Institution: Newcastle University		
Unit of Assessment: UoA1		
Title of case study: Human-derived limbal cell transplant to treat chemical burns of the eye		
Period when the underpinning research was undertaken: 2008-2017		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s):	**Role(s) (e.g. job title):**	**Period(s) employed by submitting HEI:**
Prof Francisco Figueiredo	Honorary Clinical Professor	1/12/05 to present
Prof Majlinda Lako	Professor of Stem Cell Science	1/2/03 to present
Prof Anne Dickinson	Professor of Marrow Transplant Biology	1/4/91 to present
Dr Sai Kolli	Clinical Research Associate	10/4/06 to 30/9/10
Dr Oliver Baylis	Clinical Research Associate	4/8/10 to 6/8/13
Dr Gustavo Figueiredo	Clinical Research Associate	8/2/13 to 7/2/16
Dr Sanja Bojic	Research Associate	21/10/14 to present
Dr Min Yu	Research Associate	15/9/14 to 31/5/18
Prof Che Connon	Professor of Tissue Engineering	1/9/14 to present
Dr Sajjad Ahmad	Honorary Clinical Lecturer	1/12/07 to 1/12/11
Dr Ricardo Gouveia	Research Associate	1/9/14 to present
Dr Julian de Havilland	Quality Control & Production Manager	20/4/12 to present
Dr Charles Osei Bempong	PhD Student	2011 to 2015
Dr Taty Kamarudin	PhD Student	2014 to 2017
Period when the claimed impact occurred: 2013-present		
Is this case study continued from a case study submitted in 2014? No		

1. Summary of the impact (indicative maximum 100 words)

Limbal stem cell deficiency (LSCD) is a sight-threatening rare disease most commonly caused by chemical burns through accident or attacks. LSCD also causes the failure of corneal transplants used to try to reverse the damage. Newcastle research improved existing treatments by creating the first animal-free autologous cultivated limbal epithelial stem cell transplantation (Auto-CLET) for unilateral cases, and autologous oral mucosa epithelium (Auto-OME) for bilateral cases. The technology has successfully treated all patients referred to the service from all over the UK. Of the subsequent corneal transplants, 90% remain successful 12 months post-surgery, substantially improving patient quality of life and independence.

2. Underpinning research (indicative maximum 500 words)

Limbal stem cell deficiency: cause and prevalence

Limbal stem cells are located at the corneal limbus, creating a barrier between conjunctival and corneal epithelial cells. In limbal stem cell deficiency (LSCD), the corneal epithelium cannot repair itself, leading to scarring, chronic inflammation and conjunctivalisation - invasion of the conjunctiva by epithelial cells and blood vessels (Figure 1). LSCD can affect one eye (unilateral) or both eyes (bilateral) and usually results in blindness and chronic pain.

Severe or total LCSD is most frequently caused by chemical burns to the ocular surface[1], from either strong acids or bases. Recent data found the annual incidence of LSCD caused by severe chemical injury to be 5.6 new cases per 100,000 population[2], more than half of all annual LSCD cases.

[1] Cartes C, Lako M, Figueiredo FC. (2020) Referral Patterns of Patients with Limbal Stem Cell Deficiency to a Specialized Tertiary Center in the United Kingdom. Ahead of publication. Available on request

[2] Ghosh S et al. (2019) Acute chemical eye injury and limbal stem cell deficiency - A prospective Study in the UK. *Cornea*. 38:8-12. DOI. 10.1097/ICO.0000000000001739.

LSCD is both costly and has a substantial adverse impact on a patient's quality of life. The relative rarity of LSCD means that little comprehensive patient demographic information exists. The substantial patient morbidity necessitates long-term follow-up, frequent hospital admission for intensive treatment and, in cases of bilateral LSCD, most patients are registered as severely sight impaired and require social support for vision loss.

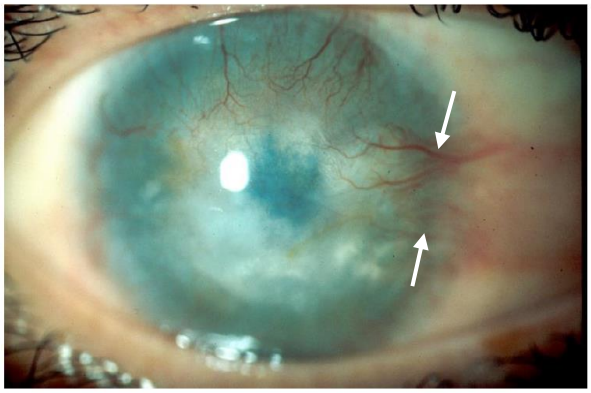

Figure 1. An eye with signs of total LSCD. Arrows indicate invasion of vessels over the cornea, exemplifying conjunctivalisation of the cornea and associated deep scar formation.

