Sections A to E (scientific part)
17 March 2016

Active substance:	1. Decellularised tracheal scaffold from a cadaveric donor. 2. Autologous mesenchymal stromal cells.
International Non-proprietary Name (INN), (accompanied by its salt or hydrate form if relevant):	Not yet known
Proposed invented name of the medicinal product (tradename):	Not yet known
Orphan indication:	Treatment of tracheal stenosis
Unique Product Identifier (UPI)	Not yet assigned
Pharmaco-therapeutic group (ATC Code):	Not yet assigned
Pharmaceutical form(s) and strength(s):	Implant
Route(s) of administration:	Surgical implantation
Sponsor (in the EEA):	Videregen Ltd Innovation Centre 1 Liverpool Science Park 131 Mount Pleasant Liverpool L3 5TF UK

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List of abbreviations

BM	Bone marrow
CT	Computerised tomography
DEM	Detergent enzymatic method
DNA	Deoxyribonucleic acid
ECM	Extracellular matrix
FBS	Foetal bovine serum
GACs	Glycosaminoglycans
G-CSF	Granulocyte colony stimulating factor
HLA	Human leukocyte antigen
HTA	Human Tissue Authority
hrEPO	Human recombinant erythropoietin
IL-6	Interleukin 6
ISCT	International Society for Cellular Therapy
ITS	Idiopathic tracheal stenosis
MHC	Major histocompatibility class
MSC	Mesenchymal stromal cell
NHS	National Health Service
TGFβ	Tumour growth factor beta

Sections A-E

A. Description of the condition

A1. Details of the condition

The information in this section is taken principally from Mostafa et al (2012).

The trachea is a flexible but rigid tube which extends from the cricotracheal ligament at the level of C6 to the carina at the level of T5. In adults it is generally 8.5 – 15 cm long and 15 – 22 mm wide. The wall of the trachea consists of three layers: mucosa, submucosa and adventitia. The mucosa is made up of epithelium (composed of predominantly of tall, columnar ciliated and goblet cells and smaller, triangular basal cells) and a basement membrane. The subepithelial tissue can be subdivided into a lamina propria, situated between the basement membrane and the muscularis mucosa, and a submucosa consisting of all the remaining airway tissue. The lamina propria consists principally of a network of capillaries, a meshwork of reticulin fibres continuous with the basement membrane and bundles of elastic and nerve fibres. The submucosa contains cartilage, muscle and other supportive connective tissue elements, as well as the major portion of the tracheobronchial glands.

Tracheal cartilage plates consist of about 16 to 20 U-shaped structures oriented in a horizontal plane with their open ends directed posteriorly. The posterior (membranous) portion of the wall is free of cartilage. The spaces between the plates contain smooth muscle, tracheal glands, and collagenous and elastic tissue.

The adventitia is mainly composed of loosely arranged collagenous fibres.

- Definition

Tracheal stenosis is narrowing of the trachea for congenital or acquired reasons.

- Aetiology

Tracheal stenosis may be encountered in both children and adults. Paediatric tracheal stenosis is usually congenital whereas in adults it is almost always acquired (Table 1).

Table 1   Causes of tracheal stenosis

Iatrogenic	Endotracheal intubation
	Tracheostomy
	Radiotherapy
	Past surgery
Congenital	
Tumours	
Auto-immune conditions	Polychondritis
	Sarcoidosis
	Wegener's granulomatosis
Bacterial infections	Tuberculosis
	Rhinolaryngoscleroma
Idiopathic	

Congenital tracheal stenosis

Congenital tracheal stenosis is a rare disorder comprising a wide range of tracheal abnormalities. There is an unexplained male preponderance (2:1). In the majority of cases, it consists of a funnel-shaped deformity of the trachea and complete circular cartilaginous tracheal rings. It may involve a variable length of the trachea and can extend to the main bronchi. It is often associated with other congenital malformations of the pulmonary, cardiovascular and gastrointestinal systems.

Adult tracheal stenosis

The most common cause of laryngotracheal stenosis continues to be trauma, which can be internal (prolonged endotracheal intubation, tracheotomy, surgery, irradiation, endotracheal burns) or external (blunt or penetrating neck trauma). In the context of endotracheal intubation, the pressure can result from a tube of inappropriate size, from the over inflated high-pressure cuff, or from friction of the tip of the tube against the tracheal wall. Inappropriately large endotracheal tubes cause subglottic stenosis; lesions in the upper third of the trachea arise from pressure from the cuff while lesions in the mid trachea result from friction against the tube end.

Other causes of adult upper tracheal stenosis are chronic inflammatory diseases (amyloidosis, sarcoidosis, polychondritis, granulomata), benign or malignant neoplasms and collagen vascular diseases (tracheopathia osteoplastica, Wegener's granulomatosis). External compression by cervical or mediastinal mases can also lead to tracheal narrowing. In some cases, no identifiable cause is found and the patient is diagnosed as having idiopathic tracheal stenosis (ITS).

Tracheomalacia is a weakness or floppiness of the trachea, which occurs when the cartilage of the trachea breaks down and can be caused by congenital disease, complications from surgery and prolonged tracheostomy. Symptoms are similar for those of stenosis.

- Specific characteristics; pathophysiological, histopathological, clinical characteristics

The process of post-intubation tracheal stenosis is best described as the laryngotracheal "bed sore". Slight and transient irritation from the endotracheal tube results in oedema of the wall which heals completely, However, pressure high enough to cause ulceration of the mucosa initiates a process of

healing which may lead to tracheal stenosis. The ischaemic injury by the tube cuff may start as early as a few hours after intubation, and the complete circumferential web-like fibrous lesion may develop after 3-6 weeks.

Microscopically, in the most affected segments, the mucosa, submucosa and cartilage cannot be distinguished and become replaced by granulation tissue in various degrees of maturation. Closer to healthy segments, there is ulceration of the mucosa, epithelial metaplasia and an inflammatory infiltrate. In some cases the cartilage may become ossified.

Two morphological changes are commonly seen:

   o   Web-like lesions: these are formed of pale avascular fibrous tissue causing circumferential narrowing of the tracheal lumen leaving a central aperture of varying sizes. The lesions are usually less than 1 cm and may be multiple. They bleed minimally.

   o   Excessive granulation tissue: These lesions appear as clumps of soft tissue aggregations, irregular in shape and extending along the tracheal wall. The lesions are vascular and bleed on manipulation. The stenotic segment is usually longer than 1 cm.

- Classification

Various classifications of tracheal stenosis have been devised but all have their shortcomings as they do not document all the relevant data necessary for evaluation.

   o   Myer-Cotton staging system: This classifies stenosis on the basis of the relative reduction of the cross sectional area – Grade I: <50%, Grade II: 51-70%, Grade III: 71-99%; Grade IV complete stenosis.

   o   McCaffrey system: this classifies stenosis on the subsites involved and the length of the stenotic segment – Stage I: subglottis/trachea and < 1 cm, Stage II isolated to subglottis and > 1 cm, Stage III Subbglottic/tracheal without glottic involvement, Stage IV: involvement of glottis.

   o   Lano's classification: This is useful to predict prognosis in adult patients and is based on the number of subsites (glottis, subglottis and trachea) involved – Stage I: one subsite, Stage II two subsites, Stage III all three subsites.

   o   Freitag classification: This is based on a detailed description of the type, location and degree of stenosis. The type of stenosis is categorised as "structural" or "dynamic", the degree of stenosis is scored on a scale from 0 to 5 and the location is assigned using five regions stretching from the upper trachea to the two main bronchi.

