Feasibility of Bioengineered Tracheal and Bronchial Reconstruction Using Stented Aortic Matrices

Emmanuel Martinod, MD, PhD; Kader Chouahnia, MD; Dana M. Radu, MD; Pascal Joudiou, MD; Yurdagul Uzunhan, MD, PhD; Morad Bensidhoum, PhD; Ana M. Santos Portela, MD; Patrice Guiraudet, MD; Marine Peretti, MD; Marie-Dominique Destable, MD; Audrey Solis, MD; Sabiha Benachi, MD; Anne Fialaire-Legendre, PharmD, PhD; Hélène Rouard, PharmD, PhD; Thierry Collon, MD; Jacques Piquet, MD; Sylvie Leroy, MD; Nicolas Vénissac, MD, PhD; Joseph Santini, MD, PhD; Christophe Tresallet, MD, PhD; Hervé Dutau, MD; Georges Sebbane, MD; Yves Cohen, MD, PhD; Sadek Beloucif, MD, PhD; Alexandre C. d’Audiffret, MD; Hervé Petite, PhD; Dominique Valeyre, MD, PhD; Alain Carpentier, MD, PhD; Eric Vicaut, MD, PhD

Importance
Airway transplantation could be an option for patients with proximal lung tumor or with end-stage tracheobronchial disease. New methods for airway transplantation remain highly controversial.

Objective
To establish the feasibility of airway bioengineering using a technique based on the implantation of stented aortic matrices.

Design, Setting, and Participants
Uncontrolled single-center cohort study including 20 patients with end-stage tracheal lesions or with proximal lung tumors requiring a pneumonectomy. The study was conducted in Paris, France, from October 2009 through February 2017; final follow-up for all patients occurred on November 2, 2017.

Exposures
Radical resection of the lesions was performed using standard surgical techniques. After resection, airway reconstruction was performed using a human cryopreserved (−80°C) aortic allograft, which was not matched by the ABO and leukocyte antigen systems. To prevent airway collapse, a custom-made stent was inserted into the allograft. In patients with proximal lung tumors, the lung-sparing intervention of bronchial transplantation was used.

Main Outcomes and Measures
The primary outcome was 90-day mortality. The secondary outcome was 90-day morbidity.

Results
Twenty patients were included in the study (mean age, 54.9 years; age range, 24-79 years; 13 men [65%]). Thirteen patients underwent tracheal (n = 5), bronchial (n = 7), or carinal (n = 1) transplantation. Airway transplantation was not performed in 7 patients for the following reasons: medical contraindication (n = 1), unavoidable pneumonectomy (n = 1), exploratory thoracotomy only (n = 2), and a lobectomy or bilobectomy was possible (n = 3). Among the 20 patients initially included, the overall 90-day mortality rate was 5% (1 patient underwent a carinal transplantation and died). No mortality at 90 days was observed among patients who underwent tracheal or bronchial reconstruction. Among the 13 patients who underwent airway transplantation, major 90-day morbidity events occurred in 4 (30.8%) and included laryngeal edema, acute lung edema, acute respiratory distress syndrome, and atrial fibrillation. There was no adverse event directly related to the surgical technique. Stent removal was performed at a postoperative mean of 18.2 months. At a median follow-up of 3 years 11 months, 10 of the 13 patients (76.9%) were alive. Of these 10 patients, 8 (80%) breathed normally through newly formed airways after stent removal. Regeneration of epithelium and de novo generation of cartilage were observed within aortic matrices from recipient cells.

Conclusions and Relevance
In this uncontrolled study, airway bioengineering using stented aortic matrices demonstrated feasibility for complex tracheal and bronchial reconstruction. Further research is needed to assess efficacy and safety.

Trial Registration
clinicaltrials.gov Identifier: NCT01331863

Published online May 20, 2018.

© 2018 American Medical Association. All rights reserved.
Over the last decade, new procedures in the field of airway transplantation have gained attention primarily through case reports or small studies. Due to the lack of prospective data, these interventions have failed to achieve standard of care status. An efficient airway replacement could potentially benefit many patients with lung cancer and eliminate the need for high-risk thoracic surgical procedures including pneumonectomies. Airway replacement also could be an option for patients with end-stage tracheobronchial disease for whom palliative treatment is often proposed.