Current LSCD treatment and development of Auto-CLET

An orphan disease due to its relative rarity, LSCD has little interest from the pharmaceutical industry because of low financial return on developed treatments. Therefore, there are currently few treatment options for LSCD patients. One treatment involves transplantation of stem cells collected from either biopsied limbal cells (for unilateral LSCD) or oral mucosa epithelium (OME; for bilateral LSCD). Both types are subsequently expanded *ex vivo* on animal-derived culture systems, which have an increased risk of graft rejection and, since they are potentially non-sterile, of pathogen transfer. Additionally, only half of LSCD patients are eligible to receive existing technology which use this culture system. In response, Newcastle investigated whether the non-human animal culture components could be replaced by good manufacturing practice (GMP) components (R1) and be used to treat those LSCD patients who currently have no available treatment options.

Newcastle research showed that both limbal and OME stem cells could be successfully cultured, expanded on human amniotic membrane and subsequently transplanted (R1, R2, R3 and R4). This resulted in the first successful transplantations of both autologous animal-free e*x-vivo* expanded human corneal epithelium (R3) and OME-containing stem cells (R4). The technology was improved further by using gamma-irradiated human amniotic membrane which significantly increased the explant expansion rate without affecting limbal stem cell phenotype, as well as providing a sterile starting substrate for explant expansion (R5).

This led to the development of the first human- and GMP-derived Auto-CLET (autologous cultivated limbal epithelial stem cell transplantation) and Auto-OME technologies, the UK patent applications for which were filed 16th February 2015 (GB1402786.6 and PCT/GB2015/050434). Newcastle was granted orphan designation status for this technology by the European Medicines Agency in 2013 (EU/3/13/1168)[3].

3. References to the research (indicative maximum of six references)

SciVal field-weighted citation impact (FWCI) as of December 2020. Newcastle researchers in **bold.**

R1. **Yu M**, **Bojic S**, **Figueiredo GS**, Rooney P, **de Havilland J**, **Dickinson A**, **Figueiredo FC**, **Lako M**. (2016) An important role for adenine, cholera toxin, hydrocortisone and triiodothyronine in the proliferation, self-renewal and differentiation of limbal stem cells in vitro. *Experimental Eye Research*. 152(1):113-122. DOI: 10.1016/j.exer.2016.09.008. FWCI: 1.38.

R2. **Kolli S**, **Lako M**, **Figueiredo F**, Mudhar H, **Ahmad S**. (2008) Loss of corneal epithelial stem cell properties in outgrowths from human limbal explants cultured on intact amniotic membrane. *Regenerative Medicine*. 3(3):329-42. DOI: 10.2217/17460751.3.3.329. FWCI: 3.06.

[3] https://www.ema.europa.eu/en/medicines/human/orphan-designations/eu3131168

R3. **Kolli S**, **Ahmad S**, **Lako M**, **Figueiredo F**. (2010) Successful Clinical Implementation of Corneal Epithelial Stem Cell Therapy for Treatment of Unilateral Limbal Stem Cell Deficiency. *Stem Cells*. 28(3):597-610. DOI: 10.1002/stem.276. FWCI: 3.74.

R4. **Kolli S**, **Ahmad S**, Mudhar HS, Meeny A, **Lako M**, **Figueiredo FC**. (2014) Successful application of ex vivo expanded human autologous oral mucosal epithelium for the treatment of total bilateral limbal stem cell deficiency. *Stem Cells*. 32(8):2135-46. DOI: 10.1002/stem.1694. FWCI: 2.76.

R5. **Figueiredo GF**, **Bojic S**, Rooney P, Wilshaw S-P, **Connon CJ**, **Gouveia RM**, Paterson C, Lepert G, Mudhar HS, **Figueiredo FC**, **Lako M**. (2017) Gamma-irradiated human amniotic membrane decellularised with sodium dodecyl sulfate is a more efficient substrate for the *ex vivo* expansion of limbal stem cells. *Acta Biomaterialia*. 61:124-133. DOI: 10.1016/j.actbio.2017.07.041. FWCI: 1.11.

4. Details of the impact (indicative maximum 750 words)

Auto-CLET and Auto-OME for the treatment of both unilateral and bilateral LSCD

The most significant impact of Auto-CLET and Auto-OME has been the treatment of LSCD patients, substantially improving their quality of life. Since August 2013, 16 patients have received Auto-CLET transplants, referred to Newcastle from across the UK (EV1). A further 4 patients were scheduled to be treated with Auto-CLET technology in 2020, but these procedures had to be rescheduled because the COVID-19 pandemic delayed the revalidation of the GMP laboratory (EV1). No transplants with Auto-OME were required in the same time frame.

Due to the rare nature of LSCD, the 25 patients treated so far, including the 9 patients in the phase I trial (R3), are all of those in the UK eligible for this treatment. The Director of NHS Newcastle Advanced Therapies, the GMP laboratory where Auto-CLET is cultured, confirms that "*This represents all LSCD patients eligible for Auto-CLET treatment (who were not eligible for any other technologies) who were referred to our service during this time period.* (EV1).

