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Limbal Stem Cell Transplantation

An Evidence-Based Analysis

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About the Medical Advisory Secretariat

The Medical Advisory Secretariat is part of the Ontario Ministry of Health and Long-Term Care. The mandate of the Medical Advisory Secretariat is to provide evidence-based policy advice on the coordinated uptake of health services and new health technologies in Ontario to the Ministry of Health and Long-Term Care and to the healthcare system. The aim is to ensure that residents of Ontario have access to the best available new health technologies that will improve patient outcomes.

The Medical Advisory Secretariat also provides a secretariat function and evidence-based health technology policy analysis for review by the Ontario Health Technology Advisory Committee (OHTAC).

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Abbreviations

AMT	Amniotic membrane transplantation
CAU	Conjunctival autologous [transplantation]
CLAU	Conjunctival-limbal autologous [transplantation]
HLA	Human leukocyte antigen
KLAL	Keratolimbal allogeneic [transplantation]
lr-CLAL	Living-related conjunctival-limbal allogeneic [transplantation]
LSCD	Limbal stem cell deficiency
LSCT	Limbal stem cell transplantation
MMC	Mitomycin C
OB	Ocular burns
OOC	Out-of-country
RCT	Randomized controlled trial
SJS	Stevens-Johnson Syndrome
VA	Visual acuity

Executive Summary

Objective

The objective of this analysis is to systematically review limbal stem cell transplantation (LSCT) for the treatment of patients with limbal stem cell deficiency (LSCD). This evidence-based analysis reviews LSCT as a primary treatment for nonpterygium LSCD conditions, and LSCT as an adjuvant therapy to excision for the treatment of pterygium.

Background

Clinical Need: Condition and Target Population

The outer surface of the eye is covered by 2 distinct cell layers: the corneal epithelial layer that overlies the cornea, and the conjunctival epithelial layer that overlies the sclera. These cell types are separated by a transitional zone known as the limbus. The corneal epithelial cells are renewed every 3 to 10 days by a population of stem cells located in the limbus.

Nonpterygium Limbal Stem Cell Deficiency

When the limbal stem cells are depleted or destroyed, LSCD develops. In LSCD, the conjunctival epithelium migrates onto the cornea (a process called conjunctivalization), resulting in a thickened, irregular, unstable corneal surface that is prone to defects, ulceration, corneal scarring, vascularization, and opacity. Patients experience symptoms including severe irritation, discomfort, photophobia, tearing, blepharospasm, chronic inflammation and redness, and severely decreased vision.

Depending on the degree of limbal stem cell loss, LSCD may be total (diffuse) or partial (local). In total LSCD, the limbal stem cell population is completed destroyed and conjunctival epithelium covers the entire cornea. In partial LSCD, some areas of the limbus are unharmed, and the corresponding areas on the cornea maintain phenotypically normal corneal epithelium.

Confirmation of the presence of conjunctivalization is necessary for LSCD diagnosis as the other characteristics and symptoms are nonspecific and indicate a variety of diseases. The definitive test for LSCD is impression cytology, which detects the presence of conjunctival epithelium and its goblet cells on the cornea. However, in the opinion of a corneal expert, diagnosis is often based on clinical assessment, and in the expert's opinion, it is unclear whether impression cytology is more accurate and reliable than clinical assessment, especially for patients with severe LSCD.

The incidence of LSCD is not well understood. A variety of underlying disorders are associated with LSCD including chemical or thermal injuries, ultraviolet and ionizing radiation, Stevens-Johnson syndrome, multiple surgeries or cryotherapies, contact lens wear, extensive microbial infection, advanced ocular cicatricial pemphigoid, and aniridia. In addition, some LSCD cases are idiopathic. These conditions are uncommon (e.g., the prevalence of aniridia ranges from 1 in 40,000 to 1 in 100,000 people).

Pterygium

Pterygium is a wing-shaped fibrovascular tissue growth from the conjunctiva onto the cornea. Pterygium

is the result of partial LSCD caused by localized ultraviolet damage to limbal stem cells. As the pterygium invades the cornea, it may cause irregular astigmatism, loss of visual acuity, chronic irritation, recurrent inflammation, double vision, and impaired ocular motility.

Pterygium occurs worldwide. Incidence and prevalence rates are highest in the "pterygium belt," which ranges from 30 degrees north to 30 degrees south of the equator, and lower prevalence rates are found at latitudes greater than 40 degrees. The prevalence of pterygium for Caucasians residing in urban, temperate climates is estimated at 1.2%.

Existing Treatments Other Than Technology Being Reviewed

Nonpterygium Limbal Stem Cell Deficiency

In total LSCD, a patient's limbal stem cells are completely depleted, so any successful treatment must include new stem cells. Autologous oral mucosal epithelium transplantation has been proposed as an alternative to LSCT. However, this procedure is investigational, and there is very limited level 4c evidence[1] to support this technique (fewer than 20 eyes examined in 4 case series and 1 case report).

For patients with partial LSCD, treatment may not be necessary if their visual axis is not affected. However, if the visual axis is conjunctivalized, several disease management options exist including repeated mechanical debridement of the abnormal epithelium; intensive, nonpreserved lubrication; bandage contact lenses; autologous serum eye drops; other investigational medical treatments; and transplantation of an amniotic membrane inlay. However, these are all disease management treatments; LSCT is the only curative option.

Pterygium

The primary treatment for pterygium is surgical excision. However, recurrence is a common problem after excision using the bare sclera technique: reported recurrence rates range from 24% to 89%. Thus, a variety of adjuvant therapies have been used to reduce the risk of pterygium recurrence including LSCT, amniotic membrane transplantation (AMT), conjunctival autologous (CAU) transplantation, and mitomycin C (MMC, an antimetabolite drug).

New Technology Being Reviewed

To successfully treat LSCD, the limbal stem cell population must be repopulated. To achieve this, 4 LSCT procedures have been developed: conjunctival-limbal autologous (CLAU) transplantation; living-related conjunctival-limbal allogeneic (lr-CLAL) transplantation; keratolimbal allogeneic (KLAL) transplantation; and ex vivo expansion of limbal stem cells transplantation. Since the ex vivo expansion of limbal stem cells transplantation procedure is considered experimental, it has been excluded from the systematic review. These procedures vary by the source of donor cells and the amount of limbal tissue used. For CLAU transplants, limbal stem cells are obtained from the patient's healthy eye. For lr-CLAL and KLAL transplants, stem cells are obtained from living-related and cadaveric donor eyes, respectively.

In CLAU and lr-CLAL transplants, 2 to 4 limbal grafts are removed from the superior and inferior limbus of the donor eye. In KLAL transplants, the entire limbus from the donor eye is used.

[1] Level 4c evidence is obtained from single-site case series.

The recipient eye is prepared by removing the abnormal conjunctival and scar tissue. An incision is made into the conjunctival tissue into which the graft is placed, and the graft is then secured to the neighbouring limbal and scleral tissue with sutures. Some LSCT protocols include concurrent transplantation of an amniotic membrane onto the cornea.

Regulatory Status

Health Canada does not require premarket licensure for stem cells. However, they are subject to Health Canada's clinical trial regulations until the procedure is considered accepted transplantation practice, at which time it will be covered by the *Safety of Human Cells, Tissues and Organs for Transplantation Regulations* (CTO Regulations).

Review Strategy

The Medical Advisory Secretariat systematically reviewed the literature to assess the effectiveness and safety of LSCT for the treatment of patients with nonpterygium LSCD and pterygium. A comprehensive search method was used to retrieve English-language journal articles from selected databases.

The GRADE approach was used to systematically and explicitly evaluate the quality of evidence and strength of recommendations.

Summary of Findings

Nonpterygium Limbal Stem Cell Deficiency

The search identified 873 citations published between January 1, 2000, and March 31, 2008. Nine studies met the inclusion criteria, and 1 additional citation was identified through a bibliography review. The review included 10 case series (3 prospective and 7 retrospective).

Patients who received autologous transplants (i.e., CLAU) achieved significantly better long-term corneal surface results compared with patients who received allogeneic transplants (lr-CLAL, $P < .001$; KLAL, $P < .001$). There was no significant difference in corneal surface outcomes between the allogeneic transplant options, lr-CLAL and KLAL ($P = .328$). However, human leukocyte antigen matching and systemic immunosuppression may improve the outcome of lr-CLAL compared with KLAL. Regardless of graft type, patients with Stevens-Johnson syndrome had poorer long-term corneal surface outcomes.

Concurrent AMT was associated with poorer long-term corneal surface improvements. When the effect of the AMT was removed, the difference between autologous and allogeneic transplants was much smaller.

Patients who received CLAU transplants had a significantly higher rate of visual acuity improvements compared with those who received lr-CLAL transplants ($P = .002$). However, to achieve adequate improvements in vision, patients with deep corneal scarring will require a corneal transplant several months after the LSCT.

No donor eye complications were observed.

Epithelial rejection and microbial keratitis were the most common long-term complications associated with LSCT (complications occurred in 6%–15% of transplantations). These complications can result in

graft failure, so patients should be monitored regularly following LSCT.

Pterygium

The search yielded 152 citations published between January 1, 2000 and May 16, 2008. Six randomized controlled trials (RCTs) that evaluated LSCT as an adjuvant therapy for the treatment of pterygium met the inclusion criteria and were included in the review.

Limbal stem cell transplantation was compared with CAU, AMT, and MMC. The results showed that CLAU significantly reduced the risk of pterygium recurrence compared with CAU (relative risk [RR], 0.09; 95% confidence interval [CI], 0.01–0.69; $P = .02$). CLAU reduced the risk of pterygium recurrence for primary pterygium compared with MMC, but this comparison did not reach statistical significance (RR, 0.48; 95% CI, 0.21–1.10; $P = .08$). Both AMT and CLAU had similar low rates of recurrence (2 recurrences in 43 patients and 4 in 46, respectively), and the RR was not significant (RR, 1.88; 95% CI, 0.37–9.5; $P = .45$). Since sample sizes in the included studies were small, failure to detect a significant difference between LSCT and AMT or MMC could be the result of type II error. Limbal stem cell transplantation as an adjuvant to excision is a relatively safe procedure as long-term complications were rare (< 2%).

GRADE Quality of Evidence

Nonpterygium Limbal Stem Cell Deficiency

The evidence for the analyses related to nonpterygium LSCD was based on 3 prospective and 7 retrospective case series. Thus, the GRADE quality of evidence is very low, and any estimate of effect is very uncertain.

Pterygium

The analyses examining LSCT as an adjuvant treatment option for pterygium were based on 6 RCTs. The quality of evidence for the overall body of evidence for each treatment option comparison was assessed using the GRADE approach. In each of the comparisons, the quality of evidence was downgraded due to serious or very serious limitations in study quality (individual study quality was assessed using the Jadad scale, and an assessment of allocation concealment and the degree of loss to follow-up), which resulted in low- to moderate-quality GRADE evidence ratings (low-quality evidence for the CLAU and AMT and CLAU and MMC comparisons, and moderate-quality evidence for the CLAU and CAU comparison).

Ontario Health System Impact Analysis

Nonpterygium Limbal Stem Cell Deficiency

Since 1999, Ontario's out-of-country (OOC) program has approved and reimbursed 8 patients for LSCTs and 1 patient for LSCT consultations. Similarly, most Canadian provinces have covered OOC or out-of-province LSCTs. Several corneal experts in Ontario have the expertise to perform LSCTs.

As there are no standard guidelines for LSCT, patients who receive transplants OOC may not receive care aligned with the best evidence. To date, many of the patients from Ontario who received OOC LSCTs received concurrent AMTs, and the evidence from this analysis questions the use of this procedure. In addition, 1 patient received a cultured LSCT, a procedure that is considered investigational.

Many patients with LSCD have bilateral disease and therefore require allogeneic transplants. These patients will require systemic and topical immunosuppression for several years after the transplant, perhaps indefinitely. Thus, systemic side effects associated with immunosuppression are a potential concern, and patients must be monitored regularly.

Amniotic membrane transplantation is a common addition to many ocular surface reconstruction procedures, including LSCT. Amniotic membranes are recovered from human placentas from planned, uneventful caesarean sections. Before use, serological screening of the donor's blood should be conducted. However, there is still a theoretical risk of disease transmission associated with this procedure.

Financial Impact

For the patients who were reimbursed for OOC LSCTs, the average cost of LSCT per eye was $18,735.20 Cdn (range, $8,219.54–$33,933.32). However, the actual cost per patient is much higher as these costs do not include consultations and follow-up visits, multiple LSCTs, and any additional procedures (e.g., corneal transplants) received during the course of treatment OOC. When these additional costs were considered, the average cost per patient was $57,583 Cdn (range, $8,219.54–$130,628.20).

The estimated average total cost per patient for performing LSCT in Ontario is $2,291.48 Cdn (range, $951.48–$4,538.48) including hospital and physician fees. This cost is based on the assumption that LSCT is technically similar to a corneal transplant, an assumption which needs to be verified. The cost does not include corneal transplantations, which some proportion of patients receiving a LSCT will require within several months of the limbal transplant.

Pterygium

Pterygium recurrence rates after surgical excision are high, ranging from 24% to 89%. However, according to clinical experts, the rate of recurrence is low in Ontario. While there is evidence that the prevalence of pterygium is higher in the "pterygium belt," there was no evidence to suggest different recurrence rates or disease severity by location or climate.

Conclusions

Nonpterygium Limbal Stem Cell Deficiency

Successful LSCTs result in corneal re-epithelialization and improved vision in patients with LSCD. However, patients who received concurrent AMT had poorer long-term corneal surface improvements. Conjunctival-limbal autologous transplantation is the treatment option of choice, but if it is not possible, living-related or cadaveric allogeneic transplants can be used. The benefits of LSCT outweigh the risks and burdens, as shown in Executive Summary Table 1. According to GRADE, these recommendations are strong with low- to very low-quality evidence.