In 1997, a research program on airway transplantation using stented aortic matrices was initiated in the laboratory of the Alain Carpentier Foundation. A series of 7 preclinical animal studies using a sheep model showed that autologous and fresh or cryopreserved allogeneic aortic grafts could be valuable airway substitutes. The regeneration of epithelium and de novo generation of cartilage were observed within the aortic matrices, thus allowing stent removal after only 6 months. Although controversial, the hypothesis that bone marrow mesenchymal stem cells play a role in this process of in vivo tissue engineering has been proposed. These favorable results led to the first human applications in patients with extensive tracheal diseases or complex tumors that would otherwise necessitate pneumonectomies.

De novo generation of cartilage within cryopreserved aortic allografts has been observed recently. This process originated from the recipient cells, which was observed in animal models. Moreover, remaining viable matrix cells have been identified to play a critical role in the regenerative process as a way to release proangiogenic, chemoattractant, proinflammatory or proimmunomodulatory cytokines, and growth factors. This prospective study was designed to evaluate the feasibility of airway bioengineering using stented cryopreserved aortic allografts as biological matrices.

**Methods**

**Study Design**

The study was sponsored by Direction de la Recherche Clinique at Assistance Publique–Hôpitaux de Paris, which is a consortium of university hospitals in Paris, France. The protocol was written by 2 of the principal investigators (E.M. and E.V.), it was approved by the French national institutional ethical review board on March 14, 2011, and it appears in Supplement 1. The initial protocol focused on bronchial transplantation to avoid a pneumonectomy in patients with extensive lung cancer.

To extend the study to all types of major (malignant or benign) lesions of the trachea and bronchi requiring airway transplantation, 3 amendments to the protocol were approved by the institutional ethical review board on March 27, 2012, October 25, 2012, and June 5, 2013. Before approval of the study and its amendments, 4 procedures had been accepted by the ethical review board. An independent data and safety monitoring board periodically reviewed the study outcomes. Written informed consent was obtained from all patients.

**Patients**

Eligible patients underwent a standard preoperative evaluation and cardiopulmonary tests. A multidisciplinary team approved the inclusion and exclusion criteria. Patients were included in the study if they (1) had proximal lung tumors requiring a surgical resection (pneumonectomy, carinal resection, or sleeve lobectomy) that may or may not have been treated with neoadjuvant chemotherapy and had adequate or compromised preoperative lung function tests; or (2) had significant major malignant or benign lesions of the trachea and bronchi untreated with conventional therapeutic approaches.

Patients were excluded from the study if they (1) were younger than 18 years; (2) were unable to give consent or not affiliated with the French Social Security System; (3) had a lung tumor requiring a standard lobectomy; (4) had nonresectable major locally invasive tumors; (5) had contralateral lymph node invasion; (6) had metastatic disease with the exception of a unique resectable brain metastasis; (7) had tracheal lesions requiring standard resection with direct anastomosis; (8) had an iodine allergy; or (9) received a preoperative evaluation indicating an inability to undergo a standard lobectomy.

**Treatment**

After enrollment in the study, patients underwent an intensive preoperative respiratory conditioning program. The human cryopreserved (~80°C) aortic allograft, which was not matched by the ABO and leukocyte antigen systems, was ordered from a certified tissue bank (Saint-Antoine, Etablissement Français du Sang, Assistance Publique–Hôpitaux de Paris). To prevent airway collapse, a custom-made, fully covered conical nitinol stent (Silmet, Novatech) or a silicone stent (Tracheobronxane Dumon, Novatech) were manufactured according to preoperative measurements obtained via computed tomography.

The first step was to evaluate whether a complete surgical resection with adequate margins was needed. The second step was to determine whether a conventional approach should be taken using direct end-to-end anastomosis for tracheal lesions or a lobectomy or bilobectomy for lung tumors. The third step was to determine whether an airway transplantation was needed.

Radical resection of the lesions was performed using standard surgical techniques. A conventional solution for airway...
reconstruction was preferred when feasible. If used, the cryopreserved aortic allografts were removed from the dry ice, then thawed in container bags for 10 minutes at room temperature, followed by 10 minutes in a water bath at 37°C. Next, the allografts were washed with a sterile saline solution for 5 minutes before implantation and were used for airway reconstruction, pulmonary artery reconstruction, or both.

