoidcystisk ca strålad och cytostatikabeh med tydlig regress. Px för att utesluta ca i planerade op.områden om 1 mån. Px1= lateralvägg hö huvudbronk ca 1 cm ovan ovanlobsavgång, px2= medialt hö huvudbronk, px3= carina, px4= vä huvudbronk medialvägg 2 cm fr carina, px5= 3 cm nedom stämbandsplanet hö, px6= 3 cm nedom stämbandet vä.
Strålbehandlad: Ja, år 2011

SVAR

Tidigare preliminärt utlåtande
2011-11-17 K18251/2011

FRYSSNITTSUNDERSÖKNING

1: Trachea huvudpreparat:

Trachearesektat inkluderande carina och proximala huvudbronkerna. Lång sutur vä huvudbronk, kort sutur hö huvudbronk. Resektatet mäter 55 mm i längd och 35 mm i tvärsnitt. Makroskopiskt väsentligen ua.

Snittkod:

A - hö huvudbronk res-rand, tangentiellt tillvaratagna snitt utmed hela circumferensen.
B - vä huvudbronk, tangentiellt tagen res-rand hela circumferensen.
C - proximal res-rand, tangentiellt snitt hela circumferensen.

Mikroskopiskt ses avgjord misstanke om kvarvarande tumör såväl i tangentiellt tillvaratagen res-rand från höger (A) som vänster (B) huvudbronk. Det rör sig om relativt betydande mängder tumör som dock återfinnes enbart perifert om bronkbrosket. Mukosala sidan normal. Det rör sig om små tubulära strukturer epitel som delvis är associerade till basalmembranliknande hyalina globuli. Återhållen atypi. Infiltration i bindväven med viss desmoplastisk reaktion. Inga cribriforma eller solida strukturer. Ingen perineural eller lymfovaskulär infiltration dokumenterad. Det hela är som sagt svårvärderat, möjligen pga given preoperativ behandling och det faktum att tillblandat har en hel del rester av benign accessorisk

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spottkörtel såväl i submucosa som i de yttre delarna av preparatet utanför brosklamellerna. Sammanfattningsvis misstanke om kvarvarande viabel tumör där det hela skulle kunna stämma med en adenoidcystisk cancer av en något ovanlig tubulär subtyp. Korrelation till tidigare biopsimaterial meddelst formell eftergranskning vore av värde vad gäller tumörtyp, subtyp och eventuell behandlingspåverkan.

Snitt från proximal res-rand ingen suspekt tumör.

2: Proximal trachealring:

Trachealring med diameter 25 mm och längd 8 mm. Suturmärkt i den proximala res-randen. Preparatet delas medelst ett tvärsnitt och den proximala res-randen bäddas i helhet för fryssnittsundersökning.

Ingen mikroskopiskt suspekt tumör. Dock något svårvärderat i fryssnittssituationen med tanke på fibros.

3: Lymfkörtelfraktion R2 och R4.

Preparatet karakteriseras makroskopiskt och mikroskopiskt efter formalinfixering. Inget fryssnitt i denna fraktion.

Tidigare diagnos
Fryssnittsdiagnos:
1: Trachea. Misstänkt tumör i distal resektionsyta avseende hö och vä huvudbronk. Snarast tubulär variant av adenoidcystisk cancer. Typbedömning på fryssnitt osäker. Proximal res-rand intet suspekt.

2: Proximal trachealring. Ingen suspekt tumör.

3: Lymfkörtlar R2, R4. Handlägges ej med fryssnitt.
BIOBANKSINFORMATION
Provet får användas för samtliga, enligt biobankslagen, godkända ändamål.
Tidigare preliminärt utlåtande
2011-11-23 K18251/2011

Paraffinsnittsundersökning:

1. Trachea huvudpreparat:

Fryssnittsdiagnos avseende A - hö huvudbronk och B - vä huvudbronk kvarstår.

Återfinner sålunda i preparaten härdar av tubulotrabekulärt strukturerad infiltrativt växande tumör perifert om brosklamellerna. Antydd bifasicitet med luminell-myoepitelial differentiering. Riklig förekomst av basalmembranglobuli. Sålunda kan fynden gå väl samman med manifestation av adenoidcystisk cancer. Beställer dock immunhistokemi för konfirmation av tumörtyp. Tumör finns som sagt i distal res-rand såväl avseende hö som vä huvudbronk i tangentialsnitt, vilket innebär att radikaliteten i longitudinell distal riktning icke kan garanteras. Vidare ses tumör mycket nära circumferentiell margin med några enstaka antydda tumörcellskomplex i artefaktbemängd diatermirand. Även betr circumferentiell margin krävs immunhistokemi för definitivt ställningstagande.

