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DOTTORATO DI RICERCA IN BIOLOGIA E MEDICINA DELLA RIGENERAZIONE

CICLO XXVI

**Effectiveness of Topical Immunosuppressants in Prevention and Treatment of
Rejection in Face Allotransplantation**

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Riassunto

Introduzione- L'impiego di immunosoppressione topica è stato episodicamente citato per il trattamento del rigetto negli Allotrapianti Compositi Vascolarizzati. Lo scopo di questa ricerca era quello di valutare l'efficacia di Tacrolimo e Clobetasolo topici nella prevenzione e nel trattamento del rigetto in un modello di trapianto di emifaccia nel ratto.

Material e Metodi- Settanta-sei trapianti emifacciali sono stati eseguiti da ratti donatori ACI (RT^{1a}) a ratti riceventi LEW (RT^{1l}). Gli animali riceventi sono stati suddivisi in 11 gruppi. Ad eccezione dei gruppi degli isograft (gruppo 1), e degli allograft (gruppo 2) che non hanno ricevuto nessun trattamento immunosoppressivo, i restanti gruppi sono stati trattati con la terapia d'induzione con l'anticorpo monoclonale anti- $\alpha\beta$ -TCR e Ciclosporina A per una settimana, seguita dal trattamento topico con Clobetasolo o Tacrolimo iniziato a 7 giorni o al primo segno di rigetto. Le biopsie della cute ed i prelievi ematici sono stati eseguiti ad intervalli regolari. Gli organi linfatici sono stati prelevati al momento dell'eutanasia degli animali. L'analisi citofluorimetrica e' stata effettuata per monitorare il rigetto e la presenza del chimerismo nel sangue periferico con i seguenti marcatori: CD3, CD4, CD8, CD45, CD11b/c, CD25, $\alpha\beta$ -TCR, $\gamma\delta$ -TCR, ACI. Gli infiltrati infiammatori sono stati caratterizzati con metodiche immunoistochimiche per la presenza di cellule CD4⁺, CD8a⁺, CD 45RA⁺, CD11b/c⁺ and CD86⁺. L'espressione

dei seguenti geni e' stata valutata con Taq-Man PCR: Interleuchina-2, Interferone- γ , Tumor Necrosis Factor- α , Transforming Growth Factor- β , Interleuchina-13 ed Interleuchina-15.

Risultati- Il trattamento immunosoppressivo topico ha prolungato la sopravvivenza degli allotrapianti in tutti i gruppi. Le sopravvivenze migliori sono state riscontrate nei gruppi trattati con Tacrolimo. L'espressione delle citochine pro-infiammatorie (Interleuchina-2, Interferone- γ , Tumor Necrosis Factor- α , Transforming Growth Factor- β) ha correlato con i segni clinici del rigetto e con la sopravvivenza del trapianto. L'applicazione del Clobetasolo e' stata associata con una marcata deplezione linfocitaria ed atrofia dell'epidermide e del derma.

Conclusioni- Entrambi i trattamenti topici si sono dimostrati efficaci nel trattamento degli episodi di rigetto acuto. I risultati migliori sono stati ottenuti quando l'applicazione della terapia topica ha seguito la terapia di induzione sistemica. Tacrolimo ha dimostrato di essere un migliore agente, come adiuvante alla terapia sistemica, in quanto il suo utilizzo non era associato a complicanze locali e sistemiche che si erano verificate con l'impiego di Clobetasolo.

Abstract

Background- The use of topical immunosuppressants has been anecdotally reported for the treatment of rejection in Vascularized Composite Allotransplantation (VCA). The aim of the present study was to evaluate the effectiveness of topical Tacrolimus and Clobetasol in the prevention and treatment of rejection.

Methods- Seventy-six hemiface allotransplants were performed, between ACI (RT1^a) donors and LEW (RT1^b) recipients. The animals were divided in 11 groups. With the exception of isografts (group 1) and allografts (group 2), the animals received systemic induction with Cyclosporine A and anti- $\alpha\beta$ -TCR antibody for one week and were subsequently treated with topical tacrolimus or clobetasol, at the end of the induction therapy or at the first sign of rejection. Skin biopsies and samples of peripheral blood were performed at regular intervals. The lymphoid organs were harvested at the time of euthanasia. Flow Cytometry analysis was performed to monitor for rejection and to detect the presence of chimerism in the peripheral blood with the following markers: CD3, CD4, CD8, CD45, CD11, CD25, $\alpha\beta$ -TCR, $\gamma\delta$ -TCR, ACI. The inflammatory infiltrate was characterized by immunostaining for presence of CD4⁺, CD8⁺, CD45⁺, CD11b/c⁺ and CD86⁺ cells. The expression of the following genes, Interleukine-2, Interleukine-13, Interleukine-15, Interferone- γ , Tumor

Necrosis- α and Transforming Growth Factor- β , was evaluated with TaqMan PCR.

Results- Topical treatment increased the survival of the allograft in all groups. Best outcomes were obtained in the groups treated with systemic therapy and topical tacrolimus. Expression of proinflammatory cytokines (Interleukine-2, Interferon- γ , Tumor Necrosis Factor- α , and Transforming Growth Factor- β) correlated with clinical signs of rejection and the final outcomes. Clobetasol application was associated with a marked depletion of lymphocytic populations, as well as dermal and epidermal atrophy.

Conclusions- Both topical tacrolimus and clobetasol were effective in treating episodes of acute rejection, and the best outcomes were achieved when their application was initiated following systemic immunosuppression. Topical tacrolimus proved to be a preferable adjunct agent to the systemic therapy by preventing both the local and systemic complications.

Introduction

Since 1998, when the first human hand allotransplantation was performed in Lyon(1,2), vascularized composite allotransplants have experienced a rapid growth, with 49 hand (3), 24 face and 9 abdominal wall allografts performed in just over a decade. Results demonstrated that these procedures were not only technically feasible, but that long-term survival of the allografts was achievable under immunosuppressive regimens used in solid organ transplantation.

Compared to solid organ allotransplantation, Vascularized Composite Allotransplantation (VCA) offered two practical advantages: the opportunity for direct clinical monitoring of rejection and the possibility of using topical immunosuppressants, either Tacrolimus or Clobetasol, for the prevention and treatment of acute rejection.

Application of topical immunosuppressants for the treatment of acute rejection in VCA was reported in the International Registry on Hand and Composite Tissue Transplantation (IRHCTT). In most cases, episodes of acute rejection were reversed by increasing the oral steroid dose or by using intravenous steroids and local immunosuppressants(3). However, neither clear indications were given, for the use of

topical immunosuppressants, nor their effectiveness or adverse effects were reported.

This experimental study was designed to address the role of topical immunosuppression in the treatment and prevention of acute rejection, using a rat model of hemiface allotransplantation. This is the first step in understanding whether this treatment modality could represent an adjunct to the current immunosuppressive regimens in VCA.

Methods

Animals

Inbred 8-10 week-old LEW (RT1^l) and ACI (RT1^a) rats weighing 190-250 g were used in this study (Harlan Sprague-Dawley, Indianapolis, IN). Animals received care in compliance with the “Guide for the Care and Use of Laboratory Animals” published by the National Institute of Health. The animals were caged individually, and maintained on a 12-hour light and dark cycle with standard access to food and water.

Hemiface transplantation and experimental design

Orthotopic hemiface transplantation was performed between ACI (RT1^a) donors and LEW (RT1^l) recipients(4).

Preparation of the Donor:

Aseptic surgical technique was used in all surgical procedures. General anesthesia was achieved with 0.15ml/100g triple cocktail (30 mg/kg Ketamine + 6 mg/kg Xylazine + 1 mg/kg Acepromazine) administered subcutaneously. Dissection of the flap and microvascular anastomosis were performed under operating microscope magnification.

The head and neck of the donor were shaved. The hemifacial flap was marked as shown in Figure 1. First, the cranial and the cervical midlines were drawn. The cervical midline was marked between the sternal notch and the lower lip.

Figure 1: Design of the flap on the donor face.



These two lines were joined with a perpendicular line at the level of the clavicle and passing approximately 0.8-1cm behind the ear. The anterior margin of the flap was marked leaving 1mm of the eyelids intact and curving ventrally along the nasomalar and nasolabial grooves. The cervical incision was performed according to the marking through the skin, subcutaneous tissue and platysma muscle. The subplatysmal dissection started from the cervical midline and proceeded

laterally. The sublingual and submandibular glands were encountered and excised after cauterization of their vascular pedicle arising from the facial artery and vein (Figure 2a).

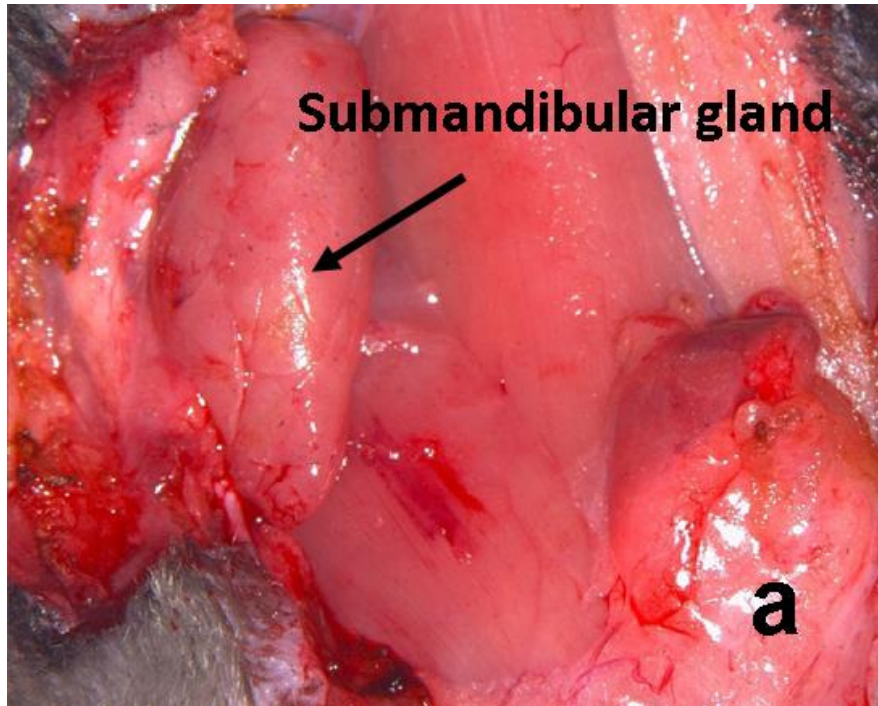


Figure 2a: After the cervical midline incision was performed submandibular gland was isolated and excised.

The external jugular vein was dissected from clavicular region to the junction of the linguofacial and maxillary veins (Figure 2b).

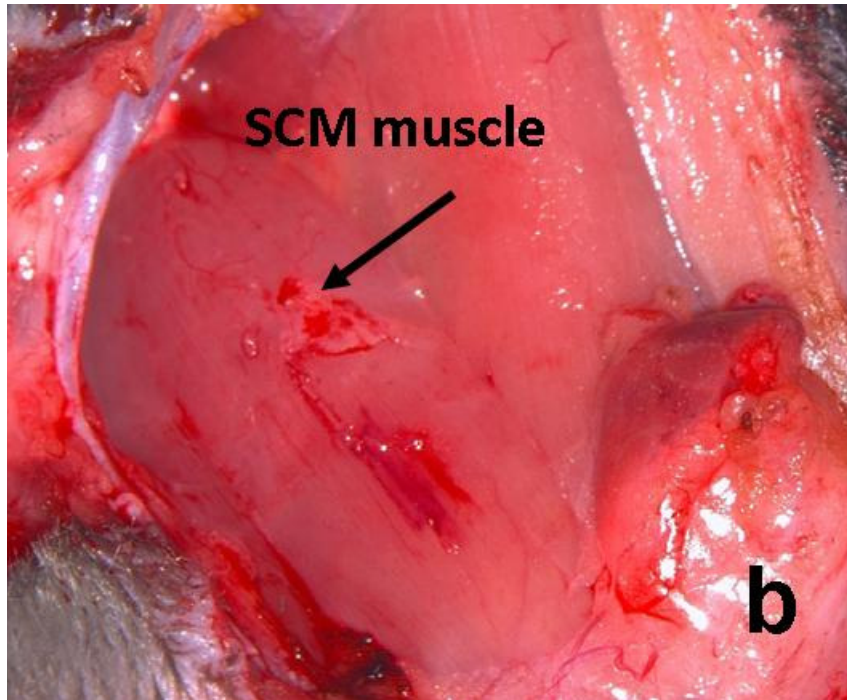


Figure 2b: The external jugular vein was dissected and the sternocleidomastoid muscle was exposed from the insertion to its origin.

The sternocleidomastoid muscle was detached from its insertions and removed, exposing the omohyoid muscle, which was excised allowing the visualization of the carotid sheath. (Figure 2c).

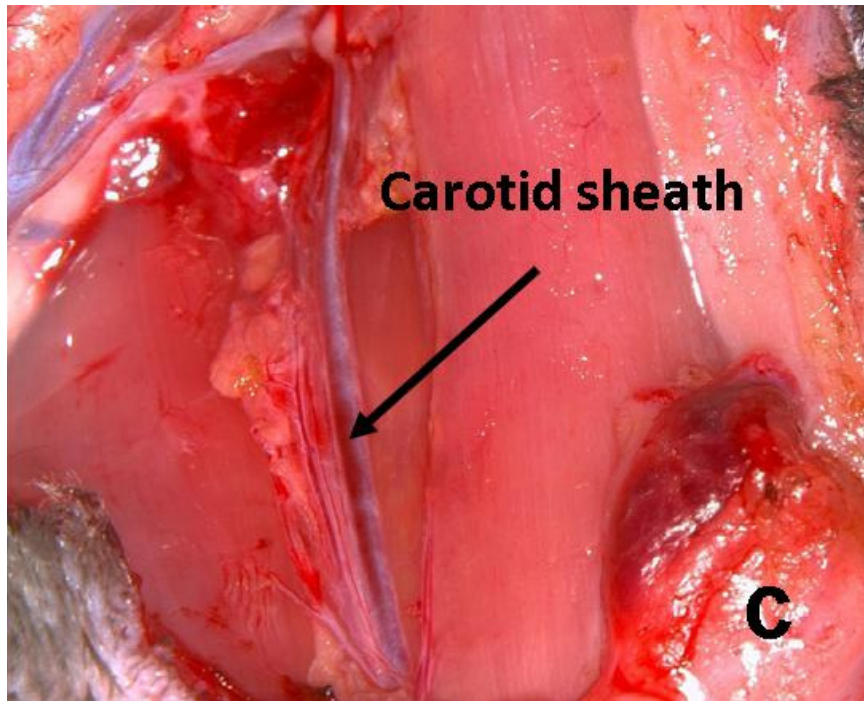


Figure 2c: Sternocleidomastoid and omohyoid muscles were removed, exposing the carotid sheath.

The common carotid artery was separated from the vagus nerve and the internal jugular vein. For better visualization of the external carotid artery and its branches, the posterior belly of the digastric muscle was removed and the greater horn of the hyoid bone was cut. Internal carotid artery and branches of external carotid artery were fully exposed (Figure 3). The internal carotid artery and all branches of the external carotid artery (superior thyroid artery, ascending pharyngeal artery, lingual artery, ascending palatine artery, facial artery and internal maxillary artery) with exception of the superficial temporal artery were ligated.

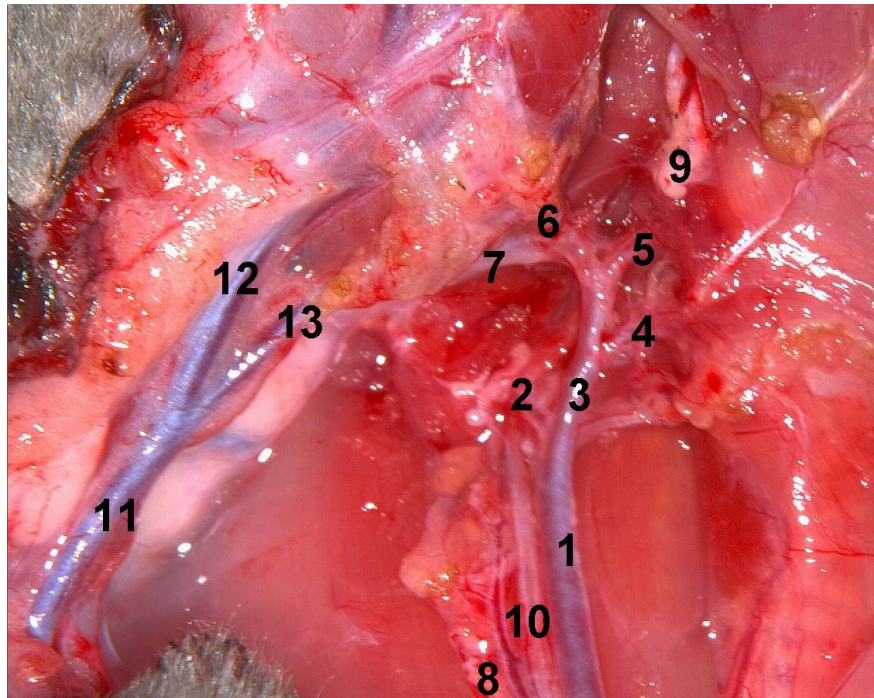


Figure 3: Omohyoid and digastric muscles, hyoid bone and glossopharyngeal nerve were removed to expose the external carotid artery and its branches. 1) Common carotid artery, 2) Internal carotid artery, 3) External carotid artery, 4) Superior thyroid artery, 5) Lingual artery, 6) Facial artery, 7) Superficial temporal artery, 8) Internal jugular vein, 9) Glossopharyngeal nerve, 10) Vagal nerve, 11) External jugular vein, 12) Linguofacial vein, 13) Maxillary vein.

The dissection of the hemiface flap was carried out above the masseter muscle elevating the branches of facial nerve with the flap. The parotid gland was included in the flap. In the retromandibular region, the maxillary vein and the vein draining the pterygoid plexus were ligated. The external

auditory canal and the facial nerve were cut at the osteo-cartilaginous junction and stylomastoid foramen respectively. At this point the cranial incision was performed. The periosteum was incised and the cranial soft tissues were raised subperiosteally up to the temporal line. Thereafter, the dissection was continued in a subfascial plan. The upper and lower eyelids were not included in the flap. At the base of the neck, the flap was raised above the cervical muscles. After transaction of platysma and levator auris longus muscles, the flap was elevated above trapezius up to the external auditory canal.

The completely raised hemifacial allograft was pedicled on the common carotid artery and external jugular vein (Figure 4). The hemiface flap was included the ear, parotid gland, facial nerve, external lacrimal gland, external auditory canal, lymph nodes and most of the hemifacial skin. Flap was left pedicled until the preparation of the recipient was completed.

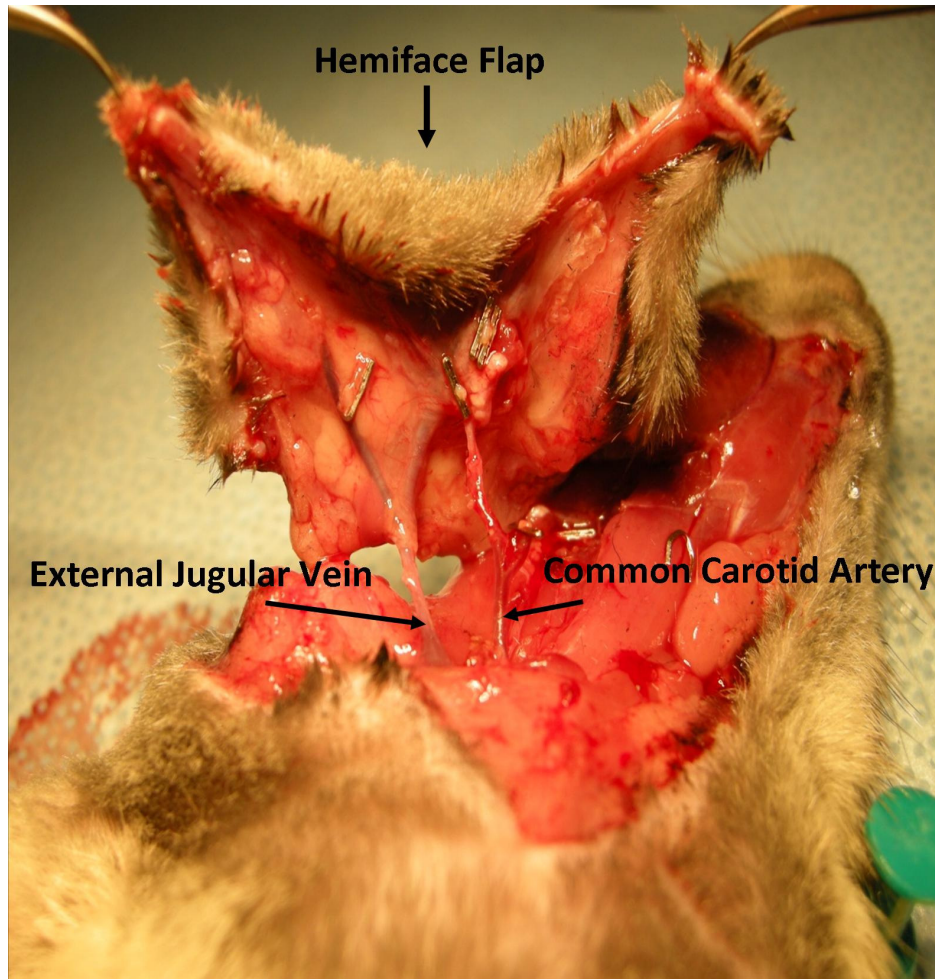


Figure 4: The hemiface flap was elevated above the muscles based on common carotid artery and external jugular vein.

Preparation of the Recipient:

The skin on the same side of the recipient face was marked as previously described for the donor and facial skin was removed as a full thickness skin graft (Figure 5).



Figure 5: Marking of the recipient hemiface for orthotopic transplantation. The skin was removed as a full thickness graft to not disturb the underlying important functional structures.

Facial nerve, the perioral and periorbital regions were spared to protect oral and visual functions. The external jugular vein was dissected and prepared for anastomosis. The sternocleidomastoid muscle was cut from its sternal insertion and mastoid origin to allow dissection of the common carotid artery. This dissection was performed very carefully to avoid stimulation of the vagal and phrenic nerves. Dissection terminated at the carotid bifurcation. After preparation of the recipient, the pedicle of the hemifacial flap was interrupted. The harvested hemiface flap was flushed with heparinized

Ringer lactate solution until the venous outflow was clear. Then the hemiface composite allograft was transplanted to the recipient orthotopically. The flap was inset for anastomosis with several 5/0 vicryl sutures. The external jugular vein of the donor was repaired end to end to recipient's external jugular vein. The arterial anastomosis was performed end-to-side between the common carotid artery of the donor and recipient (Figure 6). All anastomoses were performed under operating microscope magnification with 10/0 nylon sutures.

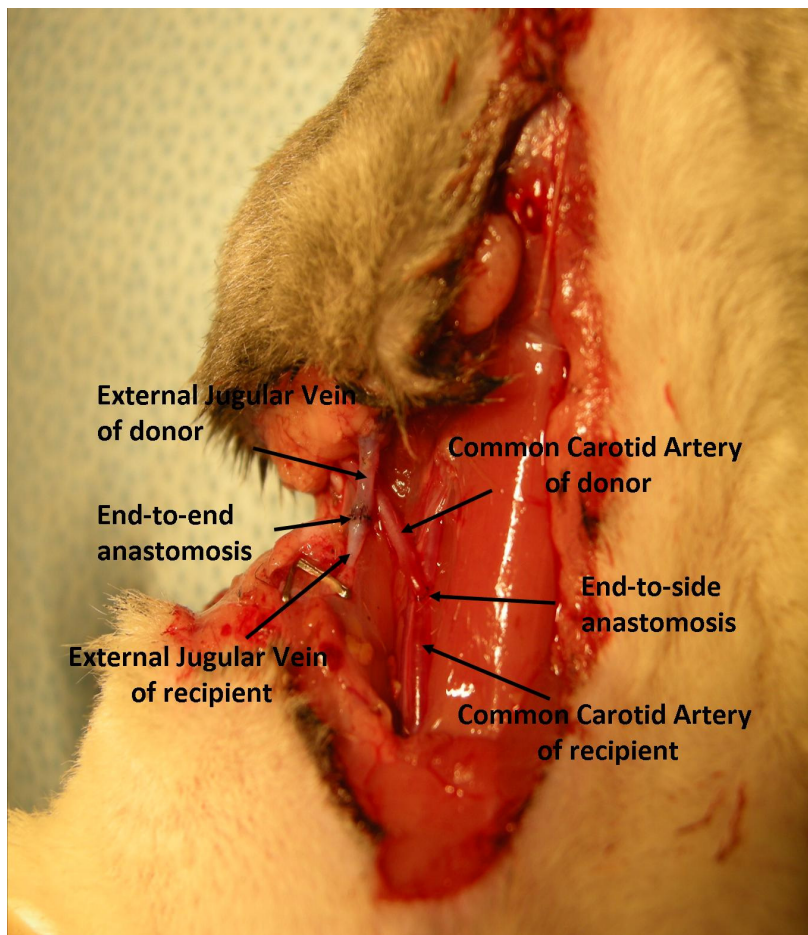


Figure 6: Vascular anastomosis: End-to-side anastomosis was performed between the common carotid artery of the donor and recipient. End-to-end anastomosis was performed between external jugular vein of the donor and recipient.

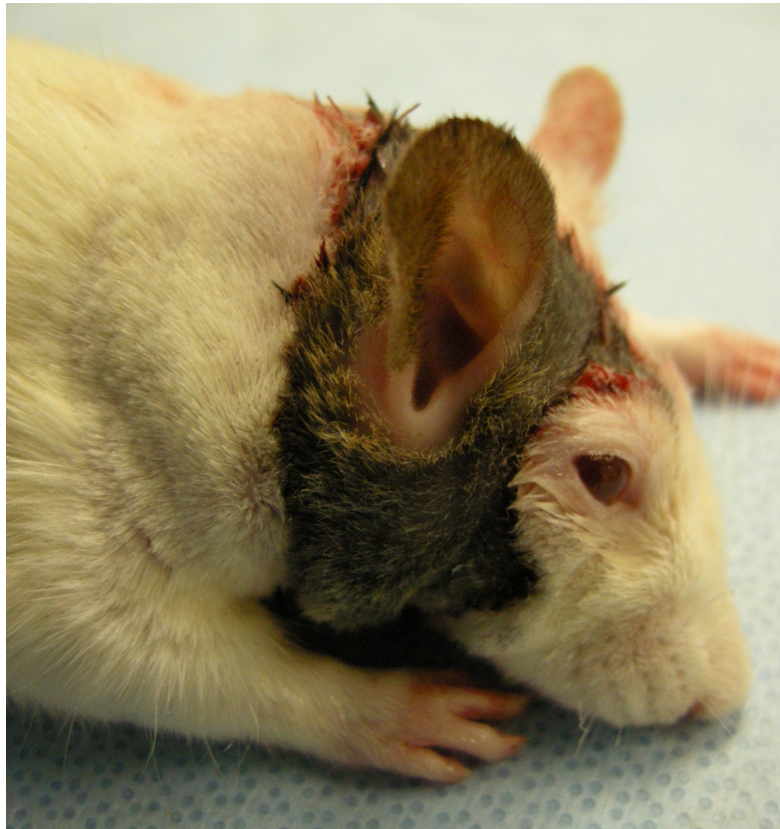


Figure 7: Hemiface allotransplantation: postoperative view after closure of the skin.

All incisions were thereafter carefully closed with 5/0 vicryl continuous suture technique (Figure 7).

The mean surgery time was 4 hours. The warm ischemia time was 45 minutes. After the operation, rats received 10 ml of

warm subcutaneous Ringer lactate solution to compensate for perioperative fluid loss and stayed on the heating pad.

Tacrolimus and Clobetasol ointments were applied to the allograft and massaged until completely absorbed.

Experimental groups

Group 1: LEW to LEW hemiface transfer (Isogenic control group).

Group 2: ACI to LEW hemiface allograft transplantation (No immunosuppression; Allogenic control group).

Group 3: ACI to LEW hemiface allograft transplantation with short-term systemic immunosuppression (7 days of immunosuppressive therapy with subcutaneous cyclosporine A (CsA, 16 mg/kg/d) and intraperitoneal anti $\alpha\beta$ -TCR monoclonal antibody (250 μ g) (clone: R73; BD Biosciences Pharmingen, Inc., San Diego, CA).

Group 4: ACI to LEW hemiface allograft transplantation with topical application of 0.7 g Tacrolimus ointment (Protopic® 0.1%, Astellas Pharma Manufacturing, Inc., Grand Island, NY) twice a day, starting on day 1.

Group 5: ACI to LEW hemiface allograft transplantation and topical application of 0.2 g Clobetasol Propionate ointment (Clobetasol Propionate ointment USP 0.05%, Taro Pharmaceuticals U.S.A., Inc., Hawthorne, NY) once a day, starting on day 1.