Executive Summary Table 1: Benefits, Risks, and Burdens – Nonpterygium Limbal Stem Cell Deficiency

Benefits	Risks	Burden
▪ Short- and long-term improvement in corneal surface (stable, normal corneal epithelium and decreased vascularization and opacity) ▪ Improvement in vision (visual acuity and functional vision)	▪ Long-term complications are experienced by 8% to 16% of patients ▪ Risks associated with long-term immunosuppression for recipients of allogeneic grafts ▪ Potential risk of induced LSCD in donor eyes	▪ High cost of treatment (average cost per patient via OOC program is $57,583; estimated cost of procedure in Ontario is $2,291.48)

Costs are expressed in Canadian dollars.

GRADE of recommendation: Strong recommendation, low-quality or very low-quality evidence.
- ➢ benefits clearly outweigh risks and burdens
- ➢ case series studies
- ➢ strong, but may change if higher-quality evidence becomes available

Pterygium

Conjunctival-limbal autologous transplantations significantly reduced the risk of pterygium recurrence compared with CAU. No other comparison yielded statistically significant results, but CLAU reduced the risk of recurrence compared with MMC. However, the benefit of LSCT in Ontario is uncertain as the severity and recurrence of pterygium in Ontario is unknown. The complication rates suggest that CLAU is a safe treatment option to prevent the recurrence of pterygium. According to GRADE, given the balance of the benefits, risks, and burdens, the recommendations are very weak with moderate quality evidence, as shown in Executive Summary Table 2.

Executive Summary Table 2: Benefits, Risks, and Burdens – Pterygium

Benefits	Risks	Burden
▪ Reduced recurrence; however, if recurrence is low in Ontario, this benefit might be minimal	▪ Long-term complications rare	▪ Increased cost

GRADE of recommendation: Very weak recommendations, moderate quality evidence.
- ➢ uncertainty in the estimates of benefits, risks, and burden; benefits, risks, and burden may be closely balanced
- ➢ RCTs
- ➢ very weak, other alternatives may be equally reasonable

Objective

The objective of this analysis is to review limbal stem cell transplantation (LSCT) for the treatment of patients with limbal stem cell deficiency (LSCD). This evidence-based analysis reviews LSCT as a primary treatment for patients with nonpterygium LSCD conditions, and LSCT as an adjuvant therapy to excision for the treatment of pterygium.

Background

Clinical Need: Condition and Target Population

The outer surface of the eye is covered by two distinct types of cells: corneal and conjunctival epithelial cells. The corneal epithelial cells are the outermost layer of the cornea, and the conjunctival epithelial cells form the layer covering the sclera and the interior of the eyelids. (1) These areas are separated by a transitional zone called the limbus. The anatomy of the outer eye is illustrated in Figure 1. The corneal epithelium comprises nonvascular, flat, nonkeratinised, stratified squamous epithelial cells; whereas the conjunctival epithelium comprises well vascularized, loosely organized mosaic-type cells and mucin-secreting goblet cells. (1)

Figure 1: Anatomy of the Outer Eye

Reprinted from J-Tec; http://www.jpte.co.jp/english/technologies/cultured_corneal_epithelium.html (Accessed June 20, 2008)

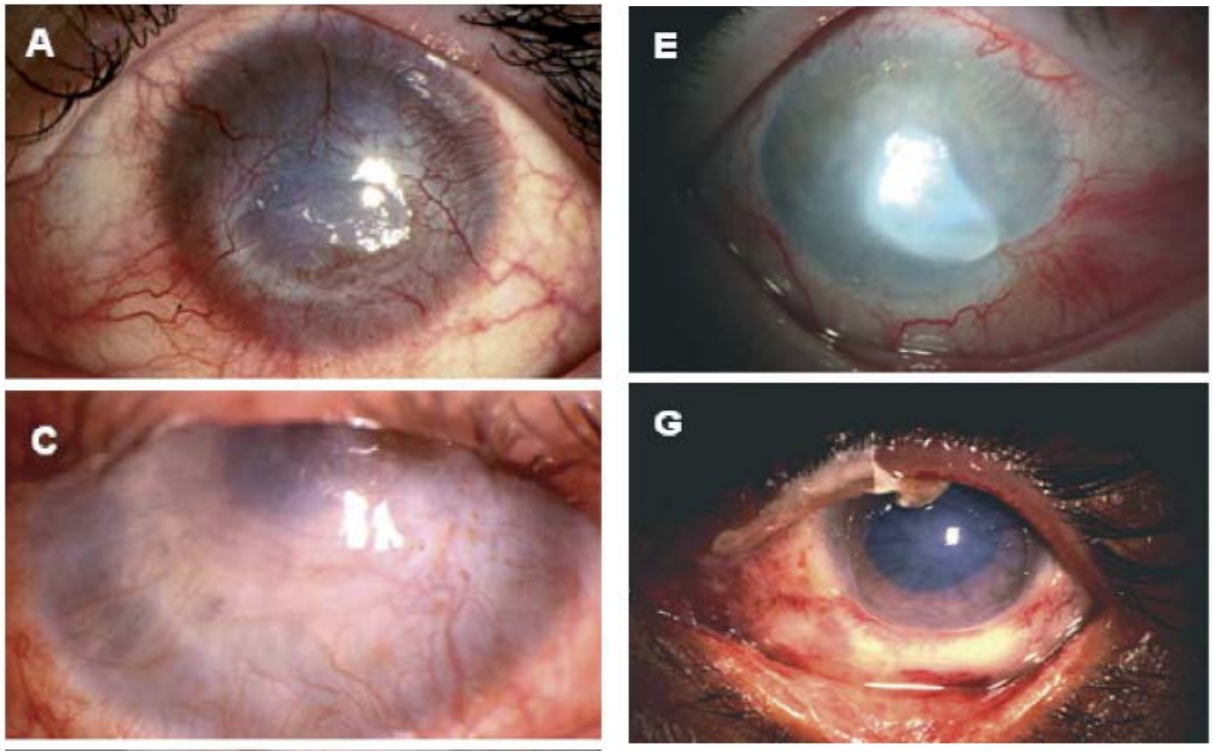
The corneal epithelium has several important roles including absorbing oxygen and nutrients; protecting the eye; and maintaining clear vision, homeostasis, and corneal integrity. (2;3) Cells are shed in the tear pool, lost by desquamation and eyelid blinking, and lost by cell death following terminal differentiation. (4) To replace the lost cells, new cells are generated by a pool of stem cells located in the limbus. The stem cells generate transient amplifying cells which migrate centripetally towards the centre of the cornea and upward from the basal layer becoming terminally differentiated nondividing cells as they move. (1)

The role of the cornea depends on the ability of corneal stem cells to successfully regenerate epithelial cells. The survival and function of any type of stem cell depend on the maintenance of an adequate microenvironment or "stem cell niche" (e.g., bone marrow for hematopoetic stem cells), which provides the cells with a protective environment. (5) For the corneal stem cells (hereafter referred to as limbal stem cells), this niche is located in the highly vascularized and innervated papilla-like columns of the Palisades of Vogt in the limbus. (6)

Nonpterygium Limbal Stem Cell Deficiency

The normal limbus acts as a functional and anatomical barrier between the conjunctival and corneal epithelium. However, when the stem cells are depleted or destroyed, this barrier is lost and LSCD develops. In LSCD, the conjunctival epithelium migrates onto the cornea (a process is called conjunctivalization). (3;7) Conjunctival epithelium cannot transdifferentiate into phenotypically normal corneal epithelium, so the cell layer retains its characteristic goblet cells, proteins, and keratins. (3) The ingrowth of conjunctival epithelium results in a thickened, irregular, unstable corneal surface that is prone to defects, ulceration, scarring, vascularization, and opacity (Figure 2). (3;4;8) Symptoms include severe irritation, discomfort, photophobia, tearing, blepharospasm, chronic inflammation and redness, and severely decreased vision. (2;4;9)

Figure 2: Eyes with Total Limbal Stem Cell Deficiency



Eyes with total LSCD caused by an acid burn (A), Stevens-Johnson syndrome (C), and alkali burn (E and G).
Reprinted from Experimental Eye Research, 78, Lavker RM, Corneal epithelial stem cells at the limbus: looking at some old problems from a new angle, Pages 433–446, Copyright 2004, with permission from Elsevier.

Limbal stem cell deficiency occurs if the surrounding microenvironment (niche) is insufficient to support the cells or, more commonly, if external factors deplete or destroy the limbal stem cells. A variety of underlying disorders are associated with LSCD including chemical or thermal injuries, ultraviolet and ionizing radiation, Stevens-Johnson syndrome (SJS), multiple surgeries or cryotherapies, contact lens wear, extensive microbial infection, advanced ocular cicatricial pemphigoid, and aniridia. As well, LSCD cases can be idiopathic. Table 1 contains a complete list of LSCD categorized by cause.

Table 1: Etiologies of Limbal Stem Cell Deficiency

Clinical Disease	Destruction of Limbal Stem Cells	Altered Limbal Stromal Microenvironment
Hereditary		
Aniridia		✓
Keratitis associated with multiple endocrine deficiency		✓
Epidermal dysplasia (extrodactyly-ectodermal dysplasia-clefting syndrome)	✓	
Acquired		
Chemical or thermal burns	✓	
Stevens-Johnson syndrome, toxic epidermal necrolysis	✓	
Multiple surgeries or cryotherapies to limbus	✓	
Contact lens-induced keratopathy	✓	
Severe microbial infection extending to limbus	✓	
Antimetabolite uses (5-FU or mitomycin C)	✓	✓
Radiation	✓	✓
Chronic limbitis (vernal, atopsy, phlyctenular)		✓
Peripheral ulcerative keratitis (Mooren's ulcer)		✓
Neurotrophic keratopathy		✓
Chronic bullous keratopathy		✓
Ocular cicatricial pemphigoid	✓	
Pterygium and Pseudopterygium	✓	✓
Herpes simplex epithelial disease		✓
Sequelae of mustard gas exposure		✓
Idiopathic	?	?

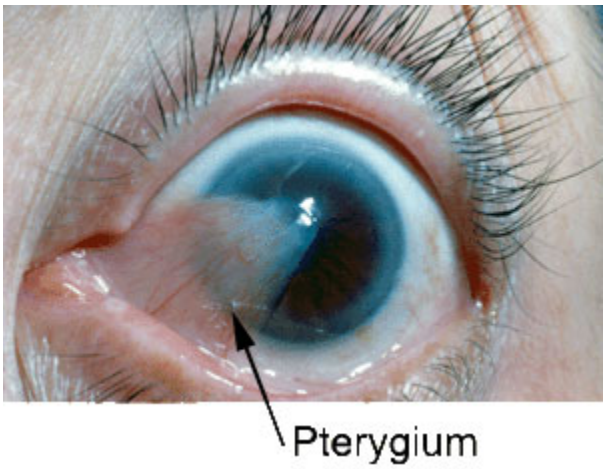
Sources (4;8;9;11-14)

Depending on the degree of limbal stem cell loss, LSCD may be total (diffuse) or partial (local). In total LSCD, the limbal stem cell population is completed destroyed, and conjunctival epithelium covers the entire cornea. (9) In partial LSCD, some areas of the limbus are unharmed, and the corresponding areas on the cornea maintain phenotypically normal corneal epithelium. Typically, there is a clear line of demarcation between the corneal and conjunctival cell types with small projections of corneal epithelium into the conjunctivalized areas. (4)

Pterygium

Pterygium is a wing-shaped fibrovascular tissue proliferation from the conjunctiva onto the cornea (Figure 3). It is the result of partial LSCD caused by localized ultraviolet damage to limbal stem cells. (15) As the pterygium invades the cornea, it may cause irregular astigmatism, loss of visual acuity (VA), chronic irritation, recurrent inflammation, double vision, and impaired ocular motility. (15;16) Asymmetric bilateral pterygium is frequently observed. (17)

Figure 3: Pterygium



Reprinted from Romanoff Vision; http://www.romanoffvision.com/eyeinfo-PingueculumAndPterygium.asp (accessed June 20, 2008).

Diagnosis

Confirmation of conjunctivalization is necessary for LSCD diagnosis, as the other characteristics and symptoms are nonspecific and could indicate other diseases. The definitive test for LSCD is impression cytology, which is used to detect the presence of conjunctival epithelium and its goblet cells on the cornea and can differentiate LSCD from other abnormalities. (4;9;11) Loss of the limbal Palisades of Vogt structures detected through a slit-lamp examination of the cornea is a clinical sign of LSCD; however, the palisade architecture is varied in the normal limbus, so this technique is limited unless the normal structure is characterized before injury. (4) In addition, late fluorescein staining is indicative of conjunctivalization because conjunctival cells are more porous than corneal epithelial cells and therefore more easily stained with fluorescein.

Incidence and Prevalence of Limbal Stem Cell Deficiency

Nonpterygium Limbal Stem Cell Deficiency

The incidence and prevalence of LSCD are not well understood. About 2.5 million people in the United States suffer an eye injury every year. (18) Of eye injuries leading to an emergency room visit, chemical burns account for 7% to 18% and thermal burns for 16%. (19) However, it is unknown what proportion of these patients will develop LSCD. The incidence of SJS is 2.6 to 7.1 cases per 1 million person-years in the United States, and 1.1 cases per 1 million person-years in Germany. (20) The incidence of ocular cicatricial pemphigoid is estimated at 1.16 cases per million population in France and 0.87 cases per million population in Germany. (21)

Pterygium

Pterygium occurs worldwide; however, the incidence and prevalence of pterygium vary by geographic location. Rates are highest in the "pterygium belt," which ranges from 30 degrees north to 30 degrees south of the equator; lower prevalence rates are found at latitudes greater than 40 degrees from the equator. For example, the prevalence of pterygium in the black population in Barbados is 23.4%, (17) whereas the prevalence of pterygium in Caucasians residing in urban, temperate climates is about 1.2%. (17)

Existing Treatments Other Than Technology Being Reviewed

Nonpterygium Limbal Stem Cell Deficiency

Total Limbal Stem Cell Deficiency

In total LSCD, a patient's limbal stem cells are completely depleted, so any successful treatment must include new stem cells. Treatment options that include transient amplifying cells or differentiated corneal epithelial cells only are temporary solutions because the LSCD phenotype will return less than 2 months after the dividing potential of these cells is exhausted. (8) Thus, traditional ophthalmological procedures such as corneal transplants are not adequate for patients with LSCD.

