Severe Ocular Graft Versus-host Disease: When Living-related Donors Can Help Twice

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INTRODUCTION

Allogenic hematopoietic stem cell transplantation (allo-HSCT) is presently the treatment of choice for a variety of malignant hematologic disorders.[1] Stem cells can be donated by relatives (when available), or by healthy volunteers. The aim of HSCT is to obtain the continued remission of the malignancy through the allo-response ("graft versus-leukemia"). Unfortunately, this phenomenon is intimately associated with graft versus-host disease (GVHD), an autoimmune-like reaction directed against a broad spectrum of recipient tissues, including the eye.

The inflammation of the ocular surface is the hallmark of chronic ocular GVHD, and similarly to other autoimmune-mediated ocular surface diseases, the inflammatory damage determines fibrotic changes of the ocular surface and adnexa, which are obstacles for the effective treatment of this condition. The most common clinical features consist of dryness, conjunctival fibrosis, punctate and/or filamentous keratopathy, superior limbic keratoconjunctivitis (SLK), recurrent erosions, chronic blepharitis, atrophy and irregularity of the eyelid margin, which may eventually induce keratinization of the tarsal conjunctiva and symblephara. In most severe cases, on one side SLK-related inflammation can potentially result in permanent limbal stem cell deficiency (LSCD), characterized by conjunctivalization and keratinization of the cornea with superficial and deep vascularization, as well as persistent epithelial defects. On the other side, immune-mediated inflammation of the bulbar conjunctiva can determine the destruction of goblet cells, leading to dry eye, sterile corneal ulcer, melting and perforation.[2] At this stage, there is no chance of visual rehabilitation through conventional management with topical anti-inflammatory treatment, including corticosteroids and cyclosporine.

Recently, our Group proposed for the first time the use of conjunctival-limbal graft obtained from the same living-related bone marrow donor for the treatment of severe GVHD patients with corneal neovascularization and/or perforation.[3] This approach is able to address both LSCD and extreme dryness utilizing the phenomenon of "chimerism" and the consequent tolerance to tissue

transplanted from the same donor at the same or later time, thus bypassing the allo-reactive response of GVHD.[4-6] Unfortunately, this technique is feasible only in GVHD patients who previously received stem cells from relatives, which account for about half of all cases.

We report herein the different clinical course and outcomes of two representative cases of patients with severe ocular GVHD, who received respectively conjunctival-limbal graft from the same living-related bone marrow donor (Patient #1), and conventional treatment (Patient #2).

CONJUNCTIVAL-LIMBAL GRAFT: SURGICAL TECHNIQUE

Westcott scissors are used to dissect from the donor eye the trapezoidal-shaped superior conjunctival tissue (about 10 x 5 mm) attached to a lamellar kerato-limbal tissue containing the limbal stem cells (about 10 x 1 mm) (Figure 1).

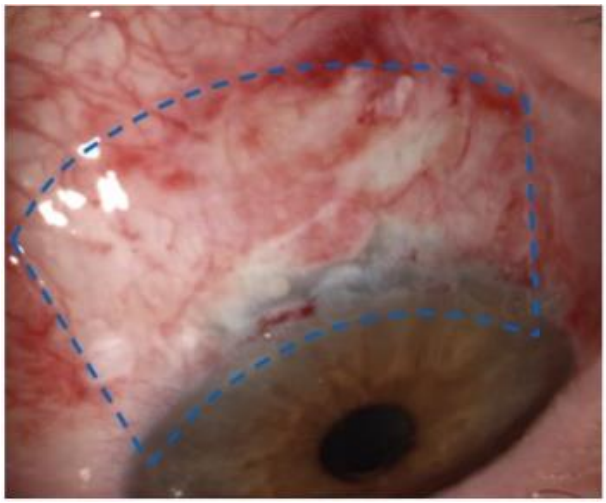

Figure 1: Clinical picture illustrating the harvest site of the donor eye outlined by the blue dotted line.

Then, the dissection and removal of a superior kerato-limbal and conjunctival lenticule of similar size is performed in the recipient eye. The harvested tissue is sutured into place using interrupted 8-0 vicryl stitches, which are reabsorbed within few weeks, taking care to maintain the orientation of the graft. Topical betamethasone/chloramphenicol/rolitetracycline ointment is applied, and the eye is pressure patched for 48 hours. No systemic immunosuppression is required either pre- or postoperatively.