Groups 6 and 7: ACI to LEW hemiface allograft transplantation, 7 days systemic immunosuppression with CsA and anti $\alpha\beta$ -TCR monoclonal antibody, topical application of 0.7 g Tacrolimus (*group 6*) or 0.2 g Clobetasol (*group 7*) starting on day 8.

Groups 8 and 9: ACI to LEW hemiface allograft transplantation, 7 days systemic immunosuppression with CsA and anti $\alpha\beta$ -TCR monoclonal antibody, topical application of 0.7 g Tacrolimus (*group 8*) or 0.2 g Clobetasol (*group 9*) starting when clinical signs of grade 1-2 rejection were detected.

Group 10: ACI to LEW hemiface allograft transplantation, 7 days systemic immunosuppression with CsA and anti $\alpha\beta$ -TCR monoclonal antibody, topical application of 0.7 g Tacrolimus on the hemiface contralateral to the allograft, starting on day 8.

Group 11: ACI to LEW hemiface allograft transplantation, 7 days systemic immunosuppression with CsA and anti $\alpha\beta$ -TCR monoclonal antibody, topical application of 0.2 g Clobetasol

on the hemiface contralateral to the allograft, starting on day 8.

Assessment of rejection

Viability of the transplant was assessed daily by gross inspection of the facial allograft for clinical signs of rejection, graded 0 (no signs of rejection), 1 (erythema), 2 (edema), 3 (epidermolysis), 4 (hair loss) and 5 (necrosis). Grade 3 rejection or 100 day survival in absence of signs of rejection, were used as the end points of the study.

Flow cytometry (FC)

Peripheral blood from LEW recipients was stained with fluorochrome-labeled monoclonal Antibodies against CD3 (BD Pharmingen, cat 554833) and CD45 RA (BD Pharmingen, cat 554884) at regular intervals post-transplantation (7, 21, 35, 63, and 100 days). Flow cytometry applied FACSCalibur system (BD Biosciences, Sparks, MD) and FlowJo software (Ashland, OR) were used for data analysis.

Tacrolimus and cortisol levels

Twenty-four hour blood Tacrolimus levels and morning serum free cortisol levels (as an index of the systemic absorption of clobetasol) were measured in blood taken from external

jugular vein during biopsy procedures and assessed by liquid chromatography-tandem mass spectrometry.

Allograft biopsies

Biopsies were performed at day 7, 21, 35, 63, and 100, and as soon as rejection was clinically suspected. The samples were processed as follows: a) fixed in 4% paraformaldehyde and paraffin embedded; b) preserved in RNALater® solution and kept overnight in 4°C; c) O.C.T. embedded and snap frozen. Donor and recipient external jugular veins and common carotid arteries were harvested, and fixed in 4% paraformaldehyde and paraffin embedded.

Histology

A pathologist graded rejection in a blinded fashion, according to Banff classification (Table 2) in Hematoxylin-Eosin-stained sections. Thickness of the epidermis and dermis was measured at different time points using Image ProPlus 7 software (Media Cybernetics, Bethesda, MD).

Table 2 Modified BANFF classification used in the grading of the biopsies

Grade	Histologic features
I	Mild to moderate lymphocytic perivascular infiltrate in the superficial to middle dermis.
II	Moderate lymphocytic perivascular infiltrate in the superficial to middle dermis.
III	Moderate to severe perivascular lymphocytic infiltrate filling the dermis and clusters of at least 2 apoptotic keratinocytes. ^a
IV	Grade III with epidermal necrosis and +/- severe dermal inflammation.

^a Addition to original BANFF classification to increase the specificity of grade II acute cellular rejection in mucosal sites.

Russel-Movat pentachrome staining

Transverse 6 µm thick sections of donor and recipient external jugular veins and common carotid arteries were stained with Russel-Movat pentachrome to assess the presence of intimal hyperplasia.

Immunohistochemical staining

Immunohistochemical labeling for CD4, CD8a, CD45RA, CD11bc, and CD86 was performed on frozen sections according to standard protocols. The cellular infiltrates were quantified as the ratio between the amount of staining and the

total area at 100x magnification (Figures 8 and 9), using Image ProPlus 7 Software (Media Cybernetics, Bethesda, MD).



Figure 8: Area in which the inflammatory infiltrate was present in dermis is measured.

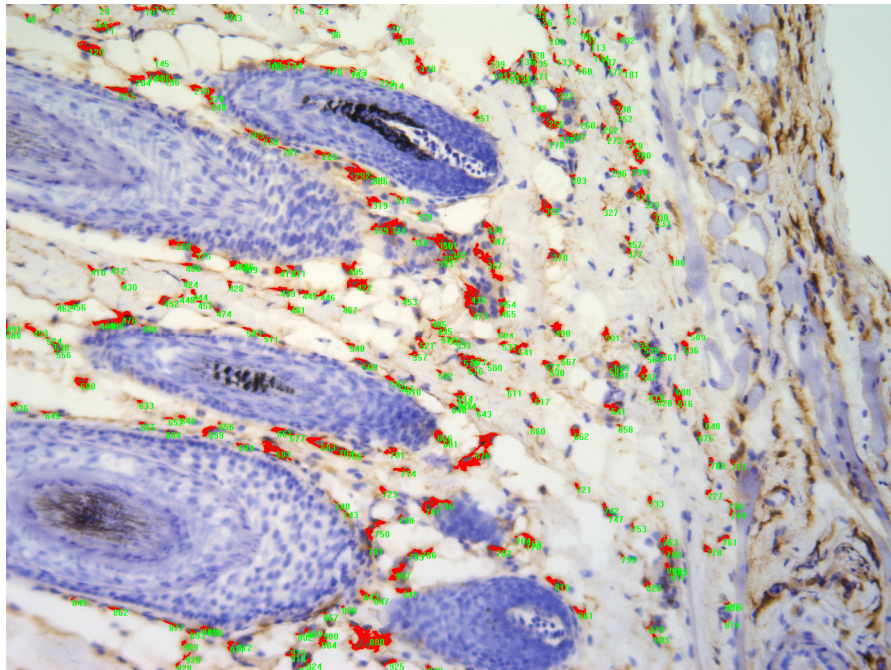


Figure 9: The stained cells are numbered in the same area as figure 8 and the ratio is obtained dividing the number of cells per stained area.

Measurement of the Thickness of Dermis, Epidermis and vascular intima

Thickness of the epidermis and dermis was measured at different time points using Image ProPlus 7 software (Media Cybernetics, Bethesda, MD) (Figure 10). The average of at least three measurements was calculated.

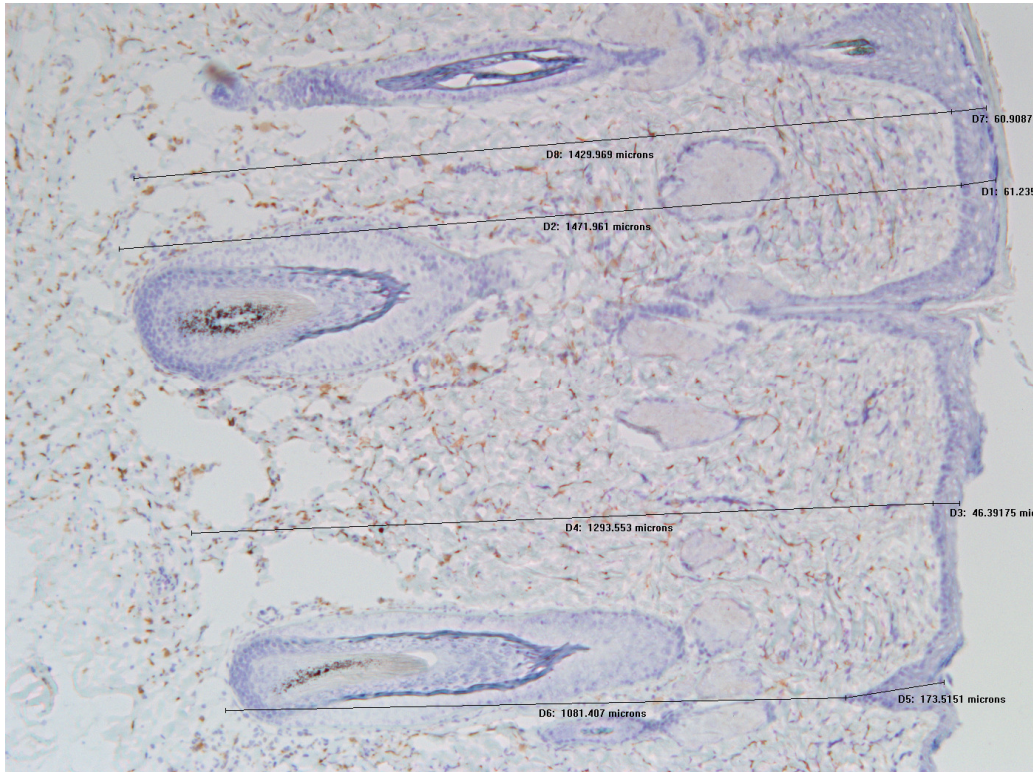


Figure 10: Measurement of dermal and epidermal thickness.

Thickness of the intima and total wall thickness of the recipient and donor carotid arteries and external jugular veins was measured at euthanasia using Image ProPlus 7 software (Media Cybernetics, Bethesda, MD).

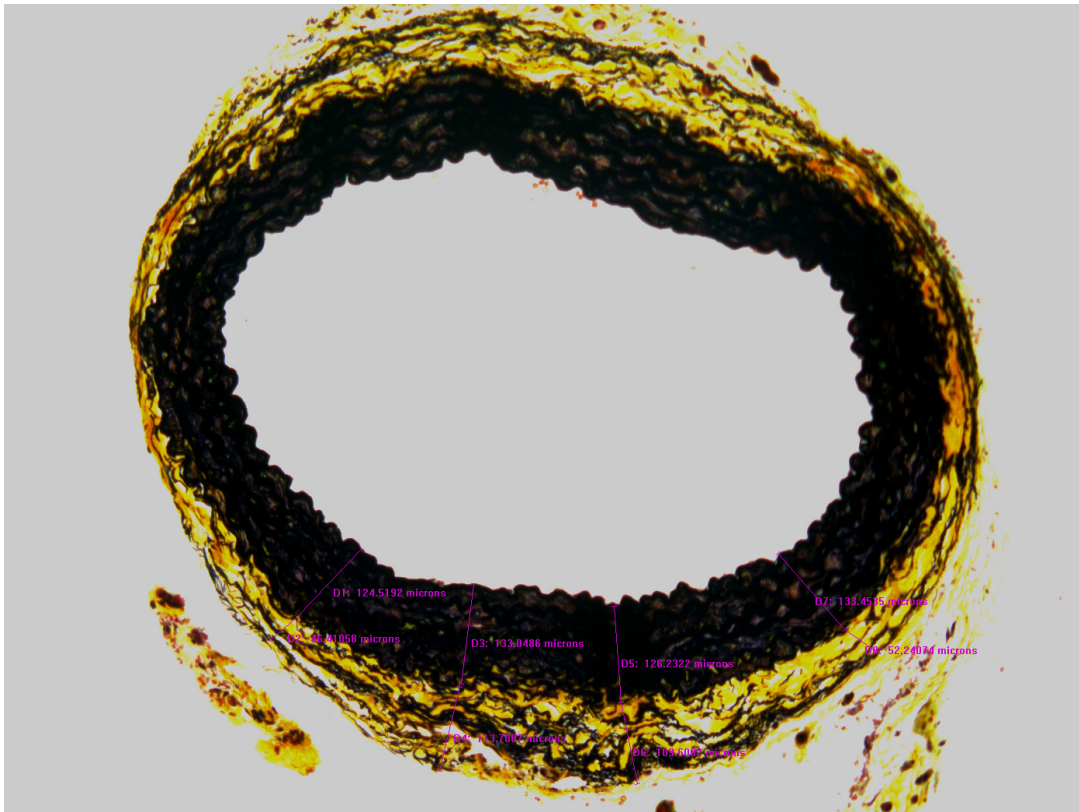


Figure 11: Measurement of intimal and arterial wall thickness in donor artery.

Real-time Polymerase Chain Reaction (Real-time PCR)

Total RNA was isolated using TRIZOL® Reagent (Invitrogen) according to the manufacturer's (Invitrogen™) instructions and was purified with RNeasy® Mini Kit.

Synthesis: 0.65 µg of total RNA was reverse-transcribed to cDNA in a total volume of 20 µl, using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems).

Relative Quantification of Gene Expression by Real-Time PCR

IL-2, IFN- γ , TNF- α , IL-13, IL-15, and TGF- β 1 genes were amplified using Real-Time PCR technique in a 7300 Real-Time PCR detection system, with 7300 System-SDS software (Applied Biosystems). Amplification was carried out in a total volume of 25 μ l, containing TaqMan Universal PCR Master Mix (2X) (Applied Biosystems), Gene Expression Assay Mix (20X), (Applied Biosystems), and 2.5 μ l cDNA under standard conditions and 40 cycles. Reported changes in expression for all examined genes were normalized to endogenous tyrosine 3-monooxygenase (Ywhaz) in each sample, and were relative to the expression of the gene in untreated skin tissue.

Statistical analysis

All data are expressed as mean \pm standard deviation. A mixed model analysis was used to assess the mean differences among experimental groups. Comparisons of the trend over time were assessed by examining the interaction between group and time. For group 2, there was only one follow-up at 7 days. The analysis of this trend over time was not performed. Therefore, the allograft group was excluded from the mixed model analysis, and a comparison between the allograft and isograft groups at 7 days was performed with the Wilcoxon's sum of

the ranks test. The comparison of group means and trends was performed for eight groups. Therefore, a Bonferroni adjusted significance level of $0.05/28=0.0018$ was used for multiple comparisons. A log-rank test was used to assess the overall difference in freedom of rejection among groups. Hazard ratios between groups were calculated from a Cox Proportional Hazard model. All analyses were performed using SAS 9.2 (Cary, NC). Results of the statistical analysis are shown in detail in appendix I.

Results

Survival

Episodes of acute rejection were observed in all groups, with the exception of isografts (Table 1). Acute rejection was invariably followed by allograft necrosis in the allograft (group 2) and in groups treated with either 1 week of systemic therapy or only topical Clobetasol (groups 3 and 5).

Allografts under topical Tacrolimus only, exhibited the most variable outcome: four allografts were rejected by 17.8 ± 8.5 days following the initial episode of acute rejection (Figure 12a), while two allografts survived the initial episode but subsequently developed hair loss, graft fibrosis and contracture on days 19 and 22 and were lost by day 60 ± 0 (Figure 12b). Two allografts recovered from three episodes of acute rejection, and reached the end point of the study (Figure 12c). Groups treated only with topical Tacrolimus or Clobetasol showed increased survival compared to untreated allografts ($p=0.005$; $p=0.030$), and under topical Tacrolimus extended survival was seen compared with topical Clobetasol group ($p<0.001$).

Table 1

	Group	Animal Number Per Group	Allograft Survival Days(SD)	Start rejection day(SD)	Number of episodes of rejection	Complications
1	Isograft	6	100±0	0±0	0±0	none
2	Allograft	8	6.8±0.6	3.6±1.1	1±0	none
3	Csa/anti-αβtcr 7 days	8	37.8±9.6	20.0±2.4	1±0	none
4	Topical Tacrolimus only	8	48.9±37.0	4±1.6	1.8±1.0	none
5	Topical Clobetasol only	8	18.3±3.4	6.5±3.2	1±0	wound dehiscence (57%)
6	Csa/anti-αβtcr 7 days+ Topical Tacrolimus from day 8	8	100±0	60.4±28.1	0.6±0.5	none
7	Csa/anti-αβtcr 7 days+ Topical Clobetasol from day 8	8	76.0±22.4	15.5±1.8	1.1±0.4	wound dehiscence (87.5%), corneal ulceration (12.5%)
8	Csa/anti-αβtcr 7 days+ Topical Tacrolimus start rejection	8	100±0	16.6±2.6	2.0±0.7	none
9	Csa/anti-αβtcr 7 days+Topical Clobetasol start rejection	8	74.1±22.1	19.5±1.6	1.1±0.4	wound dehiscence (71.4%), corneal ulceration (12.5%)
10	Csa/anti-αβtcr 7 days+ Topical Tacrolimus from day 8 on controlateral side	3	35.2±3.6	19.5±2.1	1±0	None
11	Csa/anti-αβtcr 7 days+ Topical Clobetasol from day 8 on controlateral side	3	37.4±2.8	20.1±1.8	1±0	None

Table 1: The allografts' survival in each group, the time at which the first episode of rejection was detected and the number of rejection episodes are reported as mean±standard deviation. Of note is the high prevalence of complications in groups treated with long-term Clobetasol.



Figure 12a (see next page for explanation)

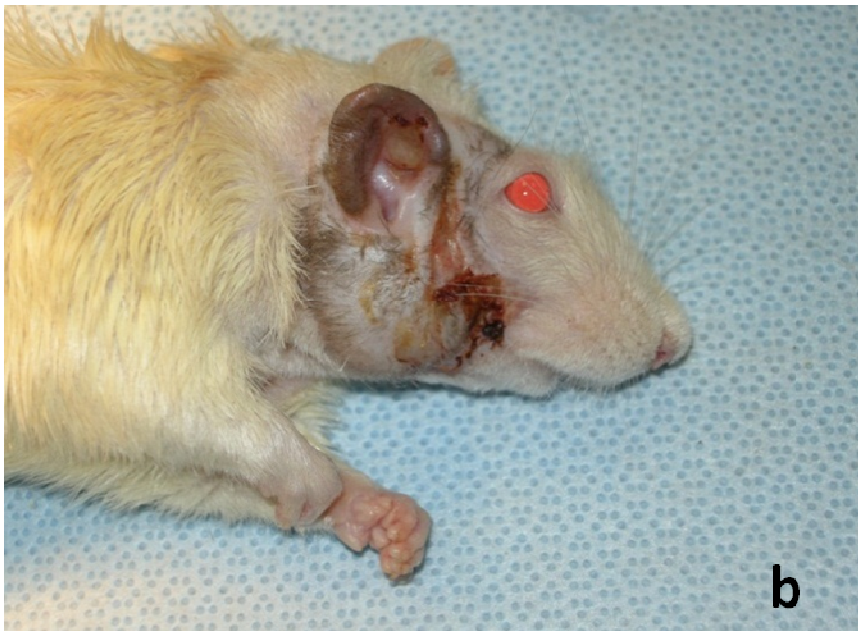


Figure 12b (see next page for explanation)



Figure 12c: Allografts in the Tacrolimus-only group displayed the most variable outcome. Following the first episode of acute rejection at an average of 4.0 ± 1.6 days (1a), four allografts were lost to full rejection; two allografts developed features of chronic rejection (1b) with hair loss, thickening, fibrosis, and progressive necrosis of the allograft at 50 days. Two allografts reached the end-point of the study without clinical signs of rejection (1c).

Improved survival was observed in groups treated with systemic therapy and topical Tacrolimus or Clobetasol applied at day 7 or at the first sign of rejection (groups 6, 7, 8 and 9 respectively $p=0.001$, 0.005 , 0.002 , 0.002) compared to the group treated only with 1 week of systemic therapy (group 3, Figure 10 a-b). Groups under 1 week of systemic therapy and topical Tacrolimus showed increased survival, compared to

systemic therapy and topical Clobetasol ($p= 0.002$; $p<0.001$, respectively). Acute rejection was lowest in allografts under systemic therapy and topical Tacrolimus (0.6 ± 0.5 episodes) (Figures 13-14).



Figure 13: Best results were achieved in the groups treated with systemic therapy, followed by application of topical Tacrolimus: the allograft appears pliable and displays normal adnexa at 100 days (13a). In contrast, allografts reaching the end-point in groups treated with systemic therapy, followed by topical Clobetasol, showed marked epidermal and dermal atrophy, wound dehiscence, and hair loss (13b).



Figure 14: Delayed episodes of acute rejection manifested with patchy hair loss in the groups treated with one week of CsA/anti- $\alpha\beta$ -TCR and topical Tacrolimus (allograft at 60 days, figure 14a). Spontaneous resolution was observed without modification of the treatment (same allograft at 78 days, figure 14b).

There was no significant survival difference between prophylactic application of the ointment and application at the first sign of rejection ($p=0.526$; $p=0.643$, respectively).

The greatest number of local complications, after topical treatment, was observed under systemic therapy and topical Clobetasol (group 7), with 87.5% wound dehiscence rate. No complications were observed in the Tacrolimus groups.

Tacrolimus and cortisol levels

Tacrolimus levels were lower than 2 ng/ml, in all groups. In both isografts and Clobetasol groups, cortisol levels were less than 1.0 $\mu\text{g/dL}$.

Peripheral blood lymphocytes

The population of CD3^+ cells decreased over time in groups under systemic therapy (Group 7) and topical Clobetasol (Group 9) (1.4% and 3.8% at euthanasia; $p<0.001$ and $p=0.002$), whereas systemic therapy alone showed significantly higher level of CD3^+ population (15.8% at euthanasia). Topical application of Clobetasol alone, without systemic induction, decreased population of the CD3^+ compared to isografts (6.3% versus 57.5% at euthanasia, $p<0.0001$). The lowest levels of CD3^+ were observed at day 7

in all groups treated with topical Tacrolimus, and increased gradually over time (49.1% group 4, 19.8% group 6 and 24.1% group 8 compared to 57.5% isograft at 100 days, $p < 0.001$) (Figure 15).

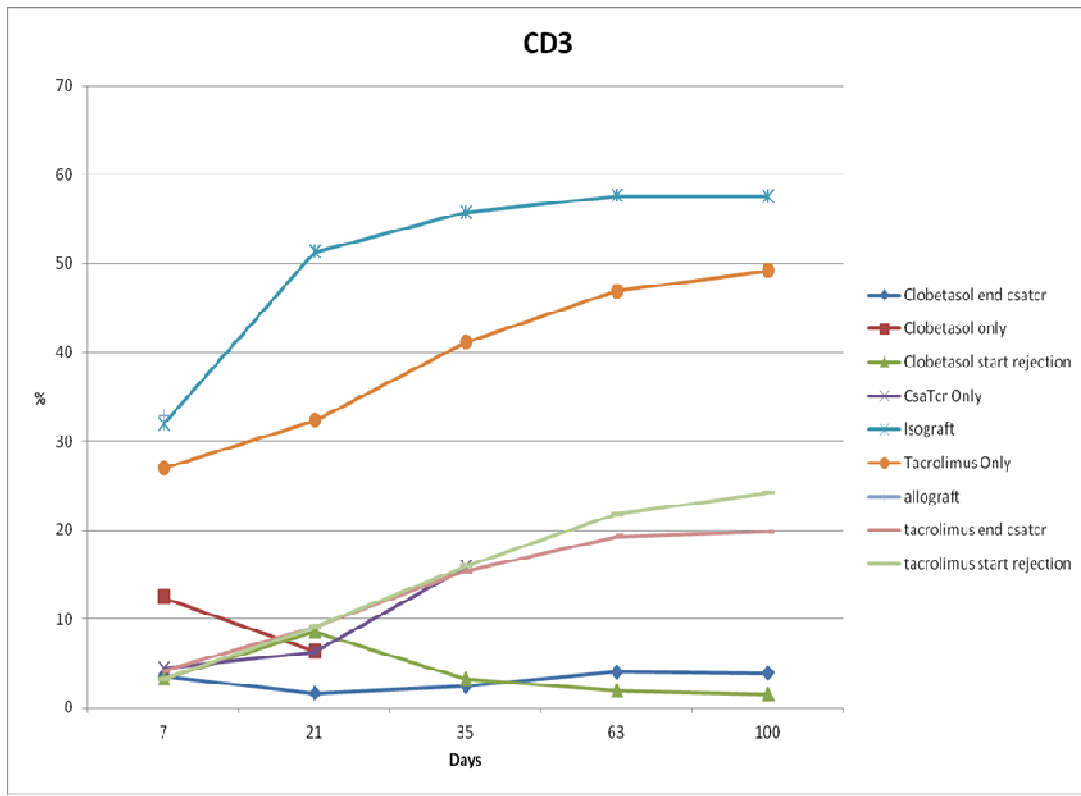


Figure 15: Flow Cytometry showed systemic depletion of CD3⁺ and in all groups compared to isografts. This effect was marked in the groups receiving Clobetasol following systemic CsA/anti- $\alpha\beta$ -TCR, where no trend toward repopulation was observed.

Assessment of CD45RA in the peripheral blood presented a pattern similar to the behavior of CD3⁺ population in

Clobetasol groups, showing decrease of CD45RA⁺ cells over time compared to the isografts (p<0.001). Groups treated with Tacrolimus showed a decreased level of CD45RA⁺ cells at day 21 (p<0.001), however at the end of the study, the values were comparable to the isograft controls (Figure 16).

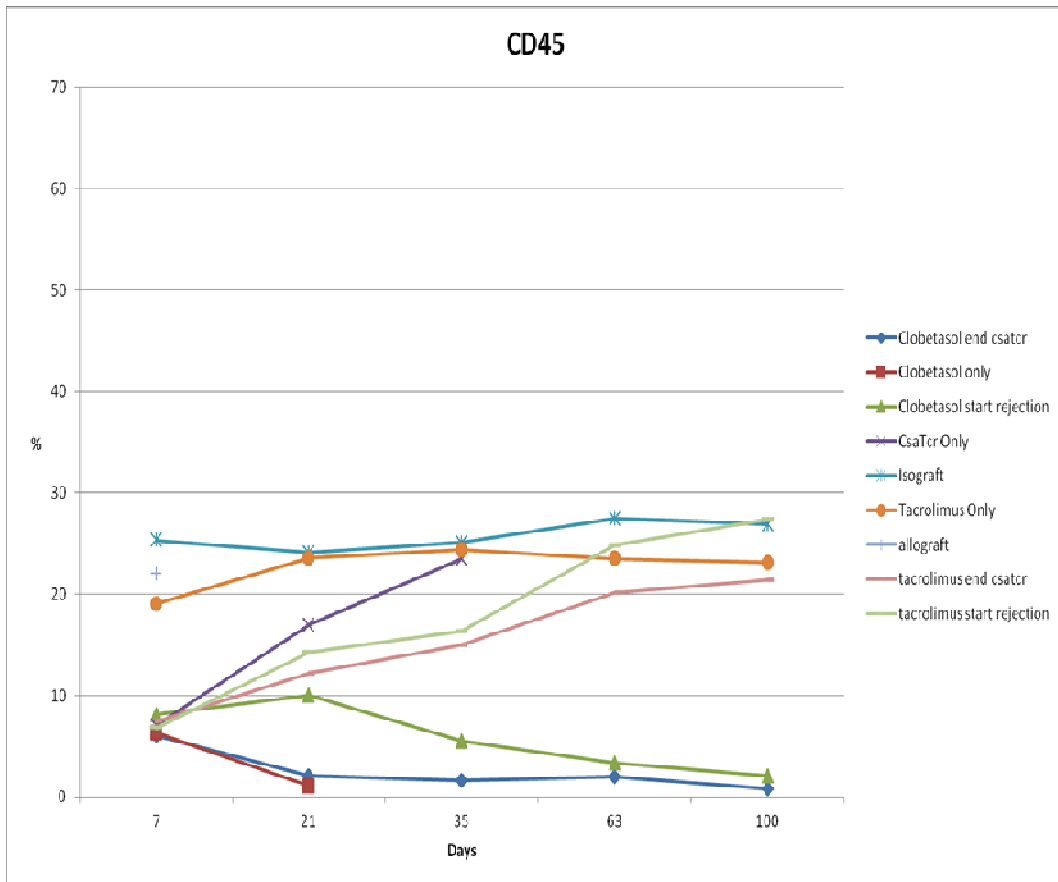


Figure 16: Flow Cytometry showed systemic depletion of CD3⁺ and in all groups compared to isografts. This effect was marked in the groups receiving Clobetasol following systemic CsA/anti- $\alpha\beta$ -TCR, where no trend toward repopulation was observed.

No evidence of chimerism was found in the peripheral blood.

Characterization of the cellular infiltrates in the skin biopsies

A rich cellular infiltrate was observed in the dermis at 7 days and was predominantly represented by CD4⁺ population in the early infiltrates scattered within the deep layer of the dermis and extending toward the surface during the rejection episodes. The number of CD4⁺ cells was significantly greater in all groups treated with Tacrolimus, compared to isografts ($p=0.002$ group 4, $p<0.001$, groups 6 and 8), and in the group treated with topical Clobetasol monotherapy ($p=0.002$).

CD8⁺ cells diffusely infiltrated the dermis at day 7 and were significantly higher in the allografts (group 2, $p=0.002$) and Clobetasol only group (group 5, $p<0.001$). The infiltrates in groups treated with systemic therapy and topical Clobetasol (groups 7 and 9) were comparable to isografts.

All groups showed decreasing CD11⁺ infiltration over time, except groups treated with systemic therapy alone or in combination with topical Clobetasol at the first sign of rejection (groups 3, 9) and the group treated only with topical Clobetasol (group 5), where diffuse infiltration was in the dermis.

CD45RA⁺ was limited to a few scattered cells throughout the dermis, or showed as small aggregates. Over time CD45RA⁺ cells decreased in all groups except for topical Clobetasol monotherapy. Isografts biopsy showed sporadically

presence of CD45RA⁺ cells. Immunostaining for CD86⁺ cells revealed rare, sparse cells, which gradually increased in all groups except for groups treated with systemic therapy or monotherapy with topical Clobetasol or Tacrolimus (groups 3, 4, 5).