Betr den proximala res-randen framkommer i HTX-varianterna ett ytterst minimalt fokus externt om och i omedelbar anslutning till brosklamellerna av likartad cancer. Här ingen relation till circumferentiell margin.

Resterande trachea mätande 4,5 x 2,7 x 2,7 cm tvärsnittas och totalbäddas 1D-1N med början distalt.

Mikroskopiskt ses i samtliga tvärskivor förekomst av vad som ter sig som adenoidcystisk cancer. På sina håll minimala cribriforma strukturer. Perineural växt dokumenterad. Merparten av tumören återfinnes dorsalt men i olika snittnivåer ses tumör i samtliga riktningar. Merparten av tumören förefaller ha gått i regress efter given preoperativ behandling med extensiv rest av fibros. Dock spridda härdar av viabel tumör i kanske 20% av det totala fibrosområdet. Tumör finns övervägande externt om brosklamellerna men i multipla nivåer även i submucosa. Ingen broskdestruktion. Betr circumferentiell margin är denna mycket svårvärderad med tanke på tumörens regress, diskontinuerliga och perineurala växt. Någon uppenbar viabel tumörcellsrest ses ej någonstädes i circumferentiell margin. I några snittnivåer finns däremot koncentrisk kollagen fibros av tumörtyp men utan viabel tumörepitelial komponent i anslutning till circumferentiell margin.

2. Proximal trachealring:

Paraffinsnitt visar här ingen säker tumörrest. Ej heller några rester av tumörfibros. Däremot ses begränsad sialometaplasi och skivepitelmetaplasi av ytslemhinnan sannolikt sekundärt till preoperativt given behandling. Utför för säkerhets skull cytokeratinfärgning även i denna fraktion.

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3. R2 och R4:

Tolv lymfkörtlar utan metastas (0/12).
Tidigare diagnos
Intermediärsvar:
1: Huvudpreparat trachea med kvarvarande till synes viabel adenoidcystisk cancer av tubulotrabekulär typ i samtliga snittnivåer. Tumör i distal tangentiell res-rand hö och vä huvudbronk. Svårvärderad circumferent margin distalt föremål för immunhistokemisk analys. Dock generellt mycket snäv circumferentiell marginal. Betydande tumörregress noteras.

2: Proximal trachealring med benign trachealvävnad med sialometaplasi. Intet tumörsuspekt.

3: Lymfkörtlar R2, R4. Tolv lymfkörtlar utan metastas (0/12).
BIOBANKSINFORMATION
Provet får användas för samtliga, enligt biobankslagen, godkända ändamål.
Tidigare preliminärt utlåtande
2011-11-25 K18251/2011

1: Trachea, huvudpreparat:

Cytokeratinfärgning F1A tangentiell res-rand hö huvudbronk visar adenoidcystiska cancerförband som fokalt förekommer i circumferentiell res-rand. In loco-radikaliteten kan sålunda icke garanteras.

I övrigt immunhistokemi väl förenlig med tubulär trabekulär variant av adenoidcystisk cancer Xanthos GI/III - EMA+, CEA-, MNF116+, CK5+, CK7+, CK14+, CK18+, CK20-, S-100+/-, SMMS-1+/-, p63+/-, GCDFP15-, AR-, ER-, PGR-, CD117+, TTF-1-, p16-/+. Inga tecken till p53-mutation. CT4 inmärkes starkt i basalmembranreduplikat i anslutning till tumör. Proliferationsfraktion cirka 5% (Ki67). Sålunda bifasisk epitelial myoepitelial grundkonstellation som tillsammans med morfologin i övrigt är väl förenligt med adenoidcystisk cancer enligt ovan.

2: Proximal trachealring:

Cytokeratinfärgning MNF116 visar inga ockulta tumörmanifestationer. Tumörfritt.

3: R2 och R4:

FRÅN

STOCKHOLMS LÄNS LANDSTING
Karolinska Universitetssjukhuset
Karolinska Universitetslaboratoriet
Klin Pat/Cyt lab

Tfn

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F: 11002-521-S03
R: 1026-8020499-1
L: K18251-11

SVAR PATOLOGI/CYTOLOGI

TILL

Karolinska Universitetssjukhuset
Thoraxkliniken, Solna
N 13/23 Thorax
171 76 Stockholm

Regnr
K18251-11

Varit i telefonkontakt med inremittenten som önskar helbäddning av fraktionen. Ber vår ansvarige ST-läkare Camilla Hilliges utföra detta.