CASE PRESENTATION - PATIENT #1

A 59-year-old man was referred to our Institution for his recalcitrant severe ocular GHVD with intense photophobia and bilateral visual impairment. In 2005, he had been treated for acute myeloid leukemia with an allo-HSCT procedure, with his sister as donor. Few years later, he developed severe ocular GVHD that was treated with topical and systemic immunosuppressants. Superficial lamellar keratectomy followed by amniotic membrane transplantation had been performed in both eyes (OU), and uneventful cataract surgery in right eye (OD). Two episodes of corneal perforations occurred in left eye (OS): the first one was treated with cyanoacrylate glue and staged penetrating keratoplasty (PK); the second one with conjunctival flap according to Gundersen technique and temporary complete tarsorraphy.

Upon presentation, slit-lamp examination revealed in OD severe meibomian gland dysfunction (MGD), intense conjunctival injection, superficial and deep corneal neovascularization involving the visual axis (Figure 2, part A). His OS presented severe MGD, intense conjunctival injection and conjunctival flap covering the corneal graft with an area of focal thinning (descemetocele) in the supero-nasal quadrant (Figure 2, part B).

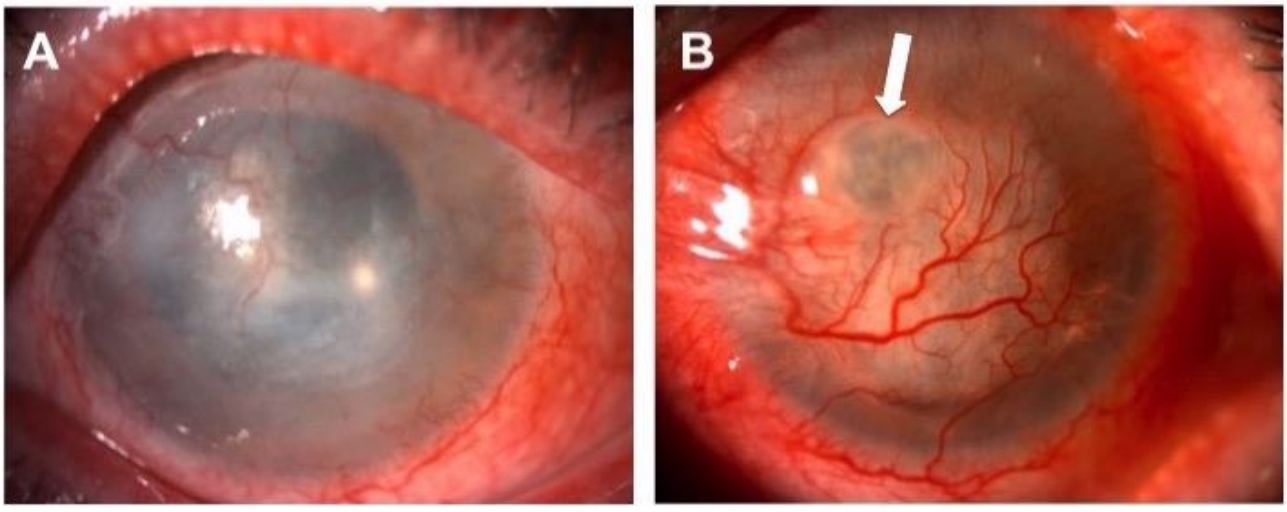

Figure 2: Clinical appearance of both eyes of Patient #1 at the time of presentation. Part A: Severe MGD, intense conjunctival injection, superficial and deep corneal neovascularization involving the visual axis in OD. Part B: Severe MGD, intense conjunctival injection, conjunctival flap covering the corneal graft. Note the area of focal thinning (descemetocele) (white arrow) in the supero-nasal quadrant.