The distribution and phenotypes of the cellular infiltrates at day 7 and at euthanasia are shown in Figures 17, 18 and 19.

Figure 17: The samples were stained with antibodies to CD4, CD8, CD11, CD45, and CD86 to assess response to treatment, along with the modification of cellular infiltrates in different groups over time: the amount of staining was expressed as stained area-ratio (SAR) for each sample. The average SARs were plotted against time for each marker (here are shown the trends for CD4, CD8, CD11, CD45).

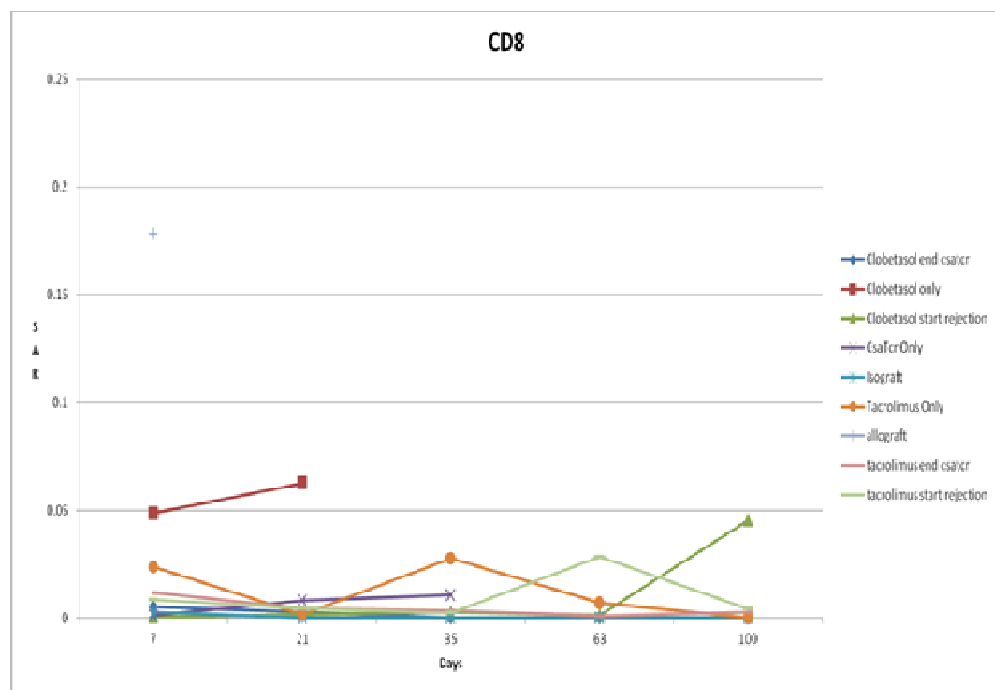
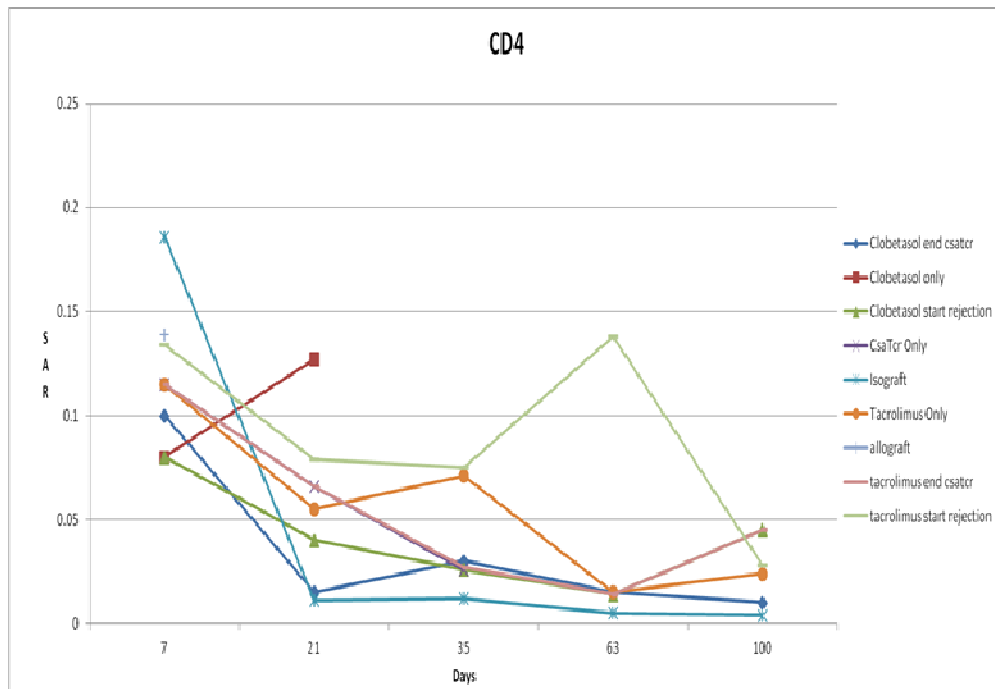


Figure 17

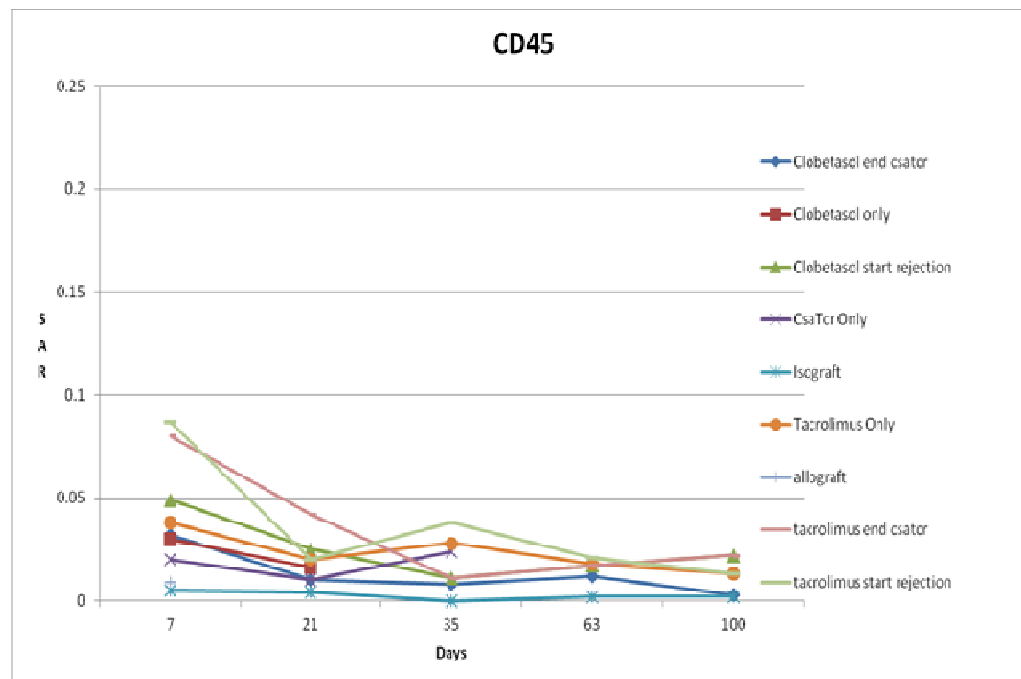
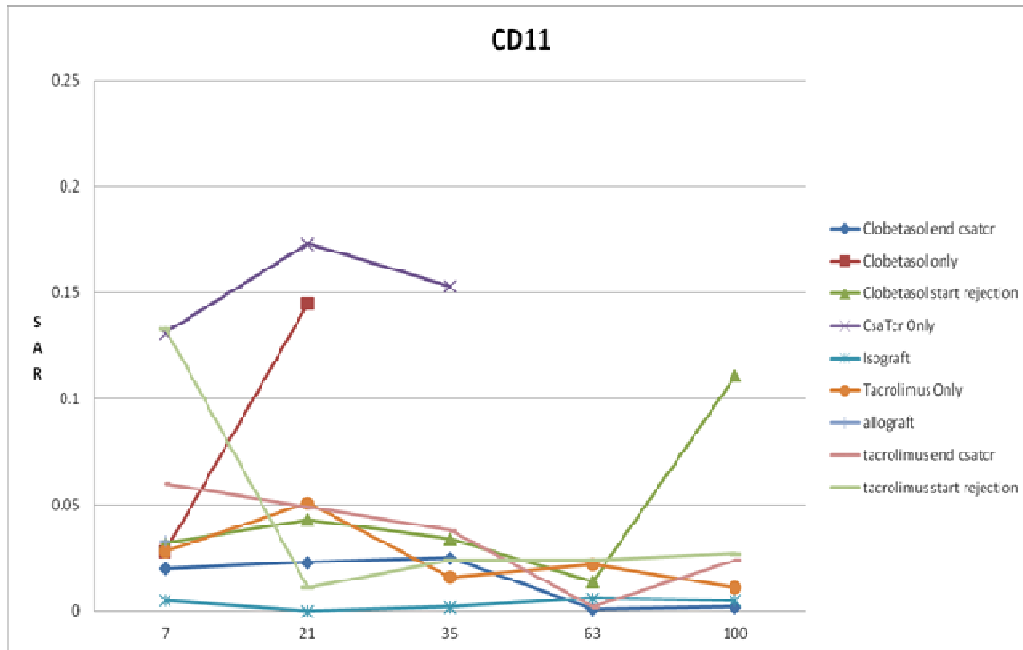


Figure 17

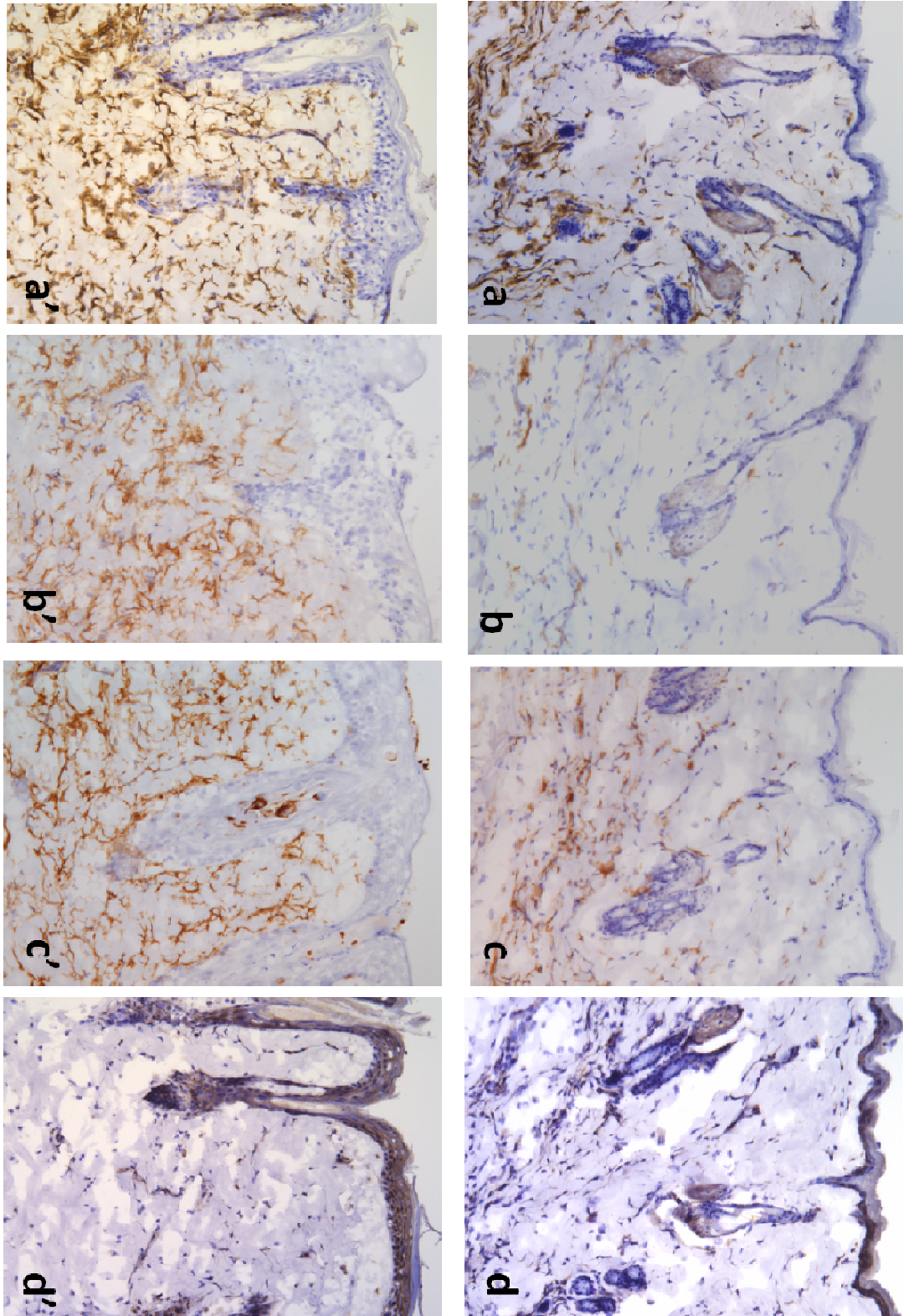


Figure 18

Figure 18: In the Clobetasol-only group, a rich cellular infiltrate dominated by CD4⁺ and CD11⁺ cells was present in the deep dermis at 7 days. The stained area for CD4⁺, CD8⁺, and CD 11⁺ cells (3a', 3b', 3c') increased with diffuse involvement of the dermal interstitium at euthanasia (18.3±3.4 days). CD45RA⁺ cells were limited to a few sparse cells or small aggregates throughout the dermis either at 7 or at euthanasia (3d and 3d').

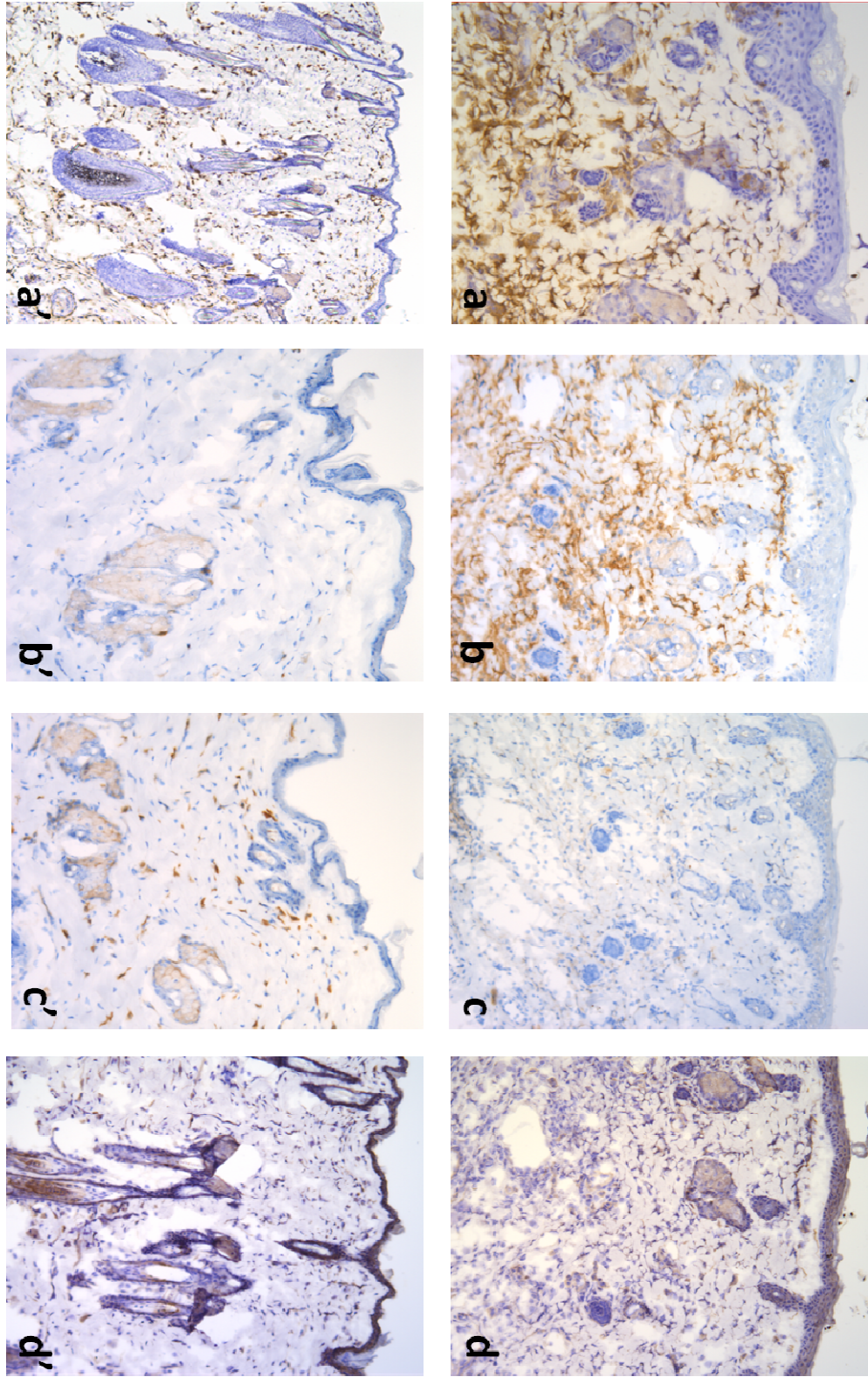


Figure 19

Figure 19: In the allografts treated with topical Tacrolimus, the cellular infiltrate at 7 days was largely positive for CD4⁺ (4a) and CD8⁺ (4b) cells. In allografts that survived up to 100 days, the infiltrate decreased over time (4a' and 4b'). The CD11⁺ and CD45⁺ cells were present moderately at 7 days (4c and 4d) and decreased toward the 100 days (4c' and 4d').

Characterization of the cytokine expression in the skin biopsies

IL-2 expression increased compared to baseline (two-fold increase) in allograft group ($p=0.002$), in with systemic therapy group ($p<0.001$) and in the groups treated with topical Tacrolimus ($p<0.001$) or Clobetasol monotherapy ($p<0.001$) (Figure 20).

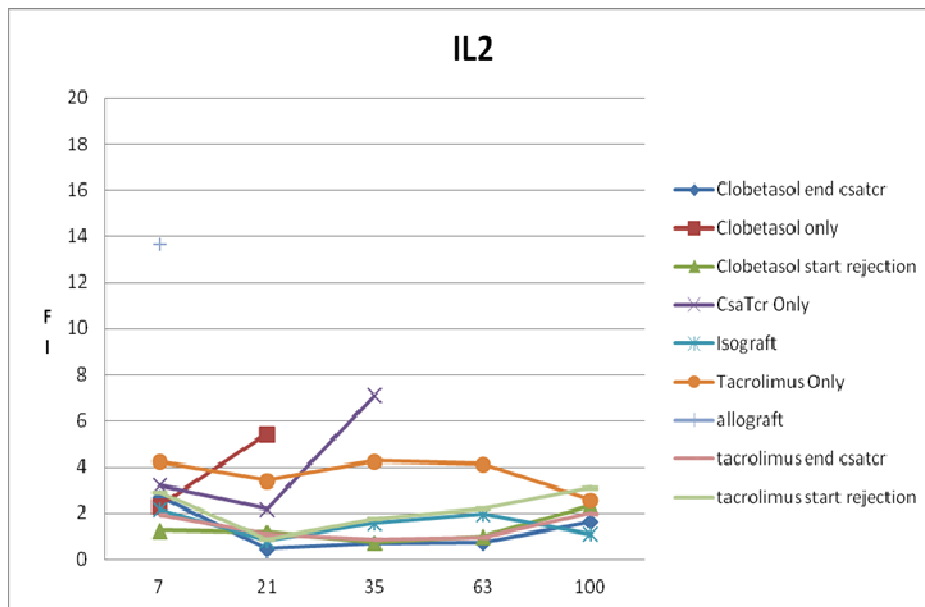


Figure 20: The expression of IL-2 is shown as the fold increase (FI), compared to the baseline.

IFN- γ levels showed an increase in allograft group ($p=0.002$) and the group treated with systemic monotherapy ($p<0.001$). The expression was higher than baseline, and increased compared to isografts in groups treated only with topical Clobetasol ($p<0.001$) or topical Clobetasol associated with systemic therapy ($p<0.001$). Increased levels of IFN- γ were recorded in the group treated with Tacrolimus monotherapy ($p=0.001$) (Figure 21).

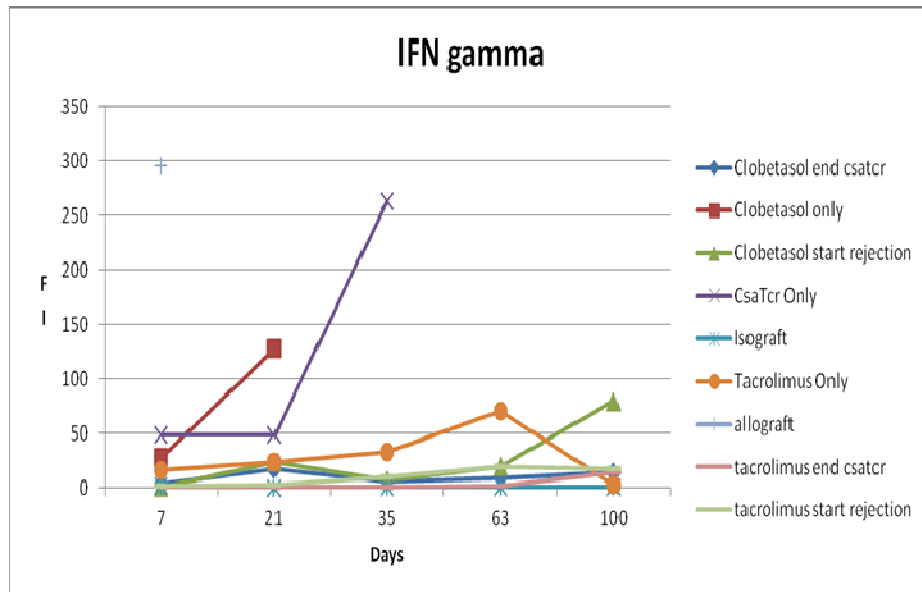


Figure 21: The expression of IFN- γ is shown as the fold increase (FI), compared to the baseline.

Groups under systemic therapy and topical tacrolimus showed increased TNF- α expression compared to baseline but no

significant differences were detected compared to isografts. Compared to isografts, TNF- α increased significantly in allograft group ($p=0.004$), in the group treated with topical Tacrolimus, in the group under systemic therapy alone ($p<0.001$) or systemic therapy with topical Clobetasol (Figure 22).

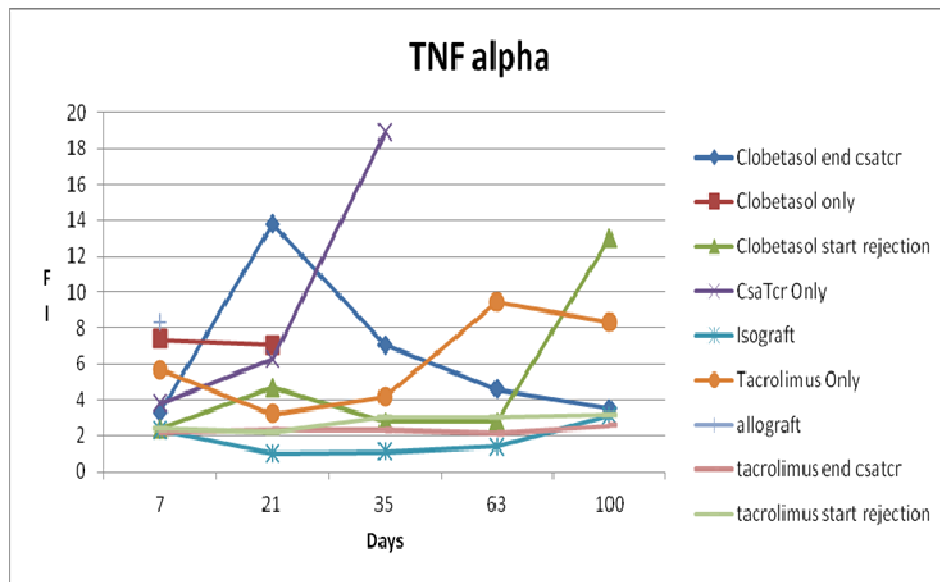


Figure 22: The expression of TNF- α is shown as the fold increase (FI), compared to the baseline.

TGF- β showed an increased expression in the allograft only group ($p=0.002$) and in the groups treated with systemic therapy only, topical Clobetasol only or systemic therapy with topical Clobetasol applied at the first sign of rejection ($p<0.001$) (Figure 23).

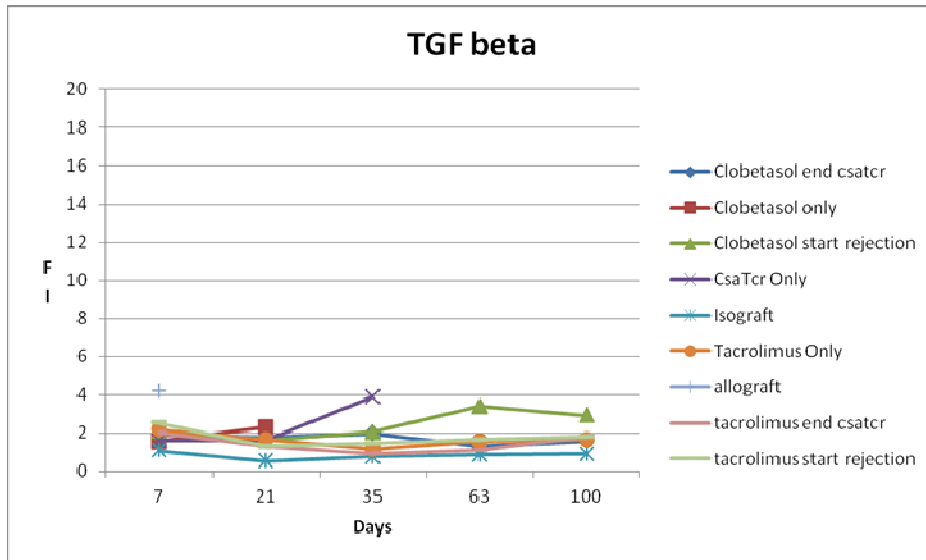


Figure 23: The expression of TGF- β is shown as the fold increase (FI), compared to the baseline.

Expression of IL-13 and IL-15 reached two-fold increase, compared to baseline.

Epidermal/dermal thickness

In all groups treated with Tacrolimus, a significant increase in dermal thickness was seen over time compared to isografts ($p < 0.001$). Allografts treated with systemic therapy and topical Clobetasol showed a decrease in dermal thickness ($p < 0.001$), whereas in allografts under topical Clobetasol alone no changes were observed over time ($p = 0.21$) and at euthanasia

dermal thickness was comparable ($p=0.30$) with isografts (Figure 24).

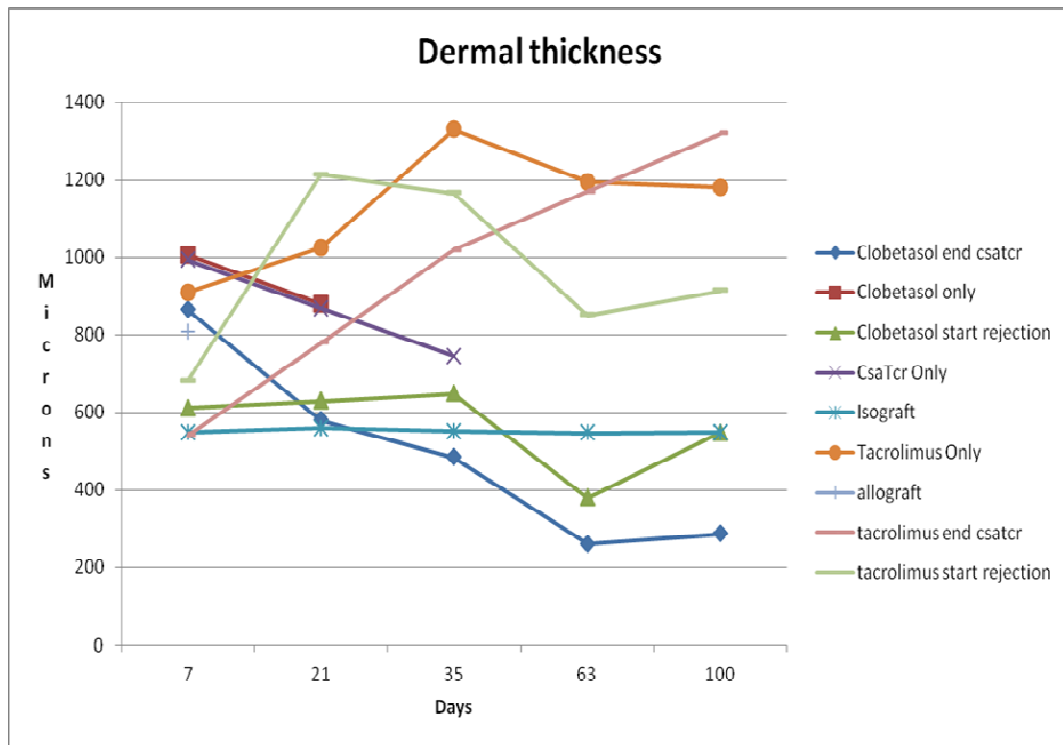


Figure 24: Modification in thickness of the dermis and epidermidis over time, was expressed in microns. In all of the groups treated with Tacrolimus, a significant increase in the dermal thickness was registered over time in comparison to the isografts ($p<0.001$), while the allografts treated with clobetasol and CsA/anti- $\alpha\beta$ -TCR showed a decrease in thickness ($p<0.001$).