- Diagnosis and symptoms

Tracheal stenosis can present very insidiously or as a catastrophic near-death episode requiring cardiopulmonary resuscitation. In many cases the condition is precipitated by an acute respiratory infection. Worsening of dyspnoea following recumbency may also result. Dyspnoea on exertion appears when about 50% of the airway is narrowed. Dyspnoea at rest occurs when 75% of the airway diameter is stenosed. Typically, in adults, exertional dyspnoea occurs when the airway diameter is reduced to about 8 mm; resting dyspnoea occurs at a diameter of 5 mm, at which point stridor also occurs.

Children with congenital tracheal stenosis present with biphasic stridor, tachypnoea, retractions, nasal flaring, apnoea, cyanosis, wheezing, noisy breathing, recurrent upper respiratory "cold symptoms", persistent croup and pneumonia. Dysphagia may occur and may be accompanied by apnoea or cyanotic spells during attempts to swallow solid food. Failure to thrive may result from poor feeding. Patients may hyperextend their heads as if to "stent" the trachea open and improve breathing.

Patients with acquired stenosis are diagnosed from a few days to 10 years or more following initial injury. The majority of cases are diagnosed within a year. Many patients are misdiagnosed with asthma and recurrent bronchitis. A high index of suspicion is warranted with the onset of respiratory symptoms following intubation, regardless of the duration of intubation. Patients may also present with hoarseness of voice due to vocal fold affection or concomitant laryngeal trauma. Aspiration and spill over may occur due to vocal fold immobility, arytenoid fixation, loss of laryngeal sensation or trachea-oesophageal fistula.

A2. Proposed orphan indication

The proposed orphan indication is:

Treatment of tracheal stenosis.

This indication statement has been translated into all the official languages of the European Union plus Icelandic and Norwegian. The translations are presented in Annex 1.

A3. Medical plausibility

A.3.1. Active substance: description of the medicinal product, pharmacological class and mode of action

The proposed product is a human-derived MSCs-seeded trachea incorporating a matrix sheet derived from human dermis (e.g. Glyaderm) and a tracheal stent. There are two active substances:

- Human allogeneic decellularised tracheal scaffold from a cadaveric donor.

- Autologous BM-derived MSCs.

The final product also incorporates:

- A Glyaderm® sheet which serves to protect the reseeded trachea during and immediately after implantation. Glyaderm is an acellular dermal collagen-elastin matrix derived from human donor skin. It is obtained from the Euro Tissue Bank.

- A standard tracheal stent which serves to support the reseeded trachea. The stent is a medical device. Stent replacement is planned at approximately 8 and 16 weeks after implant of the product followed by permanent stent removal at approximately 24 weeks.

The finished product is shown schematically in Figure 1 below. Further detail is presented in Section E.1 of this document.

Figure 1: Schematic representation of the product
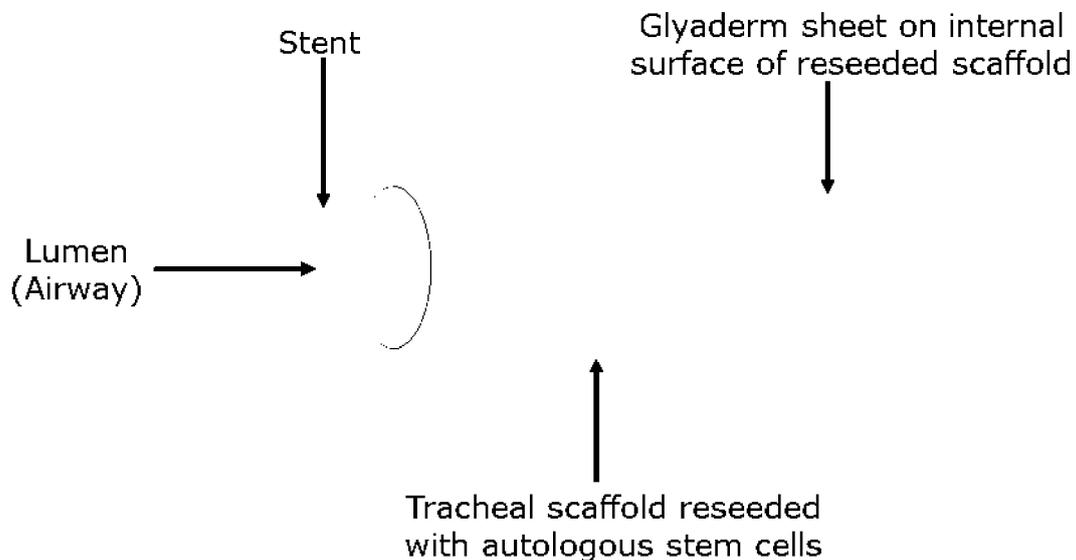
Due to the inclusion of the CE marked stent, the finished product will be classified as a combined ATMP. It has currently been designated as a non-combined ATMP, however, the need to revise this classification was identified at a recent ITF meeting. A new ATMP classification request will be filed in due course.

A.3.2. Plausibility of the orphan condition; data with the specific product as applied for designation in specific models or in patients affected the condition

Non-clinical development

Two preclinical studies have been completed in the pig; the first study examined the surgical implantation of the MSC-seeded scaffold transplanted at an orthotopic location with a four week follow up, and the second study examined the surgical implantation of the MSCs-seeded scaffold transplanted at a heterotopic location supported by the administration of a Glyaderm® sheet, in this instance preseeded with epithelial cells.

The choice of animal model for tissue engineering studies is challenging. Small animal models are not suitable as there are clear differences in tracheal size between rodents and humans. The pig model was chosen as the tracheal diameters, wall thickness and the ratio between the implant and the native trachea mimic the clinical situation and importantly the pig allowed the proposed clinical surgical procedures to be performed. However, the transplantation of the clinical product into the pig is a xenogenic situation and required immunosuppression to prevent graft rejection.

The animals, although housed in clean facilities, are not within an aseptic environment and the trachea transplanted animals are therefore subject to a range of airborne microbes with an increased risk of infection due to the immunosuppression. While anatomically similar to humans, the range of motion of the pig head and neck, the resultant biomechanical forces exerted on the trachea because of this and the quadrupedic nature of the animal, all have unknown effects on the outcomes of the implantation procedure and translation to the human clinical experience.

The first study was conducted to assess the feasibility, safety and efficacy of the first step of transplantation of a vacuum-assisted DEM decellularised human trachea seeded with human BM-derived MSCs in an orthotopic location in young adult domestic pigs. Three female pigs were assigned to the study and two underwent surgery. One tracheal scaffold was not released due to bacterial contamination prior to surgery and therefore the third animal was removed from the study.

After inducing general anaesthesia, an anterior midline cervical incision from the base of the larynx (cricoid) to the manubrium sterni was made to expose the trachea. The tracheal scaffold was trimmed according to the length of the stent to be implanted. A comparable length of native trachea was resected and the tracheal scaffold was inserted into the implantation area and the anastomoses were made. An appropriately sized stent was positioned and deployed.

The first animal was to be recovered for 36 days; however it died spontaneously at 32 days post-surgery, without predisposing symptoms or condition. The second animal was to be recovered for 28 days, however it was terminated at 12 days post-surgery due to signs of respiratory compromise incompatible with life. The study was stopped on humane grounds.