The Ocular Surface Disease Index (OSDI) score was 89, while Schirmer Test type I measured below 1 mm/5' in OU. Visual acuity was limited to 20/200 in OD and to light perception in OS. The patient's general health was good, with a normal blood cell count and no other organs involved by GVHD. The intense ocular surface inflammation was managed aggressively in OU with topical corticosteroid (0.3% hydrocortisone sodium phosphate) 4 times daily (qid), cyclosporine 1mg/mL twice daily (bid), trehalose/hyaluronate-based tear substitute every 2 hours, rolitetracycline/betamethasone/chloramphenicol ointment at bedtime, warm compresses and lid hygiene. However despite treatment, clinical picture remained approximately unchanged in OU, while anterior-segment Optical Coherence Tomography documented a progressive focal thinning of the cornea in OS. Therefore, we decided to proceed in this eye with transplantation a graft including conjunctiva and limbus obtained from the same living-related bone marrow donor (sister), according to the technique described above. Surgery was uneventful, and as early as one month postoperatively, patient's discomfort symptoms and signs of ocular surface inflammation began to improve. Six months postoperatively, the OSDI score decreased to 28, and Schirmer Test type I increased to 4 mm/5' in OS. Furthermore, conjunctival injection and peripheral corneal neovessels decreased significantly, with an increased thickness at the site of descemetocele (Figure 3, part A). Conjunctival and corneal cells with donor chromosomes (XX, female) were identified in the recipient bed by fluorescence in situ hybridization 6 months after surgery (FISH) (Figure 3, part B).

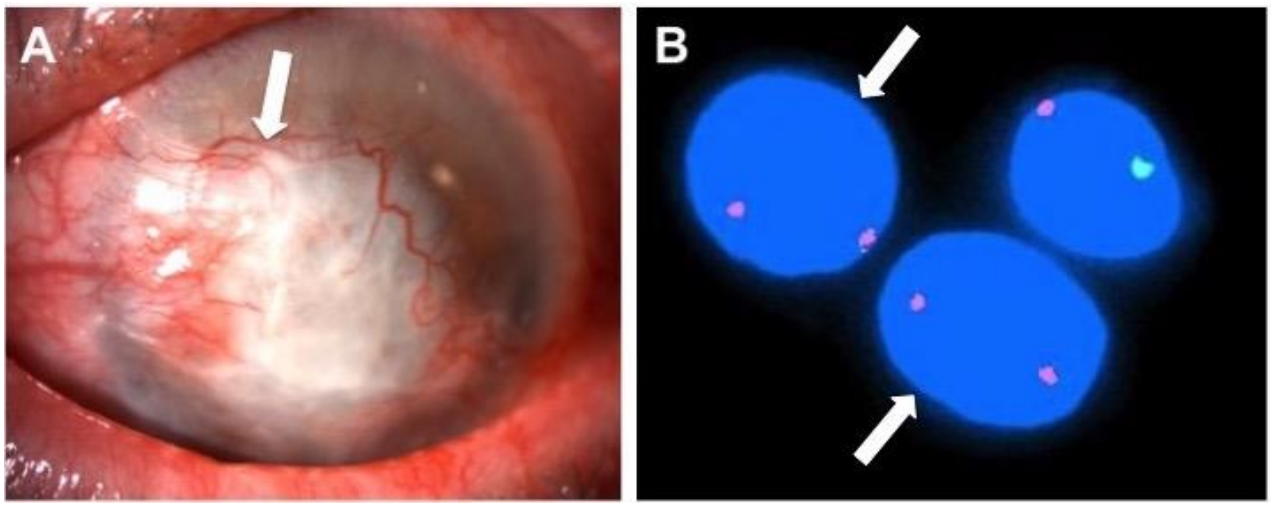

Figure 3: Part A: Clinical appearance of OS 6 months after conjunctival-limbal transplantation. Note the significant decrease of both conjunctival injection and peripheral corneal neovessels; in addition an increased thickness at the site of descemetocele was observed (white arrow). Part B: FISH analysis with chromosome-specific painting DNA probes showed the presence of cells that

expressed donor chromosomes (XX, sister; red circles) (white arrows) in the recipient bed 6 months after transplantation.

Currently, 8 months postoperatively, ocular surface condition is stable in OU, and patient is candidate for mushroom keratoplasty for optical indication in OS.

PATIENT #2

A 60-year-old man was referred to our Institution for ocular GHVD with severe bilateral visual impairment, and history of recurrent multiple corneal perforations in OS. In 2006, he had been treated for acute myeloid leukemia with an allo-HSCT obtained from a volunteer donor. Few months later, he developed ocular GVHD which worsened progressively over the subsequent years, particularly in OS where he experienced multiple corneal perforations (8 episodes), treated with different strategies including cyanoacrylate patch, tectonic PK, conjunctival flap and complete tarsorraphy.