Autologous oral mucosal epithelium transplantation has been proposed as an alternative to LSCT. Like corneal epithelium, oral mucosa consists of stratified squamous cells, and both cell types express the cell marker keratin 3. (8) It has been shown that a small sample of oral mucosa can be expanded in culture to form epithelial cell sheets, which could be transplanted onto the corneal surface. This procedure eliminates the need for allogeneic transplants and therefore the need for immunosuppression after transplantation. However, this procedure is investigational, and there is very limited level 4c evidence[2] to support this technique (fewer than 20 eyes examined in 4 case series and 1 case report). (22-25)

Partial Limbal Stem Cell Deficiency

Asymptomatic patients with partial LSCD and peripheral corneal conjunctivalization may not require treatment. However, there are several disease management options for a patient whose visual axis is conjunctivalized. A common option is mechanical debridement of the abnormal epithelium. In this procedure, a surgical blade is used to scrape away the invading conjunctival epithelium, allowing the remaining limbal stem cells to repopulate the corneal with normal epithelium. (4;12) This procedure may need to be repeated if conjunctivalization recurs.

Additional treatment options for patients with partial LSCD include intensive, nonpreserved lubrication, bandage contact lenses, autologous serum eye drops, and transplantation of an amniotic membrane inlay. However, these are all disease management options and may not be adequate. Limbal stem cell transplantation is the only curative option for these patients.

Pterygium

The primary treatment for pterygium is surgical excision. The pterygium head is removed by blunt dissection, and then it is resected from the underlying sclera. After removal, the surgeon can leave the sclera bare (known as the bare sclera technique) or use the surrounding conjunctiva to cover the sclera (known as the primary closure technique). (15) However, recurrence is reported to be an important problem after these treatment options: reported recurrence rates range from 24% to 89%. (26) Thus, to reduce the risk of pterygium recurrence, a variety of adjuvant therapies have been used, including LSCT, amniotic membrane transplantation (AMT), conjunctival autologous (CAU) transplantation, beta

[2] Level 4c evidence is obtained from single-site case series.

radiation, mitomycin C (MMC, an antimetabolite drug), and mucous membrane transplantation. This report discusses only those treatments for which the systematic review found eligible studies, namely, LSCT, AMT, CAU transplantation, and MMC.

Amniotic Membrane Transplantation

Human AMT is reported to be associated with beneficial clinical outcomes for corneal surface repair including reduced inflammation, enhanced epithelial growth, reduced vascularization, and reduced scarring. (27;28) Human placentas are obtained from planned, uneventful caesarean sections with consent from the mother. (27) The placenta is washed with balanced saline-antibiotic solution, and then using blunt dissection, the amniotic membrane is separated from the chorion. The membrane is flattened, sutured to nitrocellulose filter paper, and washed thoroughly. (27) Membranes are frozen for storage, and before use, the membrane is defrosted and removed from the nitrocellulose paper. During transplantation, the membrane inlay is placed over the bare sclera and cornea with the epithelial side facing upward, and it is secured by interrupted nylon or vicryl sutures. (15)

Conjunctival Autologous Transplantation

A conjunctival autologous transplant is obtained through blunt dissection of the conjunctiva from Tenon's capsule to the limbus area (limbal stem cells are not included in the graft). The graft is placed over the bare scleral area where the pterygium has been removed, lining up the limbus side of the graft with the limbus area, and then the graft is secured by sutures. (29)

Mitomycin C

Mitomycin C is an antibiotic isolated from *Streptomyces caespitosus* that inhibits DNA synthesis; at high concentrations it also inhibits RNA and protein synthesis. (30;31) As an adjuvant therapy to pterygium excision, MMC has been used both intraoperatively and postoperatively at a variety of concentrations. (29) However, its use in higher concentrations has been limited due to serious complications including pyogenic granuloma, dellen of the sclera, perforation of the eye, glaucoma, cataract, and corneal edema. (30)

New Technology Being Reviewed

To successfully treat LSCD, the stem cells must be repopulated. To achieve this, 4 LSCT procedures have been developed:

- conjunctival-limbal autologous (CLAU) transplantation,
- living-related conjunctival limbal allogeneic (lr-CLAL) transplantation,
- keratolimbal allogeneic (KLAL) transplantation, and
- ex vivo expansion of limbal stem cells transplantation.

Ex vivo expansion is consider investigational and so was excluded from the systematic review.

The primary difference between the transplantation methods is the source of donor cells, as shown in Table 2. The procedure choice is based on several factors, including the presence of bilateral or unilateral disease, the extent of the LSCD, patient expectations and acceptance of the procedure, risk to a healthy eye, and the availability and willingness of a living-related donor. (9)

Table 2: Summary of LSCT Methods

Limbal Stem Cell Transplantation Method	Source of Donor Stem Cells
Autologous Transplantation	
▪ Conjunctival-limbal autologous (CLAU) transplantation	Nondiseased eye
Allogeneic Transplantation	
▪ Living-related conjunctival-limbal allogeneic (lr-CLAL) transplantation	Living-related donor eye
▪ Keratolimbal allogeneic (KLAL) transplantation	Cadaveric donor eye
Autologous or Allogeneic Transplantation	
▪ Ex vivo expansion of limbal stem cells transplantation	Biopsy nondiseased (autologous) or donor eye (living-related or cadaver eye)

Conjunctival-limbal Autologous Transplantation and Living-related Conjunctival-limbal Allogeneic Transplantation

Donor Eye

The CLAU and lr-CLAL transplantation procedures are similar except for the source of donor limbal stem cells. In both procedures, limbal stem cells are obtained by removing 2 strips of conjunctival-free limbal grafts from the superior and inferior limbus of the donor eye through superficial lamellar keratectomy and blunt dissection. (11) To protect the donor eye and aid in its recovery, an amniotic membrane graft or bandage contact lens is often placed over the donor eye. Preservative-free topical antibiotics, lubricants, and corticosteroids may also be administered. (4) As there is a risk of inducing LSCD in the donor eye, it is generally accepted that less than 50% of the limbus should be removed from a donor eye. (2;4;7;32)

Recipient Eye

The risk of LSCT failure increases if the recipient has external eye diseases such as dry eye, eyelid or lid margin abnormalities, extensive conjunctival metaplasia, keratinization, corneal anaesthesia, tear film abnormalities, mucus depletion, and chronic inflammation. (2;9;33) Thus, all eyelid and ocular surface abnormalities are repaired before LSCT is undertaken.

The recipient eye is prepared by superficial keratectomy to remove the conjunctivalized tissue, and the cicatrix is removed from the subconjunctival space. A conjunctival incision is made to expose the limbus and perilimbal sclera at the graft location. The graft is put in place and sutured to the limbus and sclera using interrupted 10-0 nylon sutures and 8-0 vicryl sutures, respectively. (4;11) The surgery is performed under local or general anesthesia. Due to the clinical benefits of using amniotic membranes for ocular surface reconstruction (described above), some LSCT protocols involve the concurrent transplantation of an amniotic membrane.

Postoperative care protocols vary, but may include a protective soft contact lens, autologous serum eye drops, and a short-term course of antibiotics. To help reduce graft failure, topical and systemic immunosuppressants are prescribed for patients who receive allogeneic grafts.

Keratolimbal Allogeneic Transplantation

The source of limbal stem cells in KLAL transplants is cadaveric donor eyes. Instead of 2 small limbal grafts, a 360-degree lamellar ring that consists of the entire donor eye's limbus, most of the peripheral cornea, and a minimal portion of the scleral tissue is used. (9) The recipient eye preparation and grafting procedures are the same as those described above for the CLAU and lr-CLAL transplantation.

An advantage of the KLAL procedure is that the entire donor limbus is transplanted, which maximizes the number of transplanted stem cells without risking LSCD in the donor eye. However, the risk of graft rejection after KLAL is high because the limbus tissue is highly antigenic, and it is almost impossible to obtain immune histocompatibility between the recipient and the donor cadaver. (2;9)

Regulatory Status

Canada

Health Canada does not require premarket licensure for stem cells. However, they are subject to Health Canada's clinical trial regulations until the procedure is considered accepted transplantation practice, at which time it will be covered by the *Safety of Human Cells, Tissues and Organs for Transplantation Regulations* (CTO Regulations). (Personal communication, March 2008)

United States

On July 14, 2005, the United States Food and Drug Administration approved autologous and allogeneic limbal epithelial stem cells expanded ex vivo on human amniotic membrane (sponsor, TissueTech Inc, Florida) as an orphan-designated product[3] for the treatment of ocular surface diseases due to total LSCD.

Evidence-Based Analysis of Effectiveness

Objective

To assess the effectiveness and safety of the transplantation of limbal stem cells for the treatment of patients with LSCD.

Research Questions

- Does LSCT result in re-epithelialization of the cornea and improved vision in patients with LSCD?
- Is there a subgroup of LSCD patients among whom the procedure is more effective?
- As an adjuvant therapy to surgical excision, does LSCT reduce recurrence of pterygium compared with other adjuvant therapies (conjunctival transplantation, AMT, or MMC)?
- What is the LSCT complication rate for donor and recipient eyes?

[3] The United States Orphan Drug Act (ODA) allows the FDA to grant special status to a product to treat a rare disease or condition (prevalence of less than 20,000 people in the United States) or a product for which there is no reasonable expectation that the costs of research and development can be recovered by sales in the United States. Orphan designation qualifies the product's sponsor for a tax credit and marketing incentives. (34)

Methods

Method of Review

The Medical Advisory Secretariat completed a computer-aided search of electronic databases (OVID MEDLINE, MEDLINE In-Process and Other Non-Indexed Citations, EMBASE, Cochrane Library, and International Agency for Health Technology Assessment/Centre for Reviews and Dissemination (INAHTA/CRD) to identify evidence published between January 1, 2000, and March 31, 2008, related to LSCT for the treatment of nonpterygium LSCD. A second search using the same databases was conducted to identify evidence published between January 1, 2000, and May 16, 2008, related to LSCT as an adjuvant therapy to excision to reduce the risk of pterygium recurrence. The search strategies are detailed in Appendix 1. Studies meeting the inclusion and exclusion criteria (listed below) were identified from the search results. Additional studies were identified from the reference lists of included studies.

Inclusion Criteria

- English-language studies;
- study format: journal articles, health technology assessments, systematic reviews, meta-analyses, randomized controlled trials (RCTs), and observational studies;[4]
- studies with a mean follow-up of at least 6 months;
- studies with clearly defined design and methods;
- studies with defined population of interest and subject characteristics; and
- studies published from January 1, 2000,, to March 31, 2008 (nonpterygium LSCD analysis) or from January 1, 2000, to May 16, 2008 (pterygium analysis).

Exclusion Criteria

- studies involving fewer than 10 eyes;
- nonsystematic reviews, observational studies (excluded in the nonpterygium analysis only), letters, editorials, comments, and case reports;
- duplicate publications (superseded by another publication by the same investigator group with the same objective and data);
- animal and in vitro studies;
- studies that combine the results of multiple transplants for a single eye;
- studies that used cultured limbal stem cells; or
- studies that did not examine at least 1 of the outcomes of interest.

Outcomes of Interest

Nonpterygium Limbal Stem Cell Deficiency

- corneal re-epithelialization;
- long-term corneal surface changes (stability or maintenance of normal corneal epithelial cells and/or reduced corneal vascularization, opacity, and conjunctivalization);
- changes in VA;
- changes in functional vision;

[4] In this analysis, evidence is selected based on the highest level of evidence available. Thus, as RCTs were available for the pterygium analysis and not for the nonpterygium analysis, observational studies were included only for the nonpterygium LSCD analysis.

- donor eye complications; and
- recipient eye complications.

Pterygium

- pterygium recurrence, and
- complications.

Assessment of Quality of Evidence

The quality of the evidence was assessed as High, Moderate, Low, or Very low according to the GRADE methodology and GRADE Working Group. (35) As per GRADE the following definitions apply:

High	Further research is very unlikely to change confidence in the estimate of effect.
Moderate	Further research is likely to have an important impact on confidence in the estimate of effect and may change the estimate.
Low	Further research is very likely to have an important impact on confidence in the estimate of effect and is likely to change the estimate.
Very low	Any estimate of effect is very uncertain.

Method of Analysis

Nonpterygium Limbal Stem Cell Deficiency

Four dichotomous outcomes were identified:

- short-term corneal surface improvement,
- long-term corneal surface improvement,
- improved VA, and
- improved functional vision.

Short-term corneal surface improvement was defined as the re-epithelialization of the cornea with normal corneal epithelium after LSCT. This outcome was assessed by 2 measures: epithelial healing time and corneal re-epithelialization. Long-term corneal surface improvement was defined as an improved corneal surface observed after at least 6 months of follow-up. Long-term success was indicated by a stable layer of corneal epithelium (measured cytologically and/or clinically), increased corneal clarity, and decreased corneal vascularization and conjunctivalization. Improved VA was defined as any improvement in VA between pre- and post-transplantation measurements (e.g., improvement from light perception to counting fingers or from counting fingers to 20/200 vision). Improved functional vision was defined as an improvement in vision from below 20/200 to 20/200 or better after the transplantation.

For each of the 4 outcomes, a weighted mean was calculated for the prospective and retrospective case series separately and combined (Appendix 2). Similarly, weighted means were calculated for the stratified analyses by graft type and etiology (Appendix 2). Stratification was conducted only for strata involving at least 10 eyes, so only 3 etiologies were examined: Steven-Johnsons syndrome, ocular burns (OB), and aniridia. To reduce confounding, VA and functional vision results were excluded for those patients who received a concurrent or subsequent corneal transplant (either penetrating keratoplasty or deep lamellar keratoplasty).

A post hoc subanalysis was performed to determine the effect of concurrent AMT on limbal graft success. This analysis was also stratified by graft type and etiology. To reduce bias, only the studies in which all or no patients received an AMT were included in this subanalysis.

For each of the stratified analyses, the results of the studies were pooled, and bootstrapping methods were employed. (36) A Student *t* test was used to analyze the weighted means, and a *P* value of less than .05 was considered significant. Calculations were conducted in STATA Version 10.0 (Statacorp, College Station, TX) using 1,000 replications.