At the end of the operations, the allografts were covered circumferentially with a local muscle flap to promote neovascularization and prevent fistulization. Sterno-omo-thyrohyoid muscle flaps were used for tracheal reconstructions; pectoralis major muscle flaps were used for carinal reconstructions; and latissimus dorsi or intercostal muscle flaps were used for bronchial reconstructions. The techniques used followed guidelines established during human and other experimental studies.7-20

In Figure 1, the final aspect of the airways after surgical resection and reconstruction is shown schematically. None of the patients received immunosuppressive therapy per the usual recommendations when cryopreserved aortic allografts are used in vascular surgery. Low-molecular-weight heparin was administered for postoperative venous thromboembolism prophylaxis. Until stent removal, an aerosolized saline solution was administrated 3 times daily to prevent airway obstruction.

**Follow-up and Assessment of Outcomes**

Patients were followed up for 90 days. The outcomes of mortality and morbidity were assessed, including complications directly related to the allograft, the stent, or both. Patients were systematically examined at 30, 60, and 90 days. There was a 7-day window around each time point during which patients could have been examined (eg, on day 23 or on day 37 for the 30-day time point). If required, complementary examinations were performed such as chest radiography, computed tomography, and bronchoscopy. The primary outcome was...
90-day mortality. The secondary outcome was 90-day morbidity. After 90 days, all patients were followed up for long-term mortality and morbidity outcomes.

Biopsy specimens were obtained from cryopreserved aortic allografts after stent removal in a woman (patient 2) who received an allograft from a male donor at 15 months and in a man (patient 4) who received an allograft from a female donor at 39 months. Tissues identified as neoepipithelium, neocartilage, and granuloma were isolated and used for histological studies. Biopsy specimens from patient 2 were used for engraftment (chimerism) studies. The biological study protocols are detailed in the eMethods in Supplement 2.

For all patients, the last follow-up visit occurred on November 2, 2017. The following data were collected for each patient: sex, age, medical history, type of airway disease, tumor localization, preoperative treatment, indication for inclusion in the protocol, date of the procedure, type of operation, duration of hospitalization, 90-day mortality and morbidity, histopathological specimen examination, postoperative treatment, delayed complications (after 90 days), stent removal timing, and status at last follow-up visit.

Statistical Analysis
The predefined sample size was 20 patients. This sample size enables estimation of a 90-day survival rate with a maximal half-width of its 2-sided 95% CI equal to ± 17.5%. The reported results include assessments from all 20 patients. All analyses were made using SAS version 9.4 (SAS Institute Inc).

Results
Patients
From October 2009 through February 2017, 20 patients were included in the study. Eight patients (40%) were referred from other medical centers. There were 13 male and 7 female patients ranging in age from 24 to 79 years with a mean age of 54.9 years. The patient characteristics, types of preoperative treatments, and indications for inclusion appear in Table 1.

Fourteen patients were included to avoid a pneumonectomy for non–small cell lung cancer (n = 11), carcinoid tumor (n = 2), or rhabdomyosarcoma (n = 1). Five patients had extensive benign (n = 3) or malignant (n = 2) tracheal lesions for which previous treatment had failed. One patient presented with a carcinoid tumor extending to the distal trachea, carina, and main right bronchus.

Procedures
The interventions performed for each patient are detailed in Table 2 and Figure 2. Thirteen patients underwent a tracheal (n = 5), carinal (n = 1), or bronchial (n = 7) transplantation using a stented cryopreserved aortic allograft. Two of the patients who underwent bronchial reconstruction also underwent a partial replacement of the pulmonary artery using the same allograft to avoid a pneumonectomy. Seven patients did not receive airway transplantation for the following reasons: medical contraindication appeared after enrollment (n = 1), unavoidable pneumonectomy (n = 1), exploratory thoracotomy only (n = 2), and a lobectomy or bilobectomy was possible (n = 3).

Primary Outcome
The details regarding the primary outcome for each patient appear in Table 2. Among all 20 patients, the 90-day mortality rate was 5% (1 patient underwent a carinal transplantation and died). Among the 13 patients who underwent airway transplantation, the 90-day mortality rate was 7.7% (n = 1). The 90-day mortality rate was 0% for the patients who had a tracheal (n = 5) or bronchial transplantation (n = 7).

Secondary Outcomes
The details regarding the secondary outcomes for each patient appear in Table 2. The mean length of hospitalization was 10.5 days and ranged from 6 to 22 days. Among the 13 patients who underwent airway transplantation, major 90-day morbidity events occurred in 4 (30.8%) and included laryngeal edema, acute lung edema, acute respiratory distress syndrome, and atrial fibrillation. There was no adverse event directly related to the surgical technique. All general complications resolved except for a massive cerebrovascular accident, resulting in the death of the patient.

