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Peer Review File

Cultivated Autologous Limbal Epithelial Cell (CALEC) Transplantation for Limbal Stem Cell Deficiency: a Phase I/II clinical trial of the first xenobiotic-free, serum-free, antibiotic-free manufacturing protocol developed in the US

Corresponding Author: Dr Ula Jurkunas

This manuscript has been previously reviewed at another journal. This document only contains information relating to versions considered at Nature Communications.

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
no comments

Reviewer #4

(Remarks to the Author)
The manuscript entitled, “Cultivated Autologous Limbal Epithelial Cell (CALEC) Transplantation for Limbal Stem Cell Deficiency: a Phase I/II clinical trial” reports the outcomes of CALET for unilateral LSCD. This is a very exciting study that demonstrates the safety and feasibility of a culture method for the expansion of LSCs. The data are concisely presented. It is encouraging to show the safety of CALET under GMP condition in a prospective clinical trial. An 88% of culture success shows the robustness of the culture method. However, because the assessment of LSCD was not confirmed and standardized, the data do not support the efficacy claim. Furthermore, to truly claim the efficacy, a control group, and the criteria to stage LSCD are necessary. There are several major limitations in the trial design that reduce the significance of the study.

1. It is unclear how “conjunctivalization’ was evaluated, ie the diagnosis of LSCD. There was no test to confirm the presence of conjunctival cells. Corneal neovascularization was measured but it is not diagnostic of LSCD.
2. The definition of “Success”, “Partial Success” needs more clarification. How “improvement of integrity” was defined and assessed? It would be more meaningful if transplanted LSCs were demonstrated in the recipients.
3. There were patients with excellent vision (mild or stage I disease) before treatment. A limbal involvement of 6 clock hours or less likely do not require LSC transplant. It is unclear how the decision was reached to include these subjects.
4. Removing the corneal scars or pannus could improve the surface without CALEC. Such factor should be included in the assessment of the CALEC outcome.
5. Ocular surface optimization treatments including autologous serum and preservative-free anti-inflammatory were giving after transplantation. Such treatments alone could improve the cornea health without CALEC.
6. CD340 and CD49F were used to characterize corneal epithelial cells but not K12 marker of corneal epithelium. Were there any conjunctival cells present in the CALEC?
7. It is generally recommended to consider LSC transplant 1 year after chemical burns. What was the rationale of including some subjects with a shorter injury to treatment interval?

Reviewer #5

(Remarks to the Author)
The authors have appropriately addressed the concerns raised in the initial review.

Reviewer #6

(Remarks to the Author)
This is a manuscript presenting the results of a phase I/II clinical trial investigating the safety and efficacy of cultivated autologous limbal epithelial cell (CALEC) transplantation to treat blindness caused by unilateral limbal stem cell deficiency. The authors report that their suggested novel procedure is safe and that efficacy results are favourable. They also claim that in iATP levels they identified a marker for product release criteria. The manuscript is well-written and easy to follow. The statistical approaches have been chosen appropriately to address the objectives of the trial. The suggested procedure seems to be of clinical importance as, in contrast to other approaches, it only uses FDA-compliant materials. However, without being experts in ophthalmology, the significance of the manuscript is difficult to assess for us.

Some minor comments below that may help to further improve the manuscript:
1. As mentioned by Reviewer #3, the confidence interval for the correlation between iATP and colony forming efficiency is very wide due to the small number of patients. We acknowledge that the authors caveated this analysis as exploratory and indicate in the Discussion that it may suggest consistency with the mechanism of action with the cellular product. However, the statement in the abstract currently reads somewhat stronger and should equally be toned down.
2. In the response to Reviewer #3, the authors state that “Analyses were not done at 12 and 18 months due to the disproportionate sample sizes in the success vs partial/not a success groups.” Can they further elaborate what analyses this relates to? Is this in relation to the “Factors Related to Efficacy Outcome at 3 Months” as presented in e-Table 13?
3. It is not clear to us whether the newly added subsection on Release Criteria really presents any results or should better be moved to the Methods section.
4. The authors present an abundance of Spearman correlation coefficients in e-Table 7, investigating the correlation between biomarkers both by immunostaining and RT-PCR. Can they further elaborate on what would be expected for these correlations between different biomarkers and what the conclusions would be?
5. In e-Table 13, the last column is labelled “median difference” although it seems the difference in medians is presented. As the two can be different, can the authors please clarify? Can they also please elaborate how the 95% confidence interval was calculated?
6. The Discussion explicitly repeats the Results in several instances. This could be avoided to make the Discussion more compact, concentrating on discussing the results in light of existing literature, and any conclusions drawn.
7. The correlation coefficients in Figures 1B and 1C do not seem to show up properly for us.