Kompletterande svar följer.

Tidigare diagnos
Intermediärsvar:
1: Huvudpreparat trachea med kvarvarande till synes viabel adenoidcystisk cancer av tubulotrabekulär typ (Szanthos GI) i samtliga snittnivåer. I distal res-rand hö och vä huvudbronk tumörförekomst varför radikaliteten i longitudinell riktning distalt ej kan garanteras. Därtill tumörcellsförband i circumferentiell resektionsrand fokalt-distalt. Betydande tumörregress (90%) efter preoperativ behandling.

2: Proximal trachealring med benign trachealvävnad med sialometaplasi. Intet tumörsuspekt.

3: Lymfkörtlar R2, R4. Tolv lymfkörtlar utan metastas (0/12). Fraktionen totalbäddas för uteslutande av tumör i mjukvävnaden. Kompl svar följer.
BIOBANKSINFORMATION
Provet får användas för samtliga, enligt biobankslagen, godkända ändamål.

KOMPLETTERANDE UTLÅTANDE
2011-11-28 K18251/2011

TRE LYMFKÖRTLAR R2, R4:

Har kontrollerat vår makro-undersökning och konstaterat att allt material bäddat. Alltfort inga tecken till malignitet i fraktionen.
DIAGNOS
Intermediärsvar:
1: Huvudpreparat trachea med kvarvarande till synes viabel adenoidcystisk cancer av tubulotrabekulär typ (Szanthos GI) i samtliga snittnivåer. I distal res-rand hö och vä huvudbronk tumörförekomst varför radikaliteten i longitudinell riktning distalt ej kan garanteras. Därtill tumörcellsförband i circumferentiell resektionsrand fokalt-distalt. Betydande tumörregress (90%) efter preoperativ behandling.

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Fraktionen totalbäddas för uteslutande av tumör i mjukvävnaden. Kompl
svar följer.
BIOBANKSINFORMATION
Provet får användas för samtliga, enligt biobankslagen, godkända
ändamål.
Göran Elmberger, Överläkare 2011-11-29
---slut---

SVAR RÖNTGEN/ISOTOP

Appendix 19a

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F: 11002-303-HS1
R: 1026-7333320-3
L: 4339731801

TILL Karolinska Universitetssjukhuset
Öron-näsa-halskliniken, Danderyd
Danderyds sjukhus
182 88 Stockholm
Tel: 08-655 59 83 Fax: 08-755 72 28

Prioritet: Normal
Patienten bör undersökas på: Röntgen
Patienten kallas från: Vårdavd.

Remissdatum:	2011-06-16 12:02
Remittent:	Richard Kuylenstierna
Till sektion:	Datortomografen
Önskad undersökning:	DT thorax
Frågeställning:	Staus en vecka postop. Pnemothorax, pleuravätska, infiltrat, annat?
Anamnes, status:	Trakealtransplanerad. Pneumothorax och drän hö. Infiltrat hö. Idag ökad andningsfrekvens-visar sig beronde på sekretstagnation i transplantatet resp i huvudbronkerna. Rensuges och blir bättre. Anastomoserna ser fina ut-höger överlob dock ngt trång.Tidigare odling från bronksekret har visat candida!

Längd: 183 cm
Vikt: 78.4 kg

Kreatinin: 60 2011-06-16

SVAR

Undersökning påbörjad: 2011-06-16 13:59 avslutad: 2011-06-16 14:24 Rek. C-koder:

Undersökningskod: D49. DT Lungartärer

Utlåtande: Datortomografi lungartärer

Status post trakealtransplantation. Av operationsberättelse framkommer skada på höger pulmonalisartär som reparerats i en omgång med Dacron-graft, ånyo skada sen under operationen som ska ha reparerats med stygn.

Truncus pulmonalis noteras kontrastfylld fram till avgången för höger lungartär. Här ses ett tvärt avbrott och ingen kontrastfyllnad av huvudartären. Höger underlobsartär är dock kontrastfylld. Även artärerna till mellanloben samt ovanloben är kontrastfyllda men något tunnare. Oklar bild. Mest sannolikt ockluderat graft. Lungemboli kan övervägas men förefaller osannolikt. Ej typiskt utseende och inga lungembolier någon annanstans.

Kvarstående pneumothorax på höger sida. Som bredast ses ventralspalt på cirka 2 cm. Även lateralspalt. Pleuradränet har spetsen i hilushöjd lateralt. Små mängder pleuravätska på höger sida.