Post mortem examination revealed localised infection in both study animals. The infection was unlikely to have arisen from the seeded trachea as microbiology results at all points during manufacture were negative, including the final sample taken just prior to surgery. The one bacterial-contaminated scaffold was identified before release and was not implanted. There was no evidence of surgery-related changes in body weight or effects on haematology, clinical chemistry and coagulation parameters. IL-6 levels increased over the study period which could have been due to the bacterial infection of the tissue engineered tracheal scaffold.

It was clear from this study that minimising infection is required for successful tracheal transplantation.

A second study was conducted to assess the feasibility of administration of a Glyaderm® sheet seeded with epithelial cells into a MSC-seeded trachea using porcine tracheal scaffolds. Four female pigs were assigned to this study.

Both surgical procedures were well tolerated. There was no evidence of intolerance of the tissue engineered tracheal scaffold or epithelial sheet based on body weight measurements, haematology, clinical chemistry and coagulation parameters. Clinical observations showed the animal remained clinically healthy throughout the study. Gross necropsy findings showed no visible infections or cysts, and very few adhesions. A fibrous capsule had formed around the tissue engineered tracheal scaffolds.

The explanted heterotopically located tissue engineered tracheal scaffolds showed very little sign of immune response, and the adventitial aspect showed signs of good vascularisation with numerous small vessels. On the luminal aspect, the tissue engineered epithelial sheet did not appear to be attached to the luminal surface of the tissue engineered tracheal scaffolds, and with only a few cells seen on the tissue engineered epithelial sheet facing the lumen. Vascularisation to the luminal surface of the tissue engineered trachea is required to support the implantation of an epithelial sheet.

In summary, the general surgical procedure of tracheal transplantation at both the orthotopic and heteroptopic location was tolerated in the pig. Small changes in clinical chemistry, haematology and coagulation were observed as would be expected following surgery.

Following the successful transplantation of tissue engineered trachea to an orthotopic location, infection led to early study termination. When the tracheal scaffold was placed at a heterotopic location with minimal risk of infections, the scaffold did not become infected and retained good structural integrity. These data highlight the importance of minimising infection post tracheal transplant in a clinical setting. This can be achieved by placing an interim surface barrier (Glyaderm®) to minimise the risk of erosion of the tracheal surface due to infection.

Clinical experience

No clinical trials of this product have yet been completed.

However, tissue engineered replacement tracheas, seeded with autologous MSCs/chondrocytes, and autologous epithelial cells have been used in three named-patient clinical cases. These three cases formed the foundation and rationale for the development of the product under discussion. There were procedural differences between each case reflecting the immediate needs of the patients and the development stage of the product.

The first case was a 34-year old female with bronchial malacia (Macchiarini et al, 2008) with a decellularised tracheal allo-transplant re-populated *ex-vivo* by use of bioreactor. The patient had presented with tuberculosis infiltration of the cervical trachea and entire left main bronchus causing cough and dysphonia; a CT scan identified a circumferential, near-occlusive 3 cm airway stenosis and a hypoplastic left main bronchus with expiratory collapse. The mycobacterial infection was successfully treated but severe dyspnoea persisted, diagnosed as post-tuberculosis chronic tracheitis and secondary severe bronchomalacia of the left main bronchus.

The stenosis was successfully treated with a subglottic resection with primary end-to-end anastomosis, followed by placement of a Dumon stent in the patient's left main bronchus. However, the stent was poorly tolerated with recurrent episodes of pneumonitis in the patient's left lower lobe, untreatable cough, and mucous retention and so the stent was removed. In 2008, the patient was admitted with severe dyspnoea that was affecting her ability to do routine domestic chores. Given the high mortality rates, and perioperative and long-term morbidity of left carinal total pneumonectomy, which was the only conventional therapy available to the patient, it was decided to perform resection of the left main bronchus with tracheal replacement.

The donated trachea was decellularised over 6 weeks and re-seeded with epithelial cells (bronchial mucosa sample) and chondrocytes (BM aspirate MSCs). The chondrocytes were applied longitudinally to the external surface of the tracheal matrix. Concurrently, the internal surface of the matrix was seeded with the same density of epithelial cells. The total period of bioreactor culture was 96 hours.

The patient was monitored for 2 days post-transplant in the intensive ward and transferred to a general ward and discharged on the 10th day. Clinical assessments included rigid bronchoscopy at 4 days, and bronchoscopy and serological testing at 14 days, 1 month, and 2 months. The patient's pulmonary function tests were within normal range when assessed at 2 months and she reported improved daily living activities. There was a complete absence of antidonor HLA antibodies at 14 days, 1 month, and 2 months. At 4 days, the graft was almost indistinguishable from adjacent normal bronchial mucosa. At 14 days, no inflammatory cells were detected cytologically in the adherent layer of mucus on the graft surface. At 1 month, local mucosal bleeding was elicited when the biopsy sample was taken, indicating successful revascularisation. CT images demonstrated that the airway had been restored from near-total collapse to wide patency.

The patient is alive 5 years after surgery with improved and preserved lung function and normal cough sensitivity and expulsive force, though she has ongoing complications requiring repeated stenting procedures; a recurrent cicatricial stenosis occurred in the native trachea situated close to the

transplanted trachea anastomosis requiring regular sequential bronchoscopy. However, no adverse immunological response or serological signs of rejection despite no immunosuppressive treatment, were observed. No evidence of cancer has been found.

The second case was a 10 year old boy with long-segment congenital tracheal stenosis and pulmonary sling which were treated at 6 days old with autologous patch tracheoplasty, supported by balloon inflated stents (Elliott et al, 2012). At 3 years old, the patient had bleeding into his airway and underwent 2 tracheal stented homografts. The patient was then managed for several years with surgical interventions and stents for recurrent stenosis.

In 2010, the patient exhibited erosion of tracheal stents creating an aortotracheal fistula. As tracheal homografts were no longer being performed and a trachea allograft would mean life-long immunosuppression, it was decided to approach the parents regarding tracheal transplant similar to the prior case. However, the emergency nature of the patient's presentation meant that a more direct protocol for graft preparation was used, adapted using a method previously used in clinical trials of bone, skin and nerve regeneration.

GM-CSF was administered to the patient for 3 days to mobilise haematopoietic stem cells and endothelial progenitors and induce MSCs proliferation. The trachea was transected above the upper stent and below the lower stent leaving a 7 cm gap. Patches of trachea epithelium were removed from excised trachea and cut into stamp grafts which were added as free grafts at regular intervals within the lumen to the MSC suspension-saturated donor cadaveric decellularised scaffold. The entire construct was saturated with hrEPO and G-CSF. TGFβ was injected into tracheal rings to increase angiogenesis, improve autologous MSC recruitment and induce chondrocyte differentiation. The omentum was mobilised and interposed between the trachea and heart to reduce possibility of future fistulae and increase vascularity. Two stents, in addition to the bioabsorbable stent used to support the graft, were needed to aid ventilation; these were removed at day 26.

Bleeding on contact was observed at 1 week post-surgery demonstrating angiogenesis. Regular bronchoscopy was required to clear secretions for 8 weeks with the patient discharged at day 63. The bioabsorbable stent had dissolved 6 weeks after surgery, with mild collapse of the proximal graft so a shorter stent was implanted with regular bronchoscopy or balloon dilatation under fluoroscopy, or both, for 6 months largely due to mucus retention and crusting within the native bronchi in which there were still embedded metal stents.

After dissolution of the second stent at 5 months, overlapping, self-expanding Nitinol stents were placed in the trachea to aid the proximal graft rigidity at 6 months after the initial surgery; the patient's airway was patent and he returned to school.