Reviewer #7

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #4

(Remarks to the Author)
The authors have addressed most of the comments. There are still a couple of comments:
1. The study has not demonstrated efficacy of CALEC as there was no control group and no data supporting the biological activity of CALEC. Removal of fibrovascular pannus was performed prior to the transplantation of CALEC. As such, using the extent of fibrovascular pannus as outcome measure has falsely indicated the function/efficacy of CALEC. The authors have not shown any data supporting that CALEC improved the ocular surface. It is likely that removal of the fibrovascular pannus itself improved the ocular surface. Therefore, "preliminary efficacy" should be removed from the Abstract and Conclusion. Even without valid efficacy data, the data on feasibility and safety still support a next phase of clinical trial.

2. There are no data supporting that serum promotes differentiation of limbal stem cells. There are data supporting its use in dry eye disease and persistent epithelial defect. It is possible that serum treatment itself also improved the ocular surface and not CALEC unless the biological function of CALEC is demonstrated.

Reviewer #6

(Remarks to the Author)
The authors have sufficiently addressed my comments and I don't have any additional comments.

However, one minor thing to note relating to my original comment 2 is that looking at factors related to success versus not for 12 and 18 months is technically possible. I do agree with the authors though that such analysis may not be very meaningful given the anticipated lack of precision (i.e., wide confidence intervals) associated with the high number of successes. Only 3

patients are not regarded complete success at 12 and 18 months. In other words, the procedure will be considered a success for almost all patients at one point. I wonder whether the authors would thus want to highlight the factors identified in eTable 6 and eTable 13 as factors related to early success, which I assume is more desirable to a patient than success after 12 or 18 months.

Reviewer #7

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

AUTHOR RESPONSES TO REVIEWER COMMENTS

Reviewer #2 (Remarks to the Author):

no comments

Reviewer #4 (Remarks to the Author):

The manuscript entitled, "Cultivated Autologous Limbal Epithelial Cell (CALEC) Transplantation for Limbal Stem Cell Deficiency: a Phase I/II clinical trial" reports the outcomes of CALET for unilateral LSCD. This is a very exciting study that demonstrates the safety and feasibility of a culture method for the expansion of LSCs. The data are concisely presented. It is encouraging to show the safety of CALET under GMP condition in a prospective clinical trial. An 88% of culture success shows the robustness of the culture method.

Response: Thank you for this positive feedback.

However, because the assessment of LSCD was not confirmed and standardized, the data do not support the efficacy claim. Furthermore, to truly claim the efficacy, a control group, and the criteria to stage LSCD are necessary. There are several major limitations in the trial design that reduce the significance of the study.

Response: We acknowledge these limitations and confirm they are covered in the **Discussion** section of the paper, including that future investigations should include a randomized control design and better-defined outcomes. The primary objective of this study was to establish safety and feasibility, and the secondary objective was to investigate efficacy, as is typical in a phase 1b/2a trial. We have adjusted the conclusion in both the **Discussion** and the **Abstract** to emphasize that efficacy results are "preliminary" rather than conclusive.

- ***Abstract****: Our feasibility, safety, and preliminary efficacy results provide strong support for establishing cellular products as a viable option for patients with LSCD.*
- ***Discussion****: In conclusion, CALEC presents high manufacturing success, good safety profile, and favorable clinical outcomes in this phase I/II clinical trial. The safety, feasibility, and preliminary efficacy results of this trial provide strong support for further trials and will serve as a steppingstone for establishing cellular therapy products as viable options for patients with LSCD.*

1. It is unclear how "conjunctivalization' was evaluated, ie the diagnosis of LSCD. There was no test to confirm the presence of conjunctival cells. Corneal neovascularization was measured but it is not diagnostic of LSCD.

Response: Conjunctivalization was defined by presence of the fibrovascular pannus more than 2 mm from the limbus into the cornea for ≥6 clock hours and additionally loss of normal limbal architecture by noting lack of limbal palisades of Vogt for ≥9 clock hours. Based on the Reviewer's comment, we have added a sentence clarifying that into the **Methods** section.