Epidermal thickness decreased over time in the group under systemic therapy and topical Clobetasol applied at day 8

($p < 0.001$). An increase of epidermal thickness was observed in the groups treated with topical Tacrolimus or Clobetasol alone or under systemic therapy combined with topical Tacrolimus at signs of rejection ($p < 0.001$). During episodes of acute rejection increase in epidermal thickness was accompanied by epidermal spongiosis (Figure 25).

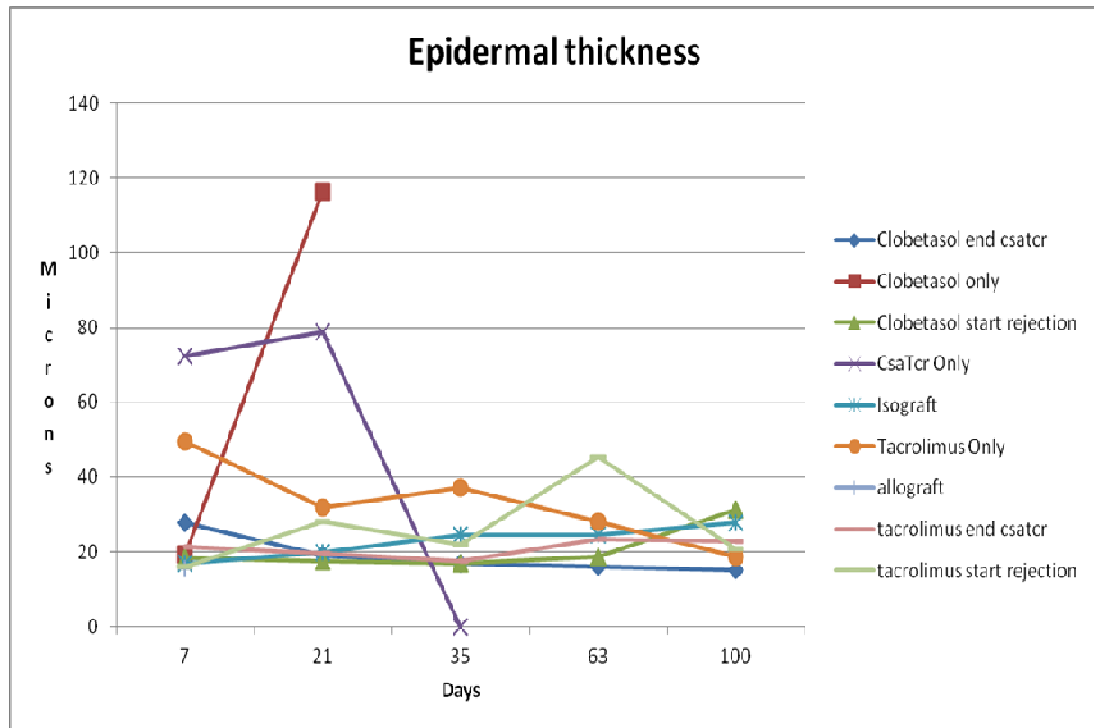


Figure 25: Modification in thickness of the dermis and epidermidis over time, was expressed in microns. Epidermal thickness decreased over time in the group treated with topical Clobetasol at the end of CsA/anti- $\alpha\beta$ -TCR ($p < 0.001$). An increase of the average epidermal thickness with treatment over time was observed in Clobetasol-only ($p < 0.001$), Tacrolimus-only ($p < 0.001$), and Tacrolimus-start rejection ($p < 0.001$).

Vessel Histology

Allograft arteries and veins showed no difference in the thickness of vessel intima, media, or adventitia compared to isografts (Figure 26).

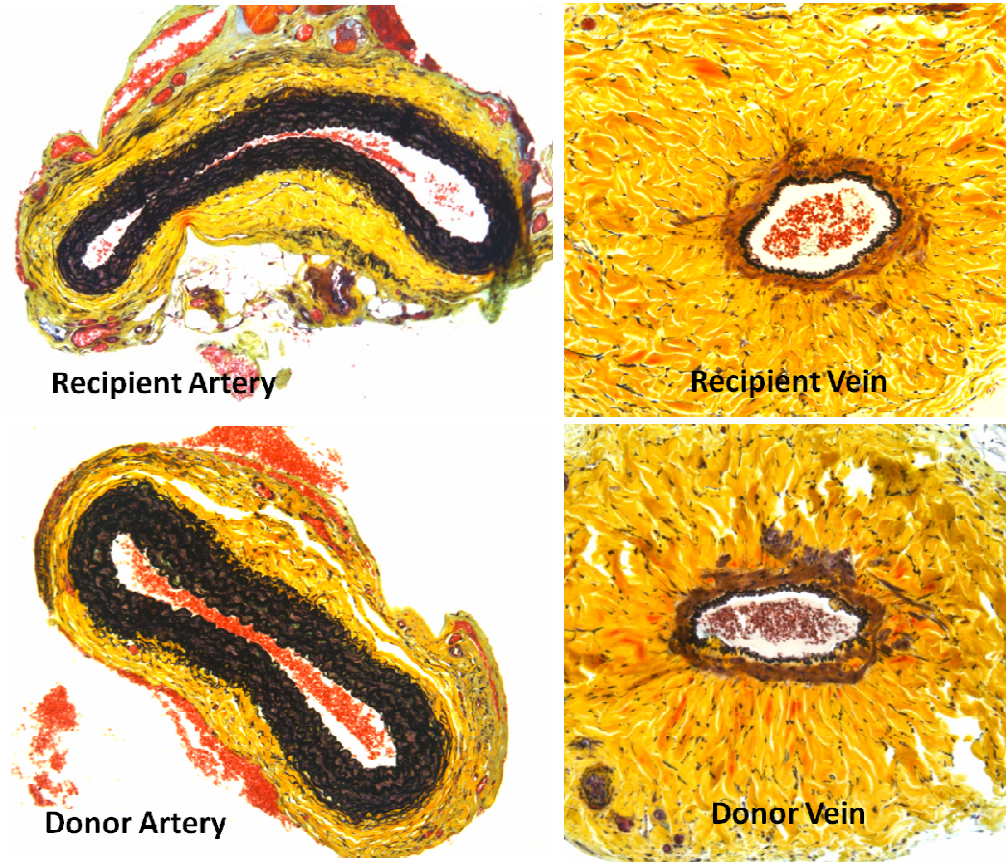


Figure 26: Histological comparison between donor and recipient arteries and veins at 100 days did not show a significant difference when compared with the isograft at 100 days (Russel-Movat pentachrome staining, 100x). Elastic Fibers: black; Collagen, Reticular Fibers: yellow; Muscle: red.

Discussion

We evaluated the effectiveness of topical immunosuppressants, Tacrolimus and Clobetasol, in the treatment and prevention of acute rejection in a face allotransplantation model.

The distribution, intensity, and phenotype of the inflammatory infiltrates of allograft were evaluated at different time points to assess if there was any correlation with the clinical outcomes under different treatment protocols. During episodes of acute rejection, composition and distribution of cellular infiltrates was similar to clinical cases of VCA(5,6). A rich, diffuse interstitial inflammatory infiltrate was found in the dermis at 7 days, with a predominance of CD4⁺ and CD11⁺ cells. This infiltrate decreased over time in all treatment groups except for Clobetasol monotherapy group (group 5) where allografts were acutely rejected. Increase in CD8⁺ cells correlated with rejection episodes. Characteristics of the observed cellular infiltrates supported the concept of a T cell mediated rejection. Graft infiltration by T cells, monocytes, macrophage and dendritic cells indicated gradual deterioration of the allograft(7). We did not observe significant differences in the subpopulation of cells infiltrating allografts under different treatment protocols, which indicates that results of biopsy may not directly correlate with recipients T cell functional responses to the allograft(8).

We assessed changes in the expression of specific cytokines to functionally characterize cytotoxic T cell infiltration, IFN- γ effects, macrophage infiltration, and tissue response to injury during rejection(7). The combined over-time and the absolute expression of IFN- γ , IL-2, TNF- α , and TGF- β levels proved to be a very sensitive tool in assessing the responses to different treatment protocols. In particular, the expression of cytokines clearly differentiated allograft rejection from surgical trauma related to the transplantation procedure. In addition, kinetics of cytokine expression provided valuable measure of cellular responses to different treatment protocols. Both the allograft controls and CsA/anti- $\alpha\beta$ -TCR-only groups exhibited a uniform increase in all measured cytokines which correlated with clinical signs of rejection. Application of topical Tacrolimus and Clobetasol was associated with decreased levels of inflammatory cytokines, however, this alone proved to be insufficient to prevent progression of the cellular damage resulting in allograft rejection in majority of animals. The combination of systemic and topical immunosuppression was most effective in suppressing the function of the infiltrating cells, by decreasing both the local production of IFN- γ and IL-2 by the effector T cells, as well as infiltration of the allograft by IFN- γ responsive macrophages.

T cell derived cytokines such as IL-15 and IL-13 have been involved in the promotion of the allograft rejection (9-12),

however we have not observed increased expression of these cytokines when compared with isografts.

Despite the fact that systemic levels of Tacrolimus were undetectable and cortisol production was not suppressed, topical application of Clobetasol and Tacrolimus was associated with lower levels of CD3⁺ and CD45RA⁺ cells, compared to isografts. Changes observed in the lymphocyte counts confirm the possibility of systemic absorption of Tacrolimus and Clobetasol.

To address the role of systemic depletion of lymphocytes on the allograft survival, topical treatment was applied to the contralateral (normal) side of the face (groups 10 and 11). These allografts were rejected at the same time as group 3 (CSA/anti $\alpha\beta$ TCR 7 days), confirming that increased allograft survival in groups 6 to 9 (systemic therapy combined with Tacrolimus or Clobetasol) was related to local immunosuppressive effects of both topical therapies, and was not influenced by systemic depletion of the lymphocytes.

Topical application of Clobetasol was associated with complete hair loss and significant dermal atrophy which correlated with duration of treatment in animals treated prophylactically (group 7 versus 9).

In this study, we observed two different patterns of rejection: a) the acute pattern (within few days after transplantation) which was characterized by edema, erythema and rapid progression to allograft necrosis and, b) a slowly progressing pattern with loss of adnexae, allograft contracture, fibrosis and ulceration. The second pattern was observed in Tacrolimus monotherapy group. It is open to discussion if the second- slowly progressing pattern- could represent evidence of chronic allograft dysfunction, due to sub-optimal immunosuppression, as evidenced by the increased levels of inflammatory cytokines in the absence of any evidence of graft vasculopathy(13).

Significant epidermal atrophy was observed only in group 7 (systemic therapy and prophylactic Clobetasol), which had the longest treatment duration, and increased epidermal thickness combined with epidermal spongiosis was observed in groups with acute rejection (3, 4, 5) and 8 (systemic therapy and Tacrolimus)(14). Although non-specific, epidermal thickness was a reliable outcome measure differentiating Clobetasol group from group 4 (tacrolimus only) group 6 and 8 (Tacrolimus and systemic therapy).

In conclusion, topical therapy with Tacrolimus and Clobetasol proved to be effective in treatment of acute episodes of rejection. Best outcomes were achieved when topical therapy

followed systemic immunosuppression with 7-day course of CsA/anti- $\alpha\beta$ -TCR. Topical Tacrolimus showed lower incidence of local and systemic complications. The combination of topical and systemic immunosuppression introduces an appealing therapeutic approach which is directly applicable for the VCA transplants.

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Appendix - Statistical Analysis

Table 0.1 Mean CD4

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	0.100(0.105)	0.015(0.004)	0.030(0.010)	0.015(0.005)	0.010(0.004)
Clobetasol only	0.080(0.013)	0.127(0.029)			
Clobetasol start rejection	0.080(0.029)	0.040(0.015)	0.026(0.014)	0.014(0.005)	0.045(0.027)
CsaTcr Only	0.115(0.015)	0.066(0.015)	0.026(0.011)		
Isograft	0.186(0.037)	0.011(0.004)	0.012(0.007)	0.005(0.003)	0.004(0.002)
Tacrolimus Only	0.115(0.057)	0.055(0.042)	0.071(0.031)	0.015(0.019)	0.024(0.009)
allograft	0.139(0.031)				
tacrolimus end csatcr	0.115(0.043)	0.066(0.016)	0.027(0.009)	0.014(0.004)	0.045(0.012)
tacrolimus start rejection	0.134(0.059)	0.079(0.034)	0.075(0.012)	0.138(0.015)	0.028(0.009)

Table 0.2 Mean CD8

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	0.0051(0.0021)	0.0026(0.0003)	0.0000(0.0000)	0.0000(0.0000)	0.0000(0.0000)
Clobetasol only	0.0487(0.0328)	0.0625(0.0230)			
Clobetasol start rejection	0.0005(0.0002)	0.0015(0.0008)	0.0027(0.0016)	0.0011(0.0010)	0.0454(0.0487)
CsaTcr Only	0.0012(0.0009)	0.0080(0.0017)	0.0106(0.0051)		
Isograft	0.0027(0.0007)	0.0000(0.0000)	0.0000(0.0000)	0.0000(0.0000)	0.0000(0.0000)
Tacrolimus Only	0.0236(0.0216)	0.0016(0.0018)	0.0276(0.0214)	0.0071(0.0086)	0.0002(0.0001)
allograft	0.1782(0.0421)				
tacrolimus end csatcr	0.0116(0.0037)	0.0045(0.0011)	0.0034(0.0027)	0.0009(0.0007)	0.0027(0.0015)
tacrolimus start rejection	0.0084(0.0041)	0.0045(0.0018)	0.0024(0.0018)	0.0284(0.0172)	0.0043(0.0030)

Table 0.3 Mean CD11

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	0.020(0.014)	0.023(0.012)	0.025(0.007)	0.001(0.000)	0.002(0.001)
Clobetasol only	0.028(0.019)	0.145(0.043)			
Clobetasol start rejection	0.032(0.014)	0.043(0.019)	0.034(0.013)	0.014(0.003)	0.111(0.030)
CsaTcr Only	0.131(0.003)	0.173(0.048)	0.153(0.071)		
Isograft	0.005(0.001)	0.000(0.000)	0.002(0.001)	0.006(0.001)	0.005(0.001)
Tacrolimus Only	0.028(0.034)	0.051(0.060)	0.016(0.011)	0.022(0.029)	0.011(0.004)
allograft	0.033(0.016)				
tacrolimus end csatcr	0.060(0.013)	0.049(0.019)	0.038(0.020)	0.002(0.001)	0.024(0.008)
tacrolimus start rejection	0.133(0.018)	0.011(0.004)	0.024(0.012)	0.024(0.009)	0.027(0.014)

Table 0.4 Mean CD45

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	0.032(0.019)	0.010(0.006)	0.008(0.002)	0.012(0.006)	0.003(0.001)
Clobetasol only	0.030(0.019)	0.016(0.004)			
Clobetasol start rejection	0.049(0.011)	0.025(0.013)	0.011(0.003)	0.017(0.008)	0.022(0.008)
CsaTcr Only	0.020(0.007)	0.010(0.002)	0.024(0.010)		
Isograft	0.005(0.001)	0.004(0.003)	0.000(0.000)	0.002(0.001)	0.002(0.001)
Tacrolimus Only	0.038(0.018)	0.020(0.007)	0.028(0.013)	0.018(0.019)	0.013(0.001)
allograft	0.009(0.003)				
tacrolimus end csatcr	0.080(0.015)	0.042(0.018)	0.011(0.003)	0.017(0.005)	0.022(0.002)
tacrolimus start rejection	0.087(0.024)	0.020(0.003)	0.038(0.009)	0.021(0.007)	0.013(0.003)

Table 0.5 Mean CD86

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	0.0178(0.0032)	0.0108(0.0046)	0.0037(0.0004)	0.0005(0.0001)	0.0001(0.0001)
Clobetasol only	0.0028(0.0015)	0.0000(0.0000)			
Clobetasol start rejection	0.0011(0.0009)	0.0026(0.0009)	0.0096(0.0017)	0.0045(0.0015)	0.0027(0.0013)
CsaTcr Only	0.0000(0.0000)	0.0046(0.0014)	0.0032(0.0011)		
Isograft	0.0005(0.0003)	0.0002(0.0002)	0.0006(0.0003)	0.0005(0.0004)	0.0004(0.0004)
Tacrolimus Only	0.0018(0.0013)	0.0012(0.0005)	0.0168(0.0296)	0.0033(0.0005)	0.0007(0.0007)
allograft	0.0000(0.0000)				
tacrolimus end csatcr	0.0016(0.0008)	0.0018(0.0015)	0.0005(0.0007)	0.0031(0.0013)	0.0026(0.0024)
tacrolimus start rejection	0.0000(0.0000)	0.0012(0.0008)	0.0012(0.0007)	0.0007(0.0004)	0.0003(0.0002)

Table 0.6 Mean Dermal Thickness

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	864.20(44.87)	579.77(15.24)	483.18(104.33)	259.87(37.42)	286.76(59.71)
Clobetasol only	1004.61(43.45)	879.95(109.47)			
Clobetasol start rejection	610.89(67.91)	629.37(46.22)	647.85(101.35)	379.82(31.70)	547.39(84.51)
CsaTcr Only	992.92(28.44)	869.11(11.71)	745.31(21.80)		
Isograft	548.41(48.69)	558.70(49.73)	550.98(48.15)	546.63(46.97)	548.36(49.05)
Tacrolimus Only	909.92(353.17)	1024.61(122.90)	1329.67(131.42)	1193.82(224.30)	1180.68(485.07)
allograft	807.44(52.38)				
tacrolimus end csatcr	541.42(61.92)	780.04(33.95)	1018.66(34.38)	1168.94(55.45)	1319.22(138.12)
tacrolimus start rejection	682.19(78.11)	1213.63(165.94)	1165.97(114.85)	851.26(151.28)	914.12(45.30)

Table 0.7 Mean epidermal thickness

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	27.62(4.26)	18.96(2.05)	16.93(3.01)	16.16(2.51)	15.20(3.05)
Clobetasol only	19.33(2.05)	116.36(29.78)			
Clobetasol start rejection	18.32(3.38)	17.57(1.24)	16.81(1.88)	18.62(3.91)	31.42(15.78)
CsaTcr Only	72.30(11.53)	78.82(21.70)	0.00(0.00)		
Isograft	17.01(2.79)	19.79(2.93)	24.60(1.75)	24.53(8.98)	27.89(5.02)
Tacrolimus Only	49.53(26.99)	31.97(21.14)	37.16(24.28)	28.15(5.40)	18.56(1.52)
allograft	15.33(1.83)				
tacrolimus end csatcr	21.27(3.41)	19.43(3.15)	17.59(3.56)	23.34(3.30)	22.85(2.85)
tacrolimus start rejection	16.16(2.51)	28.16(7.94)	21.92(3.44)	45.44(4.74)	20.73(5.29)

Table 0.8 Mean IL_2

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	2.717(3.311)	0.448(0.263)	0.707(0.619)	0.720(0.363)	1.632(0.958)
Clobetasol only	2.305(1.496)	5.452(1.821)			
Clobetasol start rejection	1.242(0.607)	1.158(0.610)	0.727(0.515)	0.988(0.571)	2.317(0.861)
CsaTcr Only	3.212(2.542)	2.185(1.193)	7.098(3.389)		
Isograft	2.150(0.556)	0.803(0.107)	1.567(0.239)	1.947(0.366)	1.100(0.210)
Tacrolimus Only	4.226(2.528)	3.423(1.802)	4.260(2.653)	4.133(1.458)	2.585(0.049)
allograft	13.672(4.699)				
tacrolimus end csatcr	1.927(1.865)	1.068(0.471)	0.877(0.368)	0.968(0.313)	2.002(0.595)
tacrolimus start rejection	2.903(2.477)	0.872(0.482)	1.752(1.084)	2.202(1.371)	3.100(0.588)

Table 0.9 Mean IL_13

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	6.230(9.248)	0.088(0.112)	0.020(0.009)	0.020(0.009)	0.018(0.008)
Clobetasol only	0.117(0.137)	0.030(0.014)			
Clobetasol start rejection	2.340(1.612)	0.333(0.462)	0.020(0.011)	0.020(0.009)	0.022(0.012)
CsaTcr Only	0.360(0.218)	1.260(0.615)	1.097(0.894)		
Isograft	1.360(0.826)	0.220(0.140)	0.510(0.192)	0.373(0.323)	0.400(0.079)
Tacrolimus Only	1.501(1.003)	0.410(0.238)	0.274(0.133)	0.605(0.484)	0.410(0.014)
allograft	1.752(0.965)				
tacrolimus end csatcr	0.802(0.635)	0.242(0.039)	0.340(0.146)	0.170(0.080)	0.708(0.429)
tacrolimus start rejection	2.038(1.063)	0.452(0.155)	0.328(0.155)	0.168(0.125)	0.527(0.029)

Table 0.10 Mean IL_15

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	0.885(0.153)	0.380(0.151)	0.410(0.159)	0.482(0.161)	0.478(0.135)
Clobetasol only	0.370(0.158)	0.660(0.257)			
Clobetasol start rejection	1.060(0.246)	0.518(0.227)	0.440(0.082)	0.382(0.306)	0.757(0.351)
CsaTcr Only	0.797(0.227)	0.908(0.174)	0.932(0.174)		
Isograft	0.583(0.121)	0.627(0.047)	1.130(0.420)	0.997(0.264)	1.000(0.057)
Tacrolimus Only	0.521(0.042)	0.534(0.172)	0.608(0.125)	0.665(0.049)	0.950(0.014)
allograft	0.538(0.080)				
tacrolimus end csatcr	0.818(0.287)	0.548(0.116)	0.507(0.115)	0.688(0.027)	0.820(0.398)
tacrolimus start rejection	1.350(0.413)	0.567(0.273)	0.628(0.279)	0.870(0.164)	0.668(0.216)

Table 0.11 Mean TGF_beta

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	1.927(0.845)	1.767(0.451)	1.980(1.038)	1.310(0.229)	1.578(0.300)
Clobetasol only	1.562(0.263)	2.317(0.245)			
Clobetasol start rejection	2.105(0.823)	1.590(0.549)	2.037(1.951)	3.392(4.757)	2.923(0.306)
CsaTcr Only	1.590(0.572)	1.620(0.677)	3.908(1.135)		
Isograft	1.080(0.179)	0.527(0.111)	0.753(0.354)	0.860(0.072)	0.900(0.283)
Tacrolimus Only	2.177(0.778)	1.657(0.292)	1.152(0.263)	1.550(0.463)	1.640(0.085)
allograft	4.180(0.787)				
tacrolimus end csatcr	2.022(0.428)	1.300(0.489)	0.928(0.197)	1.088(0.133)	1.828(0.595)
tacrolimus start rejection	2.530(0.811)	1.298(0.137)	1.472(0.473)	1.668(0.809)	1.807(0.108)

Table 0.12 Mean TNF_alpha

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	3.323(2.443)	13.778(13.446)	7.088(5.481)	4.598(2.885)	3.498(0.819)
Clobetasol only	7.368(2.564)	7.078(1.161)			
Clobetasol start rejection	2.392(0.705)	4.687(1.672)	2.790(0.986)	2.788(1.005)	13.018(7.315)
CsaTcr Only	3.802(2.675)	6.302(1.371)	18.898(10.042)		
Isograft	2.340(0.290)	0.987(0.139)	1.107(0.306)	1.380(0.132)	3.100(0.261)
Tacrolimus Only	5.684(1.785)	3.216(0.764)	4.180(2.104)	9.458(2.735)	8.300(0.014)
allograft	8.325(5.860)				
tacrolimus end csatcr	2.192(0.383)	2.312(0.698)	2.345(0.272)	2.110(0.281)	2.557(0.782)
tacrolimus start rejection	2.395(0.993)	2.215(0.738)	3.053(1.357)	3.063(1.732)	3.160(1.103)

Table 0.13 Mean IFN

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	3.982(5.853)	18.010(19.559)	6.110(3.442)	8.978(7.255)	14.137(3.487)
Clobetasol only	28.200(31.983)	127.830(38.406)			
Clobetasol start rejection	0.770(0.420)	23.637(17.977)	6.792(3.706)	19.352(11.136)	79.537(62.584)
CsaTcr Only	48.778(51.749)	48.818(24.271)	263.700(107.762)		
Isograft	1.757(0.736)	0.447(0.146)	0.520(0.102)	1.400(0.349)	1.400(0.261)
Tacrolimus Only	16.390(10.903)	24.020(23.216)	33.256(25.481)	71.000(75.036)	2.560(0.014)
allograft	295.082(244.316)				
tacrolimus end csatcr	1.193(0.812)	0.963(0.843)	1.308(0.868)	2.300(1.453)	14.138(20.449)
tacrolimus start rejection	1.800(1.167)	3.098(1.467)	10.738(15.472)	19.540(30.284)	17.583(19.313)

Table 0.14 Mean FACS_ CD3

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	3.500(2.448)	1.640(1.316)	2.398(2.410)	4.026(3.507)	3.883(2.689)
Clobetasol only	12.393(7.166)	6.313(4.307)			
Clobetasol start rejection	3.278(2.482)	8.587(0.715)	3.237(0.659)	1.903(0.414)	1.492(0.234)
CsaTcr Only	4.442(1.894)	6.268(3.592)	15.823(2.561)		
Isograft	31.875(2.382)	51.283(3.714)	55.733(8.465)	57.583(5.676)	57.565(4.406)
Tacrolimus Only	26.964(5.522)	32.306(5.986)	41.120(13.405)	46.900(8.150)	49.150(7.849)
allograft	32.783(13.713)				
tacrolimus end csatcr	4.147(1.562)	9.028(7.527)	15.378(4.995)	19.255(6.398)	19.803(11.420)
tacrolimus start rejection	3.230(0.765)	9.095(0.896)	15.942(2.758)	21.803(7.205)	24.162(1.674)

Table 0.15 Mean FACS_CD45

<i>Group</i>	<i>Mean(SD) at 7 days</i>	<i>Mean(SD) at 21 days</i>	<i>Mean(SD) at 35 days</i>	<i>Mean(SD) at 63 days</i>	<i>Mean(SD) at 100 days</i>
Clobetasol end csatcr	6.065(2.881)	2.051(2.871)	1.656(1.678)	1.983(2.065)	0.832(0.475)
Clobetasol only	6.365(1.063)	1.116(0.804)			
Clobetasol start rejection	8.107(1.341)	10.005(1.611)	5.518(1.007)	3.352(1.267)	2.053(1.202)
CsaTcr Only	7.070(3.532)	16.987(4.264)	23.452(4.423)		
Isograft	25.333(5.073)	24.100(2.434)	25.092(1.897)	27.490(2.458)	26.888(1.812)
Tacrolimus Only	19.066(4.330)	23.576(9.382)	24.412(3.946)	23.525(5.048)	23.150(0.071)
allograft	22.060(4.894)				
tacrolimus end csatcr	7.460(2.398)	12.200(3.799)	14.978(4.853)	20.140(2.442)	21.383(3.376)
tacrolimus start rejection	6.823(1.132)	14.263(3.214)	16.348(2.339)	24.830(4.728)	27.344(1.938)

Data for time to rejection analysis

Results from longitudinal data analysis

1. Immunohistochemistry responses – CD4

Table 1- Provides the estimates of intercept and slope of the regression line of CD4 over time for each group. For example, for the group “Clobetasol only”, the estimated intercept was 0.057 and the estimated slope was 0.003. The slope of 0.003 was telling that for each day increase in follow up time, the level of CD4 increased by 0.003, on average.

Table 2- Provides the comparisons on slopes of the regression lines of CD4 over time among groups. This was essentially testing whether the trend over time was the same or not among groups. The overall p value for slope comparison was 0.0015. That is to say, there were differences existing among slopes. For example, from column 3, the slope for “Clobetasol start rejection” was estimated to be 0.0003 larger than that for the “CsaTcr Only” group ($p=0.0005$). Please note, a Bonferroni adjusted significance level of $0.05/28=0.0018$ should be used here for multiple comparisons. Since there were significant differences in slopes of regression lines among groups, we were not able to compare overall group means. However, we can compare group means at a specific time point.

Table 3- Provides comparisons on mean CD4 at a specific time point (at mean follow-up days=39.8 days) among groups. The overall p value < 0.0001 was telling that there were significant differences when comparing mean CD4 at 39.8 days among groups. For example, the mean CD4 for the “Clobetasol end csatcr” group at 39.8 days was estimated to be 0.027 less than that for the “Tacrolimus Only” group at 39.8 days ($p<.0001$). Please note, a Bonferroni adjusted significance level of $0.05/28=0.0018$ should be used here for multiple comparisons. Although explanation for Table 4 – 45 were not presented, the interpretation was similar.