At 15 months, there was complete epithelisation and ciliated respiratory epithelial cells were observed.

At 18 months, the patient had his last balloon dilatation as the graft had strengthened with no additional hospital visits reported. At 2 years, the patient had normal function tests, had grown and had returned to normal activities and school. At 48 months, the patient is alive and continuing to do well (Hamilton et al, 2015).

The last case was a 15 year old girl born with a single lung and major cardiac defect, with severe tracheal stenosis due to the scarring caused by recurrent stent re-positioning and tracheotomy (Martin Birchall, personal communication). The narrowest part of the trachea was reduced to a few millimetres across. A multi-disciplinary team reviewed suitability of the tracheal replacement, given her medical need for continuous care and risk of having had a cardiac arrest 4 months prior to surgery.

The trachea was prepared similarly to that of the first case and allowed to mature in the bioreactor for 4 days.

Initial surgery was successful however the patient suffered what is assumed to be a fatal cardiovascular event 6 weeks following surgery during a bronchoscopy procedure to clear accumulating mucus that caused a tracheal obstruction and severe hypoxia. No post-mortem was performed at the family's request so cause of death cannot be confirmed.

Overall, therefore, there is a meaningful body of evidence to support the hypothesis that the tissue-engineered product is a plausible approach to the treatment of tracheal stenosis.

A4. Justification of the life-threatening or debilitating nature of the condition

Tracheal stenosis, even when surgically treated, carries a high mortality, particularly in congenital cases.

Backer et al (2002) reported a series of 61 congenital cases operated upon in the Children's Memorial Hospital, Chicago which included 3 early deaths and 8 late deaths giving an overall mortality of 18%.

Yong et al (2014) report a series of 20 congenital cases from Melbourne which included 3 early and 3 late deaths (overall mortality 30%).

Death is a less common outcome in adult patients but does occur. Friedel et al (2003) report an in-hospital mortality of 5.4% (6 of 110) in their series of adult cases while Bolca et al (2010) report a 3.8% mortality rate in their series of 107 adult cases. Bagheri et al (2013) report no deaths in their series of 20 adult patients, but early postoperative complications occurred in 4 patients (20%), 5 patients (25%) had late stenosis (4 of whom were treated with multiple dilation) and one patient needed tracheostomy and prolonged T-tube.

Patients with tracheal stenosis typically require multiple operations, laser and/or stenting procedures, with outcomes that are generally suboptimal with high morbidity and with a reduced quality and length of life. These patients often become dependent upon reiterative intervention, such as dilation (which often has a short-term treatment effect), stents (which are not often tolerated for longer than 6 months due to chronic cough, irritation, recurrent infection, halitosis, fatigue, scarring from corrosion of the tissue, movement and perforation across structures) and tracheostomy. All of these treatments significantly impair quality of life.

B. Prevalence of the condition

B1. Prevalence of the orphan disease or condition in the European Union

B1.1 Reference documentation

As an initial step, the applicant commissioned a wide-ranging literature search into tracheal disease prevalence. The research was carried out by the Royal Society of Medicine Library Search Services Team in June 2014. Embase and Medline were searched using the strategy shown in Table 2 below:

Table 2   Initial literature search strategy

Set#	Searched for	Databases
S3	(s1 and s2) and pd(1994-2014) and (la(english) or abany(yes))	Embase, Embase Alert, MEDLINE
S2	MESH.EXACT("Prevalence") OR EMB.EXACT.EXPLODE("prevalence") or prevalence	Embase, Embase Alert, MEDLINE
S1	MESH.EXACT("Tracheomalacia") OR MESH.EXACT("Tracheobronchomalacia") OR MESH.EXACT("Tracheal Neoplasms") OR MESH.EXACT("Tracheal Stenosis") OR EMB.EXACT("tracheomalacia") OR EMB.EXACT("tracheobronchomalacia") OR EMB.EXACT("trachea carcinoma") OR EMB.EXACT.EXPLODE("trachea cancer") OR EMB.EXACT("trachea tumor") OR EMB.EXACT("trachea stenosis") OR EMB.EXACT("trachea injury") or ti,ab(tracheomalacia or malacia or ((chondromalacia or bronchi or neoplasm[*1] or cancer[*1] or carcinoma[*1] or tumor[*1] or tumour[*1] or stenosis or narrow[*3] or stricture[*1] or rupture[*1] or tear[*1] or break[*3] or atresia or burn[*3] or corrosion[*1] or crush[*3] or injur[*3] or trauma[*1] or damage[*1] or congenital) near/5 (trachea or tracheal)) or tracheomalacy or "williams campbell syndrome" or "airway malacia" or "tracheo bronchomalacia" or "tracheobronchial malacia" or "carcinoma tracheae" or tracheostenosis)	Embase, Embase Alert, MEDLINE

This identified 191 publications that were then hand searched to finally yield 43 references of potential interest. These were then sifted to identify publications of direct relevance to the prevalence of tracheal stenosis. However, while several publications discussed tracheal stenosis, none offered information suitable as the basis for an estimate of the EU prevalence of the condition.

In view of this, more focused searches of PubMed were undertaken. The searches performed (which were not restricted by publication date) and the results obtained are shown in Table 3 below:

Table 3   **PubMed searches and results**

Search terms	Search scope	Hits	Retained	Comments
[Tracheal stenosis] AND [Prevalence]	Title only	0	-	-
[Tracheal stenosis] AND [Incidence]	Title only	4	None.	All 4 references referred to incidence after certain procedures, not overall incidence.
[Tracheal stenosis] AND [Epidemiology]	Title only	0	-	-
[Tracheal stenosis] AND [Prevalence]	Title and abstract	8	None.	All references referred to prevalence after certain procedures, not overall prevalence.
[Tracheal stenosis] AND [Incidence]	Title and abstract	99	Barreiro et al (2013); Godin et al (2000); Norwood et al (2000); Gaissert et al (1993).	A small number of references were rejected because of the absence of an abstract in PubMed or because the abstract was not in English language. Most however, were rejected because review of the abstract revealed them to be unlikely to contain useful information.
[Tracheal stenosis] AND [Epidemiology]	Title and abstract	3	Ywakim & El-Hakim (2012).	The remaining references referred to epidemiology in restricted settings, not of the full condition. Even Ywakim & El-Hakim concerns only congenital tracheal stenosis.

None of the retained references included a direct analysis of the overall prevalence of tracheal stenosis. The scope of most of the publications was much more limited (e.g. iatrogenic tracheal stenosis presenting as persistent asthma, Barreiro et al, 2013; tracheal stenosis after percutaneous tracheostomy, Norwood et al, 2000; incidence following inhalation injury, Gaissert et al, 1993; congenital tracheal stenosis, Ywakim & El-Hakim, 2012).

The sponsor supplemented the references identified via formal literature searching with informal internet searching using the terms "tracheal stenosis", "congenital tracheal stenosis" and "acquired tracheal stenosis". In addition, references cited as the source of epidemiological facts given in the publications identified in formal literature searching were obtained and studied. This process resulted in the following raw information:

- All authors agree that tracheal stenosis is "rare" but quantitative data are sparse.

- Zias et al (2008) state that 10-22% of all intubated patients are affected by tracheal stenosis.

- This is supported by Barreiro et al (2013) who comment that 11% of intubated patients develop tracheal stenosis. This is attributed to Stauffer et al (1981).