Pterygium

The effectiveness of LSCT as an adjuvant therapy to excision was compared with 3 other adjuvant therapy options:

- AMT,
- MMC, and
- CAU transplantation.

Meta-analyses were conducted for the comparisons for which there was more than 1 study. Post hoc power calculations were conducted to determine the potential for type II error.

The number of long-term complication episodes and the associated incidence rates were calculated for studies that prospectively monitored complications. A Student *t* test was used to analyze incidence rates of complications, and a *P* value less than .05 was considered significant.

Results of Evidence-Based Analysis

Limbal Stem Cell Transplantation for the Treatment of Nonpterygium Limbal Stem Cell Deficiency

The database search yielded 873 citations published between January 1, 2000, and March 31, 2008. The search did not identify any RCTs that evaluated LSCT for the treatment of nonpterygium LSCD. One reviewer, who was not blinded to author, institution, or journal of publication, evaluated the eligibility of the identified citations. Articles were excluded based on information in the title and abstract. The full texts of potentially relevant articles were obtained for further assessment.

Of the identified citations, 9 met the inclusion criteria. An additional citation was identified through a review of the bibliographies of the included studies. Of these 10 case series, 3 were prospective and 7 were retrospective.

Limbal Stem Cell Transplantation for the Treatment of Pterygium

The database search yielded 152 citations published between January 1, 2000, and May 16, 2008. One reviewer, who was not blinded to author, institution, or journal of publication, evaluated the eligibility of the identified citations. Articles were excluded based on information in the title and abstract. The full texts of potentially relevant articles were obtained for further assessment.

Of the identified citations, 6 met the inclusion criteria. No additional citations were identified through a review of bibliographies. The 6 studies identified were all RCTs.

For each included study, levels of evidence were assigned according to a ranking system based on the hierarchy by Goodman. (37) An additional designation "g" was added for preliminary reports of studies that had been presented to international scientific meetings. Table 3 lists the level of evidence and number of studies identified for the nonpterygium LSCD analysis and the pterygium analysis. Characteristics of the included studies were extracted and are described in Tables 4, 5, and 13.

Table 3: Quality of Evidence of Included Studies* (Adapted from Goodman (37))

Study Design	Level of Evidence	Number of Eligible Studies: Nonpterygium LSCT Studies	Number of Eligible Studies: Pterygium LSCT Studies
Large RCT, systematic review of RCTs	1		5
Large RCT unpublished but reported to an international scientific meeting	1(g)		
Small RCT	2		1†
Small RCT unpublished but reported to an international scientific meeting	2(g)		
Non-RCT with contemporaneous controls	3a		
Non-RCT with historical controls	3b		
Non-RCT presented at international conference	3(g)		
Surveillance (database or register)	4a		
Case series (multisite)	4b		
Case series (single site)	4c	10	
Retrospective review, modeling	4d		
Case series presented at international conference	4(g)		

*g refers to grey literature; LSCT, limbal stem cell transplantation; RCT, randomized controlled trial.
†A small RCT is defined as an RCT with fewer than 20 patients in each arm.

Summary of Existing Health-Technology Assessments

No health technology assessments or systematic reviews that met the inclusion criteria were identified.[5]

Systematic Review by the Medical Advisory Secretariat

Sixteen studies met the inclusion criteria: 10 case series examining LSCT for nonpterygium LSCD conditions, and 6 RCTs examining LSCT for the treatment of pterygium.

[5] A 2007 systematic review (38) and a 2006 rapid review conducted by NICE (39) were excluded as they included only studies examining the ex vivo expansion limbal stem cell transplantation procedure, which is experimental.

Nonpterygium Limbal Stem Cell Deficiency

Prospective Case Series

Three prospective case series were identified. Details of the studies are outlined in Table 4.

Table 4: Characteristics of Prospective Case Series*

Study, Country, Treatment	N Eyes Treated (N Patients)	Population Characteristics			Mean Follow-Up, Range (months)	Type of Graft, n Eyes	Outcome
		Mean Age, Range (years)	Males (%)	Etiology, n Eyes			
Dos Santos et al., 2005 (40) Brazil All received concurrent AMT	33 (31)	35	84	OB, 22 SJS, 11	33	CLAU, 10 lr-CLAL, 23	Maintenance of normal corneal epithelium VA
Ivekovic et al., 2005 (41) Croatia 4 eyes received concurrent AMT	10 (10)†	30.1, 13–62	90	OB, 10	16.2, 8–41	CLAU, 10	Epithelial healing time VA
Ozdemir et al., 2004 (42) Turkey 2 eyes received concurrent AMT 1 eye received more than 1 graft	24 (22)	32.8, 3–56	33	OB, 24	16.1, 3–29	CLAU, 15 lr-CLAL, 9	Epithelial healing time Corneal vascularity and opacity VA

*AMT refers to amniotic membrane transplantation; CLAU, conjunctival limbal autograft; lr-CLAL, living-related conjunctival limbal allograft transplantation; N or n, number; OB, ocular burn; SJS, Stevens-Johnson syndrome; VA, visual acuity.
†Patients had partial LSCD.

Retrospective Case Series

Seven retrospective case series were identified. Details of the studies are outlined in Table 5.

Table 5: Characteristics of Retrospective Case Series*

Study, Country, Treatment	N Eyes Treated (N Patients)	Population Characteristics			Mean Follow-Up, Range (months)	Type of Graft, n Eyes	Outcome
		Mean Age, Range (years)	Males (%)	Etiology, n Eyes			
Shimazaki et al., 2004 (43) Japan All eyes received concurrent AMT	32 (32)	42.2, 14–71	88	OB, 32	16.75	CLAU, 11 KLAL, 21	▪ Stable re-epithelialization of cornea ▪ Corneal clarity ▪ Complications
Holland et al., 2003 (44) USA	31 (23)	41.5, 3–72		Aniridia, 31	35.7, 12–117	KLAL, 31	▪ Corneal surface stability ▪ VA ▪ Success of subsequent PK or DLK
Solomon et al., 2002 (45) USA All eyes received concurrent AMT 11 eyes received more than 1 graft	39 (31)	40.1	68	OB, 16 SJS, 9 OCP, 2 AKC, 3 Contact lens-induced keratopathy, 2 HSK, 1 Multiple surgeries, 1 Aniridia, 1 Idiopathic, 4	34, 12–117.6	KLAL, 39	▪ Survival rate of KLAL, ambulatory vision, and PK ▪ Complications
Yao et al., 2002 (46) China All eyes received concurrent DLK	34 (34)	27.8†	87†	OB, 34	27	CLAU, 34	▪ Integrity of corneal surface ▪ Post-operative corneal epithelial stability ▪ Corneal clarity ▪ Best-corrected VA
Ilari et al., 2002 (47) England 5 eyes received concurrent AMT 10 eyes received more than 1 graft	23 (20)	45, 22–77	60	SJS, 7 OB, 8 HSK, 1 AKC, 2 AIE, 1 Trachama, 1 OCP, 2 EEC, 1	60, 15–96	KLAL, 23	▪ Re-epithelialization of cornea ▪ Epithelial healing time ▪ Corneal vascularization and conjunctivalization ▪ Reconstruction of corneal surface ▪ Pain ▪ VA
Samson et al., 2002 (48) USA 5 eyes received concurrent AMT	11 (9)	29, 5–56	33	SJS, 8 AKC, 1 Mooren's Ulcer/Sjorgren's, 1 HSK/Limbitis, 1	35, 29–51	CLAU, 1 lr-CLAL, 10	▪ Re-epithelialization of cornea ▪ Epithelial healing time ▪ Ocular function ▪ VA
Daya et al., 2001 (49) England	10 (8)	43, 30–65	25	SJS, 3 EEC, 3 OB, 2 OCP, 1 AKC, 1	26.2, 17–43	lr-CLAL, 10	▪ Restoration of corneal epithelium ▪ Corneal clarity, vascularization, and conjunctivalization ▪ Pain ▪ VA

*AIE refers to atypical ichthyosiform erythroderma; AKC, atopic keratoconjunctivitis; AMT, amniotic membrane transplantation; CLAU, conjunctival limbal autograft transplantation; DLK, deep lamellar keratoplasty; EEC, ectrodactyly, ectodermal dysplasia; HSK, herpes simplex keratitis; KLAL, keratolimbal allograft transplantation; lr-CLAL, living-related conjunctival limbal allograft transplantation; OB, ocular burn; OCP, ocular cicatricial pemphigoid; PK, penetrating keratoplasty; SJS, Stevens-Johnson syndrome; VA, visual acuity.
†Study characteristics include data for 5 patients that were excluded from the results in the study.

Corneal Surface Improvement

Limbal graft type was one of the primary causes of heterogeneity across the included studies: 81 eyes in 6 studies (3 prospective (40-42) and 3 retrospective case series (43;46;48)) received a CLAU transplant; 52 eyes received a lr-CLAL transplant in 4 case series (2 prospective (40;42) and 2 retrospective studies (48;49)); and 114 eyes in 4 retrospective case series (43-45;47) received a KLAL transplant. Two prospective case series (40;42) and 2 retrospective case series (43;48) included more than 1 type of graft. To determine the impact of graft type on transplantation success, stratified analyses of the outcome measures by graft type were conducted. The results are presented in Table 6.

Table 6: Corneal Surface Improvement Results Stratified by Graft Type*†

Results by Graft Type	Weighted Mean, %		
	CLAU	lr-CLAL	KLAL
Short-term corneal surface improvement rate	98.6[a]	89.7[ab]	59.1[b]
Long-term corneal surface improvement rate	93.4[c]	38.5[d]	30.7[d]

*CLAU refers to conjunctival-limbal autologous transplantation; KLAL, keratolimbal allogeneic transplantation; lr-CLAL, living-related conjunctival-limbal allogeneic transplantation.
†Within each row, there is a statistically significant difference ($P < .05$) between values if they do not share a superscripted letter.

Patients who received a CLAU transplant achieved significantly better long-term corneal surface results compared with patients who received a lr-CLAL ($P < .001$) or KLAL ($P < .001$) transplant. Thus, CLAU transplantation is the treatment option of choice. However, for patients with bilateral disease,[6] autologous transplants are not possible. The comparison between lr-CLAL and KLAL transplantation showed no statistically significant long-term corneal improvement differences between these allogeneic transplant options ($P = .328$).

Many of the studies included patients with a variety of LSCD etiologies, which was another source of heterogeneity. The most common causes of LSCD in the studies were chemical and thermal OB (n = 148), SJS (n = 38), and aniridia (n = 32). As the severity and prognosis of LSCD are affected by its etiology, stratified analyses of the outcome measures were conducted for these 3 etiologies.[7] The results are presented in Table 7.

Table 7: Corneal Surface Improvement Results Stratified by Etiology*†

Results by Etiology	Weighted Mean, %		
	Ocular Burns	SJS	Aniridia
Short-term corneal surface improvement rate	83.6[a]	94.4[a]	N/A
Long-term corneal surface improvement rate	58.8[b]	26.3[c]	71.9[b]

* SJS refers to Stevens-Johnson syndrome, N/A, not applicable.
† Within each row, there is a statistically significant difference ($P < .05$) between values if they do not share a superscripted letter.

Compared with patients with OB and aniridia, patients with SJS had statistically significantly poorer long-term corneal surface improvements ($P = .004$ and .003, respectively). There was no statistically significant difference between patients with OB and aniridia ($P = .212$).

[6] About 50% of patients with ocular burns and 100% of patients with aniridia or inflammatory LSCD (e.g., SJS and ocular cicatricial pemphigoid) have bilaterial LSCD. (Personal communication, corneal expert, June 6, 2008)
[7] Stratification was conducted only for strata (etiologies) with at least 10 eyes.

Human Leukocyte Antigen Matching in Living-Related Conjunctival-Limbal Allogeneic Transplants

While this analysis showed no statistical difference between lr-CLAL and KLAL transplants, there is some evidence to suggest that if a lr-CLAL transplant is closely matched (i.e., 0–1 human leukocyte antigen [HLA] mismatches), then graft survival is increased. (49-52) Two of the included case series (40;49) compared the results of HLA compatible and incompatible lr-CLAL grafts, and no difference was found in either study. Reinhard et al. (50) suggested that use of systemic immunosuppressive drugs after lr-CLAL transplantation is important regardless of HLA matching. Since the patients in the Dos Santos study (40) who received 100% HLA-matched grafts were not treated with immunosuppressive therapy, the impact of HLA matching may have been masked. It was impossible to examine HLA matching in more depth due to the lack of data regarding this factor in the included studies. However, limited evidence suggests that HLA matching and systemic immunosuppression may increase the success of lr-CLAL transplants, which may result in a significant difference between lr-CLAL and KLAL transplants.

Vision Improvements

After a successful LSCT, a patient's cornea is re-epithelialized with normal epithelium, which results in increased clarity, and reduced conjunctivalization and vascularization. These changes may improve the patient's vision. Improvement in VA and functional vision after LSCT were analyzed by graft type and etiology.[8] The results in Table 8 show that vision improvements were affected by graft type: patients who received a CLAU transplant had a significantly higher rate of improved VA compared with those who received a lr-CLAL[9] transplant ($P = .002$). Due to limited data, it was not possible to compare the vision results by LSCD etiology. However, as shown in Table 9, amongst patients with OB, visual acuity was increased to a greater extent than functional vision.

Table 8: Vision Results Stratified by Graft Type*†

Results by Graft Type	Weighted Mean, %		
	CLAU	lr-CLAL	KLAL
Improved visual acuity	96.0[a]	50.0[b]	N/A
Improved functional vision	68.0[c]	38.9[c]	N/A

*CLAU refers to conjunctival-limbal autologous transplantation; KLAL, keratolimbal allogeneic transplantation; lr-CLAL, living-related conjunctival-limbal allogeneic transplantation; N/A, not applicable.
† Within each row, there is a statistically significant difference ($P < .05$) between values if they do not share a superscripted letter.