Long-term Follow-up
Long-term follow-up did not identify any major complications specifically related to the allograft or the stent (Table 3). Minor stent-related complications (tracheal or bronchial granulomas) were found in 7 patients and required treatment management with bronchoscopy. A temporary tracheostomy, via the stented allograft, was required due to severe laryngeal edema in patient 2. Extensive granulomas required early stent removal in patient 20 at 5 months and a temporary tracheostomy from months 5 to 7 until the allograft was structurally strong enough.

Stent removal was possible in the majority of patients (n = 9; 69.2%). It was performed postoperatively between months 5 and 39 at a mean of 18.2 months. At a median follow-up of 3 years 11 months (maximal follow-up of 7 years 1 month), 10 patients (76.9%) were alive. Of these 10 patients, 8 (80%) breathed normally through newly formed airways after stent removal (Video). As of November 2, 2017, the stent was still in place in patients 3 and 17.

In biological studies, the explant cell culture demonstrated that the cells contained within the cryopreserved aortic allografts were viable and able to proliferate after thawing. The absence of SRY donor DNA 15 months after surgery in biopsy specimens from patient 2 suggested a progressive disappearance of donor cells from the cryopreserved aortic allograft in parallel to its colonization by the host’s cells. Pathological examination of superficial allograft biopsies showed the regeneration of mixed respiratory epithelium (Figure 3A).

The positive labeling of a biopsy sample from the cryopreserved aortic allograft at 39-month implantation for specific cartilage markers (type 2 collagen and Sox9) suggested the presence of cartilage-like cells (Figure 3B, C). Nonimplanted cryopreserved aortic allografts were negative for these markers.
Table 1. Patient Characteristics, Preoperative Treatment, and Indication for Inclusion in the Study

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Sex</th>
<th>Age</th>
<th>Medical History</th>
<th>Type of Airway Disease</th>
<th>Description of Localization</th>
<th>Type of Prophylactic Treatment</th>
<th>Indication for Study Inclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>78</td>
<td>Prostatic adenoma, past smoker (50 pack-years), and COPD</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung and extending to main bronchi and left atrium</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>58</td>
<td>Thyrotoxicosis</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung and extending to main bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>24</td>
<td>Severe epiglottic and tracheal obstruction</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung and extending to proximal bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>33</td>
<td></td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Left lung, upper lobe extending to main bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>59</td>
<td>Alcohol-induced diabetes, past smoker (80 pack-years), and COPD</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung and extending to proximal bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>41</td>
<td>Obesity (BMI of 37.5) and gestational diabetes</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung and extending to proximal bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>62</td>
<td>Coronary artery disease</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung and extending to proximal bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>57</td>
<td></td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung, lower lobe extending to main bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>9</td>
<td>M</td>
<td>50</td>
<td>Postintubation benign tracheal stenosis and upper airway malacia</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung and extending to proximal bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>79</td>
<td>Middle lobe stenosis and tracheal stenosis</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung and extending to proximal bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>54</td>
<td>Alcohol use disorder, past smoker (60 pack-years), and COPD</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung, lower lobe extending to main bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>41</td>
<td>Thyrotoxicosis and ablation of thyroid gland</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung, lower lobe extending to main bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>13</td>
<td>M</td>
<td>60</td>
<td>Diabetes, coronary artery disease, past smoker (80 pack-years), and COPD</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung, lower lobe extending to main bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>14</td>
<td>M</td>
<td>59</td>
<td></td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung, lower lobe extending to main bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>15</td>
<td>M</td>
<td>68</td>
<td>Hyperplasia, past smoker (60 pack-years), and COPD</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung, lower lobe extending to main bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>68</td>
<td></td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung, upper lobe extending to main bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>45</td>
<td></td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung, lower lobe extending to main bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>18</td>
<td>F</td>
<td>49</td>
<td>Amygdalotomy</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Left lung, lower lobe extending to main bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>19</td>
<td>M</td>
<td>50</td>
<td>Amygdalotomy</td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung, lower lobe extending to main bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
<tr>
<td>20</td>
<td>F</td>
<td>64</td>
<td></td>
<td>Non–small cell lung cancer, squamous cell subtype</td>
<td>Right lung, lower lobe extending to main bronchi</td>
<td>Neoadjuvant chemotherapy (3 cycles)</td>
<td>To avoid pneumonectomy</td>
</tr>
</tbody>
</table>