I trakea samt i höger stambronk ses en del slem. Höger ovanlob är till stora delar atelektatisk och mer deklivt ses även infiltrat med infektiöst utseende. Även höger underlob samt mellanlob är delvis atelektatiska.

I mediastinum ses mjukdelssvullnad samt små mängder gas i anslutning till trakealtransplantatet. Tunn pneumomediastinum, enbart ett 0,5 cm brett. Sannolikt enbart postop-förändringar.

Framställd
11-06-17 16:13

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Prioritet: Normal
Patienten bör undersökas på: Röntgen
Patienten kallas från: Vårdavd.

Ingenting anmärkningsvärt i vänster lunga.

På höger sida ses subcutant emfysem i mjukdelarna.

Subdiafragmalt finns små mängder fri gas. Enligt uppgift så har oment dragits upp i mediastinum, således förväntat fynd postoperativt.

Sammanfattning:
Oklart avbrott på höger lungartär, ockluderat graft? Infiltrat samt atelektas i höger lunga där slem ses i stambronken och distala trakea.

Bilder har demonstrerats för ansvarig IVA-läkare.

Saiepour, Laura

16:53 2011-06-16 Signering 1 Preliminärt svar: Saiepour, Laura
TILLÄGG:
Efter diskussion med en av ansvariga kirurger framkommer att Dacrongraftet är väldigt smalt, detta gör det troligt att avsaknaden av kontrastfyllnad är enbart tekniskt betingat. Ocklusion kan dock inte uteslutas.
16:13 2011-06-17 Signering 2 Slutgiltigt svar: Ripsweden, Jonaz
---slut---

SVAR RÖNTGEN/ISOTOP

Appendix 19b

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TILL
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Avdelning B82
141 86 STOCKHOLM
Tel: 08-585 877 91 Fax: 08-585 873 25

Prioritet: Akut
Patienten bör undersökas på: Röntgen
Patienten kallas från: Vårdavd.

Remissdatum: 2011-07-05 15:38
Remittent: Gert Henriksson
Till sektion: Datortomografen
Önskad undersökning: CT thorax
Frågeställning: Status post op med trachea/bronkgraft 9/6
Anamnes, status: Islänning (engelsktalande, ursprungligen fr Eritrea) som genomgått resektion av distala trachea och proximala huvudbronker 9/6 pga ca. Insytt trachea/bronkgraft. Under eftervården något mindre sekretmängder och bra andning. Innnan pat flyger hem till Island vill kirurgen Macchiarini ha uppföljand CT thorax för att bedömma slutresultatet.

Längd: 183 cm
Vikt: 73.5 kg

Kreatinin: 71 2011-06-30

SVAR

Undersökning påbörjad: 2011-07-06 08:59 avslutad: 2011-07-06 10:04 Rek. C-koder:

Undersökningskod: .

Utlåtande: Datortomografi thorax with high dose iv contrast in three phases

TRACHEAL TRANSPLANT
Status post tracheal/proximal bronchial transplant with the transplant in correct position without dislocation. Omentum is placed in the mediastinum.
There is a small air leakage from the medial part of the left bronchial anastomosis, with air (no fluid collection).

VEINS
There is abundant filling of collateral veins of the right half of the thoracic wall, probably due to technical reasons ie compression of the right subclavian vein secondary to elevation of the arm.
Oment is wrapped around the RIGHT brachiocephalic vein and the superior v cava, which are irregular and partly narrowed.

Post-op ocklusion of the LEFT brachiocephalic vein and secondary thrombosis of the left subclavian and jugular vein (upper top is not visualized).

ARTERIES
The right main pulmonary artery is ockluded in the midline. Some peripheral right pulmonary filling is noticed except to the medial part of the apical right upper lung lobe.
The arterial branches from the aortic arch are intact.

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SVAR RÖNTGEN/ISOTOP

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Tel: 08-585 877 91 Fax: 08-585 873 25

Prioritet: Akut
Patienten bör undersökas på: Röntgen
Patienten kallas från: Vårdavd.

LUNGS
No pneumothorax.
Corresponding to the medial and apical part of right upper lung lobe there is a segmental or subsegmental consolidated lung that does not enhance properly but contains some vessels and open bronchi. -Partly devascularized segment?

Nonextensive lamellar atelectasis right side.
Right pleura contains 1-1,5 cm of fluid.
Elevation of the right hemidiaphragm.

SUMMARY
Post op intact tracheo-bronchial transplant with small air leakage from the left bronchial anastomosis; ocklusion/thrombosis of LT brachiocephalic & jugular/subclavian veins.