Table 9: Vision Results Stratified by Etiology

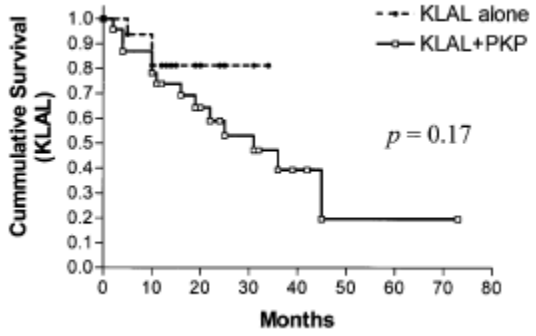
Results by Etiology	Weighted Mean, %
	Ocular Burns
Improved visual acuity	81.3
Improved functional vision	56.3

[8] To isolate the effect of LSCT on visual acuity and functional vision, these analyses were populated with data from the patients who did not receive a concurrent or subsequent corneal transplantation (the purpose of the corneal transplant is to restore vision).
[9] Due to limited data, improvement in VA and functional vision could not be assessed for KLAL.

Since a successful LSCT modifies the corneal surface only, patients with deep corneal scarring may not experience improvement in either VA or functional vision. Thus, to achieve improved vision these patients may require a corneal transplant in addition to the LSCT. When a patient requires both procedures, there are 2 options: the corneal transplant may be performed at the same time or several months after the LSCT.

There is some debate as to the safety of simultaneous LSCT and penetrating keratoplasty procedures. Solomon et al. (45) showed a nonsignificant reduction in vision and graft survival (survival of both the LSCT and corneal transplant) when the procedures were combined as shown in Figure 4 ($P = 0.17$). Other studies have compared LSCT and penetrating keratoplasty outcomes for the 2 timing options. (43;45) In Shimazaki et al., (43) corneal surface improvements were observed in 2 of 6 (33%) patients who received the 2-step procedure, and 2 of 15 (13%) patients who received simultaneous procedures, although this difference was not statistically significant ($P = .304$).[10] The rate of epithelial rejection was significantly higher in those eyes that received simultaneous transplants (66.7% vs. 0%, $P = .007$). Patients who received simultaneous procedures were more likely to require a second LSCT and/or develop persistent epithelial defects than those who had the procedures over 2 stages, but only the former outcome reached statistical significance (second LSCT, 73% vs. 0%, $P = .003$; persistent epithelial defects, 73% vs. 50%, $P = .317$).

Figure 4: Comparison of Graft Survival for Simultaneous Limbal Stem Cell Transplantation and Corneal Transplant with Limbal Stem Cell Transplantation Alone



KLAL refers to keratolimbal allogeneic transplantation; PKP, penetrating keratoplasty.
This figure was published in Ophthalmology, 109, Solomon A, Ellies P, Anderson DF, Touhami A, Grueterich M, Espana EM et al, Long-term outcome of keratolimbal allograft with or without penetrating keratoplasty for total limbal stem cell deficiency, 1159-1166, Copyright The American Academy of Ophthalmology (2002).

Overall, these results indicate that patients who received the procedures in 2 stages had fewer complications and graft rejections compared with patients who received the procedures simultaneously. This was attributed to increased inflammation due to a larger immune response generated by the combined procedures. (9;45) In contrast, patients in the study by Yao et al. (46) received simultaneous LSCT and deep lamellar keratoplasy, and the combined procedures did not affect graft success (long-term corneal surface improvement shown in 32 of 34 eyes). However, patients in this study received autologous LSCTs, so they had reduced inflammation. Thus, limited evidence indicates that patients who require an allogeneic LSCT and a corneal transplant should receive the procedures in 2 stages, separated

[10] The calculations and *P* values for the complication rates and graft survival for the Shimazaki study presented in this section are different from those in the published paper due to inconsistencies in the reported results (Medical Advisory Secretariat calculations presented).

by several months.

Subanalysis: Effect of Amniotic Membrane Transplantation on Limbal Graft Success

Amniotic membrane transplants are reported to be associated with beneficial clinical outcomes for corneal surface repair including reduced inflammation, enhanced epithelial growth, reduced vascularization, and reduced scarring. (27;28) Thus, AMT has become a common component of ocular surface repair procedures.

The use of AMT varied in the studies included in this analysis. In 3 studies (1 prospective case series (40) and 2 retrospective case series (43;45)), all of the patients received an AMT with their limbal graft. In 3 of the retrospective case series (44;46;49), none of the patients received a concurrent AMT. In the remaining 4 case series, (41;42;47;48) only a subset of the patients received a concurrent AMT; the reason for this additional procedure was not provided.

To determine the effect of concurrent AMT on limbal graft success, the studies in which all patients (n = 104) or no patients (n = 75) received a concurrent AMT were pooled. The results were stratified by graft type and etiology and are presented in Tables 10 and 11.

Table 10: Amniotic Membrane Transplantation Subanalysis Results Stratified by Graft Type*

Sub-Analysis by AMT and Graft Type (All Etiologies Included)	Weighted Mean		
	Limbal Graft + AMT, n (%)	Limbal Graft (No AMT) , n (%)	*P* Value
All Graft Types			
Short-term corneal surface improvement	32 (53)	44 (95)	< .001
Long-term corneal surface improvement	104 (20.0)	75 (84)	< .001
CLAU			
Short-term corneal surface improvement	21 (95)	35 (100)	.01
Long-term corneal surface improvement	21 (81)	35 (94)	.124
lr-CLAL			
Short-term corneal surface improvement	23 (52)	10 (80)	.234
Long-term corneal surface improvement	23 (13)	10 (80)	.192
KLAL			
Short-term corneal surface improvement	21 (33)	N/A	N/A
Long-term corneal surface improvement	21 (8.3)	31 (74)	< .001

*AMT refers to amniotic membrane transplantation; CLAU, conjunctival-limbal autologous transplantation; KLAL, keratolimbal allogeneic transplantation; lr-CLAL, living-related conjunctival-limbal allogeneic transplantation; N/A, not applicable.

Table 11: Amniotic Membrane Transplantation Subanalysis Results Stratified by Etiology*

Sub-Analysis by AMT and Etiology (all graft types included)	Weighted Mean		
	Limbal Graft + AMT, n (%)	Limbal Graft (No AMT) , n (%)	*P* Value
Short-term corneal surface improvement	32 (53.2)	70 (97.2)	< .001
Long-term corneal surface improvement	36 (34.3)	36 (91.7)	< .001

*AMT refers to amniotic membrane transplantation.

The short- and long-term corneal surface improvements were much poorer for patients who received a limbal transplant and concurrent AMT compared with those who received only a limbal transplant. This trend was consistent across all of the stratifications by graft type and etiology. In addition, the differences

between autologous and allogeneic transplants' short- and long-term corneal surface outcomes were much smaller when the studies in which patients received a concurrent AMT are removed. This suggests that allogeneic transplantation outcomes could be improved if patients do not receive a concurrent AMT. However, the results of this subanalysis should be regarded with caution because these comparisons are based on very small samples and level 4c evidence.

Immunosuppression

Limbal stem cell grafts include a large number of Langerhans cells and HLA-DR antigens, which trigger strong immune responses when transplanted into a recipient eye. (2-4;32) Thus, patients who receive an allogeneic LSCT should receive systemic immunosuppression after the transplantation. Immunosuppression protocols vary but usually consist of a combination of topical and systemic agents. Common agents are prednisone, cyclosporine A, azathioprine, and dexamethasone. Some protocols involve ongoing immunosuppression while others taper and eliminate immunosuppression after 1 or 2 years. In addition, patients who receive 75% to 100% HLA matched lr-CLAL grafts do not always receive systemic immunosuppression after the transplantation. These protocols are based on expert opinion/experience rather than clinical studies, but there is evidence that patients who receive systemic immunosuppression have statistically significantly improved outcomes compared with those who do not receive immunosuppression after allogeneic transplants, regardless of HLA matching. (32;49;50;52)

Complications

Donor Eye Complications

Four studies (42;43;46;46) prospectively monitored limbal graft donors for complications. No complications were reported.

Recipient Eye Complications

Two studies (43;45) prospectively monitored recipient eye complications, and 8 studies (40-42;44;46-48) retrospectively reported observed complications. No perioperative complications were reported. The analysis of long-term complications was limited to 4 types of complications that corneal experts identified as having an important impact on graft survival: epithelial rejection; microbial keratitis; corneal necrosis; and corneal ulceration/persistent epithelial defects. (Personal communication) The incidence of complications and the number of complication episodes are presented in Table 12.

Table 12: Long-Term Complication Episodes in Recipient Eyes*

Complication	Prospective Monitoring of Complications (N = 71) n (%)	Retrospective Monitoring of Complications (N = 176) n (%)	Combined (N = 247) n (%)
Epithelial rejection	10 (14.1)	28 (15.9)	38 (15.4)
Microbial keratitis	3 (4.2)	13 (7.4)	16 (6.5)
Corneal necrosis	0 (0)	5 (2.8)	5 (2.0)
Corneal ulceration*	0 (0)	2 (1.1)	2 (0.8)

*Graft abscesses and corneal ulcerations were included in this category. Persistent epithelial defects were excluded from this category as only some studies reported this complication while other studies used this as a category of graft failure but did not report numbers specific to persistent epithelial defects.

Epithelial rejection and microbial keratitis are the most common long-term complications of LSCT. While these complications can usually be managed by antibiotics, these complications commonly resulted in

graft failure. Thus, patients should be monitored carefully after LSCT.

Increased intraocular pressure or glaucoma was also reported in 3 studies; (43-45) however, of the patients who developed these complications, most or all had undergone 1 or more corneal transplants as well. Since corneal transplantation is a known risk factor for glaucoma, (45) it was impossible to determine the incidence of glaucoma associated with LSCT alone.

Pterygium

Six RCTs were identified. Details of the studies are outlined in Table 13. The 6 included studies compared CLAU to a variety of treatment options:

- AMT,
- MMC, and
- CAU.

Table 13: Characteristics of Randomized Controlled Trials in Pterygium

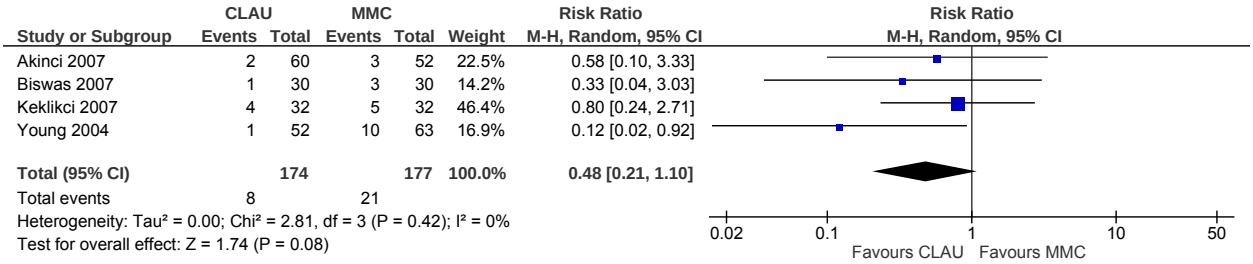
Study Country	Treatment Arm	N	Mean FU, Range (months)	Mean Age, Range (years)	Male (%)	Outcomes
Akinci et al., 2007 (53) Turkey	CLAU	60	†	43.1 (33–54)	51.7	▪ Recurrence ▪ Complications
	MMC	52	†	44.0 (35–52)	46.2	
Biswas et al., 2007 (31) India	CLAU	30	6 (3–12)	35.6 (25–60)		▪ Recurrence ▪ Visual improvement ▪ Complications
	MMC	30				
Keklikci et al., 2007 (30) Turkey	CLAU	32	24.4, 12–36	39.8, 15–55	46.9	▪ Recurrence ▪ Complications
	AMT	30	23.6, 12–36	41.8, 19–68	53.3	
	MMC (0.2 mg/mL)	32	23.4, 12–36	44.7, 20–65	56.2	
Küçükerdönmez et al., 2007 (54) Turkey	CLAU	14	13.7, 6–24	43.0, 28–59	57.1	▪ Graft vascularization ▪ Recurrence ▪ Complications
	AMT	13	14.4, 6–24	44.9, 32–65	53.8	
Young et al., 2004 (55) Hong Kong	CLAU	63	16.7	60.0, 39–81	36.5	▪ Recurrence ▪ Complications
	MMC (0.2 mg/mL)	52	16.2	59.1, 32–84	41.3	
Al Fayez et al., 2002‡ (56) Saudi Arabia	CLAU	43	49, 36–63	33.7	97.7	▪ Recurrence ▪ Improvement in VA ▪ Complications
	CAU	36	50, 36–63	32.7	94.4	

*AMT refers to amniotic membrane transplantation; CAU, conjunctival autograft transplantation (no limbal stem cells included); CLAU, conjunctival limbal autograft transplantation; FU, follow-up; MMC, mitomycin C; N, number of eyes; VA, visual acuity.
†Follow-up at 3, 6, 9, and 12 months.
‡Study includes both primary and recurrent pterygium.

Wherever more than 1 study was reported, meta-analyses were used to determine the relative risk (RR) of pterygium recurrence for excision combined with the other adjuvant treatment options. The results of these comparisons and the CLAU versus MMC meta-analysis are shown in Figure 5 and Table 14.[11]

[11] Even though 2 studies (30;54) examined the comparison between CLAU and AMT, a meta-analysis was not conducted because no events occurred in either study group in the study by Küçükerdönmez et al. (54) Thus, the relative risk for the study was non-estimable.

Figure 5: Conjunctival-Limbal Autologous Transplantation Versus Mitomycin C*
Outcome: Pterygium Recurrence



*CLAU refers to conjunctival-limbal autologous transplantation; CI, confidence interval; M-H, Mantel-Haenszel; MMC, mitomycin C.

Table 14: Results of Pterygium Analyses

Comparison	Number of Studies	RR (95% CI)	*P* Value
CLAU vs. AMT	2	1.88 (0.37, 9.50)	.45
CLAU vs. MMC	4	0.48 (0.21, 1.10)	.08
CLAU vs. CAU	1	0.09 (0.01, 0.69)	.02

*AMT refers to amniotic membrane transplantation; CAU, conjunctival autologous transplantation; CLAU, conjunctival-limbal autologous transplantation; CI, confidence interval; lr-CLAL, living-related allogeneic transplantation; MMC, mitomycin C; RR, relative risk.