Abbreviations: BMI, body mass index; COPD, chronic obstructive pulmonary disease; HSV, herpes simplex virus; ICU, intensive care unit; R2, macroscopic residual tumor. *Found during pathological examination of the resected specimen.
<table>
<thead>
<tr>
<th>Type of Intervention</th>
<th>Date of Operation</th>
<th>Patient No.</th>
<th>Hospital Length of Stay, d</th>
<th>Primary Outcome: 90-d Mortality</th>
<th>Secondary Outcome: 90-d Morbidity</th>
<th>Cancer Pathology</th>
<th>Type of Postoperative Cancer Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tracheal transplantation</td>
<td>Oct 2010</td>
<td>2</td>
<td>14</td>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tracheal transplantation</td>
<td>Jan 2011</td>
<td>3</td>
<td>11</td>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tracheal transplantation</td>
<td>Oct 2011</td>
<td>4</td>
<td>10</td>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tracheal transplantation</td>
<td>Jun 2016</td>
<td>17</td>
<td>6</td>
<td>No</td>
<td>Subcutaneous emphysema</td>
<td>R0 resection*</td>
<td>None</td>
</tr>
<tr>
<td>Tracheal transplantation</td>
<td>Feb 2017</td>
<td>20</td>
<td>10</td>
<td>No</td>
<td>Tracheal granuloma, laryngeal edema at months 2 and 3, bronchoscopy, and tracheostomy</td>
<td>R0 resection*</td>
<td>None</td>
</tr>
<tr>
<td>Venovenous ECMO, right pneumonectomy extended to trachea, and carinal transplantation</td>
<td>May 2012</td>
<td>6</td>
<td>NA</td>
<td>Yes on day 1</td>
<td>Cerebrovascular accident and intracranial carotid artery occlusion</td>
<td>Pathological T4N2M0</td>
<td>NA</td>
</tr>
<tr>
<td>Intrapерicardial upper bilobectomy and bronchial transplantation</td>
<td>Oct 2009</td>
<td>1</td>
<td>16</td>
<td>No</td>
<td>Atrial fibrillation, acute lung edema, atelectasis, and urinary retention</td>
<td>Pathological T4N0M0</td>
<td>None</td>
</tr>
<tr>
<td>Left upper lobectomy and bronchial transplantation</td>
<td>Oct 2012</td>
<td>7</td>
<td>12</td>
<td>No</td>
<td>Atelectasis</td>
<td>Clinical T2N2M0 and pathological T3N0M0</td>
<td>Radiotherapy</td>
</tr>
<tr>
<td>Left lower lobectomy and bronchial and pulmonary artery transplantation</td>
<td>Jan 2013</td>
<td>8</td>
<td>8</td>
<td>No</td>
<td>No</td>
<td>Clinical T2N2M0 and pathological T2N1M0</td>
<td>Radiotherapy</td>
</tr>
<tr>
<td>Intrapерicardial upper bilobectomy and bronchial transplantation</td>
<td>Mar 2013</td>
<td>9</td>
<td>9</td>
<td>No</td>
<td>No</td>
<td>Clinical T3N1M0 and pathological T3N1M0</td>
<td>None</td>
</tr>
<tr>
<td>Right lower lobectomy and bronchial and pulmonary artery transplantation</td>
<td>Dec 2013</td>
<td>10</td>
<td>22</td>
<td>No</td>
<td>Atrial fibrillation and acute respiratory distress syndrome</td>
<td>Clinical T3N0M0 and pathological T3N1M0</td>
<td>Radiotherapy</td>
</tr>
<tr>
<td>Lower bilobectomy and bronchial transplantation</td>
<td>Nov 2014</td>
<td>13</td>
<td>12</td>
<td>No</td>
<td>Atelectasis</td>
<td>Clinical T3N0M0 and pathological T3N1M0</td>
<td>Radiotherapy</td>
</tr>
<tr>
<td>Lower bilobectomy and bronchial transplantation</td>
<td>Jan 2015</td>
<td>14</td>
<td>9</td>
<td>No</td>
<td>Anemia necessitating blood transfusion</td>
<td>Clinical T3N0M0 and pathological T0N0M0</td>
<td>Radiotherapy</td>
</tr>
<tr>
<td>Standard lobectomy but transplantation not performed</td>
<td>Apr 2014</td>
<td>12</td>
<td>10</td>
<td>No</td>
<td>No</td>
<td>Pathological T2N0M0</td>
<td>None</td>
</tr>
<tr>
<td>Standard lobectomy but transplantation not performed</td>
<td>Mar 2016</td>
<td>15</td>
<td>6</td>
<td>No</td>
<td>No</td>
<td>Pathological T2N2M0</td>
<td>Radiochemotherapy</td>
</tr>
<tr>
<td>Standard lobectomy extended to thoracic wall but transplantation not performed</td>
<td>Jul 2016</td>
<td>19</td>
<td>9</td>
<td>No</td>
<td>No</td>
<td>Clinical T3N0M0 and pathological T3N0M0</td>
<td>Radiotherapy</td>
</tr>
<tr>
<td>Pneumonectomy but transplantation not performed</td>
<td>Apr 2016</td>
<td>16</td>
<td>11</td>
<td>No</td>
<td>Atrial fibrillation</td>
<td>Pathological T2N1M0</td>
<td>None</td>
</tr>
<tr>
<td>Exploratory thoracotomy but transplantation not performed</td>
<td>Jun 2014</td>
<td>11</td>
<td>8</td>
<td>No</td>
<td>No</td>
<td>Clinical T4N2M0</td>
<td>Radiochemotherapy</td>
</tr>
<tr>
<td>Exploratory thoracotomy but transplantation not performed</td>
<td>Jun 2016</td>
<td>18</td>
<td>6</td>
<td>No</td>
<td>No</td>
<td>Clinical T4N2M0</td>
<td>Radiochemotherapy</td>
</tr>
<tr>
<td>Myocardial ischemia but operation not performed</td>
<td>Jul 2011</td>
<td>5</td>
<td>NA</td>
<td>No</td>
<td>No</td>
<td>Clinical T3N2M0</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviations: ECMO, extracorporeal membrane oxygenation; NA, not applicable.