- ***Method:*** *Loss of normal limbal architecture as seen in LSCD was assessed by noting lack of limbal palisades of Vogt for ≥9 clock hours*.

We acknowledge that impression cytology is another, yet invasive procedure of assessing presence of conjunctivalization. While using impression cytology has specificity, it is notoriously of low-yield (low sensitivity with high false-negative rate). Additionally, the markers to diagnose LSCD from impression cytology (such as the presence of goblet cells, and various keratin markers) lack consensus.

References:

1. Donisi PM, Rama P, Fasolo A, Ponzin D. Analysis of limbal stem cell deficiency by corneal impression cytology. Cornea. 2003 Aug;22(6):533-8. doi: 10.1097/00003226-200308000-00009. PMID: 12883346.
2. Barbaro V, Ferrari S, Fasolo A, Pedrotti E, Marchini G, Sbabo A, Nettis N, Ponzin D, Di Iorio E. Evaluation of ocular surface disorders: a new diagnostic tool based on impression cytology and confocal laser scanning microscopy. Br J Ophthalmol. 2010 Jul;94(7):926-32. doi: 10.1136/bjo.2009.164152. Epub 2009 Sep 8. PMID: 19740872.
3. Poli M, Janin H, Justin V, Auxenfans C, Burillon C, Damour O. Keratin 13 immunostaining in corneal impression cytology for the diagnosis of limbal stem cell deficiency. Invest Ophthalmol Vis Sci. 2011 Dec 9;52(13):9411-5. doi: 10.1167/iovs.10-7049. PMID: 22064992.
4. Garcia I, Etxebarria J, Boto-de-Los-Bueis A, Díaz-Valle D, Rivas L, Martínez-Soroa I, Saenz N, López C, Del-Hierro-Zarzuelo A, Méndez R, Soria J, González N, Suárez T, Acera A. Comparative study of limbal stem cell deficiency diagnosis methods: detection of MUC5AC mRNA and goblet cells in corneal epithelium. Ophthalmology. 2012 May;119(5):923-9. doi: 10.1016/j.ophtha.2011.10.031. Epub 2012 Jan 31. PMID: 22297031.

2. The definition of "Success", "Partial Success" needs more clarification. How "improvement of integrity" was defined and assessed? It would be more meaningful if transplanted LSCs were demonstrated in the recipients.

Response: A more detailed definition can be found in the bottom of Table 1, pasted below for convenience. We have also provided some expanded details in the **Methods** for additional clarity, as noted below.

- ***Methods****: The primary efficacy outcome was defined as a "complete success" based on improvement in corneal epithelial surface integrity. If epithelial defect was present at baseline, improvement was defined as a decrease in epithelial defect surface area by ≥75%; otherwise, if no epithelial defect was present at baseline, improvement was defined as a decrease in corneal surface staining by ≥50%. "Partial success" was based on improvement in corneal vascularization or participant symptomatology. Detailed efficacy outcome definitions are provided in* ***Table 1****.*

Primary Efficacy Outcome Definition

1. "Complete Success" is defined as *improvement* in corneal surface integrity
2. "Partial Success" is defined as
    a. No improvement in corneal surface integrity and
    b. Improvement in either
        i. Extent of corneal vascularization or
        ii. Participant symptomatology
3. Otherwise, "Not a Success"

Improvement in each area is defined as follows, where changes are measured relative to baseline:

- Corneal surface integrity
    - If epithelial defect present at baseline, then *improvement* is defined as:
        - Decrease in epithelial defect surface area by ≥75% (based on clinical assessment by an independent investigator, not the treating surgeon)
    - If no epithelial defect present at baseline, then *improvement* is defined as:
        - No epithelial defect and
        - Decrease in corneal surface staining by ≥50% (based on clinical assessment by an independent investigator, not the treating surgeon, using NEI grading scale)
- Extent of corneal vascularization
    - Decrease in neovascular area ≥25% (based on digital slit lamp photographs, using mathematical software to calculate)
- Participant symptomatology
    - Decrease in Ocular Surface Disease Index (OSDI) score ≥25% or
    - Decrease in Symptom Assessment in Dry Eye (SANDE) score ≥25%

3. There were patients with excellent vision (mild or stage I disease) before treatment. A limbal involvement of 6 clock hours or less likely do not require LSC transplant. It is unclear how the decision was reached to include these subjects.