Upon presentation, slit-lamp examination revealed severe MGD with diffuse punctate keratopathy (Oxford score 5 out of 5) in OD (Figure 4, part A). In OS, a retracted conjunctival flap with inferior paracentral corneal perforation and a mature white cataract were visible behind the tarsorraphy (Figure 4, part B).

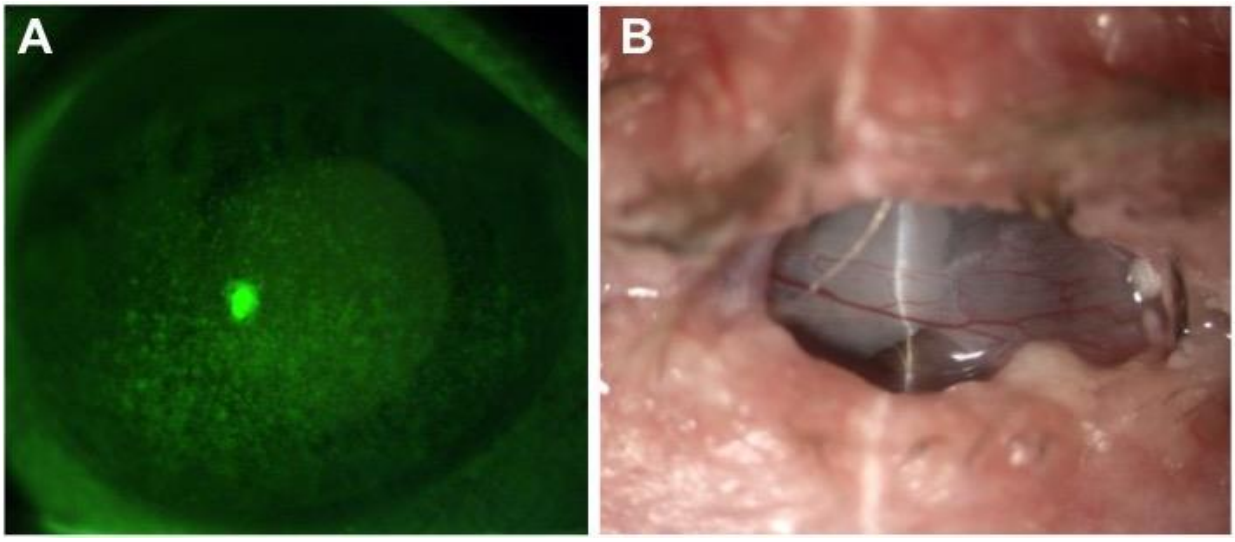

Figure 4: Part A: Slit lamp photography under cobalt blue illumination with the aid of a 7503 Boston Yellow Filter Kit showing the diffuse punctate keratopathy using 2% sodium fluorescein (Oxford score 5) in OD. Part B: Clinical appearance of OS showing retracted conjunctival flap with inferior paracentral corneal perforation and mature white cataract behind the tarsorraphy.

The OSDI score was 90, and Schirmer Test type I measured below 1 mm/5' in OU. Visual acuity was limited to 20/200 in OD due to the concomitant presence of central retinal vein occlusion, and to light perception in OS. The patient's general health was good with a normal blood cell count. The dry eye syndrome was managed only topically in OD with umbilical cord blood serum eye drops every 2 hours, corticosteroid (0.3% hydrocortisone sodium phosphate) qid, cyclosporine 1mg/mL bid, warm compresses, lid hygiene and rolitetracycline/betamethasone/chloramphenicol ointment at bedtime. This therapy allowed a good control of ocular surface inflammation in OD, thus the clinical picture remained stable over the 1-year follow-up. Furthermore, 6 intravitreal injections of anti-vascular endothelial growth factor (VEGF) combined with laser photocoagulation were performed to manage the immune-mediated retinal disease and avoid neovascular complications. Conversely, complex surgery including lensectomy, tectonic PK and conjunctival flap according to Gundersen technique was required in OS (Figure 5, part A). Although an uneventful surgery and a strong anti-inflammatory postoperative treatment (oral cyclosporine and deltacortene bid, topical prednisolone acetate qid, cyclosporine bid, short-term levofloxacin qid, lubricants and ointments every 2 hours), sterile corneal perforation occurred again requiring a new tectonic PK. Early postoperative course was regular, but two months later a persistent epithelial defect appeared and progressed rapidly to stromal ulceration and descemetocele formation (Figure 5, part B). Another PK was performed along with the injection of botulinum toxin A to induce ptosis. Currently, two months after the last surgery, corneal graft is quite clear but a large epithelial defect is still present (Figure 5, part C).