- Barreiro et al (2013) comment that iatrogenic tracheal stenosis affects 4.9 people per million per year in the general population. This is attributed to Nouraei et al (2007).

- Gelbard et al (2014) described the aetiology of laryngotracheal stenosis as 54.7% iatrogenic, 18.5% idiopathic, 18.5% auto-immune and 8% traumatic.

- Phipps et al (2006) states that fewer than 70 cases of congenital tracheal stenosis had been described in the literature by 1994. This is attributed to Hoffer et al (1994).

- Terra et al (2009) state that fewer than 200 cases of congenital tracheal stenosis had been recorded and attributes this to Lang et al (1999).

- Munzón & Martinez-Ferro (2012) report the incidence of congenital tracheal stenosis to be 1 in every 64,500 births.

- Nouraei et al (2013) report the incidence of primary tracheal cancer to be 0.9 per million per year in England. 5-year and 10-year palliation-free survival are given as 24.6% and 19.5% respectively.

- Urdaneta et al (2011) report a higher tracheal cancer incidence of 2.6 cases per million per year in the US with 5-year survival of 27%. The publication includes a graph showing that 50% of patients survive ≤1 year, 20% survive for 1-2 years and 30% survive ≥5 years.

First approach to prevalence calculation

One approach to prevalence calculation is to combine the Nouraei et al (2007) estimate that the incidence of iatrogenic tracheal stenosis is 4.9 per million per year with the Gelbard et al (2014) aetiology breakdown. Using the January 2014 estimate of the EU_{28} population of 507,416,607 (http://ec.europa.eu/eurostat), an incidence of 4.9 cases per million equates to 2,486 new cases per year in the EU. According to Gelbard et al (2014) this represents 54.7% of cases. The total annual incidence can then be estimated at 4,545 cases.

If all these cases were present at birth and if all patients lived a normal 75-year lifespan then there would be 340,875 cases within the EU at a given point in time. This, however, is clearly an overestimate:

- There is a recognised mortality associated with the condition. According to Munzón & Martinez-Ferro (2012), historically mortality was as high as 79% but has now improved to 9-21% as a result of better understanding of post-operative care and complication management. Taking the most conservative mortality figure (9%), this would reduce the number of cases by 30,679 to 310,196.

- By definition, acquired tracheal stenosis is not present throughout life. If one assumes that patients who acquire tracheal stenosis do so, on average, in mid-life and have it for the remainder of a normal life-span, then the number of cases will halve from 310,196 to 155,098.

- 155,098 equates to 3.06 per 10,000 individuals.

The adjustments for mortality and duration of disease in the above estimate are conservative, leading to a maximal estimate of prevalence. A corresponding minimal estimate can be derived by taking the higher end of the mortality figure (21%) and by assuming that most patients with iatrogenic tracheal stenosis make a full recovery. This can be represented by assigning a disease duration of 2 years to patients with iatrogenic disease.

- With 4,545 incident cases per year and 21% mortality (954 deaths), the number of surviving new cases per year is 3,591.

- The patients who do not survive therefore contribute 954 cases to the total prevalence.

- If 54.7% of the surviving patients (n=1,964) have iatrogenic disease (Gelbard et al, 2014) for a mean duration of 2 years then the contribution to prevalence is 3,928.

- If the remaining 45.3% of patients (n=1,627) acquire the condition in mid-life and continue for a normal life-span of 75 years then their contribution to prevalence will be [38 x n], i.e. 38 x 1,627 = 61,826.

- The overall prevalence is the sum of the above components, i.e. 954 + 3,928 + 61,826 = 66,708 cases in the EU.

- 66,708 cases equates to 1.31 per 10,000 individuals.

Second approach to prevalence calculation

An alternative approach to prevalence calculation is to estimate prevalence for individual aetiologies and combine them. Gelbard et al (2014) described the aetiology of laryngotracheal stenosis as 54.7% iatrogenic, 18.5% idiopathic, 18.5% auto-immune and 8% traumatic, totalling 99.7%. Congenital disease is not considered in the classification but for current purposes the remaining 0.3% could be considered congenital. This represents a conservative approach since in these patients the condition is present at birth and persists throughout life. Oncological cause is also not considered. It is assumed that this is encompassed by the idiopathic category.

The following assumptions regarding disease duration per aetiology were made:

- Mean disease duration in iatrogenic disease is 2 years (as justified above).

- Mean duration of idiopathic disease is 38 years (based on acquiring the condition in mid-life and continuing to have it for the rest of a 75-year life-span). This is conservative since this category is assumed to include oncological disease which (a) has onset late in life and (b) has a median survival in the range of only 20-30% (Nouraei et al, 2013; Urdaneta et al, 2011).

- Mean duration of autoimmune disease is 45 years (based on a typical onset of auto-immune disease at age 30 years and continuing to have it for the rest of a 75-year life-span).

- Mean duration of traumatic disease of 60 years (based on injury at age 15 years and continuing to be affected for the rest of a 75-year life-span).

- Mean duration of congenital disease is 75 years (based on living a normal 75-year life-span).

It was also assumed that mortality is 9% (the conservative end of the range reported by Munzón & Martinez-Ferro (2012).

As before, the Nouraei et al (2007) estimate that the incidence of iatrogenic tracheal stenosis is 4.9 per million per year can be combined with the Gelbard et al (2014) aetiology breakdown. Using the January 2014 estimate of the EU_{28} population of 507,416,607 (http://ec.europa.eu/eurostat), an incidence of 4.9 cases per million equates to 2,486 new cases per year in the EU. According to Gelbard et al (2014) this represents 54.7% of cases. The total annual incidence can then be estimated at 4,545 cases.

If 9% of these cases do not survive (n=409) then 4,136 cases go on to contribute to prevalence in multiple years. Table 4 below shows the contribution to prevalence per aetiology using the disease duration assumptions listed above:

Table 4   Contribution to prevalence per disease aetiology

Aetiology	% of surviving cases	n	Mean disease duration	Contribution to prevalence
Deaths	9% (of all cases)	409	1 year	409
Iatrogenic	54.7%	2,262	2 years	4,524
Idiopathic	18.5%	765	38 years	29,070
Auto-immune	18.5%	765	45 years	34,425
Traumatic	8%	331	60 years	19,860
Congenital	0.3%	12	75 years	900
Total	**100%**	**4,544**		**89,188**

This corresponds to 1.76 per 10,000 individuals.

A sensitivity analysis using the high end of the Munzón & Martinez-Ferro (2012) estimate is shown in Table 5 below:

Table 5   Contribution to prevalence per disease aetiology (sensitivity analysis 1)

Aetiology	% of surviving cases	n	Mean disease duration	Contribution to prevalence
Deaths	21% (of all cases)	954	1 year	954
Iatrogenic	54.7%	1,964	2 years	3,928
Idiopathic	18.5%	664	38 years	25,232
Auto-immune	18.5%	664	45 years	29,880
Traumatic	8%	287	60 years	17,220
Congenital	0.3%	11	75 years	825
Total	**100%**	**4,544**		**78,039**

This corresponds to 1.54 per 10,000 individuals.

It is clear that the biggest potential for influencing the prevalence calculation lies in the duration of iatrogenic disease. A sensitivity analysis in which mortality is set at 9% and iatrogenic disease duration set at 38 years (representing acquisition of disease in mid-life and continuation through the remainder of a normal life-span) results in an increased contribution to prevalence of 36 x 2,262 = 81,432. Total contributions to prevalence then become 159,471. This corresponds to an overall prevalence of 3.14 per 10,000.