Response: LSCD is a multifactorial and heterogenous disorder that has variable clinical presentation as represented in the cohort of patients enrolled into the study. All patients that received CALEC have been previously unresponsive to standard of care treatments with persistent symptoms. Ultimately participants were qualified for inclusion defined by the protocol which has been clarified in the **Methods** as follows.

- ***Methods:*** *Participants were 18 to <90 years old with unilateral LSCD as determined by conjunctivalization of the cornea defined by fibrovascular pannus more than 2 mm from the limbus into the cornea for ≥6 clock hours. Loss of normal limbal architecture as seen in LSCD was assessed by noting lack of limbal palisades of Vogt for ≥9 clock hours.*

4. Removing the corneal scars or pannus could improve the surface without CALEC. Such factor should be included in the assessment of the CALEC outcome.

Response: As mentioned in our previous response above, we acknowledge the limitations of efficacy outcome definitions, and we have couched our efficacy conclusions as "preliminary."

5. Ocular surface optimization treatments including autologous serum and preservative-free anti-inflammatory were giving after transplantation. Such treatments alone could improve the cornea health without CALEC.

Response: In all cases, ocular surface was optimized prior to CALEC treatment as much as possible, yet manifestations of disease persisted. In our study, the purpose of serum tears was to enhance the differentiation of the cultivated epithelial sheet in vivo. We added a sentence to indicate that in the **Methods** section.

- ***Methods:*** *Additionally, serum tears were employed to enhance stratification and cellular differentiation in vivo.*

6. CD340 and CD49F were used to characterize corneal epithelial cells but not K12 marker of corneal epithelium. Were there any conjunctival cells present in the CALEC?

Response: Page 5 and e-table 6 – We characterized corneal epithelial cells using the K12 marker on backup CALEC constructs, generated concurrently with primary constructs.

The backup constructs were analyzed for p63, p63α, C/EBPδ, and Krt12 for immunostaining and RT-PCR analysis using p63, p63α, C/EBPδ, Krt12, Krt14, Notch1 and ABCG2 genes.

K12, exclusive to corneal epithelium, was detected in our constructs, confirming corneal epithelial cells and not conjunctival cells. However, to specifically rule out conjunctival epithelium, Mucin 5AC expression could have been assessed, as it specifically marks conjunctival presence [1]. We have added this sentence in our limitations section.

In addition, long-term success of cultivated limbal stem cell transplantation has been correlated with >3% - p63+ve (p63-bright) holoclone-forming stem cell percentage leading to 80% success, while <3% resulted in 10% success [2,3]. Therefore, we correlated our outcomes with the limbal/epithelial markers.

1. *Ursula Schlötzer-Schrehardt, Friedrich E. Kruse. Identification and characterization of limbal stem cells. Experimental Eye Research. 2005; 81(3): 247-264.*
2. *Paolo Rama, M.D., Stanislav Matuska, M.D., Giorgio Paganoni, M.D., Alessandra Spine lli, M.D., Michele De Luca, M.D., and Graziella Pellegrini, Ph.D. Limbal Stem-Cell Therapy and Long-Term Corneal Regeneration. N Engl J Med 2010;363:147-155.*
3. *Marta Sacchetti , Paolo Rama , Alice Bruscolini , Alessandro Lambiase . Limbal Stem Cell Transplantation: Clinical Results, Limits, and Perspectives. Stem Cells Int. 2018 Oct 11:2018:8086269.*

7. It is generally recommended to consider LSC transplant 1 year after chemical burns. What was the rationale of including some subjects with a shorter injury to treatment interval?

Response: We agree that sufficient time should transpire after chemical burn for the eye to be maximally healed and non-inflamed prior to undergoing limbal stem cell transplantation. To this effect, all patients in our trial were felt to be stable to undergo surgical intervention with CALEC.

However, it should be noted that there is no consensus regarding a minimum of 1 year cutoff as the reviewer has noted. The International Limbal Stem Cell Deficiency Working Group consensus paper on management of limbal stem cell deficiency (Deng et al) does not include a cutoff for time from chemical injury to limbal stem cell transplant. Multiple studies of CLET protocols have enrolled a significant number of patients whose injury had occurred earlier than 1 year (examples below of Sangwan VS et al and Sejpal K et al). In our trial, 3 patients had an interval of less than 1 year from chemical burn to CALEC surgery.