Table1. Estimate of intercept and slope for each group - CD4

Effect	Group	Estimate(95% CI)	p value
group	Clobetasol end csatcr	0.037(0.022, 0.053)	<.0001
group	Clobetasol only	0.057(-0.051, 0.166)	0.2917
group	Clobetasol start rejection	0.024(0.008, 0.039)	0.0038
group	CsaTcr Only	0.130(0.078, 0.181)	<.0001
group	Isograft	0.028(0.013, 0.044)	0.0007
group	Tacrolimus Only	0.076(0.057, 0.095)	<.0001
group	tacrolimus end csatcr	0.037(0.022, 0.053)	<.0001
group	tacrolimus start rejection	0.116(0.101, 0.132)	<.0001
days*group	Clobetasol end csatcr	-0.000(-0.001, -0.000)	0.0080
days*group	Clobetasol only	0.003(-0.002, 0.009)	0.2184
days*group	Clobetasol start rejection	0.000(-0.000, 0.000)	0.6706
days*group	CsaTcr Only	-0.003(-0.005, -0.001)	0.0006
days*group	Isograft	-0.000(-0.001, -0.000)	0.0067
days*group	Tacrolimus Only	-0.001(-0.001, -0.000)	0.0003
days*group	tacrolimus end csatcr	-0.000(-0.000, 0.000)	0.3093
days*group	tacrolimus start rejection	-0.000(-0.001, -0.000)	0.0020

Table2. Compared trend (slope) over time among groups - CD4

response	Difference in slope	Estimate(95% CI)	p value	Overall P
CD4			.	0.0015
	difference in slope 1 vs 2	-0.0036(-0.0090, 0.0017)	0.1779	.
	difference in slope 1 vs 3	-0.0004(-0.0007, -0.0000)	0.0282	.
	difference in slope 1 vs 4	0.0027(0.0010, 0.0043)	0.0020	.
	difference in slope 1 vs 5	0.0000(-0.0003, 0.0003)	0.9624	.
	difference in slope 1 vs 6	0.0003(-0.0001, 0.0007)	0.1261	.
	difference in slope 1 vs 7	-0.0002(-0.0005, 0.0001)	0.2206	.
	difference in slope 1 vs 8	0.0001(-0.0003, 0.0004)	0.7231	.
	difference in slope 2 vs 3	0.0033(-0.0021, 0.0086)	0.2257	.
	difference in slope 2 vs 4	0.0063(0.0007, 0.0119)	0.0283	.
	difference in slope 2 vs 5	0.0036(-0.0017, 0.0090)	0.1770	.
	difference in slope 2 vs 6	0.0039(-0.0014, 0.0093)	0.1452	.
	difference in slope 2 vs 7	0.0034(-0.0019, 0.0088)	0.2030	.
	difference in slope 2 vs 8	0.0037(-0.0017, 0.0090)	0.1711	.
	difference in slope 3 vs 4	0.0030(0.0014, 0.0046)	0.0005	.
	difference in slope 3 vs 5	0.0004(0.0000, 0.0007)	0.0253	.
	difference in slope 3 vs 6	0.0007(0.0003, 0.0011)	0.0012	.
	difference in slope 3 vs 7	0.0002(-0.0002, 0.0005)	0.3086	.
	difference in slope 3 vs 8	0.0004(0.0001, 0.0008)	0.0119	.
	difference in slope 4 vs 5	-0.0026(-0.0043, -0.0010)	0.0021	.
	difference in slope 4 vs 6	-0.0023(-0.0040, -0.0007)	0.0061	.
	difference in slope 4 vs 7	-0.0029(-0.0045, -0.0012)	0.0010	.
	difference in slope 4 vs 8	-0.0026(-0.0042, -0.0010)	0.0025	.
	difference in slope 5 vs 6	0.0003(-0.0001, 0.0007)	0.1358	.
	difference in slope 5 vs 7	-0.0002(-0.0005, 0.0001)	0.2039	.
	difference in slope 5 vs 8	0.0001(-0.0003, 0.0004)	0.7586	.
	difference in slope 6 vs 7	-0.0005(-0.0009, -0.0001)	0.0127	.
	difference in slope 6 vs 8	-0.0002(-0.0006, 0.0001)	0.2141	.
	difference in slope 6 vs 9	-0.0002(-0.0006, 0.0001)	0.2141	.
	difference in slope 7 vs 8	0.0003(-0.0001, 0.0006)	0.1171	.

*1= Clobetasol end csatcr, 2= Clobetasol only, 3= Clobetasol start rejection,4= CsaTcr Only, 5= Isograft, 6= Tacrolimus Only, 7= tacrolimus end csatcr, 8= tacrolimus start rejection

Table3. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - CD4

response	Group	LS mean(95% CI)	p value	Overall p
CD4			.	<.0001
	Clobetasol end csatcr	0.024(0.017, 0.032)	<.0001	.
	Clobetasol only	0.189(0.080, 0.298)	0.0011	.
	Clobetasol start rejection	0.026(0.018, 0.033)	<.0001	.
	CsaTcr Only	0.011(-0.009, 0.032)	0.2621	.
	Isograft	0.015(0.008, 0.023)	0.0001	.
	Tacrolimus Only	0.051(0.043, 0.059)	<.0001	.
	tacrolimus end csatcr	0.033(0.025, 0.040)	<.0001	.
	tacrolimus start rejection	0.101(0.094, 0.108)	<.0001	.
	Clobetasol end csatcr - Clobetasol only	-0.164(-0.273, -0.055)	0.0041	.
	Clobetasol end csatcr - Clobetasol start rejection	-0.001(-0.012, 0.009)	0.7921	.
	Clobetasol end csatcr - CsaTcr Only	0.013(-0.009, 0.035)	0.2289	.
	Clobetasol end csatcr - Isograft	0.009(-0.001, 0.020)	0.0847	.
	Clobetasol end csatcr - Tacrolimus Only	-0.027(-0.038, -0.016)	<.0001	.
	Clobetasol end csatcr - tacrolimus end csatcr	-0.008(-0.019, 0.002)	0.1198	.
	Clobetasol end csatcr - tacrolimus start rejection	-0.077(-0.087, -0.066)	<.0001	.
	Clobetasol only - Clobetasol start rejection	0.163(0.054, 0.272)	0.0044	.
	Clobetasol only - CsaTcr Only	0.177(0.067, 0.288)	0.0024	.
	Clobetasol only - Isograft	0.173(0.064, 0.283)	0.0026	.
	Clobetasol only - Tacrolimus Only	0.138(0.028, 0.247)	0.0149	.
	Clobetasol only - tacrolimus end csatcr	0.156(0.047, 0.265)	0.0062	.
	Clobetasol only - tacrolimus start rejection	0.088(-0.022, 0.197)	0.1128	.
	Clobetasol start rejection - CsaTcr Only	0.014(-0.007, 0.036)	0.1845	.
	Clobetasol start rejection - Isograft	0.010(0.000, 0.021)	0.0486	.
	Clobetasol start rejection - Tacrolimus Only	-0.025(-0.036, -0.014)	<.0001	.
	Clobetasol start rejection - tacrolimus end csatcr	-0.007(-0.017, 0.004)	0.1931	.
	Clobetasol start rejection - tacrolimus start rejection	-0.075(-0.086, -0.065)	<.0001	.
	CsaTcr Only - Isograft	-0.004(-0.025, 0.018)	0.7157	.
	CsaTcr Only - Tacrolimus Only	-0.040(-0.062, -0.018)	0.0007	.
	CsaTcr Only - tacrolimus end csatcr	-0.021(-0.043, 0.000)	0.0533	.
	CsaTcr Only - tacrolimus start rejection	-0.090(-0.111, -0.068)	<.0001	.
	Isograft - Tacrolimus Only	-0.036(-0.047, -0.025)	<.0001	.
	Isograft - tacrolimus end csatcr	-0.017(-0.028, -0.007)	0.0017	.
	Isograft - tacrolimus start rejection	-0.086(-0.096, -0.075)	<.0001	.
	Tacrolimus Only - tacrolimus end csatcr	0.019(0.008, 0.030)	0.0015	.
	Tacrolimus Only - tacrolimus start rejection	-0.050(-0.061, -0.039)	<.0001	.
	tacrolimus end csatcr - tacrolimus start rejection	-0.068(-0.079, -0.058)	<.0001	.

2. Immunohistochemistry responses – CD8

Table4. Estimate of intercept and slope for each group - CD8

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csater	0.004(0.001, 0.007)	0.0168
group	Clobetasol only	0.042(0.020, 0.063)	0.0003
group	Clobetasol start rejection	-0.002(-0.006, 0.001)	0.1871
group	CsaTcr Only	0.001(-0.009, 0.012)	0.7893
group	Isograft	0.000(-0.003, 0.004)	0.8843
group	Tacrolimus Only	-0.006(-0.010, -0.002)	0.0073
group	tacrolimus end csater	0.006(0.003, 0.010)	0.0004
group	tacrolimus start rejection	-0.001(-0.004, 0.002)	0.5943
days*group	Clobetasol end csater	-0.000(-0.000, 0.000)	0.6669
days*group	Clobetasol only	0.001(-0.000, 0.002)	0.0866
days*group	Clobetasol start rejection	0.000(-0.000, 0.000)	0.1979
days*group	CsaTcr Only	0.000(-0.000, 0.001)	0.3913
days*group	Isograft	0.000(-0.000, 0.000)	0.8739
days*group	Tacrolimus Only	0.000(0.000, 0.000)	0.0079
days*group	tacrolimus end csater	-0.000(-0.000, 0.000)	0.5886
days*group	tacrolimus start rejection	0.001(0.000, 0.001)	<.0001

Table5. Compared trend (slope) over time among groups - CD8

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
CD8			.	<.0001
	difference in slope 1 vs 2	-0.0010(-0.0022, 0.0001)	0.0792	.
	difference in slope 1 vs 3	-0.0001(-0.0004, 0.0001)	0.2250	.
	difference in slope 1 vs 4	-0.0004(-0.0011, 0.0004)	0.3538	.
	difference in slope 1 vs 5	-0.0000(-0.0003, 0.0002)	0.6770	.
	difference in slope 1 vs 6	-0.0003(-0.0006, -0.0001)	0.0190	.
	difference in slope 1 vs 7	0.0000(-0.0002, 0.0002)	0.9374	.
	difference in slope 1 vs 8	-0.0006(-0.0008, -0.0004)	<.0001	.
	difference in slope 2 vs 3	0.0009(-0.0003, 0.0020)	0.1285	.
	difference in slope 2 vs 4	0.0007(-0.0007, 0.0020)	0.3375	.
	difference in slope 2 vs 5	0.0010(-0.0002, 0.0021)	0.0938	.
	difference in slope 2 vs 6	0.0007(-0.0004, 0.0019)	0.2217	.
	difference in slope 2 vs 7	0.0010(-0.0001, 0.0022)	0.0767	.
	difference in slope 2 vs 8	0.0004(-0.0007, 0.0016)	0.4601	.
	difference in slope 3 vs 4	-0.0002(-0.0010, 0.0006)	0.5690	.
	difference in slope 3 vs 5	0.0001(-0.0001, 0.0003)	0.4213	.
	difference in slope 3 vs 6	-0.0002(-0.0004, 0.0001)	0.1864	.
	difference in slope 3 vs 7	0.0001(-0.0001, 0.0004)	0.1972	.
	difference in slope 3 vs 8	-0.0005(-0.0007, -0.0002)	0.0003	.
	difference in slope 4 vs 5	0.0003(-0.0005, 0.0011)	0.4203	.
	difference in slope 4 vs 6	0.0000(-0.0007, 0.0008)	0.8990	.
	difference in slope 4 vs 7	0.0004(-0.0004, 0.0012)	0.3421	.
	difference in slope 4 vs 8	-0.0002(-0.0010, 0.0005)	0.5486	.
	difference in slope 5 vs 6	-0.0003(-0.0005, -0.0000)	0.0450	.
	difference in slope 5 vs 7	0.0001(-0.0002, 0.0003)	0.6208	.
	difference in slope 5 vs 8	-0.0005(-0.0008, -0.0003)	<.0001	.
	difference in slope 6 vs 7	0.0003(0.0001, 0.0006)	0.0160	.
	difference in slope 6 vs 8	-0.0003(-0.0005, -0.0000)	0.0322	.
	difference in slope 6 vs 9	-0.0003(-0.0005, -0.0000)	0.0322	.
	difference in slope 7 vs 8	-0.0006(-0.0008, -0.0004)	<.0001	.

Table6. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - CD8

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
CD8			.	<.0001
	Clobetasol end csatcr	0.003(-0.001, 0.007)	0.1666	.
	Clobetasol only	0.081(0.056, 0.106)	<.0001	.
	Clobetasol start rejection	0.002(-0.002, 0.006)	0.3168	.
	CsaTcr Only	0.014(-0.006, 0.035)	0.1640	.
	Isograft	0.001(-0.003, 0.005)	0.7003	.
	Tacrolimus Only	0.005(0.001, 0.010)	0.0290	.
	tacrolimus end csatcr	0.005(0.001, 0.009)	0.0220	.
	tacrolimus start rejection	0.021(0.018, 0.025)	<.0001	.
	Clobetasol end csatcr - Clobetasol only	-0.078(-0.103, -0.053)	<.0001	.
	Clobetasol end csatcr - Clobetasol start rejection	0.001(-0.005, 0.006)	0.7815	.
	Clobetasol end csatcr - CsaTcr Only	-0.012(-0.033, 0.009)	0.2667	.
	Clobetasol end csatcr - Isograft	0.002(-0.004, 0.008)	0.4746	.
	Clobetasol end csatcr - Tacrolimus Only	-0.003(-0.009, 0.004)	0.3959	.
	Clobetasol end csatcr - tacrolimus end csatcr	-0.002(-0.007, 0.004)	0.4958	.
	Clobetasol end csatcr - tacrolimus start rejection	-0.019(-0.024, -0.013)	<.0001	.
	Clobetasol only - Clobetasol start rejection	0.079(0.054, 0.104)	<.0001	.
	Clobetasol only - CsaTcr Only	0.067(0.034, 0.099)	0.0002	.
	Clobetasol only - Isograft	0.080(0.055, 0.105)	<.0001	.
	Clobetasol only - Tacrolimus Only	0.076(0.050, 0.101)	<.0001	.
	Clobetasol only - tacrolimus end csatcr	0.076(0.051, 0.102)	<.0001	.
	Clobetasol only - tacrolimus start rejection	0.059(0.034, 0.085)	<.0001	.
	Clobetasol start rejection - CsaTcr Only	-0.012(-0.033, 0.008)	0.2368	.
	Clobetasol start rejection - Isograft	0.001(-0.004, 0.007)	0.6604	.
	Clobetasol start rejection - Tacrolimus Only	-0.003(-0.010, 0.003)	0.2743	.
	Clobetasol start rejection - tacrolimus end csatcr	-0.003(-0.008, 0.003)	0.3394	.
	Clobetasol start rejection - tacrolimus start rejection	-0.019(-0.025, -0.014)	<.0001	.
	CsaTcr Only - Isograft	0.014(-0.007, 0.035)	0.1947	.
	CsaTcr Only - Tacrolimus Only	0.009(-0.012, 0.030)	0.3930	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
	CsaTcr Only - tacrolimus end csater	0.010(-0.011, 0.031)	0.3513	.
	CsaTcr Only - tacrolimus start rejection	-0.007(-0.028, 0.014)	0.4990	.
	Isograft - Tacrolimus Only	-0.005(-0.011, 0.002)	0.1401	.
	Isograft - tacrolimus end csater	-0.004(-0.009, 0.002)	0.1664	.
	Isograft - tacrolimus start rejection	-0.021(-0.026, -0.015)	<.0001	.
	Tacrolimus Only - tacrolimus end csater	0.001(-0.005, 0.007)	0.8101	.
	Tacrolimus Only - tacrolimus start rejection	-0.016(-0.022, -0.010)	<.0001	.
	tacrolimus end csater - tacrolimus start rejection	-0.017(-0.022, -0.011)	<.0001	.

3. Immunohistochemistry responses – CD11

Table7. Estimate of intercept and slope for each group - CD11

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csater	0.022(0.008, 0.036)	0.0037
group	Clobetasol only	-0.031(-0.060, -0.001)	0.0424
group	Clobetasol start rejection	0.017(0.003, 0.032)	0.0187
group	CsaTcr Only	0.131(0.111, 0.151)	<.0001
group	Isograft	0.005(-0.009, 0.020)	0.4604
group	Tacrolimus Only	0.033(0.019, 0.047)	<.0001
group	tacrolimus end csater	0.058(0.044, 0.072)	<.0001
group	tacrolimus start rejection	0.132(0.117, 0.146)	<.0001
days*group	Clobetasol end csater	-0.000(-0.000, -0.000)	0.0031
days*group	Clobetasol only	0.008(0.005, 0.011)	<.0001
days*group	Clobetasol start rejection	0.000(0.000, 0.000)	0.0106
days*group	CsaTcr Only	0.000(-0.001, 0.001)	0.6039
days*group	Isograft	0.000(-0.000, 0.000)	0.9820
days*group	Tacrolimus Only	-0.000(-0.000, -0.000)	0.0028
days*group	tacrolimus end csater	-0.001(-0.001, -0.001)	<.0001
days*group	tacrolimus start rejection	-0.002(-0.002, -0.001)	<.0001

Table8. Compared trend (slope) over time among groups - CD11

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
CD11			.	<.0001
	difference in slope 1 vs 2	-0.0087(-0.0116, -0.0057)	<.0001	.
	difference in slope 1 vs 3	-0.0005(-0.0008, -0.0003)	0.0002	.
	difference in slope 1 vs 4	-0.0006(-0.0017, 0.0006)	0.3091	.
	difference in slope 1 vs 5	-0.0003(-0.0006, -0.0000)	0.0306	.
	difference in slope 1 vs 6	0.0000(-0.0003, 0.0003)	0.9005	.
	difference in slope 1 vs 7	0.0004(0.0002, 0.0007)	0.0018	.
	difference in slope 1 vs 8	0.0013(0.0010, 0.0015)	<.0001	.
	difference in slope 2 vs 3	0.0081(0.0052, 0.0111)	<.0001	.
	difference in slope 2 vs 4	0.0081(0.0049, 0.0112)	<.0001	.
	difference in slope 2 vs 5	0.0084(0.0054, 0.0113)	<.0001	.
	difference in slope 2 vs 6	0.0087(0.0057, 0.0116)	<.0001	.
	difference in slope 2 vs 7	0.0091(0.0061, 0.0121)	<.0001	.
	difference in slope 2 vs 8	0.0099(0.0070, 0.0129)	<.0001	.
	difference in slope 3 vs 4	-0.0000(-0.0012, 0.0011)	0.9382	.
	difference in slope 3 vs 5	0.0002(-0.0000, 0.0005)	0.0676	.
	difference in slope 3 vs 6	0.0006(0.0003, 0.0008)	0.0002	.
	difference in slope 3 vs 7	0.0010(0.0007, 0.0012)	<.0001	.
	difference in slope 3 vs 8	0.0018(0.0015, 0.0021)	<.0001	.
	difference in slope 4 vs 5	0.0003(-0.0008, 0.0014)	0.6113	.
	difference in slope 4 vs 6	0.0006(-0.0005, 0.0017)	0.2961	.
	difference in slope 4 vs 7	0.0010(-0.0001, 0.0021)	0.0791	.
	difference in slope 4 vs 8	0.0018(0.0007, 0.0030)	0.0021	.
	difference in slope 5 vs 6	0.0003(0.0000, 0.0006)	0.0258	.
	difference in slope 5 vs 7	0.0007(0.0005, 0.0010)	<.0001	.
	difference in slope 5 vs 8	0.0016(0.0013, 0.0018)	<.0001	.
	difference in slope 6 vs 7	0.0004(0.0001, 0.0007)	0.0032	.
	difference in slope 6 vs 8	0.0012(0.0010, 0.0015)	<.0001	.
	difference in slope 6 vs 9	0.0012(0.0010, 0.0015)	<.0001	.

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
	difference in slope 7 vs 8	0.0008(0.0006, 0.0011)	<.0001	.

Table9. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - CD11

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
CD11				. <.0001
	Clobetasol end csatcr	0.011(0.003, 0.018)	0.0060	.
	Clobetasol only	0.302(0.209, 0.395)	<.0001	.
	Clobetasol start rejection	0.027(0.020, 0.035)	<.0001	.
	CsaTcr Only	0.142(0.110, 0.175)	<.0001	.
	Isograft	0.005(-0.002, 0.013)	0.1441	.
	Tacrolimus Only	0.021(0.014, 0.028)	<.0001	.
	tacrolimus end csatcr	0.029(0.022, 0.037)	<.0001	.
	tacrolimus start rejection	0.070(0.063, 0.077)	<.0001	.
	Clobetasol end csatcr - Clobetasol only	-0.292(-0.385, -0.198)	<.0001	.
	Clobetasol end csatcr - Clobetasol start rejection	-0.017(-0.027, -0.006)	0.0022	.
	Clobetasol end csatcr - CsaTcr Only	-0.132(-0.165, -0.099)	<.0001	.
	Clobetasol end csatcr - Isograft	0.005(-0.005, 0.015)	0.3251	.
	Clobetasol end csatcr - Tacrolimus Only	-0.010(-0.021, -0.000)	0.0482	.
	Clobetasol end csatcr - tacrolimus end csatcr	-0.019(-0.029, -0.008)	0.0007	.
	Clobetasol end csatcr - tacrolimus start rejection	-0.060(-0.070, -0.049)	<.0001	.
	Clobetasol only - Clobetasol start rejection	0.275(0.181, 0.368)	<.0001	.
	Clobetasol only - CsaTcr Only	0.160(0.061, 0.258)	0.0022	.
	Clobetasol only - Isograft	0.297(0.203, 0.390)	<.0001	.
	Clobetasol only - Tacrolimus Only	0.281(0.188, 0.375)	<.0001	.
	Clobetasol only - tacrolimus end csatcr	0.273(0.179, 0.366)	<.0001	.
	Clobetasol only - tacrolimus start rejection	0.232(0.138, 0.326)	<.0001	.
	Clobetasol start rejection - CsaTcr Only	-0.115(-0.149, -0.082)	<.0001	.
	Clobetasol start rejection - Isograft	0.022(0.011, 0.032)	0.0001	.
	Clobetasol start rejection - Tacrolimus Only	0.006(-0.004, 0.017)	0.2248	.
	Clobetasol start rejection - tacrolimus end csatcr	-0.002(-0.012, 0.008)	0.6916	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
	Clobetasol start rejection - tacrolimus start rejection	-0.043(-0.053, -0.032)	<.0001	.
	CsaTcr Only - Isograft	0.137(0.104, 0.171)	<.0001	.
	CsaTcr Only - Tacrolimus Only	0.122(0.088, 0.155)	<.0001	.
	CsaTcr Only - tacrolimus end csatcr	0.113(0.080, 0.147)	<.0001	.
	CsaTcr Only - tacrolimus start rejection	0.072(0.039, 0.106)	<.0001	.
	Isograft - Tacrolimus Only	-0.016(-0.026, -0.005)	0.0042	.
	Isograft - tacrolimus end csatcr	-0.024(-0.034, -0.014)	<.0001	.
	Isograft - tacrolimus start rejection	-0.065(-0.075, -0.054)	<.0001	.
	Tacrolimus Only - tacrolimus end csatcr	-0.008(-0.019, 0.002)	0.1101	.
	Tacrolimus Only - tacrolimus start rejection	-0.049(-0.059, -0.039)	<.0001	.
	tacrolimus end csatcr - tacrolimus start rejection	-0.041(-0.051, -0.030)	<.0001	.

4. Immunohistochemistry responses – CD45

Table 10. Estimate of intercept and slope for each group - CD45

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csatcr	0.017(0.012, 0.022)	<.0001
group	Clobetasol only	0.038(-0.000, 0.076)	0.0511
group	Clobetasol start rejection	0.021(0.016, 0.026)	<.0001
group	CsaTcr Only	0.005(-0.019, 0.028)	0.7019
group	Isograft	0.003(-0.002, 0.008)	0.3018
group	Tacrolimus Only	0.031(0.025, 0.036)	<.0001
group	tacrolimus end csatcr	0.036(0.031, 0.041)	<.0001
group	tacrolimus start rejection	0.044(0.039, 0.049)	<.0001
days*group	Clobetasol end csatcr	-0.000(-0.000, -0.000)	<.0001
days*group	Clobetasol only	-0.001(-0.003, 0.001)	0.2837
days*group	Clobetasol start rejection	-0.000(-0.000, 0.000)	0.7276
days*group	CsaTcr Only	0.001(-0.000, 0.001)	0.2532
days*group	Isograft	-0.000(-0.000, 0.000)	0.8313
days*group	Tacrolimus Only	-0.000(-0.000, -0.000)	0.0002
days*group	tacrolimus end csatcr	-0.000(-0.000, -0.000)	<.0001
days*group	tacrolimus start rejection	-0.000(-0.000, -0.000)	<.0001

Table11. Compared trend (slope) over time among groups - CD45

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
CD45			.	<.0001
	difference in slope 1 vs 2	0.0009(-0.0010, 0.0029)	0.3455	.
	difference in slope 1 vs 3	-0.0001(-0.0002, -0.0000)	0.0056	.
	difference in slope 1 vs 4	-0.0006(-0.0015, 0.0003)	0.1549	.
	difference in slope 1 vs 5	-0.0001(-0.0002, -0.0000)	0.0043	.
	difference in slope 1 vs 6	0.0000(-0.0001, 0.0001)	0.4647	.
	difference in slope 1 vs 7	0.0000(-0.0000, 0.0001)	0.2968	.
	difference in slope 1 vs 8	0.0002(0.0001, 0.0003)	0.0001	.
	difference in slope 2 vs 3	-0.0011(-0.0030, 0.0009)	0.2884	.
	difference in slope 2 vs 4	-0.0016(-0.0037, 0.0006)	0.1505	.
	difference in slope 2 vs 5	-0.0011(-0.0030, 0.0009)	0.2866	.
	difference in slope 2 vs 6	-0.0009(-0.0029, 0.0011)	0.3645	.
	difference in slope 2 vs 7	-0.0009(-0.0029, 0.0011)	0.3678	.
	difference in slope 2 vs 8	-0.0008(-0.0027, 0.0012)	0.4396	.
	difference in slope 3 vs 4	-0.0005(-0.0014, 0.0004)	0.2451	.
	difference in slope 3 vs 5	-0.0000(-0.0001, 0.0001)	0.9237	.
	difference in slope 3 vs 6	0.0002(0.0001, 0.0003)	0.0030	.
	difference in slope 3 vs 7	0.0002(0.0001, 0.0002)	0.0003	.
	difference in slope 3 vs 8	0.0003(0.0002, 0.0004)	<.0001	.
	difference in slope 4 vs 5	0.0005(-0.0004, 0.0014)	0.2486	.
	difference in slope 4 vs 6	0.0007(-0.0002, 0.0016)	0.1340	.
	difference in slope 4 vs 7	0.0007(-0.0002, 0.0016)	0.1296	.
	difference in slope 4 vs 8	0.0008(-0.0001, 0.0017)	0.0734	.
	difference in slope 5 vs 6	0.0002(0.0001, 0.0003)	0.0024	.
	difference in slope 5 vs 7	0.0002(0.0001, 0.0002)	0.0002	.
	difference in slope 5 vs 8	0.0003(0.0002, 0.0004)	<.0001	.
	difference in slope 6 vs 7	0.0000(-0.0001, 0.0001)	0.8947	.
	difference in slope 6 vs 8	0.0001(0.0000, 0.0002)	0.0094	.
	difference in slope 6 vs 9	0.0001(0.0000, 0.0002)	0.0094	.

<i>response</i>	<i>Differnce in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
	difference in slope 7 vs 8	0.0001(0.0000, 0.0002)	0.0031	.