Ocklusion of the right main pulmonary artery.
Partly devascularized segment upper RT lobe??

Leidner, Bertil

16:22 2011-07-06 Signering 1 Preliminärt svar: Leidner, Bertil

16:47 2011-07-22 Signering 2 Slutgiltigt svar: Cederlund, Kerstin
--slut--

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SVAR RÖNTGEN/ISOTOP

Appendix 19c

Prioritet: Normal
Patienten bör undersökas på: Röntgen
Patienten kallas från: Vårdavd.

Remissdatum: 2011-11-16 13:33 Remittent: Jan-Erik Juto
Till sektion: Datortomografen
Önskad undersökning: CT hals och thorax. med kontrast
Frågeställning: Utredning inför bronkoskopiop 21/11 HS, luftvägar ua i övr? parenkym?? lungartärfunktion hö???
Anamnes, status: I överenskommelse med Dr Bertil Leidner.
undersökning innan op. den 21/11 planerad till efter kl 10.00
Radikalop juni 2011 med resektion av trachea och del av huvudbronker och inop av plastgraf.
I efterförloppet lite trångt in i höger ovanlob.
Nu utvecklat granulationsvävnad i luftvägen distalt om graft i höjd med hö ovanlobsbronks avgång.

Kreatinin: 71 2011-06-30

SVAR

Undersökning påbörjad: 2011-11-22 07:47 avslutad: 2011-11-22 09:06 Rek. C-koder:

Undersökningskod: .

Utlåtande:

Datortomografi thorax och övre buk
Undersökningen är utförd efter i.v. kontrast.

Undersökningen är utförd i arteriell och venös fas.
Jämförelse gjord med tidigare undersökning 2011.07.06.
Status efter op med plastgraft i distala trachea inkluderande proximala huvudbronker bilateralt.
Jämfört tidigare undersökning har det tillkommit metallstent i vardera huvudbronken, på höger sida går metallstentet förbi avgången för höger ovanlob, och når ner till precis avgången för mellanlobsbronken. Något trångt precis vid avgången för mellanlobsbronken vilket är väsentligen oförändrat jämfört tidigare undersökning.
Mängden fri gas i mediastinum framförallt subcarinalt och ventralt av carinanivå har minskat jämfört tidigare undersökning, dock finns små mängder kvar och ligger även lateralt om högra intermediärbronken och sträcker sig upp längs plastgraftets högra omfång 3,5 cm.
Oförändrat ses ocklusion av höger pulmonalisartär precarinalt, tillkommit ocklusion av vena cava superior från strax nedom konfluens vena braciocephalica sinistra, återfyllnad av kärlet omedelbart ovan höger förmak sannolikt retrograd fyllnad. Ingen kontrastfyllnad av vena azygos intill distala vena cava superior.

I höger lunga ses vid dagens undersökning ett mer tätt närmast atelektatiskt parenkym apikomedialt i höger ovanlob oförändrat tät

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SVAR RÖNTGEN/ISOTOP

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Prioritet:	Normal
Patienten bör undersökas på:	Röntgen
Patienten kallas från:	Vårdavd.

infiltrat apikoventralt i höger ovanlob. Tillkommit en del smärre
infiltrat i mellanlob och i höger underlob.
Ökad mängd pleuravätska dorsalt höger sida med även vätska in i
interlobärfåran.
Tillkommit närmast peribronkiellt anordnat glest infiltrat i vänster
underlob jämfört tidigare.

SUMMARY:
1. GRAFT: Granulation tissue in the upper tracheal anastomosis. Metal stents in both main bronchi. Remaining free mediastinal air (most likely from lower graft/carinal area) partly within a small cavity. It may be a risk for abscess formation when stent now is covering this leakage area.
2. VESSELS: New occlusion of the superior vena cava, partial recanalisation of left subclavian and subclavian/jugular vein. Collateral filling of the azygos vein and superficial veins of thorax and abdomen. Remaining ocklusion of the right pulmonary artery.
3. LUNGS: Central and right upper lobe consolidation with open airways possibly due to radiation reaction. In right lower lobe more extensive chronic type of inflammation/infection possibly due to secret stagnation. In LT lower lobe peribronchial infection of more recent/acute type.
4. Up to 5 cm LT pleural fluid - possible to drain!

Images have been shown to dr Juto interactively.

Wallier, Egon/ Bertil Leidner

14:43 2011-11-23 Signering 1 Preliminärt svar: Leidner, Bertil

20:29 2011-12-06 Signering 2 Slutgiltigt svar: Cederlund, Kerstin
---slut---