-Deng SX, Kruse F, Gomes JA, Chan CC, Daya S, Dana R, Figueiredo FC, Kinoshita S, Rama P, Sangwan V, Slomovic AR. Global consensus on the management of limbal stem cell deficiency. Cornea. 2020 Oct 1;39(10):1291-302.

-Sangwan VS, Basu S, Vemuganti GK, Sejpal K, Subramaniam SV, Bandyopadhyay S, Krishnaiah S, Gaddipati S, Tiwari S, Balasubramanian D. Clinical outcomes of xeno-free autologous cultivated limbal epithelial transplantation: a 10-year study. British Journal of Ophthalmology. 2011 Nov 1;95(11):1525-9.

-Sejpal K, Ali MH, Maddileti S, Basu S, Ramappa M, Kekunnaya R, Vemuganti GK, Sangwan VS. Cultivated limbal epithelial transplantation in children with ocular surface burns. JAMA ophthalmology. 2013 Jun 1;131(6):731-6.

Reviewer #5 (Remarks to the Author):

The authors have appropriately addressed the concerns raised in the initial review.

Response: Thank you for your prior constructive feedback to improve the manuscript.

Reviewer #6 (Remarks to the Author):

This is a manuscript presenting the results of a phase I/II clinical trial investigating the safety and efficacy of cultivated autologous limbal epithelial cell (CALEC) transplantation to treat blindness caused by unilateral limbal stem cell deficiency. The authors report that their suggested novel procedure is safe and that efficacy results are favourable. They also claim that in iATP levels they identified a marker for product release criteria. The manuscript is well-written and easy to follow. The statistical approaches have been chosen appropriately to address the objectives of the trial. The suggested procedure seems to be of clinical importance as, in contrast to other approaches, it only uses FDA-compliant materials. However, without being experts in ophthalmology, the significance of the manuscript is difficult to assess for us.

Response: Thank you for this positive feedback.

Some minor comments below that may help to further improve the manuscript:
1. As mentioned by Reviewer #3, the confidence interval for the correlation between iATP and colony forming efficiency is very wide due to the small number of patients. We acknowledge that the authors caveated this analysis as exploratory and indicate in the Discussion that it may suggest consistency with the mechanism of action with the cellular product. However, the statement in the abstract currently reads somewhat stronger and should equally be toned down.

Response: We have toned down the **Abstract** language as follows

- ***Abstract:*** *After first stage manufacturing, intracellular adenosine triphosphate levels correlated with colony forming efficiency (r=0.65, 95% CI [0.04, 0.89]), an exploratory result that may suggest novel product release criteria.*

2. In the response to Reviewer #3, the authors state that "Analyses were not done at 12 and 18 months due to the disproportionate sample sizes in the success vs partial/not a success groups." Can they further elaborate what analyses this relates to? Is this in relation to the "Factors Related to Efficacy Outcome at 3 Months" as presented in e-Table 13?

Response: This refers to both e-Table 6 and e-Table 13. The reason we did not perform analyses at 12 and 18 months for these is there were too many successes (~80%) to look at factors related to success versus not. At 3 months, the success rate was 50%, so it was possible to look at factors related to success versus not.

3. It is not clear to us whether the newly added subsection on Release Criteria really presents any results or should better be moved to the Methods section.

Response: We agree and have moved this section to the **Methods.**

4. The authors present an abundance of Spearman correlation coefficients in e-Table 7, investigating the correlation between biomarkers both by immunostaining and RT-PCR. Can they further elaborate on what would be expected for these correlations between different biomarkers and what the conclusions would be?

Response: We have added the following sentences to the **Results** and **Discussion** sections.