Table 12. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - CD45

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
CD45			.	<.0001
	Clobetasol end csatcr	0.011(0.008, 0.015)	<.0001	.
	Clobetasol only	-0.004(-0.047, 0.038)	0.8348	.
	Clobetasol start rejection	0.021(0.018, 0.024)	<.0001	.
	CsaTcr Only	0.025(0.012, 0.037)	0.0003	.
	Isograft	0.002(-0.001, 0.006)	0.1441	.
	Tacrolimus Only	0.024(0.021, 0.028)	<.0001	.
	tacrolimus end csatcr	0.029(0.026, 0.032)	<.0001	.
	tacrolimus start rejection	0.032(0.029, 0.035)	<.0001	.
	Clobetasol end csatcr - Clobetasol only	0.016(-0.027, 0.058)	0.4540	.
	Clobetasol end csatcr - Clobetasol start rejection	-0.009(-0.014, -0.005)	0.0001	.
	Clobetasol end csatcr - CsaTcr Only	-0.013(-0.026, -0.000)	0.0478	.
	Clobetasol end csatcr - Isograft	0.009(0.005, 0.014)	0.0002	.
	Clobetasol end csatcr - Tacrolimus Only	-0.013(-0.017, -0.008)	<.0001	.
	Clobetasol end csatcr - tacrolimus end csatcr	-0.017(-0.022, -0.013)	<.0001	.
	Clobetasol end csatcr - tacrolimus start rejection	-0.021(-0.025, -0.016)	<.0001	.
	Clobetasol only - Clobetasol start rejection	-0.025(-0.068, 0.017)	0.2344	.
	Clobetasol only - CsaTcr Only	-0.029(-0.073, 0.015)	0.1911	.
	Clobetasol only - Isograft	-0.007(-0.049, 0.036)	0.7501	.
	Clobetasol only - Tacrolimus Only	-0.028(-0.071, 0.014)	0.1831	.
	Clobetasol only - tacrolimus end csatcr	-0.033(-0.076, 0.009)	0.1202	.
	Clobetasol only - tacrolimus start rejection	-0.037(-0.079, 0.006)	0.0898	.
	Clobetasol start rejection - CsaTcr Only	-0.004(-0.017, 0.009)	0.5687	.
	Clobetasol start rejection - Isograft	0.019(0.014, 0.023)	<.0001	.
	Clobetasol start rejection - Tacrolimus Only	-0.003(-0.008, 0.002)	0.1870	.
	Clobetasol start rejection - tacrolimus end csatcr	-0.008(-0.012, -0.003)	0.0009	.
	Clobetasol start rejection - tacrolimus start rejection	-0.011(-0.016, -0.007)	<.0001	.
	CsaTcr Only - Isograft	0.022(0.009, 0.035)	0.0013	.
	CsaTcr Only - Tacrolimus Only	0.001(-0.013, 0.014)	0.9258	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
	CsaTcr Only - tacrolimus end csater	-0.004(-0.017, 0.009)	0.5129	.
	CsaTcr Only - tacrolimus start rejection	-0.007(-0.020, 0.006)	0.2569	.
	Isograft - Tacrolimus Only	-0.022(-0.026, -0.017)	<.0001	.
	Isograft - tacrolimus end csater	-0.027(-0.031, -0.022)	<.0001	.
	Isograft - tacrolimus start rejection	-0.030(-0.034, -0.025)	<.0001	.
	Tacrolimus Only - tacrolimus end csater	-0.005(-0.010, -0.000)	0.0413	.
	Tacrolimus Only - tacrolimus start rejection	-0.008(-0.013, -0.003)	0.0012	.
	tacrolimus end csater - tacrolimus start rejection	-0.003(-0.008, 0.001)	0.1619	.

5. Immunohistochemistry responses – CD86

Table13. Estimate of intercept and slope for each group - CD86

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csatcr	0.018(0.017, 0.019)	<.0001
group	Clobetasol only	0.004(0.002, 0.007)	0.0008
group	Clobetasol start rejection	0.001(0.000, 0.002)	0.0133
group	CsaTcr Only	-0.002(-0.004, 0.000)	0.0852
group	Isograft	0.000(-0.000, 0.001)	0.4365
group	Tacrolimus Only	0.002(0.001, 0.002)	0.0001
group	tacrolimus end csatcr	0.002(0.001, 0.002)	0.0006
group	tacrolimus start rejection	0.000(-0.000, 0.001)	0.2988
days*group	Clobetasol end csatcr	-0.000(-0.000, -0.000)	<.0001
days*group	Clobetasol only	-0.000(-0.000, -0.000)	0.0248
days*group	Clobetasol start rejection	0.000(0.000, 0.000)	<.0001
days*group	CsaTcr Only	0.000(0.000, 0.000)	0.0009
days*group	Isograft	0.000(-0.000, 0.000)	0.7574
days*group	Tacrolimus Only	0.000(-0.000, 0.000)	0.2715
days*group	tacrolimus end csatcr	0.000(-0.000, 0.000)	0.1079
days*group	tacrolimus start rejection	0.000(-0.000, 0.000)	0.6572

Table14. Compared trend (slope) over time among groups - CD86

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
CD86			.	<.0001
	difference in slope 1 vs 2	-0.0000(-0.0002, 0.0001)	0.6094	.
	difference in slope 1 vs 3	-0.0003(-0.0003, -0.0003)	<.0001	.
	difference in slope 1 vs 4	-0.0005(-0.0007, -0.0004)	<.0001	.
	difference in slope 1 vs 5	-0.0002(-0.0003, -0.0002)	<.0001	.
	difference in slope 1 vs 6	-0.0003(-0.0003, -0.0002)	<.0001	.
	difference in slope 1 vs 7	-0.0003(-0.0003, -0.0002)	<.0001	.
	difference in slope 1 vs 8	-0.0002(-0.0003, -0.0002)	<.0001	.
	difference in slope 2 vs 3	-0.0003(-0.0004, -0.0001)	0.0046	.
	difference in slope 2 vs 4	-0.0005(-0.0007, -0.0003)	0.0002	.
	difference in slope 2 vs 5	-0.0002(-0.0004, -0.0000)	0.0238	.
	difference in slope 2 vs 6	-0.0002(-0.0004, -0.0000)	0.0194	.
	difference in slope 2 vs 7	-0.0002(-0.0004, -0.0000)	0.0179	.
	difference in slope 2 vs 8	-0.0002(-0.0004, -0.0000)	0.0231	.
	difference in slope 3 vs 4	-0.0002(-0.0004, -0.0001)	0.0067	.
	difference in slope 3 vs 5	0.0001(0.0000, 0.0001)	<.0001	.
	difference in slope 3 vs 6	0.0000(0.0000, 0.0001)	0.0002	.
	difference in slope 3 vs 7	0.0000(0.0000, 0.0001)	0.0001	.
	difference in slope 3 vs 8	0.0001(0.0000, 0.0001)	<.0001	.
	difference in slope 4 vs 5	0.0003(0.0001, 0.0005)	0.0010	.
	difference in slope 4 vs 6	0.0003(0.0001, 0.0005)	0.0014	.
	difference in slope 4 vs 7	0.0003(0.0001, 0.0005)	0.0015	.
	difference in slope 4 vs 8	0.0003(0.0001, 0.0005)	0.0011	.
	difference in slope 5 vs 6	-0.0000(-0.0000, 0.0000)	0.5246	.
	difference in slope 5 vs 7	-0.0000(-0.0000, 0.0000)	0.3516	.
	difference in slope 5 vs 8	-0.0000(-0.0000, 0.0000)	0.9238	.
	difference in slope 6 vs 7	-0.0000(-0.0000, 0.0000)	0.8209	.
	difference in slope 6 vs 8	0.0000(-0.0000, 0.0000)	0.5833	.
	difference in slope 6 vs 9	0.0000(-0.0000, 0.0000)	0.5833	.

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
	difference in slope 7 vs 8	0.0000(-0.0000, 0.0000)	0.4024	.

Table15. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - CD86

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
CD86				. <.0001
	Clobetasol end csatcr	0.008(0.008, 0.008)	<.0001	.
	Clobetasol only	-0.004(-0.008, 0.001)	0.1143	.
	Clobetasol start rejection	0.003(0.003, 0.004)	<.0001	.
	CsaTcr Only	0.010(0.005, 0.014)	<.0001	.
	Isograft	0.000(0.000, 0.001)	0.0221	.
	Tacrolimus Only	0.002(0.002, 0.002)	<.0001	.
	tacrolimus end csatcr	0.002(0.002, 0.002)	<.0001	.
	tacrolimus start rejection	0.001(0.000, 0.001)	0.0024	.
	Clobetasol end csatcr - Clobetasol only	0.012(0.007, 0.017)	<.0001	.
	Clobetasol end csatcr - Clobetasol start rejection	0.005(0.004, 0.005)	<.0001	.
	Clobetasol end csatcr - CsaTcr Only	-0.002(-0.006, 0.003)	0.4602	.
	Clobetasol end csatcr - Isograft	0.008(0.007, 0.008)	<.0001	.
	Clobetasol end csatcr - Tacrolimus Only	0.006(0.006, 0.007)	<.0001	.
	Clobetasol end csatcr - tacrolimus end csatcr	0.006(0.006, 0.007)	<.0001	.
	Clobetasol end csatcr - tacrolimus start rejection	0.008(0.007, 0.008)	<.0001	.
	Clobetasol only - Clobetasol start rejection	-0.007(-0.012, -0.002)	0.0040	.
	Clobetasol only - CsaTcr Only	-0.014(-0.020, -0.007)	0.0001	.
	Clobetasol only - Isograft	-0.004(-0.009, 0.001)	0.0816	.
	Clobetasol only - Tacrolimus Only	-0.006(-0.011, -0.001)	0.0175	.
	Clobetasol only - tacrolimus end csatcr	-0.006(-0.011, -0.001)	0.0177	.
	Clobetasol only - tacrolimus start rejection	-0.004(-0.009, 0.000)	0.0716	.
	Clobetasol start rejection - CsaTcr Only	-0.006(-0.011, -0.002)	0.0064	.
	Clobetasol start rejection - Isograft	0.003(0.002, 0.003)	<.0001	.
	Clobetasol start rejection - Tacrolimus Only	0.001(0.001, 0.002)	<.0001	.
	Clobetasol start rejection - tacrolimus end csatcr	0.001(0.001, 0.002)	<.0001	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
	Clobetasol start rejection - tacrolimus start rejection	0.003(0.002, 0.003)	<.0001	.
	CsaTcr Only - Isograft	0.009(0.005, 0.014)	0.0001	.
	CsaTcr Only - Tacrolimus Only	0.008(0.003, 0.012)	0.0012	.
	CsaTcr Only - tacrolimus end csatcr	0.008(0.003, 0.012)	0.0012	.
	CsaTcr Only - tacrolimus start rejection	0.009(0.005, 0.014)	0.0002	.
	Isograft - Tacrolimus Only	-0.002(-0.002, -0.001)	<.0001	.
	Isograft - tacrolimus end csatcr	-0.002(-0.002, -0.001)	<.0001	.
	Isograft - tacrolimus start rejection	-0.000(-0.001, 0.000)	0.5457	.
	Tacrolimus Only - tacrolimus end csatcr	0.000(-0.001, 0.001)	0.9429	.
	Tacrolimus Only - tacrolimus start rejection	0.001(0.001, 0.002)	<.0001	.
	tacrolimus end csatcr - tacrolimus start rejection	0.001(0.001, 0.002)	<.0001	.

6. PCR – IL-2

Table 16. Estimate of intercept and slope for each group - IL_2

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csatcr	0.264(-0.396, 0.924)	0.4233
group	Clobetasol only	0.732(-2.607, 4.070)	0.6604
group	Clobetasol start rejection	0.399(-0.261, 1.059)	0.2293
group	CsaTcr Only	-1.447(-3.137, 0.242)	0.0911
group	Isograft	1.517(0.857, 2.177)	<.0001
group	Tacrolimus Only	3.765(3.021, 4.509)	<.0001
group	tacrolimus end csatcr	0.594(-0.066, 1.254)	0.0767
group	tacrolimus start rejection	0.718(0.058, 1.379)	0.0337
days*group	Clobetasol end csatcr	0.015(0.008, 0.023)	0.0002
days*group	Clobetasol only	0.225(0.046, 0.403)	0.0149
days*group	Clobetasol start rejection	0.017(0.009, 0.025)	<.0001
days*group	CsaTcr Only	0.221(0.149, 0.292)	<.0001
days*group	Isograft	0.001(-0.007, 0.009)	0.8073
days*group	Tacrolimus Only	0.002(-0.010, 0.014)	0.7679
days*group	tacrolimus end csatcr	0.013(0.006, 0.021)	0.0011
days*group	tacrolimus start rejection	0.027(0.019, 0.035)	<.0001

Table17. Compared trend (slope) over time among groups - II_2

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
II_2			.	<.0001
	difference in slope 1 vs 2	-0.2095(-0.3882, -0.0308)	0.0227	.
	difference in slope 1 vs 3	-0.0017(-0.0126, 0.0091)	0.7468	.
	difference in slope 1 vs 4	-0.2053(-0.2772, -0.1335)	<.0001	.
	difference in slope 1 vs 5	0.0143(0.0035, 0.0252)	0.0110	.
	difference in slope 1 vs 6	0.0135(-0.0007, 0.0276)	0.0615	.
	difference in slope 1 vs 7	0.0020(-0.0089, 0.0128)	0.7176	.
	difference in slope 1 vs 8	-0.0117(-0.0225, -0.0008)	0.0355	.
	difference in slope 2 vs 3	0.2078(0.0291, 0.3865)	0.0238	.
	difference in slope 2 vs 4	0.0042(-0.1881, 0.1965)	0.9651	.
	difference in slope 2 vs 5	0.2238(0.0451, 0.4025)	0.0154	.
	difference in slope 2 vs 6	0.2230(0.0441, 0.4019)	0.0158	.
	difference in slope 2 vs 7	0.2115(0.0328, 0.3902)	0.0215	.
	difference in slope 2 vs 8	0.1978(0.0192, 0.3765)	0.0309	.
	difference in slope 3 vs 4	-0.2036(-0.2755, -0.1317)	<.0001	.
	difference in slope 3 vs 5	0.0160(0.0052, 0.0269)	0.0047	.
	difference in slope 3 vs 6	0.0152(0.0011, 0.0294)	0.0358	.
	difference in slope 3 vs 7	0.0037(-0.0071, 0.0145)	0.4946	.
	difference in slope 3 vs 8	-0.0099(-0.0208, 0.0009)	0.0716	.
	difference in slope 4 vs 5	0.2196(0.1478, 0.2915)	<.0001	.
	difference in slope 4 vs 6	0.2188(0.1464, 0.2913)	<.0001	.
	difference in slope 4 vs 7	0.2073(0.1354, 0.2792)	<.0001	.
	difference in slope 4 vs 8	0.1937(0.1218, 0.2655)	<.0001	.
	difference in slope 5 vs 6	-0.0008(-0.0150, 0.0133)	0.9075	.
	difference in slope 5 vs 7	-0.0123(-0.0232, -0.0015)	0.0267	.
	difference in slope 5 vs 8	-0.0260(-0.0368, -0.0151)	<.0001	.
	difference in slope 6 vs 7	-0.0115(-0.0257, 0.0026)	0.1079	.
	difference in slope 6 vs 8	-0.0252(-0.0393, -0.0110)	0.0009	.
	difference in slope 6 vs 9	-0.0252(-0.0393, -0.0110)	0.0009	.

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
	difference in slope 7 vs 8	-0.0136(-0.0245, -0.0028)	0.0150	.

Table18. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - II_2

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
II_2				. <.0001
	Clobetasol end csatcr	0.870(0.370, 1.370)	0.0011	.
	Clobetasol only	9.666(5.726, 13.606)	<.0001	.
	Clobetasol start rejection	1.074(0.574, 1.574)	<.0001	.
	CsaTcr Only	7.321(5.775, 8.866)	<.0001	.
	Isograft	1.554(1.054, 2.054)	<.0001	.
	Tacrolimus Only	3.835(3.306, 4.363)	<.0001	.
	tacrolimus end csatcr	1.122(0.622, 1.622)	<.0001	.
	tacrolimus start rejection	1.788(1.288, 2.288)	<.0001	.
	Clobetasol end csatcr - Clobetasol only	-8.796(-12.768, -4.825)	<.0001	.
	Clobetasol end csatcr - Clobetasol start rejection	-0.204(-0.911, 0.503)	0.5634	.
	Clobetasol end csatcr - CsaTcr Only	-6.451(-8.075, -4.826)	<.0001	.
	Clobetasol end csatcr - Isograft	-0.684(-1.391, 0.023)	0.0575	.
	Clobetasol end csatcr - Tacrolimus Only	-2.965(-3.692, -2.237)	<.0001	.
	Clobetasol end csatcr - tacrolimus end csatcr	-0.252(-0.959, 0.456)	0.4765	.
	Clobetasol end csatcr - tacrolimus start rejection	-0.918(-1.625, -0.211)	0.0122	.
	Clobetasol only - Clobetasol start rejection	8.592(4.621, 12.564)	<.0001	.
	Clobetasol only - CsaTcr Only	2.346(-1.887, 6.578)	0.2696	.
	Clobetasol only - Isograft	8.112(4.140, 12.084)	0.0002	.
	Clobetasol only - Tacrolimus Only	5.832(1.856, 9.807)	0.0051	.
	Clobetasol only - tacrolimus end csatcr	8.545(4.573, 12.516)	<.0001	.
	Clobetasol only - tacrolimus start rejection	7.878(3.906, 11.849)	0.0003	.
	Clobetasol start rejection - CsaTcr Only	-6.247(-7.871, -4.622)	<.0001	.
	Clobetasol start rejection - Isograft	-0.480(-1.187, 0.227)	0.1775	.
	Clobetasol start rejection - Tacrolimus Only	-2.761(-3.488, -2.033)	<.0001	.
	Clobetasol start rejection - tacrolimus end csatcr	-0.048(-0.755, 0.659)	0.8926	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
	Clobetasol start rejection - tacrolimus start rejection	-0.714(-1.421, -0.007)	0.0478	.
	CsaTcr Only - Isograft	5.766(4.142, 7.391)	<.0001	.
	CsaTcr Only - Tacrolimus Only	3.486(1.852, 5.120)	<.0001	.
	CsaTcr Only - tacrolimus end csatcr	6.199(4.575, 7.824)	<.0001	.
	CsaTcr Only - tacrolimus start rejection	5.532(3.908, 7.157)	<.0001	.
	Isograft - Tacrolimus Only	-2.280(-3.008, -1.553)	<.0001	.
	Isograft - tacrolimus end csatcr	0.433(-0.274, 1.140)	0.2235	.
	Isograft - tacrolimus start rejection	-0.234(-0.941, 0.473)	0.5075	.
	Tacrolimus Only - tacrolimus end csatcr	2.713(1.986, 3.441)	<.0001	.
	Tacrolimus Only - tacrolimus start rejection	2.046(1.319, 2.774)	<.0001	.
	tacrolimus end csatcr - tacrolimus start rejection	-0.667(-1.374, 0.040)	0.0639	.

7. PCR – TGF-Beta

Table 19. Estimate of intercept and slope for each group - TGF_beta

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csatcr	1.859(1.537, 2.181)	<.0001
group	Clobetasol only	1.184(0.110, 2.258)	0.0315
group	Clobetasol start rejection	1.496(1.174, 1.817)	<.0001
group	CsaTcr Only	0.351(-0.424, 1.126)	0.3662
group	Isograft	0.626(0.304, 0.948)	0.0003
group	Tacrolimus Only	1.767(1.459, 2.076)	<.0001
group	tacrolimus end csatcr	1.377(1.055, 1.699)	<.0001
group	tacrolimus start rejection	1.571(1.249, 1.893)	<.0001
days*group	Clobetasol end csatcr	-0.002(-0.005, 0.001)	0.2739
days*group	Clobetasol only	0.054(-0.007, 0.115)	0.0804
days*group	Clobetasol start rejection	0.015(0.012, 0.018)	<.0001
days*group	CsaTcr Only	0.079(0.040, 0.118)	0.0002
days*group	Isograft	0.004(0.001, 0.007)	0.0192
days*group	Tacrolimus Only	0.001(-0.004, 0.005)	0.8355
days*group	tacrolimus end csatcr	0.005(0.002, 0.008)	0.0031
days*group	tacrolimus start rejection	0.004(0.001, 0.008)	0.0086

Table20. Compared trend (slope) over time among groups - TGF_beta

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
TGF_beta			.	<.0001
	difference in slope 1 vs 2	-0.0557(-0.1165, 0.0051)	0.0716	.
	difference in slope 1 vs 3	-0.0168(-0.0214, -0.0122)	<.0001	.
	difference in slope 1 vs 4	-0.0810(-0.1199, -0.0421)	0.0001	.
	difference in slope 1 vs 5	-0.0057(-0.0103, -0.0011)	0.0162	.
	difference in slope 1 vs 6	-0.0023(-0.0082, 0.0036)	0.4364	.
	difference in slope 1 vs 7	-0.0069(-0.0115, -0.0023)	0.0045	.
	difference in slope 1 vs 8	-0.0062(-0.0109, -0.0016)	0.0091	.
	difference in slope 2 vs 3	0.0389(-0.0219, 0.0997)	0.2038	.
	difference in slope 2 vs 4	-0.0253(-0.0973, 0.0468)	0.4831	.
	difference in slope 2 vs 5	0.0500(-0.0108, 0.1108)	0.1046	.
	difference in slope 2 vs 6	0.0534(-0.0075, 0.1144)	0.0841	.
	difference in slope 2 vs 7	0.0489(-0.0120, 0.1097)	0.1125	.
	difference in slope 2 vs 8	0.0495(-0.0114, 0.1103)	0.1081	.
	difference in slope 3 vs 4	-0.0642(-0.1031, -0.0253)	0.0018	.
	difference in slope 3 vs 5	0.0111(0.0065, 0.0157)	<.0001	.
	difference in slope 3 vs 6	0.0145(0.0086, 0.0204)	<.0001	.
	difference in slope 3 vs 7	0.0100(0.0053, 0.0146)	<.0001	.
	difference in slope 3 vs 8	0.0106(0.0060, 0.0152)	<.0001	.
	difference in slope 4 vs 5	0.0752(0.0363, 0.1141)	0.0003	.
	difference in slope 4 vs 6	0.0787(0.0396, 0.1177)	0.0002	.
	difference in slope 4 vs 7	0.0741(0.0352, 0.1130)	0.0004	.
	difference in slope 4 vs 8	0.0747(0.0358, 0.1136)	0.0004	.
	difference in slope 5 vs 6	0.0034(-0.0025, 0.0093)	0.2490	.
	difference in slope 5 vs 7	-0.0011(-0.0058, 0.0035)	0.6178	.
	difference in slope 5 vs 8	-0.0005(-0.0051, 0.0041)	0.8191	.
	difference in slope 6 vs 7	-0.0046(-0.0105, 0.0013)	0.1261	.
	difference in slope 6 vs 8	-0.0039(-0.0099, 0.0020)	0.1848	.
	difference in slope 6 vs 9	-0.0039(-0.0099, 0.0020)	0.1848	.

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
	difference in slope 7 vs 8	0.0006(-0.0040, 0.0052)	0.7866	.

Table21. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - TGF_beta

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
TGF_beta			.	<.0001
	Clobetasol end csater	1.788(1.536, 2.040)	<.0001	.
	Clobetasol only	3.328(1.914, 4.741)	<.0001	.
	Clobetasol start rejection	2.093(1.841, 2.345)	<.0001	.
	CsaTcr Only	3.498(2.630, 4.366)	<.0001	.
	Isograft	0.782(0.530, 1.034)	<.0001	.
	Tacrolimus Only	1.788(1.537, 2.038)	<.0001	.
	tacrolimus end csater	1.579(1.327, 1.831)	<.0001	.
	tacrolimus start rejection	1.748(1.496, 2.000)	<.0001	.
	Clobetasol end csater - Clobetasol only	-1.540(-2.976, -0.104)	0.0361	.
	Clobetasol end csater - Clobetasol start rejection	-0.305(-0.662, 0.051)	0.0911	.
	Clobetasol end csater - CsaTcr Only	-1.711(-2.614, -0.807)	0.0004	.
	Clobetasol end csater - Isograft	1.006(0.649, 1.362)	<.0001	.
	Clobetasol end csater - Tacrolimus Only	-0.000(-0.355, 0.355)	0.9999	.
	Clobetasol end csater - tacrolimus end csater	0.208(-0.148, 0.565)	0.2445	.
	Clobetasol end csater - tacrolimus start rejection	0.040(-0.317, 0.396)	0.8238	.
	Clobetasol only - Clobetasol start rejection	1.235(-0.201, 2.671)	0.0899	.
	Clobetasol only - CsaTcr Only	-0.170(-1.829, 1.488)	0.8368	.
	Clobetasol only - Isograft	2.546(1.110, 3.982)	0.0009	.
	Clobetasol only - Tacrolimus Only	1.540(0.105, 2.976)	0.0361	.
	Clobetasol only - tacrolimus end csater	1.749(0.313, 3.184)	0.0182	.
	Clobetasol only - tacrolimus start rejection	1.580(0.144, 3.016)	0.0319	.
	Clobetasol start rejection - CsaTcr Only	-1.405(-2.309, -0.502)	0.0031	.
	Clobetasol start rejection - Isograft	1.311(0.955, 1.667)	<.0001	.
	Clobetasol start rejection - Tacrolimus Only	0.305(-0.050, 0.660)	0.0901	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
	Clobetasol start rejection - tacrolimus end csater	0.514(0.157, 0.870)	0.0058	.
	Clobetasol start rejection - tacrolimus start rejection	0.345(-0.012, 0.701)	0.0575	.
	CsaTcr Only - Isograft	2.716(1.813, 3.620)	<.0001	.
	CsaTcr Only - Tacrolimus Only	1.711(0.807, 2.614)	0.0004	.
	CsaTcr Only - tacrolimus end csater	1.919(1.015, 2.823)	0.0001	.
	CsaTcr Only - tacrolimus start rejection	1.750(0.846, 2.654)	0.0003	.
	Isograft - Tacrolimus Only	-1.006(-1.361, -0.651)	<.0001	.
	Isograft - tacrolimus end csater	-0.797(-1.154, -0.441)	<.0001	.
	Isograft - tacrolimus start rejection	-0.966(-1.323, -0.610)	<.0001	.
	Tacrolimus Only - tacrolimus end csater	0.208(-0.147, 0.563)	0.2429	.
	Tacrolimus Only - tacrolimus start rejection	0.040(-0.316, 0.395)	0.8231	.
	tacrolimus end csater - tacrolimus start rejection	-0.169(-0.525, 0.188)	0.3443	.

8. PCR – TNF-alpha

Table22. Estimate of intercept and slope for each group - TNF_alpha

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csatcr	4.797(3.469, 6.124)	<.0001
group	Clobetasol only	7.513(3.873, 11.154)	0.0002
group	Clobetasol start rejection	1.061(-0.266, 2.389)	0.1142
group	CsaTcr Only	-0.655(-2.769, 1.460)	0.5353
group	Isograft	1.948(0.621, 3.276)	0.0050
group	Tacrolimus Only	5.210(3.868, 6.552)	<.0001
group	tacrolimus end csatcr	2.139(0.811, 3.467)	0.0023
group	tacrolimus start rejection	2.314(0.986, 3.642)	0.0011
days*group	Clobetasol end csatcr	0.005(-0.018, 0.028)	0.6736
days*group	Clobetasol only	-0.021(-0.404, 0.363)	0.9137
days*group	Clobetasol start rejection	0.065(0.042, 0.088)	<.0001
days*group	CsaTcr Only	0.563(0.420, 0.707)	<.0001
days*group	Isograft	-0.003(-0.026, 0.020)	0.8128
days*group	Tacrolimus Only	0.047(0.016, 0.079)	0.0046
days*group	tacrolimus end csatcr	0.002(-0.021, 0.025)	0.8913
days*group	tacrolimus start rejection	0.010(-0.013, 0.033)	0.3643

Table23. Compared trend (slope) over time among groups - TNF_alpha

<i>response</i>	<i>Differnce in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
TNF_alpha			.	<.0001
	difference in slope 1 vs 2	0.0255(-0.3587, 0.4097)	0.8939	.
	difference in slope 1 vs 3	-0.0600(-0.0925, -0.0276)	0.0006	.
	difference in slope 1 vs 4	-0.5586(-0.7036, -0.4136)	<.0001	.
	difference in slope 1 vs 5	0.0075(-0.0249, 0.0400)	0.6419	.
	difference in slope 1 vs 6	-0.0426(-0.0818, -0.0033)	0.0344	.
	difference in slope 1 vs 7	0.0033(-0.0292, 0.0357)	0.8403	.
	difference in slope 1 vs 8	-0.0056(-0.0381, 0.0268)	0.7291	.
	difference in slope 2 vs 3	-0.0856(-0.4698, 0.2986)	0.6552	.
	difference in slope 2 vs 4	-0.5842(-0.9935, -0.1748)	0.0063	.
	difference in slope 2 vs 5	-0.0180(-0.4022, 0.3662)	0.9250	.
	difference in slope 2 vs 6	-0.0681(-0.4529, 0.3167)	0.7226	.
	difference in slope 2 vs 7	-0.0223(-0.4065, 0.3619)	0.9074	.
	difference in slope 2 vs 8	-0.0311(-0.4153, 0.3530)	0.8708	.
	difference in slope 3 vs 4	-0.4986(-0.6436, -0.3536)	<.0001	.
	difference in slope 3 vs 5	0.0676(0.0351, 0.1000)	0.0001	.
	difference in slope 3 vs 6	0.0175(-0.0218, 0.0568)	0.3739	.
	difference in slope 3 vs 7	0.0633(0.0309, 0.0958)	0.0003	.
	difference in slope 3 vs 8	0.0544(0.0220, 0.0869)	0.0016	.
	difference in slope 4 vs 5	0.5662(0.4211, 0.7112)	<.0001	.
	difference in slope 4 vs 6	0.5161(0.3694, 0.6627)	<.0001	.
	difference in slope 4 vs 7	0.5619(0.4169, 0.7069)	<.0001	.
	difference in slope 4 vs 8	0.5530(0.4080, 0.6980)	<.0001	.
	difference in slope 5 vs 6	-0.0501(-0.0894, -0.0108)	0.0137	.
	difference in slope 5 vs 7	-0.0043(-0.0367, 0.0282)	0.7918	.
	difference in slope 5 vs 8	-0.0131(-0.0456, 0.0193)	0.4185	.
	difference in slope 6 vs 7	0.0458(0.0065, 0.0851)	0.0233	.
	difference in slope 6 vs 8	0.0370(-0.0023, 0.0762)	0.0644	.
	difference in slope 6 vs 9	0.0370(-0.0023, 0.0762)	0.0644	.