The results of the comparisons and meta-analyses showed that CLAU significantly reduced the risk of pterygium recurrence for both primary and recurrent pterygium compared with CAU (RR, 0.09; 95% confidence interval [CI], 0.01–0.69; *P* = .02). CLAU reduced the risk of pterygium recurrence for primary pterygium compared with MMC although this comparison did not reach statistical significance (RR, 0.48; 95% CI, 0.21–1.10; *P* = .08). AMT and CLAU had similar low rates of recurrence (2 recurrences in 43 patients and 4 in 46, respectively), and the relative risk was not significant (RR, 1.88; 95% CI, 0.37–9.5; *P* = .45).

All of the included RCTs had relatively small sample sizes, so failure to detect a significant difference between 2 options may be explained by a type II error. Post hoc power calculations were calculated for each study, and only the study by Küçükerdönmez et al. (54) was appropriately powered (β = 0.93). The power for the CLAU-MMC meta-analysis was also calculated, and despite combing the studies, this comparison was still underpowered (β = .63). Thus, type II error is a potential concern.

Complications

Five of the 6 studies (83%) prospectively monitored and reported on complications associated with the 4 adjuvant treatment options. No perioperative complications were reported for any procedure option. A corneal expert identified 4 severe long-term complications of potential importance: corneal epithelial defects; necrosis; symblephara; and glaucoma. The complication rates and the number of complication episodes for the LSCT and MMC procedures[12] are presented in Table 15. Important long-term complications associated with LSCT were rare (< 2%).

[12] None of the 4 long-term complications were observed in the AMT and CAU treatment groups.

Table 15: Long-Term Complications for Pterygium Adjuvant Therapy Treatments*

Complication	Complications, n (%)		
	CLAU (N = 187)	MMC (N = 147)	*P* Value
Corneal epithelial defects	3 (1.6)	18 (12.2)	< .001
Symblephara	3 (1.6)	3 (2.0)	.766
Necrosis	0 (0.0)	1 (0.68)	.259
Glaucoma	0 (0.0)	1 (0.68)	.259

* CLAU refers to conjunctival-limbal autologous transplantation; MMC, mitomycin C.

GRADE Quality of Evidence

The quality of the trials was examined according to the GRADE Working Group Criteria. (35;57)

Quality refers to criteria such as the adequacy of allocation concealment, blinding, and follow-up.

Consistency refers to the similarity of estimates effect across studies. If there is important, unexplained inconsistency in the results, our confidence in the estimate for that outcome decreases. Differences in the direction of effect, the size of the differences in effect, and the significance of the differences guide the decision about whether important consistency exists.

Directness refers to the extent to which the interventions and outcome measures are similar to those of interest.

As stated by the GRADE Working Group, the following definitions were used in grading the quality of the evidence.

High	Further research is unlikely to change our confidence in the estimate of effect.
Moderate	Further research is likely to have an important impact on our confidence in the estimate of effect and may change the estimate.
Low	Further research is very likely to have an important impact on our confidence in the estimate of effect and is likely to change the estimate.
Very Low	Any estimate of effect is very uncertain.

Nonpterygium Limbal Stem Cell Deficiency

The evidence for the analyses related to nonpterygium LSCD was based on 3 prospective and 7 retrospective case series. Thus, the GRADE quality of evidence is very low, and any estimate of effect is very uncertain.

Pterygium

The analyses examining LSCT as an adjuvant treatment option for pterygium were based on 6 RCTs. The GRADE quality of evidence was assessed for each treatment option comparison. The parameter “quality of the studies” was based on a combination of the Jadad Scale (58) and an assessment of allocation concealment and the degree of loss to follow-up. All 6 studies scored 3 or lower on the Jadad scale: 3

studies scored 1 point; 2 studies scored 2 points; and 1 study scored 3 points. The results are presented below in Tables 16 to 19.

Table 16: GRADE Quality of Evidence – CLAU Versus AMT Comparison*

Number of Studies	Design	Quality of the Studies	Consistency	Directness	Other Modifying Factors	RR (95% CI)	Overall Quality of Evidence
2	RCT	Serious limitations	No inconsistency	No limitations	Sparse Data	1.88 (0.37, 95)	Low
	High	Moderate	Moderate	Moderate	Low		

*AMT refers to amniotic membrane transplantation; CI, confidence interval; CLAU, conjunctival-limbal autologous; RR, relative risk; RCT, randomized controlled trial.

Table 17: GRADE Quality of Evidence – CLAU Versus MMC Comparison*

Number of Studies	Design	Quality of the Studies	Consistency	Directness	Other Modifying Factors	RR (95% CI) (Random Effects Meta-Analysis)	Overall Quality of Evidence
4	RCT	Very serious limitations	No inconsistency	Yes	Not applicable	0.48 (0.21, 1.10)	Low
	High	Low	Low	Low	Low		

*CI refers to confidence interval; CLAU, conjunctival-limbal autologous; MMC, mitomycin C; RR, relative risk; RCT, randomized controlled trial.

Table 18: GRADE Quality of Evidence – CLAU Versus CAU Comparison*

Number of Studies	Design	Quality of the Studies	Consistency	Directness	Other Modifying Factors	RR (95% CI)	Overall Quality of Evidence
1	RCT	Serious limitations	No inconsistency	No limitations	N/A	0.09 (0.01, 0.69)	Moderate
	High	Moderate	Moderate	Moderate	Moderate		

*CI refers to confidence interval; CAU, conjunctival autologous transplant; CLAU, conjunctival-limbal autologous; N/A, not applicable; RR, relative risk; RCT, randomized controlled trial.

Existing Guidelines

No guidelines for LSCT were found.

Ontario Health System Impact Analysis

Diffusion

Diffusion in Ontario

Nonpterygium Limbal Stem Cell Deficiency

Since 1999, Ontario's out-of-country (OOC) program has approved and reimbursed 8 patients for LSCTs

and 1 patient for LSCT consultations. (E-mail communication, May 2008) However, several corneal experts in Ontario have the expertise to perform LSCTs.

Pterygium

Over the past 5 fiscal years, the mean number of pterygium excisions performed per year in Ontario was 3,181 (range, 2,642–4,587).[13] Two adjuvant therapy options are included in the Ontario Schedule of Benefits: CAU transplantation; and mucous membrane transplantation. However, between 28% and 37%[14] of the patients who had a pterygium excision also received one of the listed adjuvant therapies during this period. It is possible that some patients are receiving nonlisted adjuvant therapies such as MMC, but this cannot be tracked.

Diffusion in Other Provinces

Most Canadian provinces and territories have covered LSCTs OOC or out-of-province. Details are found in Table 19.

Table 19: Status of LSCT in Canadian Provinces/Territories*

Province/Territory	Funding Status
Alberta	LSCTs are not included in the schedule of medical benefits; however, Alberta has approved OOC payments for several patients.
British Columbia	No reply to date.
Manitoba	This procedure is not being offered by ophthalmologists in Manitoba and is not listed in the insured services.
New Brunswick	LSC transplant service is not available, but Nova Scotia has funded 1 procedure OOP/OOC.
Newfoundland and Labrador	Covered OOP on the basis of specialist recommendation in a case where it was the only remaining option in a case of conjunctival carcinoma.
Northwest Territories	No reply to date.
Nova Scotia	LSCT is insured OOP and OOC (in extremely rare cases). Some physicians are capable of performing LSCT in province, but it is not certain whether they do any, perhaps because of low volume.
Nunavut	No reply to date.
Ontario	Ontario has approved and paid for 8 patients to receive OOC LSCTs and 1 patient for LSCT consultations.
Prince Edward Island	LSCT is not performed on the Island, but an OOP service has been paid for.
Quebec	Quebec has authorized 1 LSCT out of Canada in 2004, but would consider it insured when some criteria are met.
Saskatchewan	One doctor performs some LSCTs and bills “by report” as Saskatchewan has no fee codes for LSCT.
Yukon	No reply to date.

*OOC refers to out-of-country; OOP, out-of-province; LSCT, limbal stem cell transplantation.

Diffusion Outside Canada

Table 20 summarizes the use coverage of LSCT in the United States.

[13] Source: *The Ministry of Health and Long Term Care, Provincial Health Planning Database.*
[14] Source: *The Ministry of Health and Long Term Care, Provincial Health Planning Database.*

Table 20: Coverage of LSCT in the United States

Insurer	Funding Status
Aetna (59)	Coverage of limbal transplantation is described under the clinical policy for *Corneal Graft with Amniotic Membrane Transplantation* (Number: 0293). The procedure is considered medically necessary in members with limbal deficiency (hypofunction or total loss of stem cells) refractory to conventional treatment when the member has any of the following conditions: Total loss of stem cells: (one eye involvement only) ▪ Chemical/thermal injuries of the ocular surface ▪ Stevens-Johnson syndrome ▪ Multiple surgeries or cryotherapies to the limbal region ▪ Contact lens-induced keratopathy or toxic effects from lens-cleaning solutions Hypofunction of stem cells: (one or both eyes can be involved) ▪ Aniridia (hereditary) ▪ Keratitis associated with multiple endocrine deficiency (hereditary) ▪ Neurotrophic keratopathy (neuronal or ischemic) ▪ Chronic limbitis ▪ Peripheral corneal ulcerative keratitis ▪ Pterygium and pseudopterygium
Cigna (60)	Coverage of limbal transplantation is described under the coverage position for *Amniotic Membrane Transplant for the Treatment of Ocular Conditions*. Amniotic membrane transplant is considered medically necessary for the treatment of ocular conditions when there is failure, contraindication, or intolerance to medical therapy (e.g., lubricants/artificial tears, topical and systemic steroids and antibiotics, eyelid taping, patches). References to ocular conditions in the coverage position document include LSCD (partial or total, combined with stem cell graft)

Considerations

Nonpterygium Limbal Stem Cell Deficiency

Many patients with LSCD have bilateral disease so require allogeneic LSCT. These patients will require systemic and topical immunosuppression for several years after the transplant, perhaps indefinitely. Thus, systemic side effects associated with immunosuppression are a potential concern, and patients must be monitored regularly. (9)

Amniotic membrane transplantation is a common addition to many ocular surface reconstruction procedures, including LSCT. Amniotic membranes are recovered from human placentas from planned, uneventful caesarean sections. (27) Before use, serological screening of the donor's blood for syphilis, human immunodeficiency virus, and hepatitis virus B and C should be conducted. However, there is a theoretical risk of disease transmission associated with this procedure. (33)

As there are no standard guidelines for LSCT, patients who receive transplants OOC may not receive care aligned with the best evidence. For example, to date many of the patients sent OOC for LSCTs received concurrent AMTs, and the evidence from this analysis questions the use of this procedure. In addition, 1 patient received a cultured LSCT, a procedure that is considered investigational.

Pterygium

Pterygium recurrence rates after surgical excision without adjuvant therapy are high, ranging from 24% to 89%. (26) However, less than 40% of patients who have a pterygium excision in Ontario receive a CAU

or mucous membrane graft[15] (the adjuvant therapy options included in the Ontario Schedule of Benefits). These data suggest that a substantial fraction of patients with pterygium in Ontario do not receive adjuvant therapy with pterygium excision. According to Ontario clinical experts, the need for adjuvant therapies is limited because the rate of pterygium recurrence is low in Ontario. (Personal communication) While there is evidence that the prevalence of pterygium is higher in the "pterygium belt", (17) there is no evidence from the literature to suggest that recurrence rates or disease severity vary by location or climate.

Financial Impact

There are no costing or cost-effectiveness studies for LSCT to date.

Nonpterygium Limbal Stem Cell Deficiency

Since 1999, Ontario has reimbursed OOC LSCT for 8 patients.[16] The average cost of LSCT per eye[17] was $18,735.20 Cdn (range, $8,219.54–$33,933.32). (E-mail communication) Out-patient facility fees account for 55% to 67% of these costs. In addition, such costs may be inflated, as it was not always possible to determine if unilateral or bilateral transplants were performed, and some costs combined additional procedures (e.g., corneal transplants or AMTs) with the LSCT. However, the actual cost per patient is much higher, as these costs do not include consultations and follow-up visits, and because most patients received multiple LSCTs as well as additional procedures (e.g., corneal transplants) during their course of treatment OOC. When these additional costs were considered, the average cost per patient was $57,583 Cdn (range, $8,219.54–$130,628.20).

The estimated average total cost for performing LSCT in Ontario is $2,291.48 Cdn (range, $951.48–$4,538.48) per patient including hospital and physician fees.[18] This cost is based on the assumption that LSCT is technically similar to a corneal transplant,[19] which needs to be verified. The cost does not include the cost of a corneal transplant, which some proportion of patients receiving a LSCT will require within several months of the limbal transplant.

System Pressures

Nonpterygium Limbal Stem Cell Deficiency

The demand for LSCT in Ontario was estimated by corneal experts at 20 to 50 procedures per year. Over the past 9 years, 8 patients have received LSCTs through the OOC program. Thus, there may be unmet

[15] While mucous membrane grafts are included in the Ontario Schedule of Benefits as a possible adjuvant therapy for pterygium excision, this technology was not found in the literature examined for this analysis.
[16] One patient was reimbursed for OOC LSCT consultations, but they did not receive a transplant.
[17] Based on the amount paid by the Ontario Ministry of Health and Long-Term Care Provider Services OOC Program.
[18] Average total cost per patient was based on results from the Ontario Case Costing Initiative for Day Surgery patients with CCI's 1CC85LAXXK and 1CC85LAXXH and Ontario Health Insurance (OHIP) Schedule of Benefits and Fees. Average total hospital costs include both direct costs (costs that are directly related to the provision of care to the patient and include Nursing (incl. Operating Room, ICU), Diagnostic Imaging, Pharmacy and Labs) and indirect costs (overhead expense relating to the running of hospitals and include administration, finance, human resources, plant operations etc). Source: Ontario Case Costing Initiative, Accessed June 4, 2008: http://www.occp.com/. Physician costs were based on the Ontario Health Insurance (OHIP) Schedule of Benefits and Fees (April 1, 2008). Costs are for a penetrating corneal transplant (fee code: E121) and include assistant and anesthetist fees.
[19] Based on conversation with clinical experts in Ontario. (Personal communication)

demand for LSCT, assuming that some of these procedures are not being performed in Ontario. Since there is no fee code for the procedure, it is not possible to track LSCTs in Ontario.