- ***Results:*** *The largest negative correlations were between Krt14 and Krt12 (r = -0.64) and Krt12 and Notch 1 (-0.48) . The largest positive correlations were between C/EBPδ and p63 (r=0.77), C/EBPδ and p63α (r=0.63), C/EBPδ and Krt12 (r=0.65), C/EBPδ and Notch1 (r=0.69), p63 and p63α (r=0.61), p63 and ABCG2 (r=0.64), and Krt14 and Notch 1 (r=0.81), as would be expected (e-Table 7).*
- ***Discussion:*** *We noted correlations between several biomarkers tested. The negative correlation between Krt12 and Krt14 and Notch1 reveals the balance between limbal stem cell presence and corneal differentiation. Presence of C/EBPδ, p63α, and Notch1 support self-renewal in limbal stem cells, while ABCG2 cells confirm putative stem cell*

properties; thus, taken together, CALEC cultures showed dynamic expression of stem cell and epithelial markers, indicating active differentiation and self-renewal.

5. In e-Table 13, the last column is labelled "median difference" although it seems the difference in medians is presented. As the two can be different, can the authors please clarify? Can they also please elaborate how the 95% confidence interval was calculated?

Response: Difference in medians were calculated and tables titles have been updated accordingly. Confidence intervals for the difference in medians between two groups were calculated using the SAS PROC QUANTREG procedure using inverting rank-score test method.

6. The Discussion explicitly repeats the Results in several instances. This could be avoided to make the Discussion more compact, concentrating on discussing the results in light of existing literature, and any conclusions drawn.

Response: We have trimmed down some repetition throughout the **Discussion**.

7. The correlation coefficients in Figures 1B and 1C do not seem to show up properly for us.

Response: This has been corrected.

Reviewer #7 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Response: Thank you for your efforts to improve the manuscript.

Reviewer #4 Comments:
"The authors have addressed most of the comments. There are still a couple of comments:
1. The study has not demonstrated efficacy of CALEC as there was no control group and no data supporting the biological activity of CALEC. Removal of fibrovascular pannus was performed prior to the transplantation of CALEC. As such, using the extent of fibrovascular pannus as outcome measure has falsely indicated the function/efficacy of CALEC. The authors have not shown any data supporting that CALEC improved the ocular surface. It is likely that removal of the fibrovascular pannus itself improved the ocular surface. Therefore, "preliminary efficacy" should be removed from the Abstract and Conclusion. Even without valid efficacy data, the data on feasibility and safety still support a next phase of clinical trial.

Response: This was the first trial of the CALEC method in the United States. In a Phase I/II trial it is not uncommon to lack a control group, but to still gather preliminary data to investigate how well the treatment works. Regarding removal of fibrovascular pannus, we are including our prior answer, below. We previously removed efficacy from the abstract conclusion in the prior draft, noted below. We have further removed "and favorable clinical outcomes" in the conclusion of the discussion so that efficacy is fully removed from our conclusions.

- **Current abstract conclusion** – "Our results provide strong support that CALEC transplantation is safe and feasible and further studies are needed to evaluate therapeutic efficacy."
- **Revised discussion conclusion** – "In conclusion, CALEC presents high manufacturing success and good safety profile ~~and favorable clinical outcomes~~ in this phase I/II clinical trial. The results of this trial provide strong support for further trials and will serve as a steppingstone for establishing cellular therapy products as viable options for patients with LSCD."
- **Prior answer regarding fibrovascular pannus -** Extensive LSCD cannot be fully treated with just fibrovascular pannus excision without stem cell replacement, due to risk for pannus recurrence or delated healing/re-epithelialization. To this effect, the International Limbal Stem Cell Deficiency Working Group management consensus paper has suggested pannus excision without stem cell transplant to be contraindicated in LSCD patients with poor stem cell reserve.

References:

1. Deng SX, Kruse F, Gomes JA, Chan CC, Daya S, Dana R, Figueiredo FC, Kinoshita S, Rama P, Sangwan V, Slomovic AR. Global consensus on the management of limbal stem cell deficiency. Cornea. 2020 Oct 1;39(10):1291-302.
2. Dua HS. Sequential Sectoral Conjunctival Epitheliectomy. In: Mannis MJ, Holland EJ, editors. Ocular Surface Disease: Medical and Surgical Management [Internet]. New York, NY: Springer; p. 168–74. Available from: https://link.springer.com/chapter/10.1007/0-387-21570-0_14
3. Kate A, Basu S. A review of the diagnosis and treatment of limbal stem cell deficiency. Frontiers in Medicine. 2022 May 25;9:836009.
4. Konomi K, Satake Y, Shimmura S, Tsubota K, Shimazaki J. Long-term results of amniotic membrane transplantation for partial limbal deficiency. Cornea. 2013 Aug 1;32(8):1110-5.