* Indicates no residual tumor was found during pathological examination of the resected specimens.
Figure 2. Schematic Illustrations of the Intervention Performed for Each of the 20 Patients With End-Stage Tracheal Lesions or Proximal Lung Tumors

- Tracheal transplantation
- Carinal transplantation
- Bronchial transplantation
- Lobectomy
- Pneumonectomy
- Exploratory thoracotomy
- Medical treatment

In patients 12, 15, 16, and 19, dashed lines indicate bronchial sutures. In patients 5, 11, and 18, gray irregular shapes represent unresectable proximal lung tumors.
In this uncontrolled study, airway bioengineering using stented aortic matrices demonstrated feasibility for complex tracheal and bronchial reconstruction. Prospective protocols for surgical innovations before their introduction into clinical practice remain the exception rather than the rule despite international recommendations. In the field of airway transplantation, recent advances have been associated with important scientific and ethical controversies. In 2014, the International Society of Cell Therapy in association with other regulators and ethics experts proposed specific recommendations for human airway bioengineering. Since the establishment of this research initiative in 1997, all investigators have strictly adhered to the international scientific and ethical principles of surgical innovation.

In this study, the 90-day mortality rate was low among patients undergoing the innovative surgical approach. The 90-day mortality rate was used as the primary outcome rather than the 30-day mortality rate because it is more suitable for the evaluation of surgical interventions. The only death reported during the study was observed after a complex carinal reconstruction with extracorporeal membrane oxygenation. However, the mechanism of the cerebrovascular event remains unclear. Despite the fact that the death was not directly associated with the surgical technique, carinal reconstruction should be approached with extreme caution. In a retrospective study of 12 patients who had autologous tracheal substitution, the 90-day mortality rate was 16.6%; 2 patients who underwent carinal resection died.

In the present study, the 90-day mortality rate was 0% in the subgroup of patients who underwent bronchial transplantation to avoid a high-risk pneumonectomy. The mortality rate was lower than the rate observed in a retrospective series of patients who required pneumonectomies (varying from 10.5% to 26% at 90 days). This is important to note because pneumonectomy remains a frequent operation in the United States and in Europe. Ta et al developed a closed technique of lung-sparing pulmonary resection of malignant tumors using a different type of tissue-engineered bronchus.

In the subgroup of patients who had tracheal transplantation (n = 5) in the present study, the 90-day mortality rate was 0%. Regarding this mortality rate, it is challenging to compare the results from the present study with those obtained by retrospective case reports or series. Since the review of airway transplantation by Grillo, recent advances have been observed and include the development of modern tissue engineering techniques. The results obtained by Macchiarini et al have been critically debated following the revelation that the majority of patients died after the implantation of tissue-engineered airways, which is in contrast to the initial reports.