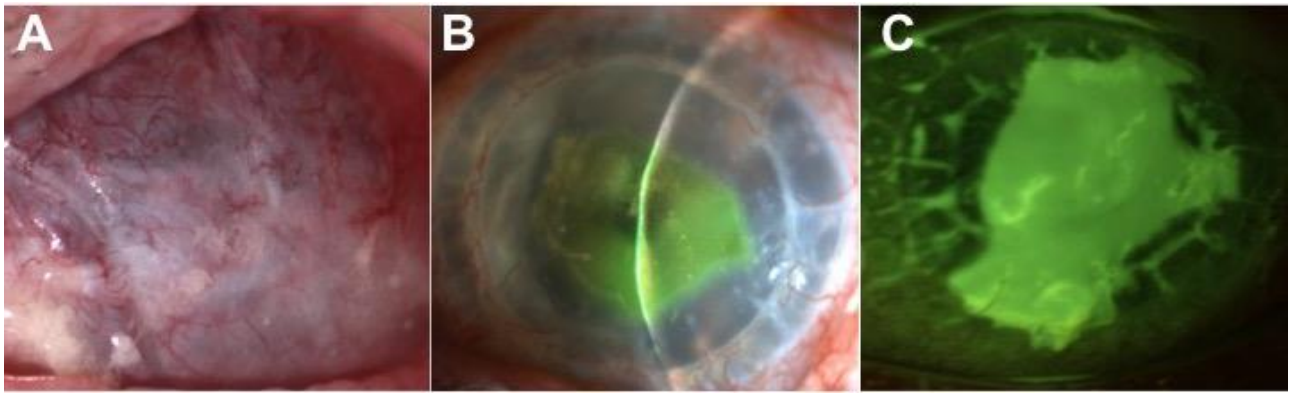

Figure 5: Clinical appearance of OS during the course of the disease. Part A: Normally-vascularized conjunctival Gundersen flap few days after surgery. Part B: Persistent epithelial

defect of the PK graft progressed to stromal melting and central descemetocele. Part C: Persistent epithelial defect of the last PK graft 2 months after surgery.

Theoretically, this case would benefit from conjunctival-limbal graft (like Patient #1), but since HSCT was obtained by an anonymous volunteer donor, this technique is not feasible. However, we sent an official request to the Bone Marrow Donor Registry of our Country, and we are still waiting the authorization for asking the same donor to donate again. If conjunctival-limbal donation from the same bone marrow donor will be not practicable for legislative issues, we will evaluate other possible options for the challenging management of this patient, including Boston Keratoprosthesis, oral mucosa graft or conjunctival flap plus complete tarsorraphy.

DISCUSSION

Ocular GVHD is a major complication following allo-HSCT occurring in a significant percentage of transplanted patients. The intense inflammation of the ocular surface determines LSCD with corneal conjuctivalization and neovascularization; in addition, the extreme dryness causes the onset of persistent epithelial defect that often progresses to corneal ulceration, melting and perforation.

Recently, our Group proposed for the first time the transplantation of a conjunctival-limbal graft obtained from the same living-related bone marrow donor in the setting of ocular GVHD. On one side, the conjunctival graft provides to the recipient bed the goblet cells that are essential for the production of the mucus layer of the tear film. On the other side, the limbal graft provides the amount of stem cells fundamental for the regeneration of the corneal surface, the recovery of corneal transparency and the healing of corneal ulcer. This approach is based on the hypothesis formulated by Starzl (pioneering transplant surgeon and Nobel prize recipient) that the bone-marrow transplantation induces chimerism and consequent tolerance to tissue transplanted from the same donor at the same or later time (Figure 6, parts A-B).[4,5]