Pterygium

Over the past 5 fiscal years, the mean number of pterygium excisions performed per year in Ontario was 3181 (range, 2,642–4,587).[20] Mucous membrane transplants and CAU transplants are the only adjuvant therapy options for pterygium excision included in the Ontario Schedule of Benefits, and less than 40%[21] of the patients who had a pterygium excision also received 1 of these listed adjuvant therapies.

Conclusions

Nonpterygium Limbal Stem Cell Deficiency

Successful LSCTs result in corneal re-epithelialization and improved vision in patients with LSCD. However, patients who received concurrent AMT had poorer long-term corneal surface improvements. Conjunctival-limbal autologous transplantation is the treatment option of choice, but if it is not possible, living-related or cadaveric allogeneic transplants can be used. Overall, the benefits of LSCT outweigh the risks and burdens thus resulting in strong recommendations with low to very low-quality evidence as shown in Table 21. (61)

Table 21: Benefits, Risks, and Burdens – Nonpterygium Limbal Stem Cell Deficiency

Benefits	Risks	Burden
▪ Short- and long-term improvement in corneal surface (stable, normal corneal epithelium and decreased vascularization and opacity) ▪ Improvement in vision (visual acuity and functional vision)	▪ Long-term complications are experienced by 8% to 16% of patients ▪ Risks associated with long-term immunosuppression for recipients of allogeneic grafts ▪ Potential risk of induced LSCD in donor eyes	▪ High cost of treatment (average cost per patient via OOC program is $57,583; estimated cost of procedure in Ontario is $2,291.48)

Costs are expressed in Canadian dollars.

GRADE of recommendation: Strong recommendation, low-quality or very low-quality evidence
- benefits clearly outweigh risks and burdens
- case series studies
- strong, but may change if higher-quality evidence becomes available

Pterygium

Conjunctival-limbal autologous transplantation significantly reduced the risk of pterygium recurrence compared with CAU. No other comparison yielded statistically significant results, but CLAU reduced the risk of recurrence compared with MMC. However, the benefit of LSCT in Ontario is uncertain, as the severity and recurrence of pterygium is unknown in Ontario. Risks associated with CLAU transplantation as an adjuvant therapy for pterygium excision are low, as the complication rates suggest that CLAU is a

[20] Source: *The Ministry of Health and Long Term Care, Provincial Health Planning Database.*
[21] Source: *The Ministry of Health and Long Term Care, Provincial Health Planning Database.*

safe treatment option to prevent the recurrence of pterygium. According to GRADE, the recommendations are very weak with moderate-quality evidence[22] as shown in Table 22. (61)

Table 22: Benefits, Risks, and Burdens – Pterygium

Benefits	Risks	Burden
▪ Reduced recurrence; however, if recurrence is low in Ontario, this benefit might be minimal	▪ Long-term complications rare	▪ Increased cost

GRADE of recommendation: Very weak recommendations, moderate-quality evidence

- uncertainty in the estimates of benefits, risks, and burden; benefits, risks, and burden may be closely balanced
- RCTs
- very weak, other alternatives may be equally reasonable

[22] While the evidence in the CLAU versus AMT and CLAU versus MMC comparisons is of low quality, the recommendation is based on the statistically significant reduction in pterygium recurrence observed in the CLAU versus CAU comparison, which based on moderate-quality evidence.

Glossary

Allocation concealment: the process in which the people responsible for recruiting individuals into a study are unaware of the group to which a participant will be allocated, should that person agree to be in the study, thereby avoiding conscious and unconscious selection of individuals into the study

Allogeneic stem cell transplants (also known as a stem cell allograft): transplants in which stem cells collected from one person are transplanted into another person

Aniridia: a genetic condition characterized by the incomplete formation of the iris

Autologous stem cell transplants (also known as a stem cell autograft): transplants in which stem cells collected from one person are transplanted back into the same person

Blepharospasm: spasm or twitching of the eyelid muscle resulting in closure of the eye

Conjunctivalization: the ingrowth of conjunctival epithelium onto the cornea

Deep lamellar keratoplasty: a type of corneal transplantation.

Dellen: shallow cavities in the eye.

Functional vision: vision of 20/200 or better.

Limbal stem cell deficiency: a condition that develops when the limbal stem cells are depleted or destroyed so normal corneal epithelium is no longer produced and is replaced with conjunctival epithelium

Ocular cicatricial pemphidoid: a condition characterized by the development of blisters of mucous membranes

Penetrating keratoplasty: a common type of corneal transplantation

Photophobia: sensitivity to light

Pterygium: a fibrovascular growth on the eye that may extend from the conjunctiva onto the cornea

Pyogenic granuloma: a small growth of granulation tissue that can occur in the eye, in the mouth, or on the skin

Stevens-Johnson syndrome: a disease affecting a person's skin and mucous membranes, which is characterized by painful rashes and blisters and is caused by a drug reaction that separates the upper and lower layers of these body surfaces from each other

Vascularization: the development of new blood vessels

Appendices

Appendix 1 – Search Strategies

Nonpterygium Limbal Stem Cell Deficiency

Search date: April 3, 2008
Databases searched: OVID MEDLINE, MEDLINE In-Process and Other Non-Indexed Citations, EMBASE, Cochrane Library, INAHTA/CRD

Database: Ovid MEDLINE(R) <1996 to March Week 4 2008>
Search Strategy:

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1 exp Eye Diseases/ (127724)
2 exp Eye Injuries/ (4201)
3 exp Limbus Corneae/ (646)
4 exp Cornea/ (15935)
5 (stem cell$ adj2 deficien$).mp. [mp=title, original title, abstract, name of substance word, subject heading word] (352)
6 or/1-5 (135836)
7 exp Stem Cell Transplantation/ (24656)
8 Epithelial Cells/tr [Transplantation] (257)
9 (stem cell$ adj2 (therap$ or transplant$)).mp. [mp=title, original title, abstract, name of substance word, subject heading word] (28111)
10 exp Epithelium, Corneal/tr [Transplantation] (90)
11 exp Amnion/tr [Transplantation] (437)
12 or/7-11 (28669)
13 6 and 12 (919)
14 limit 13 to (english language and humans and yr="2000 - 2008") (597)
15 exp Models, Animal/ (129322)
16 *Cataract/ (4558)
17 14 not (15 or 16) (574)
18 exp Technology Assessment, Biomedical/ or exp Evidence-based Medicine/ (31629)
19 (meta analy$ or metaanaly$ or pooled analysis or (systematic$ adj2 review$)).mp. or (published studies or published literature or medline or embase or data synthesis or data extraction or cochrane).ab. (59824)
20 17 and (18 or 19) (4)
21 17 (574)
22 limit 21 to (case reports or comment or editorial or letter or "review") (325)
23 21 not 22 (249)
24 20 or 23 (251)

Database: EMBASE <1980 to 2008 Week 13>
Search Strategy:

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1 exp Eye Disease/ (303011)
2 exp Eye Injury/ (13214)
3 exp Cornea/ (20259)

4 (stem cell$ adj2 deficien$).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer name] (383)
5 or/1-4 (312664)
6 exp Stem Cell Transplantation/ (26673)
7 ((stem cell$ or amnion or amniotic) adj2 (therap$ or transplant$)).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer name] (30013)
8 6 or 7 (30013)
9 5 and 8 (1634)
10 limit 9 to (human and english language and yr="2000 - 2008") (1194)
11 *cataract/ or *aftercataract/ or *radiation cataract/ or *senile cataract/ or *subcapsular cataract/ or *traumatic cataract/ or *congenital cataract/ (11411)
12 10 not 11 (1189)
13 Animal Model/ (458642)
14 12 not 13 (1165)
15 exp Biomedical Technology Assessment/ or exp Evidence Based Medicine/ (281603)
16 (meta analy$ or metaanaly$ or pooled analysis or (systematic$ adj2 review$) or published studies or published literature or medline or embase or data synthesis or data extraction or cochrane).ti,ab. (57049)
17 15 or 16 (315401)
18 14 and 17 (82)
19 14 (1165)
20 limit 19 to (editorial or letter or note or "review") (470)
21 19 not 20 (695)
22 18 or 21 (743)

Pterygium

Search date: May 16, 2008
Databases searched: OVID MEDLINE, MEDLINE In-Process and Other Non-Indexed Citations, EMBASE, Cochrane Library, INAHTA/CRD

Database: Ovid MEDLINE(R) <1996 to May Week 1 2008>
Search Strategy:
---
1 (pterygium$ or pterygia).mp. or exp Pterygium/ (852)
2 exp Transplantation, Autologous/ (11105)
3 exp Corneal Transplantation/ (3773)
4 exp Conjunctiva/tr [Transplantation] (168)
5 (transplant$ or autograft$ or allograft$).mp. [mp=title, original title, abstract, name of substance word, subject heading word] (190021)
6 transplantation.fs. (36977)
7 or/2-6 (206443)
8 1 and 7 (223)
9 limit 8 to (english language and humans and yr="2000 - 2008") (142)
10 limit 9 to (case reports or comment or editorial or letter) (37)
11 9 not 10 (105)

Database: EMBASE <1980 to 2008 Week 20>
Search Strategy:
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1 (pterygium$ or pterygia).mp. or exp PTERYGIUM/ (1504)
2 exp Autotransplantation/ (5576)
3 exp AUTOGRAFT/ (5825)
4 exp Cornea Transplantation/ (4256)
5 (transplant$ or autograft$ or allograft$).mp. [mp=title, abstract, subject headings, heading word, drug trade name, original title, device manufacturer, drug manufacturer name] (274526)
6 or/2-5 (275807)
7 1 and 6 (310)
8 limit 7 to (human and english language and yr="2000 - 2008") (161)
9 limit 8 to (editorial or letter or note) (26)
10 Case Report/ (990053)
11 8 not (9 or 10) (118)

Appendix 2 – Breakdown of Results by Study

Overall Results (All Graft Types and Etiologies Combined)

Table 1: Corneal Surface Improvement Results – All Graft Types and Etiologies Included

Study	Short-Term Corneal Surface Improvement*	Long-Term Corneal Surface Improvement*
Prospective Case Series		
Dos Santos et al., 2005 (40)		11/33 (33%)
Ivekovic et al., 2005† (41)	10/10 (100%)	10/10 (100%)
Ozdemir et al., 2004 (42)	24‡/24 (100%)	18§/24 (75%)
Weighted Mean Corneal Surface Improvement	**100%**	**58.21%**
Retrospective Case Series		
Shimazaki et al., 2004 (43)	17/32 (53%)	14/32 (44%)
Holland et al., 2003 (44)		23/31 (74%)
Solomon et al., 2002‖ (45)		0/39 (0%)
Yao et al., 2002¶ (46)	34/34 (100%)	32/34 (94%)
Ilari et al., 2002# (47)	19/23 (83%)	7/23 (30%)
Samson et al., 2002 (48)	10/11 (91%)	6/11 (55%)
Daya et al., 2001 (49)	8/10 (80%)	8/10 (80%)
Weighted Mean Corneal Surface Improvement	**80%**	**50%**
Combined Weighted Mean Corneal Surface Improvement	**84.72%**	**52.23%**

*Number successful / number eyes treated (%), success was defined by improved corneal surface, which includes stable corneal epithelium, increased clarity, and decreased vascularization of the cornea.
†Results exclude 5 patients who received AMT only.
‡Two eyes took longer to heal and required AMT to aid in healing (days to re-epithelialize: 210 and 150).
§One eye recorded as a success in the paper received a second graft after 1 year, so counted as long-term failure in this analysis.
‖Only results for first limbal graft included, 11 eyes received more than 1 graft because the initial graft failed.
¶Study presented results for only 34 of 39 patients (34 eyes) due to change of procedure or incomplete follow-up.
#Only results for first limbal graft included,10 eyes received more than 1 graft because the initial graft failed.

Table 2: Improvement in Vision Results – All Graft Types and Etiologies Included*

Study	Improvement in VA (Any)†	Improvement in Functional Vision ($\geq$ 0.1)†
Prospective Case Series		
Ivekovic et al., 2005‡ (41)	10/10 (100%)	6/10 (60%)
Ozdemir et al., 2004 (42)	16/21 (76%)	12/21 (57%)
Weighted mean proportion patients with improved vision	**84%**	**58%**
Retrospective Case Series		
Samson et al., 2002 (48)	1/5 (20%)	1/5 (20%)
Daya et al., 2001 (49)	6/7 (86%)	5/7 (71%)
Weighted mean proportion patients with improved vision	**58%**	**50%**
Combined weighted mean proportion patients with improved vision	**76.74%**	**55.81%**

*VA refers to visual acuity.
†N eyes with improved VA or vision / number eyes treated (%).
‡Results exclude 5 patients who received AMT only.

Results Stratified by Graft Type

Conjunctival-Limbal Autograft

Table 3: Improvement in Corneal Surface by Graft Type: Conjunctival-Limbal Autologous Transplantation

Study	Short-Term Corneal Surface Improvement*	Long-Term Corneal Surface Improvement*
Prospective Case Series		
Dos Santos et al., 2005 (40)		8/10 (80%)
Ivekovic et al., 2005† (41)	10/10 (100%)	10/10 (100%)
Ozdemir et al., 2004 (42)	15‡/15 (100%)	14§/15 (93%)
Weighted Mean Corneal Surface Improvement	**100%**	**91.43%**
Retrospective Case Series		
Shimazaki et al., 2004 (43)	10/11 (91%)	9/11 (82%)
Yao et al., 2002‖ (46)	34/34 (100%)	32/34 (94%)
Samson et al., 2002 (48)	1/1 (100%)	1/1 (100%)
Weighted Mean Corneal Surface Improvement	**98%**	**91%**
Combined Weighted Mean Corneal Surface Improvement	**98.59%**	**91.36%**

*Number successful / number eyes treated (%), success defined by improved corneal surface includes stable corneal epithelium, increased clarity, and decreased vascularization of the cornea.
†Results exclude 5 patients who received AMT only.
‡2 eyes took longer to heal and required AMT to aid in healing (days to re-epithelialize: 210 and 150).
§1 eye recorded as a success in the paper received a second graft after 1 year, so counted as long-term failure in this analysis.
‖Study presented results for only 34 of 39 patients (34 eyes) due to change of procedure or incomplete follow-up.