Furthermore, in 2018 an agreement with the NHS was reached where all new LSCD patients in Cumbria, Northumberland and Tyne and Wear would be offered limbal stem cell treatment as part of a commissioned service from Newcastle Hospitals (EV2). This was recognised as "*great news for the patients who will benefit from the treatment and is testament to the great working relationships across Newcastle Hospitals and Newcastle University to be able to translate research into outstanding, cutting edge clinical care.*" (EV2).

Auto-CLET for the avoidance of corneal transplant rejection/failure and improved visual acuity

Reduced vision in LSCD patients is exclusively due to corneal pathology as the rest of eye is otherwise healthy. While traditional corneal transplant, or penetrating keratoplasty (PKP), would restore vision in these patients, the successful outcome of PKP transplantation in LSCD patients is extremely poor without prior stabilisation of the limbal stem cell environment[4]. Since Auto-CLET corrects LSCD, the environment becomes suitable for subsequent PKP, with consequent surgical success and significant improvement in sight (EV3).

Follow up of Auto-CLET patients from a 2015 phase II trial (EU Clinical Trials Registry Number: 2011-000608) showed that 91% of Auto-CLET-treated LSCD patients retained functioning corneal transplants 12 months after receiving PKP. Other studies investigating the survival of high-risk PKP grafts without equivalent Auto-CLET treatment report a 12 month graft survival rate of 80% and 87%[5]. The epithelium remains stable after PKP, indicating that the Auto-CLET graft is not damaged by subsequent corneal transplants (EV3), at 32 months after PKP transplant 72% of transplants are retained in Auto-CLET patients (EV3, Figure 2).

[4] Sacchetti et al. (2018) Limbal Stem Cell Transplantation: Clinical Results, Limits, and Perspectives. *Stem Cells International*. DOI: https://doi.org/10.1155/2018/8086269

[5] Basu et al. (2011) Clinical Outcomes of Penetrating Keratoplasty After Autologous Cultivated Limbal Epithelial Transplantation for Ocular Surface Burns. *American Journal of Ophthalmology*. 152(6): P917-924.E1. DOI: https://doi.org/10.1016/j.ajo.2011.05.019

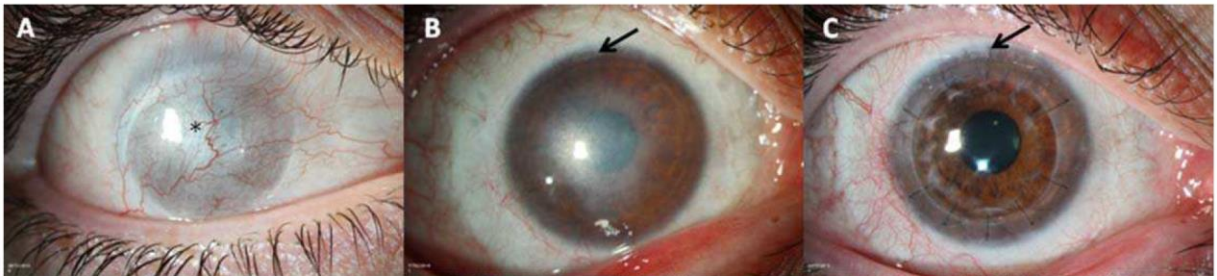

Figure 2. LCSD patient eye (A) before Auto-CLET treatment, (B) 12 months after Auto-CLET with central deep corneal scar and poor sight despite successful LSC transplantation and (C) 36 months post-PKP with clear cornea, normal sight and comfortable eye. The arrow in B and C indicates the location of Auto-CLET transplantation (EV3).

Follow-up of patients from the phase II clinical trial also assessed changes in visual acuity every 6 months following Auto-CLET and PKP. The best-corrected visual acuity of patients significantly improved from a mean 1.46 logMAR to 0.74 logMAR. This is the equivalent of reading 36 additional letters on a logMAR chart, the widely-used chart containing letters of decreasing size. Of significance, after PKP 60% of patients had regained sufficient visual acuity to meet UK driving standards (EV3), offering a substantial increase in independence, and most patients return to work shortly after Auto-CLET transplant and recovery (EV1). Restoring sight and removing the need for pain management not only improves patient quality of life but dramatically reduces the socio-economic burden of the disease.

Summary
UK LSCD patients who have received Auto-CLET transplants have longer lasting retention of subsequent corneal transplants. This in turn measurably improves patient visual acuity and reduces chronic pain. As the first LSCD treatment developed using all non-animal components, Auto-CLET and Auto-OME, developed by Newcastle University, represents a vital treatment of patients with this very rare orphan disease.

5. Sources to corroborate the impact (indicative maximum of 10 references)

EV1. Letter of support from the Director of Newcastle Advanced Therapies. PDF.
EV2. Letter of support from Russell Watkins confirming that Limbal stem cell transplantation commissioning from Newcastle Hospitals for Cumbria, Northumberland and Tyne & Wear Local Area Teams. PDF.
EV3. Figueiredo GS, et al. (2018) Outcomes of penetrating keratoplasty following autologous cultivated limbal epithelial stem cell transplantation. *Stem Cells*. 36(6):925-931. DOI: 10.1002/stem.2803.