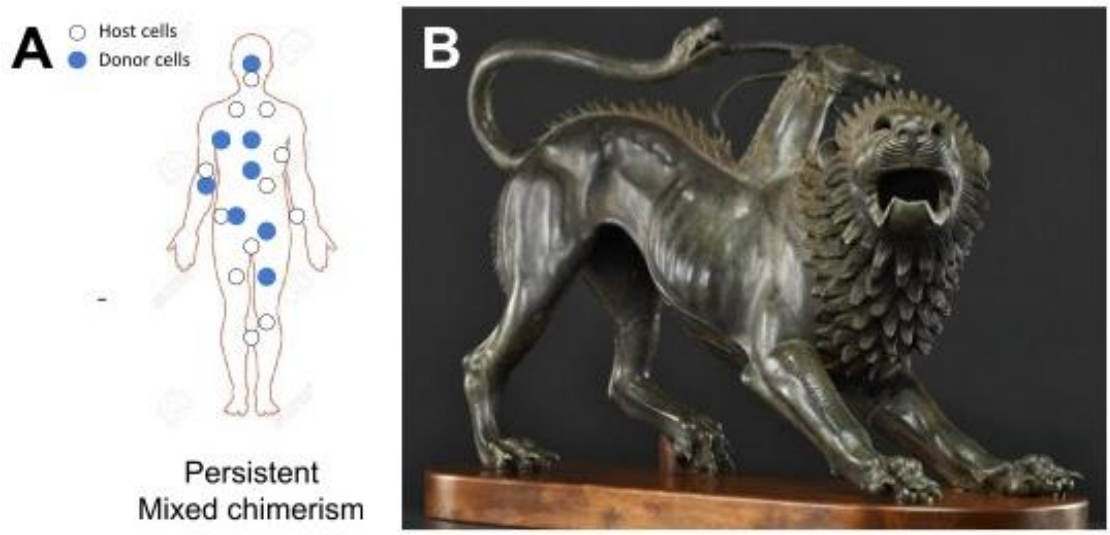

Figure 6: Part A: Persistent mixed chimerism induced after HSCT (from Oura T et al. Clin Exp Immunol. 2017); Part B: The "Chimera of Arezzo" is an Etruscan bronze of 400 BC that was found in 1553 in Arezzo, and brought to Florence to join the collections of the Grand Duke Cosimo I de' Medici and set up in the hall of Leo X in Palazzo Vecchio. In 1871 it was transferred to the Archaelogical Museum of Florence.

Based on this hypothesis, Kawai and co-Authors were also able to discontinue all immunosuppressive therapy in four of five patients receiving a combined transplantation of HLA-mismatched bone marrow and kidney.[6]

The initial results of our technique are extremely encouraging. The procedure improved tear secretion, decreasing ocular surface inflammation and causing a marked regression of corneal neovessels. Transplanted cells survived in the recipient environment for at least 6 months, as demonstrated by FISH analysis performed in the case with donor-recipient sex mismatch (Patient #1). It must be highlighted that our surgical approach succeeded without any sign of rejection in the absence of any systemic immunosuppression. Conversely, in the past HLA matched (identical or haplo-identical) allograft conjunctival transplantation obtained from relatives has been used for treating severe bilateral dry eye conditions including Stevens-Johnson and Lyell syndrome as well as chemical burns. However, episodes of rejection were noticed within the first year after surgery in a significant percentage of cases.[7] Furthermore, combined conjunctival limbal allografts and keratolimbal allografts have been proposed for the treatment of severe ocular surface failure secondary to Steven-Johnson syndrome, ocular cicatricial pemphigoid, chemical/thermal injuries,

and severe atopic keratoconjunctivitis. However, systemic and/or topical long-term immunosuppressive medications were required throughout the entire follow-up period.[8]

Our procedure can eventually be repeated safely even more times in case of "exhaustion" of the transplanted tissue, since the recovery of normal anatomy and function has been demonstrated to occur within 1 year from harvesting in the donor eye.

Unfortunately, only half of all GVHD patients received HSCT from a sibling, and thus can benefit from this technique. On the contrary, patients who received stem cell from volunteer donor are not eligible (at least in our Country) since the Bone Marrow Donor Registry does not allow any direct contact between donors and recipients throughout the entire course of care.

CONCLUSIONS

Transplantation from the living-related bone marrow donor of both conjunctiva and limbal epithelial stem cells is effective for the treatment of patients with severe ocular GVHD. In these cases, the intense ocular surface inflammation determines LSCD with corneal neovascularization, while the extreme dryness causes the onset of persistent epithelial defect that often progresses to corneal ulceration, melting and perforation. At this stage, topical anti-inflammatory therapy alone cannot restore ocular surface condition, while conjunctival-limbal graft is able to address both LSCD and extreme dryness, without the need for systemic immunosuppressants thanks to the induced-chimerism.

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