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
	difference in slope 7 vs 8	-0.0089(-0.0413, 0.0236)	0.5843	.

Table24. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - TNF_alpha

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
TNF_alpha			.	<.0001
	Clobetasol end csatcr	4.988(3.978, 5.998)	<.0001	.
	Clobetasol only	6.690(-5.253, 18.633)	0.2645	.
	Clobetasol start rejection	3.640(2.629, 4.650)	<.0001	.
	CsaTcr Only	21.743(17.538, 25.948)	<.0001	.
	Isograft	1.841(0.831, 2.851)	0.0007	.
	Tacrolimus Only	7.094(5.973, 8.215)	<.0001	.
	tacrolimus end csatcr	2.201(1.191, 3.211)	<.0001	.
	tacrolimus start rejection	2.728(1.718, 3.739)	<.0001	.
	Clobetasol end csatcr - Clobetasol only	-1.702(-13.688, 10.284)	0.7758	.
	Clobetasol end csatcr - Clobetasol start rejection	1.349(-0.080, 2.777)	0.0637	.
	Clobetasol end csatcr - CsaTcr Only	-16.755(-21.079, -12.431)	<.0001	.
	Clobetasol end csatcr - Isograft	3.147(1.719, 4.576)	<.0001	.
	Clobetasol end csatcr - Tacrolimus Only	-2.106(-3.615, -0.597)	0.0074	.
	Clobetasol end csatcr - tacrolimus end csatcr	2.787(1.358, 4.216)	0.0003	.
	Clobetasol end csatcr - tacrolimus start rejection	2.260(0.831, 3.688)	0.0027	.
	Clobetasol only - Clobetasol start rejection	3.050(-8.936, 15.036)	0.6100	.
	Clobetasol only - CsaTcr Only	-15.053(-27.715, -2.391)	0.0210	.
	Clobetasol only - Isograft	4.849(-7.137, 16.835)	0.4186	.
	Clobetasol only - Tacrolimus Only	-0.404(-12.400, 11.592)	0.9461	.
	Clobetasol only - tacrolimus end csatcr	4.489(-7.497, 16.475)	0.4538	.
	Clobetasol only - tacrolimus start rejection	3.961(-8.024, 15.947)	0.5082	.
	Clobetasol start rejection - CsaTcr Only	-18.103(-22.428, -13.779)	<.0001	.
	Clobetasol start rejection - Isograft	1.799(0.370, 3.228)	0.0149	.
	Clobetasol start rejection - Tacrolimus Only	-3.454(-4.963, -1.945)	<.0001	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
	Clobetasol start rejection - tacrolimus end csater	1.439(0.010, 2.867)	0.0485	.
	Clobetasol start rejection - tacrolimus start rejection	0.911(-0.518, 2.340)	0.2050	.
	CsaTcr Only - Isograft	19.902(15.578, 24.227)	<.0001	.
	CsaTcr Only - Tacrolimus Only	14.649(10.298, 19.001)	<.0001	.
	CsaTcr Only - tacrolimus end csater	19.542(15.218, 23.867)	<.0001	.
	CsaTcr Only - tacrolimus start rejection	19.015(14.690, 23.339)	<.0001	.
	Isograft - Tacrolimus Only	-5.253(-6.762, -3.744)	<.0001	.
	Isograft - tacrolimus end csater	-0.360(-1.789, 1.069)	0.6134	.
	Isograft - tacrolimus start rejection	-0.888(-2.316, 0.541)	0.2167	.
	Tacrolimus Only - tacrolimus end csater	4.893(3.384, 6.402)	<.0001	.
	Tacrolimus Only - tacrolimus start rejection	4.365(2.856, 5.874)	<.0001	.
	tacrolimus end csater - tacrolimus start rejection	-0.527(-1.956, 0.901)	0.4602	.

9. PCR – IL-13

Table 25. Estimate of intercept and slope for each group - IL_13

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csatcr	0.134(-0.088, 0.357)	0.2305
group	Clobetasol only	0.160(-4.739, 5.059)	0.9478
group	Clobetasol start rejection	0.278(0.055, 0.501)	0.0157
group	CsaTcr Only	1.498(0.870, 2.126)	<.0001
group	Isograft	0.268(0.046, 0.491)	0.0194
group	Tacrolimus Only	0.422(0.163, 0.681)	0.0021
group	tacrolimus end csatcr	-0.131(-0.354, 0.092)	0.2418
group	tacrolimus start rejection	0.167(-0.055, 0.390)	0.1369
days*group	Clobetasol end csatcr	-0.001(-0.004, 0.002)	0.3972
days*group	Clobetasol only	-0.006(-0.239, 0.227)	0.9577
days*group	Clobetasol start rejection	-0.003(-0.006, 0.000)	0.0828
days*group	CsaTcr Only	-0.012(-0.034, 0.011)	0.3034
days*group	Isograft	0.001(-0.002, 0.004)	0.4186
days*group	Tacrolimus Only	0.001(-0.003, 0.006)	0.5171
days*group	tacrolimus end csatcr	0.008(0.005, 0.011)	<.0001
days*group	tacrolimus start rejection	0.003(0.000, 0.006)	0.0346

Table26. Compared trend (slope) over time among groups - II_13

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
II_13			.	0.0006
	difference in slope 1 vs 2	0.0049(-0.2282, 0.2380)	0.9663	.
	difference in slope 1 vs 3	0.0014(-0.0028, 0.0056)	0.5177	.
	difference in slope 1 vs 4	0.0103(-0.0123, 0.0329)	0.3627	.
	difference in slope 1 vs 5	-0.0025(-0.0067, 0.0017)	0.2437	.
	difference in slope 1 vs 6	-0.0027(-0.0080, 0.0026)	0.3130	.
	difference in slope 1 vs 7	-0.0092(-0.0134, -0.0050)	<.0001	.
	difference in slope 1 vs 8	-0.0045(-0.0087, -0.0003)	0.0375	.
	difference in slope 2 vs 3	-0.0035(-0.2367, 0.2296)	0.9756	.
	difference in slope 2 vs 4	0.0054(-0.2288, 0.2396)	0.9631	.
	difference in slope 2 vs 5	-0.0074(-0.2405, 0.2258)	0.9494	.
	difference in slope 2 vs 6	-0.0076(-0.2407, 0.2256)	0.9480	.
	difference in slope 2 vs 7	-0.0141(-0.2472, 0.2190)	0.9033	.
	difference in slope 2 vs 8	-0.0094(-0.2425, 0.2237)	0.9356	.
	difference in slope 3 vs 4	0.0089(-0.0137, 0.0315)	0.4289	.
	difference in slope 3 vs 5	-0.0038(-0.0080, 0.0004)	0.0737	.
	difference in slope 3 vs 6	-0.0040(-0.0093, 0.0013)	0.1311	.
	difference in slope 3 vs 7	-0.0106(-0.0148, -0.0064)	<.0001	.
	difference in slope 3 vs 8	-0.0058(-0.0101, -0.0016)	0.0077	.
	difference in slope 4 vs 5	-0.0128(-0.0354, 0.0098)	0.2606	.
	difference in slope 4 vs 6	-0.0130(-0.0358, 0.0098)	0.2576	.
	difference in slope 4 vs 7	-0.0195(-0.0421, 0.0031)	0.0889	.
	difference in slope 4 vs 8	-0.0148(-0.0374, 0.0078)	0.1938	.
	difference in slope 5 vs 6	-0.0002(-0.0055, 0.0051)	0.9361	.
	difference in slope 5 vs 7	-0.0067(-0.0109, -0.0025)	0.0024	.
	difference in slope 5 vs 8	-0.0020(-0.0062, 0.0022)	0.3388	.
	difference in slope 6 vs 7	-0.0065(-0.0118, -0.0012)	0.0169	.
	difference in slope 6 vs 8	-0.0018(-0.0071, 0.0035)	0.4946	.
	difference in slope 6 vs 9	-0.0018(-0.0071, 0.0035)	0.4946	.

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
	difference in slope 7 vs 8	0.0047(0.0005, 0.0089)	0.0290	.

Table27. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - II_13

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
II_13				. 0.0003
	Clobetasol end csatcr	0.084(-0.056, 0.224)	0.2314	.
	Clobetasol only	-0.085(-4.467, 4.296)	0.9688	.
	Clobetasol start rejection	0.174(0.034, 0.314)	0.0162	.
	CsaTcr Only	1.039(0.657, 1.420)	<.0001	.
	Isograft	0.316(0.176, 0.456)	<.0001	.
	Tacrolimus Only	0.478(0.329, 0.627)	<.0001	.
	tacrolimus end csatcr	0.185(0.045, 0.324)	0.0109	.
	tacrolimus start rejection	0.295(0.156, 0.435)	0.0001	.
	Clobetasol end csatcr - Clobetasol only	0.169(-4.214, 4.553)	0.9382	.
	Clobetasol end csatcr - Clobetasol start rejection	-0.090(-0.287, 0.108)	0.3654	.
	Clobetasol end csatcr - CsaTcr Only	-0.954(-1.361, -0.548)	<.0001	.
	Clobetasol end csatcr - Isograft	-0.232(-0.430, -0.034)	0.0226	.
	Clobetasol end csatcr - Tacrolimus Only	-0.394(-0.598, -0.190)	0.0004	.
	Clobetasol end csatcr - tacrolimus end csatcr	-0.100(-0.298, 0.097)	0.3109	.
	Clobetasol end csatcr - tacrolimus start rejection	-0.211(-0.409, -0.013)	0.0369	.
	Clobetasol only - Clobetasol start rejection	-0.259(-4.643, 4.125)	0.9056	.
	Clobetasol only - CsaTcr Only	-1.124(-5.522, 3.274)	0.6086	.
	Clobetasol only - Isograft	-0.401(-4.785, 3.982)	0.8542	.
	Clobetasol only - Tacrolimus Only	-0.563(-4.948, 3.821)	0.7965	.
	Clobetasol only - tacrolimus end csatcr	-0.270(-4.654, 4.114)	0.9017	.
	Clobetasol only - tacrolimus start rejection	-0.381(-4.765, 4.003)	0.8617	.
	Clobetasol start rejection - CsaTcr Only	-0.865(-1.271, -0.458)	0.0001	.
	Clobetasol start rejection - Isograft	-0.142(-0.340, 0.055)	0.1535	.
	Clobetasol start rejection - Tacrolimus Only	-0.304(-0.508, -0.100)	0.0044	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
	Clobetasol start rejection - tacrolimus end csater	-0.011(-0.209, 0.187)	0.9122	.
	Clobetasol start rejection - tacrolimus start rejection	-0.122(-0.319, 0.076)	0.2213	.
	CsaTcr Only - Isograft	0.722(0.316, 1.129)	0.0009	.
	CsaTcr Only - Tacrolimus Only	0.561(0.151, 0.970)	0.0085	.
	CsaTcr Only - tacrolimus end csater	0.854(0.448, 1.260)	0.0001	.
	CsaTcr Only - tacrolimus start rejection	0.743(0.337, 1.150)	0.0006	.
	Isograft - Tacrolimus Only	-0.162(-0.366, 0.042)	0.1167	.
	Isograft - tacrolimus end csater	0.132(-0.066, 0.329)	0.1866	.
	Isograft - tacrolimus start rejection	0.021(-0.177, 0.219)	0.8332	.
	Tacrolimus Only - tacrolimus end csater	0.293(0.089, 0.498)	0.0059	.
	Tacrolimus Only - tacrolimus start rejection	0.183(-0.021, 0.387)	0.0780	.
	tacrolimus end csater - tacrolimus start rejection	-0.111(-0.309, 0.087)	0.2645	.

10. PCR – IL-15

Table28. Estimate of intercept and slope for each group - IL_15

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csatcr	0.665(0.546, 0.785)	<.0001
group	Clobetasol only	0.225(-0.187, 0.637)	0.2769
group	Clobetasol start rejection	0.667(0.548, 0.786)	<.0001
group	CsaTcr Only	0.800(0.548, 1.053)	<.0001
group	Isograft	0.520(0.401, 0.640)	<.0001
group	Tacrolimus Only	0.457(0.315, 0.600)	<.0001
group	tacrolimus end csatcr	0.672(0.553, 0.792)	<.0001
group	tacrolimus start rejection	1.104(0.985, 1.223)	<.0001
days*group	Clobetasol end csatcr	-0.002(-0.005, 0.000)	0.0936
days*group	Clobetasol only	0.021(-0.006, 0.047)	0.1257
days*group	Clobetasol start rejection	-0.001(-0.003, 0.002)	0.5172
days*group	CsaTcr Only	0.004(-0.009, 0.016)	0.5590
days*group	Isograft	0.007(0.004, 0.009)	<.0001
days*group	Tacrolimus Only	0.004(0.000, 0.008)	0.0304
days*group	tacrolimus end csatcr	0.001(-0.002, 0.003)	0.4685
days*group	tacrolimus start rejection	-0.004(-0.007, -0.002)	0.0018

Table29. Compared trend (slope) over time among groups - II_15

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
II_15			.	<.0001
	difference in slope 1 vs 2	-0.0229(-0.0497, 0.0040)	0.0936	.
	difference in slope 1 vs 3	-0.0013(-0.0049, 0.0022)	0.4565	.
	difference in slope 1 vs 4	-0.0058(-0.0186, 0.0070)	0.3653	.
	difference in slope 1 vs 5	-0.0088(-0.0123, -0.0052)	<.0001	.
	difference in slope 1 vs 6	-0.0062(-0.0106, -0.0018)	0.0074	.
	difference in slope 1 vs 7	-0.0030(-0.0066, 0.0005)	0.0909	.
	difference in slope 1 vs 8	0.0020(-0.0015, 0.0056)	0.2603	.
	difference in slope 2 vs 3	0.0215(-0.0054, 0.0484)	0.1135	.
	difference in slope 2 vs 4	0.0171(-0.0125, 0.0466)	0.2502	.
	difference in slope 2 vs 5	0.0141(-0.0128, 0.0409)	0.2968	.
	difference in slope 2 vs 6	0.0167(-0.0104, 0.0437)	0.2202	.
	difference in slope 2 vs 7	0.0198(-0.0071, 0.0467)	0.1445	.
	difference in slope 2 vs 8	0.0249(-0.0020, 0.0517)	0.0690	.
	difference in slope 3 vs 4	-0.0045(-0.0172, 0.0083)	0.4841	.
	difference in slope 3 vs 5	-0.0075(-0.0110, -0.0039)	0.0001	.
	difference in slope 3 vs 6	-0.0049(-0.0093, -0.0004)	0.0321	.
	difference in slope 3 vs 7	-0.0017(-0.0053, 0.0018)	0.3331	.
	difference in slope 3 vs 8	0.0033(-0.0002, 0.0069)	0.0654	.
	difference in slope 4 vs 5	-0.0030(-0.0158, 0.0098)	0.6378	.
	difference in slope 4 vs 6	-0.0004(-0.0135, 0.0126)	0.9494	.
	difference in slope 4 vs 7	0.0027(-0.0100, 0.0155)	0.6669	.
	difference in slope 4 vs 8	0.0078(-0.0050, 0.0206)	0.2245	.
	difference in slope 5 vs 6	0.0026(-0.0019, 0.0070)	0.2463	.
	difference in slope 5 vs 7	0.0057(0.0022, 0.0093)	0.0022	.
	difference in slope 5 vs 8	0.0108(0.0072, 0.0143)	<.0001	.
	difference in slope 6 vs 7	0.0032(-0.0013, 0.0076)	0.1591	.
	difference in slope 6 vs 8	0.0082(0.0038, 0.0126)	0.0006	.
	difference in slope 6 vs 9	0.0082(0.0038, 0.0126)	0.0006	.

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
	difference in slope 7 vs 8	0.0051(0.0015, 0.0086)	0.0064	.

Table30. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - II_15

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
II_15				. <.0001
	Clobetasol end csatcr	0.581(0.498, 0.663)	<.0001	.
	Clobetasol only	1.048(0.361, 1.736)	0.0037	.
	Clobetasol start rejection	0.635(0.552, 0.717)	<.0001	.
	CsaTcr Only	0.946(0.637, 1.254)	<.0001	.
	Isograft	0.785(0.702, 0.867)	<.0001	.
	Tacrolimus Only	0.619(0.525, 0.713)	<.0001	.
	tacrolimus end csatcr	0.708(0.626, 0.791)	<.0001	.
	tacrolimus start rejection	0.939(0.857, 1.022)	<.0001	.
	Clobetasol end csatcr - Clobetasol only	-0.468(-1.161, 0.225)	0.1799	.
	Clobetasol end csatcr - Clobetasol start rejection	-0.054(-0.171, 0.062)	0.3526	.
	Clobetasol end csatcr - CsaTcr Only	-0.365(-0.685, -0.045)	0.0262	.
	Clobetasol end csatcr - Isograft	-0.204(-0.321, -0.087)	0.0010	.
	Clobetasol end csatcr - Tacrolimus Only	-0.038(-0.164, 0.087)	0.5388	.
	Clobetasol end csatcr - tacrolimus end csatcr	-0.128(-0.245, -0.011)	0.0324	.
	Clobetasol end csatcr - tacrolimus start rejection	-0.359(-0.476, -0.242)	<.0001	.
	Clobetasol only - Clobetasol start rejection	0.414(-0.279, 1.106)	0.2347	.
	Clobetasol only - CsaTcr Only	0.103(-0.651, 0.857)	0.7843	.
	Clobetasol only - Isograft	0.264(-0.429, 0.956)	0.4461	.
	Clobetasol only - Tacrolimus Only	0.429(-0.265, 1.124)	0.2186	.
	Clobetasol only - tacrolimus end csatcr	0.340(-0.353, 1.033)	0.3273	.
	Clobetasol only - tacrolimus start rejection	0.109(-0.584, 0.802)	0.7521	.
	Clobetasol start rejection - CsaTcr Only	-0.311(-0.630, 0.009)	0.0564	.
	Clobetasol start rejection - Isograft	-0.150(-0.266, -0.033)	0.0132	.
	Clobetasol start rejection - Tacrolimus Only	0.016(-0.110, 0.141)	0.8001	.
	Clobetasol start rejection - tacrolimus end csatcr	-0.074(-0.190, 0.043)	0.2098	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
	Clobetasol start rejection - tacrolimus start rejection	-0.305(-0.421, -0.188)	<.0001	.
	CsaTcr Only - Isograft	0.161(-0.159, 0.481)	0.3151	.
	CsaTcr Only - Tacrolimus Only	0.327(0.004, 0.649)	0.0476	.
	CsaTcr Only - tacrolimus end csatcr	0.237(-0.083, 0.557)	0.1418	.
	CsaTcr Only - tacrolimus start rejection	0.006(-0.313, 0.326)	0.9690	.
	Isograft - Tacrolimus Only	0.166(0.040, 0.291)	0.0109	.
	Isograft - tacrolimus end csatcr	0.076(-0.041, 0.193)	0.1949	.
	Isograft - tacrolimus start rejection	-0.155(-0.271, -0.038)	0.0106	.
	Tacrolimus Only - tacrolimus end csatcr	-0.089(-0.215, 0.036)	0.1573	.
	Tacrolimus Only - tacrolimus start rejection	-0.320(-0.446, -0.195)	<.0001	.
	tacrolimus end csatcr - tacrolimus start rejection	-0.231(-0.348, -0.114)	0.0003	.

11. PCR – IFN-gamma

Table31. Estimate of intercept and slope for each group - IFN_gamma

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csatcr	12.629(5.557, 19.700)	0.0008
group	Clobetasol only	-21.615(-57.360, 14.130)	0.2290
group	Clobetasol start rejection	10.148(3.077, 17.220)	0.0060
group	CsaTcr Only	-29.022(-44.421, -13.622)	0.0005
group	Isograft	2.449(-4.622, 9.520)	0.4882
group	Tacrolimus Only	28.029(19.668, 36.390)	<.0001
group	tacrolimus end csatcr	-0.013(-7.084, 7.059)	0.9971
group	tacrolimus start rejection	-1.057(-8.129, 6.014)	0.7642
days*group	Clobetasol end csatcr	-0.013(-0.281, 0.254)	0.9207
days*group	Clobetasol only	7.116(4.845, 9.387)	<.0001
days*group	Clobetasol start rejection	0.675(0.407, 0.943)	<.0001
days*group	CsaTcr Only	5.295(4.711, 5.878)	<.0001
days*group	Isograft	0.001(-0.267, 0.268)	0.9966
days*group	Tacrolimus Only	0.185(-0.201, 0.572)	0.3387
days*group	tacrolimus end csatcr	0.146(-0.122, 0.414)	0.2766
days*group	tacrolimus start rejection	0.193(-0.074, 0.461)	0.1523

Table32. Compared trend (slope) over time among groups - IFN_gamma

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
IFN_gamma			.	<.0001
	difference in slope 1 vs 2	-7.1297(-9.4164, -4.8430)	<.0001	.
	difference in slope 1 vs 3	-0.6881(-1.0668, -0.3094)	0.0007	.
	difference in slope 1 vs 4	-5.3080(-5.9500, -4.6659)	<.0001	.
	difference in slope 1 vs 5	-0.0138(-0.3925, 0.3648)	0.9415	.
	difference in slope 1 vs 6	-0.1985(-0.6686, 0.2716)	0.3988	.
	difference in slope 1 vs 7	-0.1595(-0.5381, 0.2192)	0.3999	.
	difference in slope 1 vs 8	-0.2067(-0.5853, 0.1720)	0.2767	.
	difference in slope 2 vs 3	6.4416(4.1549, 8.7283)	<.0001	.
	difference in slope 2 vs 4	1.8218(-0.5230, 4.1665)	0.1243	.
	difference in slope 2 vs 5	7.1159(4.8292, 9.4026)	<.0001	.
	difference in slope 2 vs 6	6.9312(4.6276, 9.2348)	<.0001	.
	difference in slope 2 vs 7	6.9702(4.6835, 9.2569)	<.0001	.
	difference in slope 2 vs 8	6.9230(4.6363, 9.2097)	<.0001	.
	difference in slope 3 vs 4	-4.6199(-5.2619, -3.9778)	<.0001	.
	difference in slope 3 vs 5	0.6742(0.2956, 1.0529)	0.0009	.
	difference in slope 3 vs 6	0.4896(0.0195, 0.9597)	0.0416	.
	difference in slope 3 vs 7	0.5286(0.1499, 0.9073)	0.0074	.
	difference in slope 3 vs 8	0.4814(0.1028, 0.8601)	0.0140	.
	difference in slope 4 vs 5	5.2941(4.6521, 5.9361)	<.0001	.
	difference in slope 4 vs 6	5.1095(4.4096, 5.8093)	<.0001	.
	difference in slope 4 vs 7	5.1485(4.5064, 5.7905)	<.0001	.
	difference in slope 4 vs 8	5.1013(4.4592, 5.7433)	<.0001	.
	difference in slope 5 vs 6	-0.1846(-0.6547, 0.2854)	0.4322	.
	difference in slope 5 vs 7	-0.1456(-0.5243, 0.2330)	0.4417	.
	difference in slope 5 vs 8	-0.1928(-0.5715, 0.1858)	0.3097	.
	difference in slope 6 vs 7	0.0390(-0.4311, 0.5091)	0.8678	.
	difference in slope 6 vs 8	-0.0082(-0.4783, 0.4619)	0.9720	.
	difference in slope 6 vs 9	-0.0082(-0.4783, 0.4619)	0.9720	.

<i>response</i>	<i>Differnce in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
	difference in slope 7 vs 8	-0.0472(-0.4259, 0.3315)	0.8025	.

Table33. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - IFN_gamma

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
IFN_gamma			.	<.0001
	Clobetasol end csatcr	12.101(-0.197, 24.399)	0.0536	.
	Clobetasol only	261.272(203.096, 319.447)	<.0001	.
	Clobetasol start rejection	36.973(24.675, 49.271)	<.0001	.
	CsaTcr Only	181.448(162.444, 200.452)	<.0001	.
	Isograft	2.471(-9.827, 14.769)	0.6870	.
	Tacrolimus Only	35.391(20.542, 50.240)	<.0001	.
	tacrolimus end csatcr	5.799(-6.499, 18.097)	0.3465	.
	tacrolimus start rejection	6.631(-5.667, 18.929)	0.2826	.
	Clobetasol end csatcr - Clobetasol only	-249.171(-308.632, -189.710)	<.0001	.
	Clobetasol end csatcr - Clobetasol start rejection	-24.872(-42.264, -7.480)	0.0062	.
	Clobetasol end csatcr - CsaTcr Only	-169.347(-191.983, -146.711)	<.0001	.
	Clobetasol end csatcr - Isograft	9.629(-7.763, 27.022)	0.2700	.
	Clobetasol end csatcr - Tacrolimus Only	-23.290(-42.571, -4.010)	0.0191	.
	Clobetasol end csatcr - tacrolimus end csatcr	6.302(-11.091, 23.694)	0.4685	.
	Clobetasol end csatcr - tacrolimus start rejection	5.470(-11.922, 22.862)	0.5289	.
	Clobetasol only - Clobetasol start rejection	224.299(164.838, 283.760)	<.0001	.
	Clobetasol only - CsaTcr Only	79.824(18.623, 141.024)	0.0118	.
	Clobetasol only - Isograft	258.800(199.339, 318.261)	<.0001	.
	Clobetasol only - Tacrolimus Only	225.881(165.840, 285.921)	<.0001	.
	Clobetasol only - tacrolimus end csatcr	255.473(196.012, 314.934)	<.0001	.
	Clobetasol only - tacrolimus start rejection	254.641(195.180, 314.102)	<.0001	.
	Clobetasol start rejection - CsaTcr Only	-144.475(-167.111, -121.839)	<.0001	.
	Clobetasol start rejection - Isograft	34.501(17.109, 51.894)	0.0003	.
	Clobetasol start rejection - Tacrolimus Only	1.582(-17.699, 20.862)	0.8692	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
	Clobetasol start rejection - tacrolimus end csater	31.174(13.781, 48.566)	0.0008	.
	Clobetasol start rejection - tacrolimus start rejection	30.342(12.950, 47.734)	0.0011	.
	CsaTcr Only - Isograft	178.977(156.341, 201.612)	<.0001	.
	CsaTcr Only - Tacrolimus Only	146.057(121.940, 170.174)	<.0001	.
	CsaTcr Only - tacrolimus end csater	175.649(153.013, 198.285)	<.0001	.
	CsaTcr Only - tacrolimus start rejection	174.817(152.181, 197.453)	<.0001	.
	Isograft - Tacrolimus Only	-32.920(-52.200, -13.639)	0.0013	.
	Isograft - tacrolimus end csater	-3.328(-20.720, 14.064)	0.7012	.
	Isograft - tacrolimus start rejection	-4.159(-21.552, 13.233)	0.6317	.
	Tacrolimus Only - tacrolimus end csater	29.592(10.311, 48.873)	0.0035	.
	Tacrolimus Only - tacrolimus start rejection	28.760(9.480, 48.041)	0.0044	.
	tacrolimus end csater - tacrolimus start rejection	-0.832(-18.224, 16.560)	0.9235	.