A third sensitivity analysis involves discarding the contribution from congenital disease and replacing it with an estimate of congenital disease prevalence based on published data. Munzón & Martinez-Ferro gave the birth incidence of tracheal stenosis as 1 per 64,500. If one assumes that the condition is present throughout a normal lifespan the prevalence of congenital tracheal stenosis in the EU_{28} would be 7,867 cases. Applying the conservative 9% mortality figure reduces this to 7,159 cases. This figure is an order of magnitude higher than that presented in the base calculation. Nevertheless, it corresponds to only 0.14 per 10,000, showing that the extent of congenital disease has little impact on overall prevalence.

Similar calculations can be done to estimate the impact of tracheal cancer on overall disease prevalence. Nouraei et al estimated the incidence of tracheal cancer in England at 0.9 cases per million per year. If extrapolated to the entire EU, this would result in 456 new cases per year. The authors report palliation-free survival rather than overall survival. However, Urdaneta et al (2011) give a 5-year overall survival figure of 27% and provide a graph which shows that around 50% of patients survive for <1 year. The contribution to prevalence due to tracheal cancer is therefore probably in the region of 1,000 cases, equivalent to 0.02 per 10,000. The main value of this calculation is that it shows that it is plausible that tracheal cancer is encompassed within the 18.5% of cases which Gelbard et al (2014) categorise as idiopathic. It also provides reassurance that even if the authors took no account of tracheal cancer, the contribution to prevalence from this aetiology is extremely small.

Summary

The two prevalence estimates based on "worst-case" assumptions yield estimates of 3.06 and 3.14 per 10,000. The degree of error in the methodology does not justify estimation to 2 decimal places. It is more reasonable to conclude that both estimates are in the region of 3.1 per 10,000. These serve to provide a high level of reassurance that the true prevalence lies below 5 per 10,000.

The two calculations which are intended to be realistic (rather than highly conservative) yield prevalence estimates of 1.31 and 1.76 per 10,000. Again, it would be more reasonable to round these estimates to 1.3 and 1.8 per 10,000 respectively. Sensitivity analyses show that the realistic prevalence estimates vary by less than 0.25 per 10,000. It is not possible to say whether 1.3 or 1.8 per 10,000 is closer to the true figure. The applicant has therefore opted for "not more than 1.8 per 10,000" as the formal estimate of the prevalence of tracheal stenosis.

B1.2 Information from databases on rare diseases

No relevant information from databases on rare diseases was identified.

B2. Prevalence and incidence of the condition in the European Union

Not applicable.

C. Potential for return on investment

Not applicable.

D. Other methods for diagnosis, prevention or treatment of the condition

D1. Details of any existing diagnosis, prevention or treatment methods

Treatment is essentially surgical and may be considered under two headings:

- ***Open surgery:*** A number of open surgical techniques are available including:

  o Tracheal resection anastomosis, which is becoming the standard of care in many centres and gives the most consistent results in both adult and paediatric patients. It is indicated for tracheal stenosis involving less than two-thirds of the tracheal length. The procedure consists of resection of the stenotic portion and end to end anastomosis of healthy tracheal segments.

  o Laryngotracheoplasty, in which the goal is to replace the damaged tracheal wall by graft in order to restore the lumen and guarantee a certain rigidity. Partial replacement can be performed using cartilage, bone, dermo-epidermic grafts and delto-pectoral flaps or pericardium. The technique is indicated in symptomatic patients with greater than 50% stenosis and a long segment with marked peritracheal scarring and failure of mobilisation.

  o Slide tracheoplasty, which is indicated for the correction of congenital long-segment tracheal stenosis with complete tracheal rings. The length of the trachea is reduced, the circumference doubled and the transverse section quadrupled.

  o Titanium mesh augmentation, which makes use of a shaped titanium mesh over a silastic stent. A composite septal cartilage-titanium ring graft can also be used to augment the anterior tracheal wall.

- ***Endoscopic treatment:*** This involves two key stages – luminal restoration and maintenance of patency. The aim of luminal restoration is to dilate the stenotic segment to match as closely as possible the normal proximal and distal diameters. This may be achieved by a variety of techniques including mechanical dilatation, laser, diathermy, argon plasma, cryosurgery or balloons. In most cases following endoluminal dilatation, the restored lumen tends to restenose. This may be addressed by the use of steroids or mitomycin C (neither of which are approved in this indication), by brachytherapy and by stents.

D2. Justification as to why methods are not satisfactory

In addition to the mortality associated with current surgical treatments (see Section A.4), complications (particularly restenosis) leading to poor short- or long-term outcome are common (Bagheri et al, 2013; Hecker & Volmerig, 2014).

Hence patients with tracheal stenosis often require multiple operations, laser and/or stenting procedures, with outcomes that are generally suboptimal with high morbidity with a reduced quality and length of life.

There are also cases in which primary anastomosis is not possible, such as after extensive burns, trauma, tumour resection, or post-intubation injuries, or where earlier surgical attempts at cure have failed. Additionally, the treatment of congenital tracheal stenosis can be hindered by the lack of sufficient tissue for surgical reconstruction, as the length of trachea involved may be extensive.

These patients often become dependent upon reiterative intervention, such as dilation (which often has a short-term treatment effect), stents (which are not often tolerated for longer than 6 months due to chronic cough, irritation, recurrent infection, halitosis, fatigue, scarring from corrosion of the tissue, movement and perforation across structures) and tracheostomy. All of these treatments significantly impair quality of life.

As discussed in Section D.1, steroids and mitomycin C are sometimes used to reduce the risk of restenosis after dilatation surgery. However, there are no pharmaceutical products which carry an indication for this purpose; they are used off-label. Commission Communication 2003/C 178/02 states that authorised medicinal products used off-label are not considered "satisfactory". Therefore significant benefit vs these drugs does not have to be argued / demonstrated.

D3. Justification of significant benefit

Not applicable.

E. Description of the stage of development

E1. Summary of the development of the product

Quality aspects

The information provided here is complementary to the summary description provided in Section A.3.1.

The individual components of the product are discussed individually below and are combined as demonstrated schematically (Figure 2) to produce the final product for implantation.