Other case studies have been reported by multiple groups using various conduit types including a Marlex mesh patch with spiral rings covered by a collagen sponge (Omor et al; n = 7),

<table>
<thead>
<tr>
<th>Patient</th>
<th>Tracheal Transplantation</th>
<th>Bronchial Transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>Types of Complications During Long-Term Follow-up</td>
<td>Stent Removed?</td>
</tr>
<tr>
<td>2</td>
<td>Laryngeal edema and stent bacterial infection</td>
<td>Yes at 15 mo</td>
</tr>
<tr>
<td>17</td>
<td>Tracheal granuloma related to the stent requiring bronchoscopy</td>
<td>No</td>
</tr>
<tr>
<td>20</td>
<td>Tracheal granuloma related to the stent requiring bronchoscopy and tracheostomy during months 5-7</td>
<td>Yes at 5 mo</td>
</tr>
<tr>
<td>1</td>
<td>Bronchial granuloma related to the stent requiring bronchoscopy</td>
<td>No</td>
</tr>
<tr>
<td>8</td>
<td>None</td>
<td>Yes at 22 mo</td>
</tr>
<tr>
<td>14</td>
<td>None</td>
<td>Yes at 14 mo</td>
</tr>
</tbody>
</table>

a From the date of the operation.
tracheal allograft after withdrawal of immunosuppressive therapy (Delaere et al2,6,35; n = 6), and a decellularized cadaveric trachea (Elliott et al4, Hamilton et al36 and Steinke et al37; n = 2). These reports have led some groups to start prospective observational studies.24 The 90-day morbidity rate in the present series was not directly linked to the surgical technique but rather to common complications of thoracic surgery. Importantly, there were no major complications related to the allograft or the stent.

The present study demonstrated positive long-term results. No death was related to airway transplantation using stented cryopreserved aortic allografts. Furthermore, stent removal was feasible in the majority of patients in this study as well as in preclinical studies2,3,7 and in a recent report.12 The majority of patients were breathing and speaking normally without a tracheostomy or stent at long-term follow-up. Moreover, postoperative examinations did not detect a definitive malacia of the cryopreserved aortic allografts after stent removal.

The present study confirmed prior biological observations.12 The regeneration of a mixed respiratory epithelium was observed on superficial allograft biopsies. Immunodetection of type 2 collagen, Sox9-specific markers, and engraftment (chimerism) studies from samples of neotissues demonstrated de novo generation of cartilage within the aortic allografts from recipient cells. Aortic matrices played a significant role in this observation as illustrated by the release of proangiogenic, chemoattractant, proinflammatory and immunomodulatory cytokines, and growth factors.12 The main hypothesis was that this phenomenon promoted progenitor or stem cell homing followed by de novo generation of cartilage. In this scenario, the human body was used as a natural bioreactor and allowed in vivo airway tissue engineering.

The role of stem cells in airway tissue regeneration has been widely debated in recent years. Some have argued that airway regeneration should be regarded as hypothetical and scientifically unfounded.35 To date, there has been no further explanation of the process observed since the beginning of the experiments. The mechanism of epithelium regeneration is less controversial. Epithelial cells have gradually repopulated the allograft lumen by direct migration, by expansion from adjacent native airways (as observed after epithelium destruction), or by both.38

The technique of in vivo bioengineering using an aortic matrix has been applied to the replacement of a small bowel segment with encouraging results in a porcine model.39 Compared with other techniques, the present solution did not require the use of decellularized cadaveric tracheal allografts, recipient cells, artificial bioreactors, or immunosuppressive treatment.7,24,30-32

Areas for additional research include the possibility to accelerate de novo generation of cartilage for early stent removal; study of mechanisms of airway regeneration within cryopreserved aortic matrices; assessment of long-term quality of life among patients after receiving a transplantation; and development of clinical applications using multicenter studies, in particular for patients with end-stage tracheal lesions or proximal lung tumors requiring a pneumonectomy.
Feasibility of Airway Transplantation Using a Bioengineered Stented Aortic Allograft

Preliminary Communication

Research

Limitations
This study has several limitations. First, this is a feasibility study with a limited number of patients. Larger studies are needed for a validated estimate of this technique. Second, along with many surgical innovations, this a monocentric study, which implies that the results cannot be generalized without further evaluation involving a larger number of centers. Third, this is a noncomparative study. Considering these limitations, further studies and multicenter randomized clinical trials are necessary to evaluate the benefit-risk balance of this approach for specific indications such as end-stage tracheal diseases, locally advanced thyroid cancer, and proximal lung cancer.

