**Peer Review File**

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This group has published several papers reporting results of their projects on vascularized composite allografts (VCA) in swine and nonhuman primate (NHP) models. In this paper, they introduced subcutaneous implantable disks to optimize of FK506 delivery in their NHP VCA model.

The study consisted of two groups; Group 1 tested two different regimens of FK506 delivery via subcutaneous implanted disks. Animals in Group 2 included BM Tx in the maintenance period for the induction of tolerance.

Unfortunately, results presented in the current manuscript lacks convincing data. Authors cannot readily report on the "optimization of FK506 delivery via subcutaneous implanted disks to abrogate VCA rejection", as stated in the Abstract.

Thank you for your comment. We have modified the abstract as advised (see Page 3, line 58-59): "Herein, we report the first utilization of FK506 delivery via subcutaneously implanted discs to mitigate VCA rejection in NHPs ".

The results do not support their conclusion in the Abstract that "This study shows proof-of-concept of local FK506 implants in potentially reducing the overall immunosuppressive load, as well as mitigating VCA rejection for tolerance protocols based on DBMT."

Thank you for pointing out this overstatement. We have modified the abstract as advised (see Page 3, line 83-85): "This study shows preliminary results of local FK506 implants in potentially reducing VCA acute rejection for tolerance protocols based on mixed chimerism approach".

Specific comments are as follows:

1. Only one animal reached the end-point in Group 1 and the duration was too short. They used four NHPs. The first animal was euthanized at POD 4 due to technical failure and the second animal was euthanized at POD 12 associated with high FK506 levels. The authors changed the regimen of the device for the 3rd and 4th animals. One was euthanized due to technical failure and the other reached the end-point at Day 13.

Therefore, only one animal reached the end point, not enough to be used as a thorough assessment of Group 1's aim. In addition, the end-point at POD 13 was too short to optimize the regimen. In fact, all three animals in Group 2 had very high FK506 blood levels, 60, 79 and 122 ng/ml that required cessation of IM FK506 administration. These animals also developed PTLD.

If my understanding is correct, they developed this delivery system to reduce rejection of the VCA by increased FK506 local dose while reducing systemic side effects. However, data presented in this manuscript clearly indicated that the authors have not achieved this balance in their regimen with implantable disks.

Yes, we agree with the reviewer's comments; one of the hard-to-control effects of local FK506 was the systemic absorption of tacrolimus. In some ways, this is not unexpected given the proximity of the subdermal plexus to the site of implantation i.e., subcutaneous tissue. On the other hand, none of the NHPs developed an episode of acute rejection in this study, which is contrary to our last report without such local FK506 (Lellouch et al., PRS 2020).

2. Subtitle "Successful Delayed Mixed Chimerism after DTIP but PTLD Remained Rampant" is overstated. All animals were euthanized before 1000 days after VCA Tx and only one animal had high chimerism. All animals had > 20ng/ml of blood FK506 levels throughout experimental period. That does not assess immune tolerance. In addition, in vitro data showing anti-donor and third-party responses were missing. The authors included assessment of anti-donor antibody (DSA) in Methods. However, no results of DSA were shown.

Yes, this reviewer's observation is accurate. While we detected donor cells in only one NHP (M6), the levels were still far higher (>90% donor granulocytes, >10% donor lymphocytes) than that in other NHP studies by other groups (usually ~ 0.5%). In fact, our group has previously shown in swine studies that the low levels of such *transient* chimerism reported by other groups does not establish a state of clinical tolerance (see Leto Barone et al. (2015) Effects of Transient Donor Chimerism on Rejection of MHC-Mismatched Vascularized Composite Allografts in Swine, Vascularized Composite Allotransplantation, 2:1,1-8). Additionally, following DBMT, there is a generalized state of *in vitro* unresponsiveness including absence of DSA (which we have also reported in our previous paper in Lellouch et al., PRS 2020) but clinically, VCA rejection still developed. However, we do not report DSA in this manuscript, and have removed this description from the methods. We do agree with the reviewer's

suggestions and have thus modified the sub-heading as advised (see Page 13, line 300) to “Detection of Donor Cells after DTIP but PTLD Remained Rampant”.

3. Important controls are missing from this study: (1) animals without implantable disks, were not included

The historical control (DTIP without FK506 discs) was published in this report: Lellouch AG, Ng ZY, Rosales IA, et al. Toward Development of the Delayed Tolerance Induction Protocol for Vascularized Composite Allografts in Nonhuman Primates. Plast Reconstr Surg. 2020;145(4):757e-768e. This has been added to in Page 10.

(2) FK 506 blood levels were high and (3) all animals except one animal in Group 1 (euthanized due to protocol endpoint at POD 13) died from FK506 toxicity including PTLD, and technical failure. Data offered in this study does not permit one to evaluate the effects of the implantable disks.

Group 2 animals in this study treated with local FK implants did not acutely reject their VCAs whereas historical controls that were treated with an identical protocol, but not local FK implant, did. Lellouch AG, Ng ZY, Rosales IA, et al. Toward Development of the Delayed Tolerance Induction Protocol for Vascularized Composite Allografts in Nonhuman Primates. Plast Reconstr Surg. 2020;145(4):757e-768e.

Other comments:

1.Target levels of FK506 in this study is missing.

The target level was 20-30ng/ml as established in previous studies (see below) and mentioned previously in Lines 243 and 374 on Pages 10 and 15.

2. Needed a description of the indication/criteria: of adding systemic FK506 administration.

Our experience of managing FK level is based on our laboratory’s previous experience. Initially we did not expect there to be high spillover of the FK506 into the bloodstream. Therefore, we used our previous protocol as described in this report: Lellouch AG, Ng ZY, Rosales IA, et al. Toward Development of the Delayed Tolerance Induction Protocol for Vascularized Composite Allografts in Nonhuman Primates. Plast Reconstr Surg. 2020;145(4):757e-768e. Essentially, topical FK506 was insufficient to prevent VCA rejection and required additional systemic FK506. This was mentioned in brief previously in Lines 198-201.

3. Figure 1 included “CyA”. However, there is no description of CyA in the manuscript.

Thank you for noticing this detail. CyA was mistakenly included from our previous work. Although use of CyA and FK506 are interchangeable after the DTIP, we updated Figure 1 image and legend to remove CyA since only FK506 was given in this study.

4. Figure 2: It is not clear to me where the implant disks were placed.

The implant was placed subcutaneously, just under the skin (subcutaneous pocket). Figure 8A and B edited