Figure 2: Production and implantation of the replacement trachea
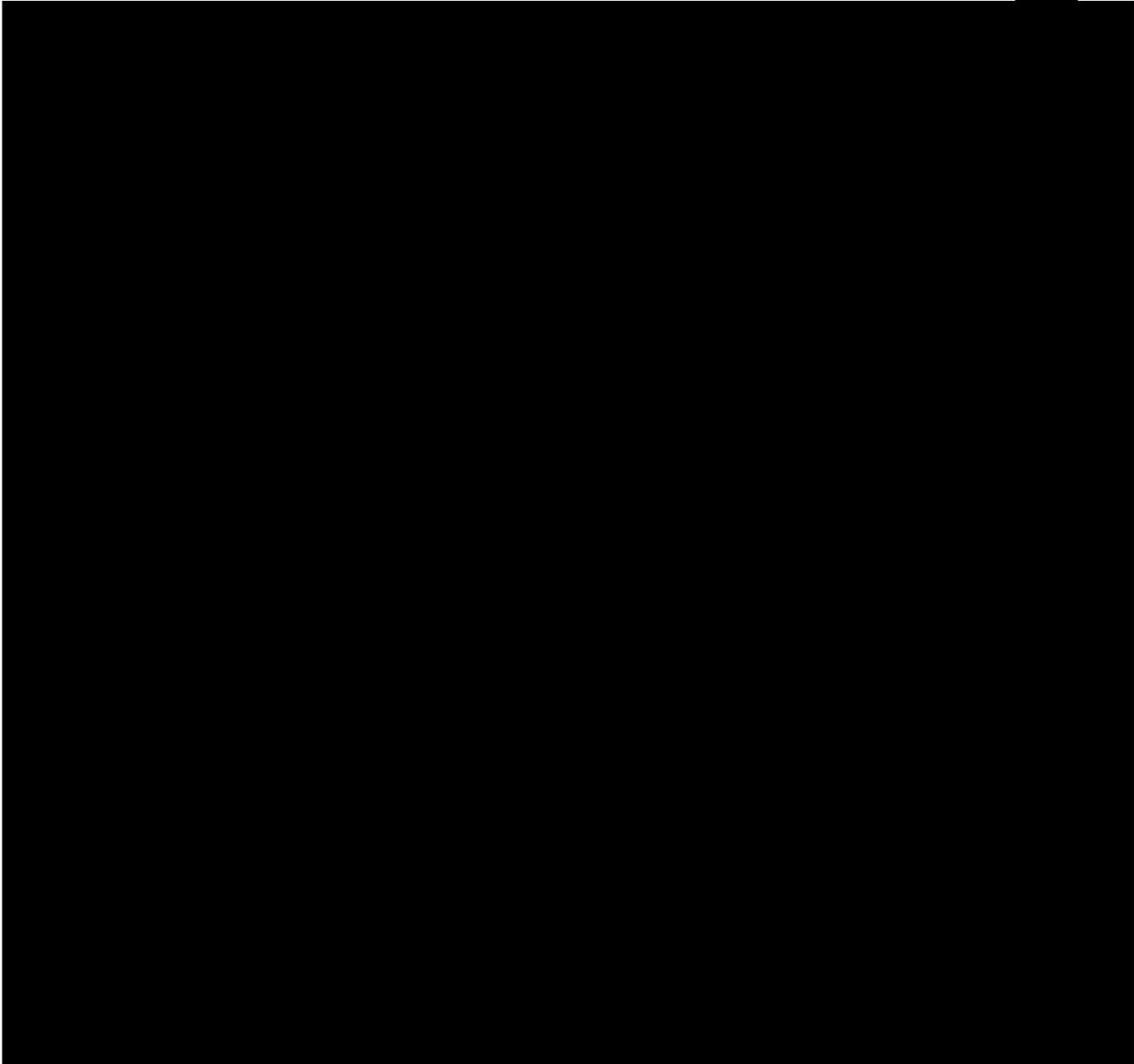

Decellularised human trachea

Suitably consented human decellularised trachea scaffold (approximately 6-10 cm) from a cadaveric donor is sourced from a Human Tissue Authority (HTA)-approved tissue bank (NHSBT) which has prepared it fit for clinical use.

The trachea undergoes a decellularisation process using validated vacuum-assisted methods which have been shown to effectively reduce the residual DNA content in keeping with ranges published by others (Crapo et al, 2011); the product is then irradiated ███████ as a microbiological contamination reduction step.
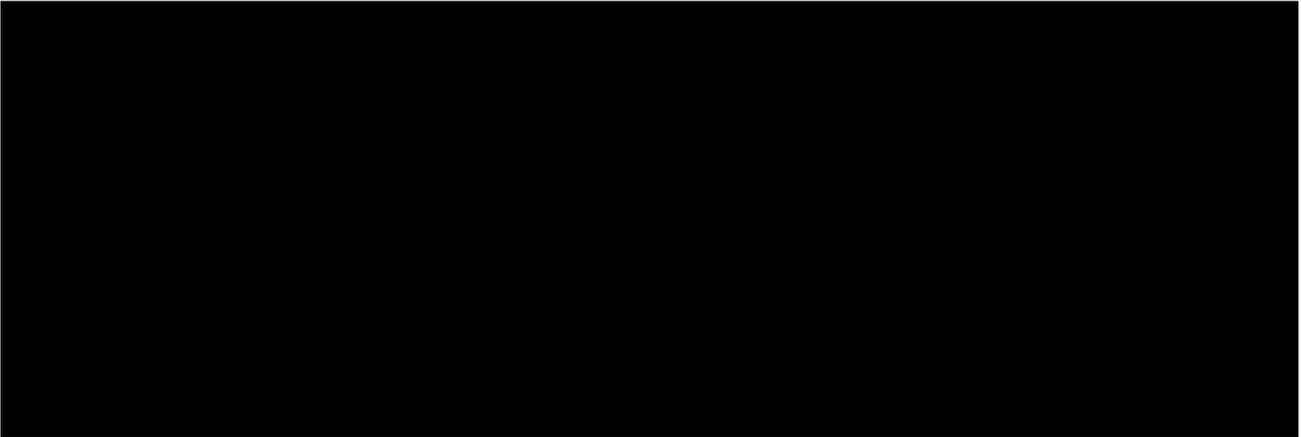
Glyaderm® sheet

Glyaderm® is an acellular dermal collagen-elastin matrix derived from human donor skin and obtained from the Euro Tissue Bank (Stichting Euro Skin Bank - Huidbank, Beverwijk, The Netherlands).

Non-clinical aspects

A series of nonclinical studies have been performed to develop a tissue engineered replacement human trachea comprising decellularised tracheal scaffold from a cadaveric donor recellularised with autologous MSCs on the outer surface.

In vitro studies were performed to assess the physiological and biomechanical properties of the decellularised scaffold and showed that the vacuum assisted decellularisation technique enhanced tracheal decellularisation without adversely impacting the biomechanical properties.

Studies in rats have examined the immunogenicity of the decellularised material and shown that the degree of decellularisation achieved in the vacuum-assisted process efficiently removes cells and abrogates the immune response following transplantation in a xenogeneic model.

Feasibility and safety studies (described in detail in Section A.3) were performed in the pig as these allowed the proposed clinical surgical procedures to be performed. In summary, these feasibility studies in pigs have shown that the general surgical procedure of tracheal transplantation was tolerated by the animals. In addition, minimising infection is required to prevent scaffold surface erosion and adequate revascularisation may be advantageous to support epithelialisation of the luminal surface.

Proof-of concept in relevant model

These studies are presented in Section A.3.

Pharmacology

Physiological and biomechanical properties of porcine and human tracheal scaffolds

Tracheas were harvested after euthanasia from eleven Large-White / Landrace crossbreed pigs and decellularised using either a detergent enzymatic method (DEM) or a vacuum-assisted DEM method. Seven porcine tracheas (3-5 cm in length) were decellularised using the vacuum assisted DEM and four controls underwent the DEM protocol under normal atmospheric pressure. In addition, two donated cadaveric human tracheas (NHS Blood and Transplant) from donors without known previous airway

disease were decellularised using a vacuum assisted DEM. The following assessments were performed on the trachea; histology, quantitative testing of DNA, glycosaminoglycan and collagen content analysis and assessment of mechanical properties.

The clearance of intact nuclei by the vacuum assisted DEM was demonstrated in both human and porcine trachea by histological analysis. In the vacuum-assisted DEM processed pig trachea, haematoxylin and eosin staining demonstrated clearance of all intact nuclei within the luminal epithelium, sub-mucosal glands, trachealis muscle and the outer adventitia. In addition, vacuum-assisted DEM efficiently removed all chondrocytes from within the cartilage lacunae, whereas trachea prepared using the DEM decellularisation under normal pressure retained intact chondrocytes within some lacunae. Both human tracheal scaffolds showed clearance of all nuclear material.