Conclusions
In this uncontrolled study, airway bioengineering using stented aortic matrices demonstrated feasibility for complex tracheal and bronchial reconstruction. Further research is needed to assess efficacy and safety.

ARTICLE INFORMATION

Accepted for Publication: April 10, 2018.
Published Online: May 20, 2018.

Author Affiliations: Assistance Publique-Hôpitaux de Paris, Hôpitaux Universitaires Paris Seine-Saint-Denis, Hôpital Avicenne, Chirurgie Thoracique et Vascularise, Université Paris 13, Sorbonne Paris Cité, UFR Santé, Médecine et Biologie Humaine, Bobigny, France (Martino); Assistance Publique-Hôpitaux de Paris, Hôpitaux Universitaires Paris Seine-Saint-Denis, Hôpital Avicenne, Chirurgie Thoracique et Vascularise, Université Paris 13, Sorbonne Paris Cité, UFR Santé, Médecine et Biologie Humaine, Bobigny, France (Vicaut); Assistance Publique-Hôpitaux de Paris, Hôpitaux Universitaires Paris Seine-Saint-Denis, Hôpital Avicenne, Oncologie, Université Paris 13, Sorbonne Paris Cité, UFR Santé, Médecine et Biologie Humaine, Bobigny, France (Chouahnia); Assistance Publique-Hôpitaux de Paris, Hôpitaux Universitaires Paris Seine-Saint-Denis, Hôpital Avicenne, Pneumologie, Université Paris 13, Sorbonne Paris Cité, UFR Santé, Médecine et Biologie Humaine, Bobigny, France (Martinod).

Statistical analysis: Drs Martinod and Vicaut

Critical revision of the manuscript for important intellectual content: Martinod, Chouahnia, Radu, Joudiou, Uznun, Guiraudet, Peretti, Solis, Benachi, Rouard, Collon, Piquet, Leroy, Venissac, Santini, Tresallet, Dutau, Sebbane, Cohen, Beloucif, d’Audiffret, Petite, Valeyre, Carpentier, Vicaut.

Drafting of the manuscript: Martinod, Chouahnia, Radu, Joudiou, Uznun, Guiraudet, Peretti, Solis, Benachi, Rouard, Collon, Piquet, Leroy, Venissac, Santini, Tresallet, Dutau, Sebbane, Cohen, Beloucif, d’Audiffret, Petite, Valeyre, Carpentier, Vicaut.

Administrative, technical, or material support: Martinod, Radu, Benischdoum, Guiraudet, Peretti, Rouard, Leroy, Beloucif, Vicaut.

Supervision: Martinod, Chouahnia, Destable, Tresallet, Dutau, Beloucif, Petite, Valeyre, Carpentier, Vicaut.

Conflict of Interest Disclosures: The authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest.

Dr Uznun reported receiving personal fees from Roche; personal fees and nonfinancial support from Boehringer Ingelheim; and nonfinancial support from Oxivy. Dr Valeyre reported being a member of advisory boards on idiopathic pulmonary fibrosis treatment that are supported by Roche and Boehringer Ingelheim; receiving personal fees from AstraZeneca and Isis France; and receiving travel support to attend scientific meetings from Roche and Boehringer Ingelheim. Dr Carpentier reported being a cofounder and shareholder of Carmat SA. Dr Vicaut reported receiving grant support from Bristol-Myers Squibb; and personal fees from Bristol-Myers Squibb, Pfizer, Novartis, Pierre Fabre, and Ottobock. No other disclosures were reported.

Funding/Support: Funding for the study was provided by the Direction de la Recherche Clinique at Assistance Publique-Hôpitaux de Paris.

Role of the Funder/Sponsor: The sponsor had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.


Additional Contributions: We thank the medical and nursing teams from Assistance Publique-Hôpitaux de Paris, Hôpitaux Universitaires Paris Seine-Saint-Denis for their participation in the care of patients; the Clinical Research Unit team from Saint Louis-Lariboisière-Fernand Widal Hospital, Paris Diderot University for the control of the study; and Andrei Bedusc, PhD (University of Arts and Design, Cluj-Napoca, Romania), for providing Figures 1 and 2. Dr Bedusc was not compensated for his contribution.

REFERENCES