Table 4: Improvement in Vision Results by Graft Type: Conjunctival-Limbal Autologous Transplantation* (Prospective Case Series Only)

Study	Improvement in VA (Any)†	Improvement in Functional Vision ($\geq$ 0.1)†
Ivekovic et al., 2005‡ (41)	10/10 (100%)	6/10 (60%)
Ozdemir et al., 2004 (42)	14/15 (93%)	11/15 (73%)
Weighted mean proportion patients with improved vision	**96.00%**	**68.00%**

*VA refers to visual acuity.
†N eyes with improved VA or vision / number eyes treated (%).
‡Results exclude 5 patients who received AMT only.

Living-Related Conjunctival-Limbal Allograft

Table 5: Improvement in Corneal Surface by Graft Type: Living-Related Conjunctival-Limbal Allogeneic Transplantation

Study	Short-Term Corneal Surface Improvement*	Long-Term Corneal Surface Improvement*
Prospective Case Series		
Dos Santos et al, 2005 (40)		3/23 (13%)
Ozdemir et al., 2004 (42)	9/9 (100%)	4/9 (44%)
Weighted Mean Corneal Surface Improvement	**100%**	**21.88%**
Retrospective Case Series		
Samson et al., 2002 (48)	9/10 (90%)	5/10 (50%)
Daya et al., 2001 (49)	8/10 (80%)	8/10 (80%)
Weighted Mean Corneal Surface Improvement	**85%**	**65%**
Combined Weighted Mean Corneal Surface Improvement	**89.66%**	**38.46%**

*Number successful / number eyes treated (%), success defined by improved corneal surface includes stable corneal epithelium, increased clarity, and decreased vascularization of the cornea.

Table 6: Improvement in Vision Results by Graft Type: Living-Related Conjunctival-Limbal Allogeneic Transplantation*

Study	Improvement in VA (Any)†	Improvement in Functional Vision ($\geq$ 0.1)†
Prospective Case Series		
Ozdemir et al., 2004 (42)	**2/6 (33%)**	**1/6 (17%)**
Retrospective Case Series		
Samson et al., 2002 (48)	1/5 (20%)	1/5 (20%)
Daya et al., 2001 (49)	6/7 (86%)	5/7 (71%)
Weighted mean proportion patients with improved vision	**58.33%**	**50.00%**
Combined weighted mean proportion patients with improved vision	**50.00%**	**38.89%**

*VA refers to visual acuity.
†N eyes with improved VA or vision / number eyes treated (%).

Keratolimbal Allograft

Table 7: Improvement in Corneal Surface by Graft Type: Keratolimbal Allogeneic Transplantation (Retrospective Case Series Only)

Study	Short-Term Corneal Surface Improvement*	Long-Term Corneal Surface Improvement*
Shimazaki et al., 2004 (43)	7/21 (33%)	5/21 (24%)
Holland et al., 2003 (44)		23/31 (74%)
Solomon et al., 2002† (45)		0/39 (0%)
Ilari et al., 2002‡ (47)	19/23 (83%)	7/23 (30%)
Weighted Mean Corneal Surface Improvement	**59.09%**	**30.70%**

*Number successful / number eyes treated (%), success defined by improved corneal surface includes stable corneal epithelium, increased clarity, and decreased vascularization of the cornea.
†Only results for first limbal graft included, 11 eyes received more than 1 graft because the initial graft failed.
‡Only results for first limbal graft included, 10 eyes received more than 1 graft because the initial graft failed.

Results Stratified by Etiology

Ocular Burns

Table 8: Improvement in Corneal Surface by Etiology: Ocular Burns

Study	Short-Term Corneal Surface Improvement*	Long-Term Corneal Surface Improvement*
Prospective Case Series		
Dos Santos et al., 2005 (40)		10/22 (45%)
Ivekovic et al., 2005† (41)	10/10 (100%)	10/10 (100%)
Ozdemir et al., 2004 (42)	24‡/24 (100%)	18§/24 (75%)
Weighted Mean Corneal Surface Improvement	100.00%	67.86%
Retrospective Case Series		
Shimazaki et al., 2004 (43)	17/32 (53%)	14/32 (44%)
Solomon et al., 2002‖ (45)		0/16 (0%)
Yao et al., 2002¶ (46)	34/34 (100%)	32/34 (94%)
Ilari et al., 2002# (47)	6/8 (75%)	2/8 (25%)
Daya et al., 2001 (49)	1/2 (50%)	1/2 (50%)
Weighted Mean Corneal Surface Improvement	76.32%	53.26%
Combine Weighted Mean Corneal Surface Improvement	83.64%	58.78%

*Number successful / number eyes treated (%), success defined by improved corneal surface includes stable corneal epithelium, increased clarity, and decreased vascularization of the cornea.
†Results exclude 5 patients who received AMT only.
‡2 eyes took longer to heal and required AMT to aid in healing (days to re-epithelialize: 210 and 150).
§1 eye recorded as a success in the paper received a second graft after 1 year, so counted as long-term failure in this analysis.
‖Only results for first limbal graft included, 5 eyes received more than 1 graft because the initial graft failed.
¶Study presented results for only 34 of 39 patients (34 eyes) due to change of procedure or incomplete follow-up.
#Only results for first limbal graft included, 4 eyes received more than 1 graft because the initial graft failed.

Table 9: Improvement in Vision Results by Etiology: Ocular Burns*

Study	Improvement in VA (Any)†	Improvement in Functional Vision (≥ 0.1)†
Prospective Case Series		
Ivekovic et al., 2005‡ (41)	10/10 (100%)	6/10 (60%)
Ozdemir et al., 2004 (42)	16/21 (76%)	12/21 (57%)
Weighted mean proportion patients with improved vision	**83.87%**	**58.06%**
Retrospective Case Series		
Daya et al., 2001 (49)	**0/1 (0%)**	**0/1 (0%)**
Combined weighted mean proportion patients with improved vision	**81.25%**	**56.25%**

*VA refers to visual acuity.
†N eyes with improved VA or vision / number eyes treated (%).
‡Results exclude 5 patients who received AMT only.

Stevens-Johnson Syndrome

Table 10: Improvement in Corneal Surface by Etiology: Stevens-Johnson Syndrome

Study	Short-Term Corneal Surface Improvement*	Long-Term Corneal Surface Improvement*
Prospective Case Series		
Dos Santos et al., 2005 (40)		**1/11 (9%)**
Retrospective Case Series		
Solomon et al., 2002† (45)		0/9 (0%)
Ilari et al., 2002‡ (47)	6/7 (86%)	2/7 (29%)
Samson et al., 2002 (48)	8/8 (100%)	4/8 (50%)
Daya et al., 2001 (49)	3/3 (100%)	3/3 (100%)
Weighted Mean Corneal Surface Improvement	**94.44%**	**33.33%**
Combined Weighted Mean Corneal Surface Improvement	**94.44%**	**26.32%**

*Number successful / number eyes treated (%), success defined by improved corneal surface includes stable corneal epithelium, increased clarity, and decreased vascularization of the cornea.
†Only results for first graft included, 2 eyes received more than 1 graft.
‡Only results for first graft included, 4 eyes received more than 1 graft.

Table 11: Improvement in Vision Results by Etiology: Stevens-Johnson Syndrome (Retrospective Case Series Only)*

Study	Improvement in VA (Any)†	Improvement in Functional Vision (≥ 0.1)†
Samson et al., 2002 (48)	1/4 (25%)	1/4 (25%)
Daya et al., 2001 (49)	3/3 (100%)	2/3 (67%)
Weighted mean proportion patients with improved vision	**57.14%**	**42.86%**

*VA refers to visual acuity.
†N eyes with improved VA or vision / number eyes treated (%).

Aniridia

Table 12: Improvement in Corneal Surface by Etiology: Aniridia (Retrospective Case Series Only)

Study	Short-Term Corneal Surface Improvement*	Long-Term Corneal Surface Improvement*
Holland et al., 2003 (44)		23/31 (74%)
Solomon et al., 2002† (45)		0/1 (0%)
Weighted Mean Corneal Surface Improvement		**71.88%**

*Number successful / number eyes treated (%), success defined by improved corneal surface includes stable corneal epithelium, increased clarity, and decreased vascularization of the cornea.
†Only results for first graft included.

Results Stratified by Amniotic Membrane Transplantation

Amniotic Membrane Transplantation Overall Results (All Graft Types and Etiologies Combined)

Table 13: Improvement in Corneal Surface by Amniotic Membrane Transplantation

Study	Short-Term Corneal Surface Improvement*	Long-Term Corneal Surface Improvement*
AMT + Limbal Graft		
Prospective Case Series		
Dos Santos et al., 2005 (40)		**11/33 (33%)**
Retrospective Case Series		
Shimazaki et al., 2004 (43)	17/32 (53%)	14/32 (44%)
Solomon et al., 2002† (45)		0/39 (0%)
Weighted Mean Corneal Surface Improvement	**53.13%**	**19.72%**
Combined Weighted Mean Corneal Surface Improvement	**53.13%**	**24.04%**
Limbal Graft Alone (No AMT)		
Retrospective Case Series		
Holland et al., 2003 (44)		23/31 (74%)
Yao et al., 2002‡ (46)	34/34 (100%)	32/34 (94%)
Daya et al., 2001 (49)	8/10 (80%)	8/10 (80%)
Weighted Mean Corneal Surface Improvement	**95.45%**	**84.00%**

*Number successful / number eyes treated (%), success defined by improved corneal surface includes stable corneal epithelium, increased clarity, and decreased vascularization of the cornea.
†Only results for first graft included, 11 eyes received more than 1 graft.
‡Study presented results for only 34 of 39 patients (34 eyes) due to change of procedure or incomplete follow-up.

Amniotic Membrane Transplantation Analysis Results Stratified by Graft Type

Conjunctival-Limbal Autograft

Table 14: Improvement in Corneal Surface by Amniotic Membrane Transplantation and Graft Type: Conjunctival-Limbal Autologous Transplantation

Study	Short-Term Corneal Surface Improvement*	Long-Term Corneal Surface Improvement*
Limbal Graft + Amniotic Membrane Transplantation		
Prospective Case Series		
Dos Santos, et al., 2005 (40)	**10/10 (100%)**	**8/10 (80%)**
Retrospective Case Series		
Shimazaki et al., 2004 (43)	**10/11 (91%)**	**9/11 (82%)**
Combined Weighted Mean Corneal Surface Improvement	**95%**	**81%**
Limbal Graft Alone (No Amniotic Membrane Transplantation)		
Retrospective Case Series		
Yao et al., 2002† (46)	34/34 (100%)	32/34 (94%)
Samson et al., 2002 (48)	1/1 (100%)	1/1 (100%)
Weighted Mean Corneal Surface Improvement	**100%**	**94.29%**

*Number successful / number eyes treated (%), success defined by improved corneal surface includes stable corneal epithelium, increased clarity, and decreased vascularization of the cornea.
†Study presented results for only 34 of 39 patients (34 eyes) due to change of procedure or incomplete follow-up.

Living-Related Conjunctival-Limbal Allograft

Table 15: Improvement in Corneal Surface by Amniotic Membrane Transplantation and Graft Type: Living-Related Conjunctival-Limbal Allogeneic Transplantation

Study	Short-Term Corneal Surface Improvement*	Long-Term Corneal Surface Improvement*
Limbal Graft + Amniotic Membrane Transplantation		
Prospective Case Series		
Dos Santos et al., 2005 (40)	**12/23 (52%)**	**3/23 (13%)**
Limbal Graft Alone (No Amniotic Membrane Transplantation)		
Retrospective Case Series		
Daya et al., 2001 (49)	**8/10 (80%)**	**8/10 (80%)**

*Number successful / number eyes treated (%), success defined by improved corneal surface includes stable corneal epithelium, increased clarity, and decreased vascularization of the cornea.

Keratolimbal Allograft

Table 16: Improvement in Corneal Surface by Amniotic Membrane Transplantation and Graft Type: Keratolimbal Allogeneic Transplantation

Study	Short-Term Corneal Surface Improvement*	Long-Term Corneal Surface Improvement*
Limbal Graft + Amniotic Membrane Transplantation		
Retrospective Case Series		
Shimazaki et al., 2004 (43)	7/21 (33%)	5/21 (24%)
Solomon et al., 2002† (45)		0/39 (0%)
Weighted Mean Corneal Surface Improvement	**33.33%**	**8.33%**
Limbal Graft Alone (No Amniotic Membrane Transplantation)		
Retrospective Case Series		
Holland et al., 2003 (44)		**23/31 (74%)**

*Number successful / number eyes treated (%), success defined by improved corneal surface includes stable corneal epithelium, increased clarity, and decreased vascularization of the cornea.
†Only results for first graft included, 11 eyes received more than 1 graft.

Amniotic Membrane Transplantation Analysis Results by Etiology

Ocular Burns

Table 17: Improvement in Corneal Surface by Amniotic Membrane Transplantation and Etiology: Ocular Burns

Study	Short-Term Corneal Surface Improvement*	Long-Term Corneal Surface Improvement*
Limbal Graft + Amniotic Membrane Transplantation		
Prospective Case Series		
Dos Santos et al., 2005 (40)		**10/22 (45%)**
Retrospective Case Series		
Shimazaki et al., 2004 (43)	17/32 (53%)	14/32 (44%)
Solomon et al., 2002 (45)		0/16 (0%)
Weighted Mean Success Rate	**53.13%**	**29.17%**
Combined Weighted Mean Corneal Surface Improvement	**53.13%**	**34.29%**
Limbal Graft Alone (No Amniotic Membrane Transplantation)		
Retrospective Case Series		
Yao et al., 2002 (46)	34/34 (100%)	32/34 (94%)
Daya et al., 2002 (49)	1/2 (50%)	1/2 (50%)
Weighted Mean Corneal Surface Improvement	**97.22%**	**91.67%**

*Number successful / number eyes treated (%), success defined by improved corneal surface includes stable corneal epithelium, increased clarity, and decreased vascularization of the cornea.

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