The amount of residual DNA following the application of a vacuum to DEM decellularisation compared to the standard DEM decellularisation protocol was assessed. Molecular quantification of DNA content extracted from minced wet tissue of fresh and decellularised tracheal tissue (porcine and human) showed a significant reduction in DNA content following vacuum-assisted decellularisation. Only trace residual amounts of DNA ($\leq$50 ng/mg wet tissue) were detectable in porcine and human ECM, which is consistent with published data for allogeneic scaffolds to demonstrate adequate decellularisation (Crapo et al, 2011).

The collagen content following the application of a vacuum-assisted DEM decellularisation protocol compared to the standard DEM decellularisation protocol was assessed. The collagen content of fresh and decellularised human and porcine trachea was qualitatively assessed by histological analysis (Picro-Sirius red and Miller's elastin staining). Evaluation of the tissue showed overall good preservation and morphology of the cartilage and collagen although there was some decrease in collagen fibre thickness. This change was predominantly influenced by the chemical treatment alone and was not further exacerbated by the application of the vacuum technology. Collagen content was quantified by the Sircol collagen assay. No significant difference was observed in collagen quantity between scaffolds and control tissue of both species prepared using either decellularisation method.

Glycosaminoglycans (GAGs) have an important role in facilitating cell migration during tissue morphogenesis and repair and also have a role in chemical signalling between cells, including the support for cellular proliferation. The impact of the vacuum-assisted DEM decellularisation process on GAG content was assessed. GAG content was quantified using the Blyscan GAG assay kit. The loss of GAG staining in decellularised samples, with and without the use of vacuum, was confirmed by molecular analysis. In the porcine tissue approximately 30% of native GAG levels were retained. There was no reduction in GAGs in human tissue due to the low content of GAGs in the starting material as evidenced in the control untreated tissue. The human tracheas had been initially stored in solution at -80°C following retrieval. GAGs are sensitive to freeze/thaw procedures as well as maintenance in solution and therefore may have been altered in the control tissue (Baiguera et al., 2012). Therefore a similar reduction in GAG content as in porcine tissue is considered to be more likely from freshly excised tissue.

Mechanical analysis was performed to assess the impact of applying negative pressure during the vacuum-assisted DEM decellularisation process. Biomechanical strength was assessed by subjecting porcine or human specimens to uniaxial tension until failure, the loss of load and the appearance of tears in the tissue. For each test one open tracheal ring (pig or human, fresh or decellularised) was used. Specimens in the form of flat rectangular pieces with a maximum length of 33 mm were clamped into sample holders and loaded at a constant tension rate of 100 mm/min and a maximum force of 500 N. The tests were performed with the application of uniaxial tension with an Instron In-Spec 2200 Benchtop Portable Tester at room temperature.

Application for orphan medicinal product designation

The vacuum-decellularised tracheal tissue showed no significant difference between control tissue and processed tissue.

Overall the vacuum assisted decellularisation technique enhanced tracheal decellularisation without adversely impacting the biomechanical properties.

Immunogenicity properties of porcine and human tracheal scaffolds

To address the potential for an immunogenic reaction to the extracellular matrix scaffold a series of *in vitro* and *in vivo* tests were performed.

Porcine and human untreated control and vacuum assisted DEM decellularised tracheas were assessed for immunogenic properties via major histocompatibility complex class I (MHC-I) immunostaining. Immunohistochemistry analysis was performed on both frozen and paraffin sections of decellularised pig and human trachea using anti porcine MHC-1 and anti-human MHC 1 antibodies respectively. Both porcine and human control trachea showed intense staining of the membranes and only mild staining in the cartilaginous part consistent with prior description in the literature (Shaari et al, 1998). The decellularised tissue however was negative for MHC-I immunostaining supporting the non-immunogenic characteristics of vacuum assisted-decellularised trachea.

Vacuum assisted decellularised human tracheal samples were implanted into Sprague Dawley rats (n=6) in small pockets between the skin and muscle created either sides of a midline incision in the abdominal wall (12 implants in total) (xenogeneic transplantation). Gross analysis of the tissue two weeks post-surgery showed no signs of rejection. The explanted scaffolds were morphologically intact and the structure of the collagen fibres viewed with Picro-Sirius-Red stain was normal. Histological analysis indicated integration of the tissue and the presence of neovascularisation. A thin fibrous capsule with scattered neutrophils was also present, indicating that the scaffolds elicited a mild inflammatory response, typical and consistent with a normal wound healing response and unlikely to be of clinical significance.

In summary, decellularisation mitigates the risk of immunogenicity by the removal of allogeneic cellular and nuclear antigens. Overall the risk of immunogenicity is low.

Distribution

MSCs are found in various tissues and organs of the body. Chemokines, cytokines, and growth factors released upon injury provide migratory cues for administered or resident stem cells to mobilize and recruit to the damaged site, where they proliferate and differentiate, eventually replacing the damaged tissues. The small number of autologous administered cultured MSCs (approximately ███████ ██████ are retained on and are not expected to distribute beyond the tracheal scaffold as they respond to the local signals of tissue damage. Whilst some normal cellular distribution will occur there are no expected biodistribution risk.

Tumourigenicity

No evidence for tumour formation has been reported in preclinical tests (typically immunocompromised rodents) of tumourigenicity of bone marrow- or adipose tissue- derived MSC (Barkholt et al, 2013). Although the current models may not be fully predictive and the risk of tumourigenicity cannot be ruled out completely, the risk is very small. For an autologous MSC product the risk of tumourigenicity is associated with the accumulation of chromosomal aberrations during extended culture. The MSCs used for seeding the vacuum decellularised tracheal scaffold will have been expanded for ██ population doublings to avoid chromosomal abnormalities (ID test calls must be <2% positive for CD45, 34, 14 and HLA DR). Additional support data for the low risk of tumourigenicity arises from a study in pigs

examining the safety and efficacy of reconstructing the larynx using similar technology to that proposed here.

Human cells on a decellularised scaffold were administered to six immunosuppressed pigs (cyclosporine 10mg/kg/day) and followed for 6 months. There was no evidence of tumour formation at study termination in the examined tissues (larynx, kidney, liver, lung, spleen).

Clinical aspects

Proof-of concept

No clinical trials have yet been completed. However, support for the concept in human patients has emerged from the three named-patient clinical cases detailed in Section A.3.

Planned clinical studies

The next step in development will be a "first in human" Phase 1 trial that will be conducted in 4 adult patients with stent- or tracheostomy-dependent tracheal stenosis or tracheomalacia for whom further conventional therapies are no longer adequate. The primary objective of this study will be to assess the safety of the tracheal implant procedure and the tolerability of the tracheal implant 8 months following surgery.

E2. Details of current regulatory status and marketing history in the EU and non EU countries

Sponsor's position:

No application for marketing authorisation has previously been submitted, for any indication, in the EU.

The product was not authorised in any country inside or outside the EU at the time of submission of the application.

No scientific advice on the product has been given by the CHMP.

A meeting with EMA's ITF Committee took place on 20 January 2016. The sponsor's meeting minutes have been approved by EMA.

US orphan drug status has not been granted or requested.

Regulatory authority and ethics committee approval for the first-in-human phase 1 trial (which will take place in UK) has been obtained. The study is scheduled to start shortly.

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