12. FACS – CD-3

Table34. Estimate of intercept and slope for each group - FACS_CD3

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csatcr	2.403(0.014, 4.792)	0.0487
group	Clobetasol only	15.433(8.850, 22.017)	<.0001
group	Clobetasol start rejection	6.662(4.273, 9.050)	<.0001
group	CsaTcr Only	-0.873(-4.482, 2.736)	0.6278
group	Isograft	35.909(33.521, 38.298)	<.0001
group	Tacrolimus Only	25.514(23.203, 27.824)	<.0001
group	tacrolimus end csatcr	2.932(0.544, 5.321)	0.0174
group	tacrolimus start rejection	1.687(-0.702, 4.075)	0.1614
days*group	Clobetasol end csatcr	0.022(-0.024, 0.067)	0.3436
days*group	Clobetasol only	-0.434(-0.933, 0.064)	0.0861
days*group	Clobetasol start rejection	-0.063(-0.109, -0.017)	0.0080
days*group	CsaTcr Only	0.466(0.196, 0.736)	0.0012
days*group	Isograft	0.218(0.173, 0.264)	<.0001
days*group	Tacrolimus Only	0.239(0.171, 0.307)	<.0001
days*group	tacrolimus end csatcr	0.184(0.138, 0.229)	<.0001
days*group	tacrolimus start rejection	0.241(0.196, 0.287)	<.0001

Table35. Compared trend (slope) over time among groups - FACS_CD3

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
FACS_CD3			.	<.0001
	difference in slope 1 vs 2	0.4560(-0.0449, 0.9568)	0.0732	.
	difference in slope 1 vs 3	0.0847(0.0201, 0.1493)	0.0114	.
	difference in slope 1 vs 4	-0.4441(-0.7178, -0.1704)	0.0021	.
	difference in slope 1 vs 5	-0.1966(-0.2612, -0.1320)	<.0001	.
	difference in slope 1 vs 6	-0.2174(-0.2992, -0.1355)	<.0001	.
	difference in slope 1 vs 7	-0.1621(-0.2267, -0.0975)	<.0001	.
	difference in slope 1 vs 8	-0.2196(-0.2842, -0.1550)	<.0001	.
	difference in slope 2 vs 3	-0.3713(-0.8721, 0.1296)	0.1420	.
	difference in slope 2 vs 4	-0.9000(-1.4671, -0.3330)	0.0026	.
	difference in slope 2 vs 5	-0.6526(-1.1534, -0.1518)	0.0119	.
	difference in slope 2 vs 6	-0.6733(-1.1767, -0.1700)	0.0100	.
	difference in slope 2 vs 7	-0.6180(-1.1189, -0.1172)	0.0168	.
	difference in slope 2 vs 8	-0.6756(-1.1764, -0.1747)	0.0094	.
	difference in slope 3 vs 4	-0.5288(-0.8025, -0.2551)	0.0003	.
	difference in slope 3 vs 5	-0.2813(-0.3459, -0.2167)	<.0001	.
	difference in slope 3 vs 6	-0.3021(-0.3839, -0.2202)	<.0001	.
	difference in slope 3 vs 7	-0.2468(-0.3114, -0.1822)	<.0001	.
	difference in slope 3 vs 8	-0.3043(-0.3689, -0.2397)	<.0001	.
	difference in slope 4 vs 5	0.2474(-0.0263, 0.5211)	0.0752	.
	difference in slope 4 vs 6	0.2267(-0.0516, 0.5050)	0.1076	.
	difference in slope 4 vs 7	0.2820(0.0083, 0.5557)	0.0438	.
	difference in slope 4 vs 8	0.2245(-0.0492, 0.4982)	0.1053	.
	difference in slope 5 vs 6	-0.0207(-0.1026, 0.0611)	0.6116	.
	difference in slope 5 vs 7	0.0346(-0.0300, 0.0992)	0.2862	.
	difference in slope 5 vs 8	-0.0230(-0.0876, 0.0416)	0.4769	.
	difference in slope 6 vs 7	0.0553(-0.0266, 0.1371)	0.1799	.
	difference in slope 6 vs 8	-0.0022(-0.0841, 0.0796)	0.9566	.
	difference in slope 6 vs 9	-0.0022(-0.0841, 0.0796)	0.9566	.

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
	difference in slope 7 vs 8	-0.0575(-0.1221, 0.0071)	0.0795	.

Table36. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - FACS_CD3

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
FACS_CD3				. <.0001
	Clobetasol end csatcr	3.264(1.071, 5.457)	0.0045	.
	Clobetasol only	-1.830(-15.693, 12.033)	0.7911	.
	Clobetasol start rejection	4.156(1.963, 6.349)	0.0004	.
	CsaTcr Only	17.641(9.425, 25.856)	<.0001	.
	Isograft	44.587(42.395, 46.780)	<.0001	.
	Tacrolimus Only	35.016(32.387, 37.646)	<.0001	.
	tacrolimus end csatcr	10.237(8.044, 12.430)	<.0001	.
	tacrolimus start rejection	11.277(9.085, 13.470)	<.0001	.
	Clobetasol end csatcr - Clobetasol only	5.094(-8.941, 19.130)	0.4677	.
	Clobetasol end csatcr - Clobetasol start rejection	-0.892(-3.993, 2.210)	0.5646	.
	Clobetasol end csatcr - CsaTcr Only	-14.377(-22.880, -5.873)	0.0015	.
	Clobetasol end csatcr - Isograft	-41.323(-44.424, -38.222)	<.0001	.
	Clobetasol end csatcr - Tacrolimus Only	-31.752(-35.176, -28.328)	<.0001	.
	Clobetasol end csatcr - tacrolimus end csatcr	-6.973(-10.074, -3.871)	<.0001	.
	Clobetasol end csatcr - tacrolimus start rejection	-8.013(-11.114, -4.912)	<.0001	.
	Clobetasol only - Clobetasol start rejection	-5.986(-20.021, 8.049)	0.3941	.
	Clobetasol only - CsaTcr Only	-19.471(-35.585, -3.357)	0.0191	.
	Clobetasol only - Isograft	-46.417(-60.453, -32.382)	<.0001	.
	Clobetasol only - Tacrolimus Only	-36.846(-50.957, -22.736)	<.0001	.
	Clobetasol only - tacrolimus end csatcr	-12.067(-26.102, 1.968)	0.0900	.
	Clobetasol only - tacrolimus start rejection	-13.108(-27.143, 0.928)	0.0664	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
	Clobetasol start rejection - CsaTcr Only	-13.485(-21.988, -4.982)	0.0026	.
	Clobetasol start rejection - Isograft	-40.431(-43.533, -37.330)	<.0001	.
	Clobetasol start rejection - Tacrolimus Only	-30.860(-34.284, -27.436)	<.0001	.
	Clobetasol start rejection - tacrolimus end csatcr	-6.081(-9.182, -2.980)	0.0003	.
	Clobetasol start rejection - tacrolimus start rejection	-7.121(-10.223, -4.020)	<.0001	.
	CsaTcr Only - Isograft	-26.947(-35.450, -18.443)	<.0001	.
	CsaTcr Only - Tacrolimus Only	-17.375(-26.002, -8.749)	0.0002	.
	CsaTcr Only - tacrolimus end csatcr	7.404(-1.099, 15.907)	0.0861	.
	CsaTcr Only - tacrolimus start rejection	6.363(-2.140, 14.867)	0.1384	.
	Isograft - Tacrolimus Only	9.571(6.147, 12.995)	<.0001	.
	Isograft - tacrolimus end csatcr	34.351(31.249, 37.452)	<.0001	.
	Isograft - tacrolimus start rejection	33.310(30.209, 36.411)	<.0001	.
	Tacrolimus Only - tacrolimus end csatcr	24.780(21.355, 28.204)	<.0001	.
	Tacrolimus Only - tacrolimus start rejection	23.739(20.315, 27.163)	<.0001	.
	tacrolimus end csatcr - tacrolimus start rejection	-1.041(-4.142, 2.061)	0.5018	.

13. FACS – CD – 45

Table37. Estimate of intercept and slope for each group - FACS_CD45

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csatcr	5.135(2.814, 7.457)	<.0001
group	Clobetasol only	8.990(4.342, 13.638)	0.0003
group	Clobetasol start rejection	8.928(6.606, 11.250)	<.0001
group	CsaTcr Only	3.569(0.387, 6.750)	0.0288
group	Isograft	25.215(22.893, 27.537)	<.0001
group	Tacrolimus Only	20.527(18.257, 22.797)	<.0001
group	tacrolimus end csatcr	8.779(6.457, 11.101)	<.0001
group	tacrolimus start rejection	8.601(6.279, 10.923)	<.0001
days*group	Clobetasol end csatcr	-0.041(-0.070, -0.012)	0.0064
days*group	Clobetasol only	-0.375(-0.715, -0.035)	0.0317
days*group	Clobetasol start rejection	-0.067(-0.096, -0.038)	<.0001
days*group	CsaTcr Only	0.573(0.446, 0.700)	<.0001
days*group	Isograft	0.014(-0.015, 0.043)	0.3469
days*group	Tacrolimus Only	0.030(-0.008, 0.068)	0.1185
days*group	tacrolimus end csatcr	0.116(0.087, 0.145)	<.0001
days*group	tacrolimus start rejection	0.174(0.145, 0.203)	<.0001

Table38. Compared trend (slope) over time among groups - FACS_CD45

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
FACS_CD45			.	<.0001
	difference in slope 1 vs 2	0.3336(-0.0080, 0.6752)	0.0554	.
	difference in slope 1 vs 3	0.0256(-0.0155, 0.0667)	0.2155	.
	difference in slope 1 vs 4	-0.6139(-0.7443, -0.4836)	<.0001	.
	difference in slope 1 vs 5	-0.0551(-0.0962, -0.0140)	0.0099	.
	difference in slope 1 vs 6	-0.0712(-0.1188, -0.0235)	0.0044	.
	difference in slope 1 vs 7	-0.1577(-0.1989, -0.1166)	<.0001	.
	difference in slope 1 vs 8	-0.2154(-0.2565, -0.1743)	<.0001	.
	difference in slope 2 vs 3	-0.3080(-0.6496, 0.0336)	0.0759	.
	difference in slope 2 vs 4	-0.9475(-1.3108, -0.5842)	<.0001	.
	difference in slope 2 vs 5	-0.3886(-0.7302, -0.0471)	0.0268	.
	difference in slope 2 vs 6	-0.4048(-0.7472, -0.0623)	0.0217	.
	difference in slope 2 vs 7	-0.4913(-0.8329, -0.1497)	0.0059	.
	difference in slope 2 vs 8	-0.5490(-0.8906, -0.2074)	0.0023	.
	difference in slope 3 vs 4	-0.6396(-0.7699, -0.5092)	<.0001	.
	difference in slope 3 vs 5	-0.0807(-0.1218, -0.0396)	0.0003	.
	difference in slope 3 vs 6	-0.0968(-0.1444, -0.0491)	0.0002	.
	difference in slope 3 vs 7	-0.1834(-0.2245, -0.1422)	<.0001	.
	difference in slope 3 vs 8	-0.2410(-0.2821, -0.1999)	<.0001	.
	difference in slope 4 vs 5	0.5589(0.4285, 0.6893)	<.0001	.
	difference in slope 4 vs 6	0.5428(0.4102, 0.6753)	<.0001	.
	difference in slope 4 vs 7	0.4562(0.3258, 0.5866)	<.0001	.
	difference in slope 4 vs 8	0.3985(0.2682, 0.5289)	<.0001	.
	difference in slope 5 vs 6	-0.0161(-0.0638, 0.0315)	0.4982	.
	difference in slope 5 vs 7	-0.1027(-0.1438, -0.0616)	<.0001	.
	difference in slope 5 vs 8	-0.1603(-0.2015, -0.1192)	<.0001	.
	difference in slope 6 vs 7	-0.0866(-0.1342, -0.0389)	0.0007	.
	difference in slope 6 vs 8	-0.1442(-0.1919, -0.0966)	<.0001	.
	difference in slope 6 vs 9	-0.1442(-0.1919, -0.0966)	<.0001	.

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
	difference in slope 7 vs 8	-0.0577(-0.0988, -0.0166)	0.0071	.

Table39. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - FACS_CD45

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
FACS_CD45			.	<.0001
	Clobetasol end csater	3.491(2.019, 4.963)	<.0001	.
	Clobetasol only	-5.915(-15.640, 3.810)	0.2263	.
	Clobetasol start rejection	6.266(4.794, 7.738)	<.0001	.
	CsaTcr Only	26.330(23.069, 29.590)	<.0001	.
	Isograft	25.759(24.287, 27.231)	<.0001	.
	Tacrolimus Only	21.712(20.194, 23.231)	<.0001	.
	tacrolimus end csater	13.405(11.933, 14.877)	<.0001	.
	tacrolimus start rejection	15.520(14.048, 16.992)	<.0001	.
	Clobetasol end csater - Clobetasol only	9.406(-0.430, 19.242)	0.0604	.
	Clobetasol end csater - Clobetasol start rejection	-2.775(-4.856, -0.693)	0.0102	.
	Clobetasol end csater - CsaTcr Only	-22.838(-26.416, -19.261)	<.0001	.
	Clobetasol end csater - Isograft	-22.268(-24.349, -20.186)	<.0001	.
	Clobetasol end csater - Tacrolimus Only	-18.221(-20.336, -16.106)	<.0001	.
	Clobetasol end csater - tacrolimus end csater	-9.914(-11.996, -7.832)	<.0001	.
	Clobetasol end csater - tacrolimus start rejection	-12.028(-14.110, -9.947)	<.0001	.
	Clobetasol only - Clobetasol start rejection	-12.181(-22.016, -2.345)	0.0165	.
	Clobetasol only - CsaTcr Only	-32.245(-42.502, -21.987)	<.0001	.
	Clobetasol only - Isograft	-31.674(-41.510, -21.838)	<.0001	.
	Clobetasol only - Tacrolimus Only	-27.627(-37.470, -17.784)	<.0001	.
	Clobetasol only - tacrolimus end csater	-19.320(-29.156, -9.484)	0.0003	.
	Clobetasol only - tacrolimus start rejection	-21.434(-31.270, -11.599)	<.0001	.
	Clobetasol start rejection - CsaTcr Only	-20.064(-23.641, -16.486)	<.0001	.
	Clobetasol start rejection - Isograft	-19.493(-21.575, -17.411)	<.0001	.
	Clobetasol start rejection - Tacrolimus Only	-15.446(-17.561, -13.332)	<.0001	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
	Clobetasol start rejection - tacrolimus end csatcr	-7.139(-9.221, -5.058)	<.0001	.
	Clobetasol start rejection - tacrolimus start rejection	-9.254(-11.335, -7.172)	<.0001	.
	CsaTcr Only - Isograft	0.571(-3.007, 4.148)	0.7490	.
	CsaTcr Only - Tacrolimus Only	4.617(1.021, 8.214)	0.0131	.
	CsaTcr Only - tacrolimus end csatcr	12.924(9.347, 16.502)	<.0001	.
	CsaTcr Only - tacrolimus start rejection	10.810(7.233, 14.388)	<.0001	.
	Isograft - Tacrolimus Only	4.047(1.932, 6.161)	0.0004	.
	Isograft - tacrolimus end csatcr	12.354(10.272, 14.435)	<.0001	.
	Isograft - tacrolimus start rejection	10.239(8.158, 12.321)	<.0001	.
	Tacrolimus Only - tacrolimus end csatcr	8.307(6.192, 10.422)	<.0001	.
	Tacrolimus Only - tacrolimus start rejection	6.193(4.078, 8.307)	<.0001	.
	tacrolimus end csatcr - tacrolimus start rejection	-2.114(-4.196, -0.032)	0.0467	.

14. Derma-thickness

Table40. Estimate of intercept and slope for each group - derma_thickness

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csatcr	763.066(717.786, 808.346)	<.0001
group	Clobetasol only	1066.945(789.499, 1344.392)	<.0001
group	Clobetasol start rejection	699.379(654.099, 744.658)	<.0001
group	CsaTcr Only	1054.819(862.985, 1246.654)	<.0001
group	Isograft	554.755(509.475, 600.034)	<.0001
group	Tacrolimus Only	961.051(909.915, 1012.188)	<.0001
group	tacrolimus end csatcr	633.794(588.514, 679.073)	<.0001
group	tacrolimus start rejection	1101.888(1056.608, 1147.168)	<.0001
days*group	Clobetasol end csatcr	-7.632(-8.337, -6.927)	<.0001
days*group	Clobetasol only	-8.905(-23.175, 5.366)	0.2147
days*group	Clobetasol start rejection	-2.684(-3.389, -1.979)	<.0001
days*group	CsaTcr Only	-8.843(-16.113, -1.573)	0.0184
days*group	Isograft	-0.048(-0.753, 0.657)	0.8912
days*group	Tacrolimus Only	6.826(5.715, 7.937)	<.0001
days*group	tacrolimus end csatcr	8.975(8.270, 9.680)	<.0001
days*group	tacrolimus start rejection	0.578(-0.127, 1.283)	0.1054

Table41. Compared trend (slope) over time among groups - derma_thickness

<i>response</i>	<i>Differnce in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
derma_thickness			.	<.0001
	difference in slope 1 vs 2	1.2729(-13.0153, 15.5611)	0.8581	.
	difference in slope 1 vs 3	-4.9477(-5.9446, -3.9507)	<.0001	.
	difference in slope 1 vs 4	1.2115(-6.0925, 8.5155)	0.7394	.
	difference in slope 1 vs 5	-7.5835(-8.5805, -6.5866)	<.0001	.
	difference in slope 1 vs 6	-14.4574(-15.7731, -13.1417)	<.0001	.
	difference in slope 1 vs 7	-16.6068(-17.6037, -15.6098)	<.0001	.
	difference in slope 1 vs 8	-8.2096(-9.2065, -7.2126)	<.0001	.
	difference in slope 2 vs 3	-6.2206(-20.5088, 8.0676)	0.3844	.
	difference in slope 2 vs 4	-0.0614(-16.0773, 15.9544)	0.9939	.
	difference in slope 2 vs 5	-8.8565(-23.1447, 5.4317)	0.2177	.
	difference in slope 2 vs 6	-15.7303(-30.0443, -1.4164)	0.0321	.
	difference in slope 2 vs 7	-17.8797(-32.1679, -3.5915)	0.0155	.
	difference in slope 2 vs 8	-9.4825(-23.7707, 4.8057)	0.1875	.
	difference in slope 3 vs 4	6.1591(-1.1449, 13.4631)	0.0961	.
	difference in slope 3 vs 5	-2.6359(-3.6328, -1.6389)	<.0001	.
	difference in slope 3 vs 6	-9.5098(-10.8254, -8.1941)	<.0001	.
	difference in slope 3 vs 7	-11.6591(-12.6561, -10.6622)	<.0001	.
	difference in slope 3 vs 8	-3.2619(-4.2589, -2.2650)	<.0001	.
	difference in slope 4 vs 5	-8.7950(-16.0990, -1.4910)	0.0195	.
	difference in slope 4 vs 6	-15.6689(-23.0232, -8.3146)	0.0001	.
	difference in slope 4 vs 7	-17.8183(-25.1223, -10.5143)	<.0001	.
	difference in slope 4 vs 8	-9.4211(-16.7251, -2.1171)	0.0127	.
	difference in slope 5 vs 6	-6.8739(-8.1896, -5.5582)	<.0001	.
	difference in slope 5 vs 7	-9.0232(-10.0202, -8.0263)	<.0001	.
	difference in slope 5 vs 8	-0.6260(-1.6230, 0.3709)	0.2119	.
	difference in slope 6 vs 7	-2.1494(-3.4651, -0.8337)	0.0020	.
	difference in slope 6 vs 8	6.2478(4.9322, 7.5635)	<.0001	.
	difference in slope 6 vs 9	6.2478(4.9322, 7.5635)	<.0001	.

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
	difference in slope 7 vs 8	8.3972(7.4003, 9.3941)	<.0001	.

Table42. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - derma_thickness

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall lp</i>
derma_thickness			.	<.0001
	Clobetasol end csatcr	459.702(410.799, 508.604)	<.0001	.
	Clobetasol only	712.980(404.045, 1021.915)	<.0001	.
	Clobetasol start rejection	592.689(543.787, 641.592)	<.0001	.
	CsaTcr Only	703.297(590.778, 815.815)	<.0001	.
	Isograft	552.845(503.943, 601.748)	<.0001	.
	Tacrolimus Only	1232.387(1180.856, 1283.918)	<.0001	.
	tacrolimus end csatcr	990.569(941.667, 1039.472)	<.0001	.
	tacrolimus start rejection	1124.865(1075.962, 1173.767)	<.0001	.
	Clobetasol end csatcr - Clobetasol only	-253.279(-566.060, 59.503)	0.1096	.
	Clobetasol end csatcr - Clobetasol start rejection	-132.988(-202.147, -63.829)	0.0004	.
	Clobetasol end csatcr - CsaTcr Only	-243.595(-366.281, -120.909)	0.0003	.
	Clobetasol end csatcr - Isograft	-93.144(-162.303, -23.985)	0.0095	.
	Clobetasol end csatcr - Tacrolimus Only	-772.685(-843.727, -701.644)	<.0001	.
	Clobetasol end csatcr - tacrolimus end csatcr	-530.868(-600.027, -461.709)	<.0001	.
	Clobetasol end csatcr - tacrolimus start rejection	-665.163(-734.322, -596.004)	<.0001	.
	Clobetasol only - Clobetasol start rejection	120.291(-192.491, 433.072)	0.4418	.
	Clobetasol only - CsaTcr Only	9.684(-319.104, 338.471)	0.9529	.
	Clobetasol only - Isograft	160.135(-152.647, 472.916)	0.3072	.
	Clobetasol only - Tacrolimus Only	-519.407(-832.610, -206.204)	0.0017	.
	Clobetasol only - tacrolimus end csatcr	-277.589(-590.371, 35.192)	0.0805	.
	Clobetasol only - tacrolimus start rejection	-411.884(-724.666, -99.103)	0.0111	.
	Clobetasol start rejection - CsaTcr Only	-110.607(-233.294, 12.079)	0.0760	.
	Clobetasol start rejection - Isograft	39.844(-29.315, 109.003)	0.2514	.
	Clobetasol start rejection - Tacrolimus Only	-639.698(-710.739, -568.656)	<.0001	.
	Clobetasol start rejection - tacrolimus end csatcr	-397.880(-467.039, -328.721)	<.0001	.
	Clobetasol start rejection - tacrolimus start rejection	-532.175(-601.334, -463.016)	<.0001	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall l p</i>
	CsaTer Only - Isograft	150.451(27.765, 273.138)	0.0175	.
	CsaTer Only - Tacrolimus Only	-529.090(-652.848, -405.333)	<.0001	.
	CsaTer Only - tacrolimus end csater	-287.273(-409.959, -164.586)	<.0001	.
	CsaTer Only - tacrolimus start rejection	-421.568(-544.254, -298.882)	<.0001	.
	Isograft - Tacrolimus Only	-679.542(-750.583, -608.500)	<.0001	.
	Isograft - tacrolimus end csater	-437.724(-506.883, -368.565)	<.0001	.
	Isograft - tacrolimus start rejection	-572.019(-641.178, -502.860)	<.0001	.
	Tacrolimus Only - tacrolimus end csater	241.817(170.776, 312.859)	<.0001	.
	Tacrolimus Only - tacrolimus start rejection	107.522(36.481, 178.564)	0.0039	.
	tacrolimus end csater - tacrolimus start rejection	-134.295(-203.454, -65.136)	0.0003	.

15. Epidermal-thickness

Table43. Estimate of intercept and slope for each group - epidermal_thickness

<i>Effect</i>	<i>Group</i>	<i>Estimate(95% CI)</i>	<i>p value</i>
group	Clobetasol end csatcr	25.267(22.151, 28.384)	<.0001
group	Clobetasol only	-29.184(-49.166, -9.202)	0.0052
group	Clobetasol start rejection	12.823(9.706, 15.940)	<.0001
group	CsaTcr Only	113.341(103.452, 123.229)	<.0001
group	Isograft	17.120(14.003, 20.237)	<.0001
group	Tacrolimus Only	49.934(45.302, 54.567)	<.0001
group	tacrolimus end csatcr	21.174(18.057, 24.291)	<.0001
group	tacrolimus start rejection	30.762(27.645, 33.879)	<.0001
days*group	Clobetasol end csatcr	-0.134(-0.206, -0.062)	0.0006
days*group	Clobetasol only	6.931(5.397, 8.464)	<.0001
days*group	Clobetasol start rejection	0.144(0.071, 0.216)	0.0002
days*group	CsaTcr Only	-3.392(-3.648, -3.137)	<.0001
days*group	Isograft	0.115(0.042, 0.187)	0.0026
days*group	Tacrolimus Only	-0.356(-0.470, -0.242)	<.0001
days*group	tacrolimus end csatcr	0.018(-0.054, 0.090)	0.6178
days*group	tacrolimus start rejection	0.052(-0.020, 0.125)	0.1511

Table44. Compared trend (slope) over time among groups - epidermal_thickness

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
epidermal_thickness			.	<.0001
	difference in slope 1 vs 2	-7.0645(-8.5997, -5.5293)	<.0001	.
	difference in slope 1 vs 3	-0.2776(-0.3797, -0.1755)	<.0001	.
	difference in slope 1 vs 4	3.2584(2.9929, 3.5239)	<.0001	.
	difference in slope 1 vs 5	-0.2486(-0.3507, -0.1465)	<.0001	.
	difference in slope 1 vs 6	0.2218(0.0866, 0.3570)	0.0019	.
	difference in slope 1 vs 7	-0.1520(-0.2541, -0.0499)	0.0045	.
	difference in slope 1 vs 8	-0.1863(-0.2884, -0.0842)	0.0007	.
	difference in slope 2 vs 3	6.7869(5.2517, 8.3221)	<.0001	.
	difference in slope 2 vs 4	10.3229(8.7683, 11.8775)	<.0001	.
	difference in slope 2 vs 5	6.8159(5.2808, 8.3511)	<.0001	.
	difference in slope 2 vs 6	7.2863(5.7486, 8.8241)	<.0001	.
	difference in slope 2 vs 7	6.9125(5.3774, 8.4477)	<.0001	.
	difference in slope 2 vs 8	6.8782(5.3430, 8.4134)	<.0001	.
	difference in slope 3 vs 4	3.5360(3.2706, 3.8015)	<.0001	.
	difference in slope 3 vs 5	0.0291(-0.0730, 0.1312)	0.5685	.
	difference in slope 3 vs 6	0.4995(0.3642, 0.6347)	<.0001	.
	difference in slope 3 vs 7	0.1257(0.0236, 0.2278)	0.0171	.
	difference in slope 3 vs 8	0.0913(-0.0108, 0.1934)	0.0782	.
	difference in slope 4 vs 5	-3.5070(-3.7724, -3.2415)	<.0001	.
	difference in slope 4 vs 6	-3.0366(-3.3165, -2.7567)	<.0001	.
	difference in slope 4 vs 7	-3.4104(-3.6758, -3.1449)	<.0001	.
	difference in slope 4 vs 8	-3.4447(-3.7102, -3.1792)	<.0001	.
	difference in slope 5 vs 6	0.4704(0.3352, 0.6056)	<.0001	.
	difference in slope 5 vs 7	0.0966(-0.0055, 0.1987)	0.0631	.
	difference in slope 5 vs 8	0.0623(-0.0398, 0.1644)	0.2251	.
	difference in slope 6 vs 7	-0.3738(-0.5090, -0.2386)	<.0001	.
	difference in slope 6 vs 8	-0.4081(-0.5433, -0.2729)	<.0001	.
	difference in slope 6 vs 9	-0.4081(-0.5433, -0.2729)	<.0001	.

<i>response</i>	<i>Difference in slope</i>	<i>Estimate(95% CI)</i>	<i>p value</i>	<i>Overall P</i>
	difference in slope 7 vs 8	-0.0343(-0.1364, 0.0678)	0.5010	.

Table45. Estimate of LS means of groups and difference among groups at mean follow-up days (39.8 days) - epidermal_thickness

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
epidermal_thickness			.	<.0001
	Clobetasol end csatcr	19.941(18.083, 21.799)	<.0001	.
	Clobetasol only	246.313(202.627, 289.999)	<.0001	.
	Clobetasol start rejection	18.533(16.675, 20.391)	<.0001	.
	CsaTcr Only	-21.511(-30.542, -12.481)	<.0001	.
	Isograft	21.674(19.816, 23.532)	<.0001	.
	Tacrolimus Only	35.790(33.537, 38.043)	<.0001	.
	tacrolimus end csatcr	21.889(20.031, 23.747)	<.0001	.
	tacrolimus start rejection	32.841(30.983, 34.699)	<.0001	.
	Clobetasol end csatcr - Clobetasol only	-226.372(-270.097, -182.646)	<.0001	.
	Clobetasol end csatcr - Clobetasol start rejection	1.408(-1.220, 4.035)	0.2856	.
	Clobetasol end csatcr - CsaTcr Only	41.452(32.233, 50.672)	<.0001	.
	Clobetasol end csatcr - Isograft	-1.733(-4.361, 0.894)	0.1902	.
	Clobetasol end csatcr - Tacrolimus Only	-15.849(-18.769, -12.929)	<.0001	.
	Clobetasol end csatcr - tacrolimus end csatcr	-1.948(-4.576, 0.680)	0.1420	.
	Clobetasol end csatcr - tacrolimus start rejection	-12.900(-15.528, -10.272)	<.0001	.
	Clobetasol only - Clobetasol start rejection	227.780(184.054, 271.505)	<.0001	.
	Clobetasol only - CsaTcr Only	267.824(223.214, 312.434)	<.0001	.
	Clobetasol only - Isograft	224.639(180.913, 268.364)	<.0001	.
	Clobetasol only - Tacrolimus Only	210.523(166.779, 254.267)	<.0001	.
	Clobetasol only - tacrolimus end csatcr	224.424(180.698, 268.149)	<.0001	.
	Clobetasol only - tacrolimus start rejection	213.472(169.746, 257.197)	<.0001	.
	Clobetasol start rejection - CsaTcr Only	40.045(30.825, 49.264)	<.0001	.
	Clobetasol start rejection - Isograft	-3.141(-5.768, -0.513)	0.0203	.
	Clobetasol start rejection - Tacrolimus Only	-17.257(-20.177, -14.336)	<.0001	.

<i>response</i>	<i>Group</i>	<i>LS mean(95% CI)</i>	<i>p value</i>	<i>Overall p</i>
	Clobetasol start rejection - tacrolimus end csatcr	-3.356(-5.983, -0.728)	0.0136	.
	Clobetasol start rejection - tacrolimus start rejection	-14.308(-16.935, -11.680)	<.0001	.
	CsaTcr Only - Isograft	-43.185(-52.405, -33.966)	<.0001	.
	CsaTcr Only - Tacrolimus Only	-57.301(-66.609, -47.994)	<.0001	.
	CsaTcr Only - tacrolimus end csatcr	-43.400(-52.620, -34.181)	<.0001	.
	CsaTcr Only - tacrolimus start rejection	-54.352(-63.572, -45.133)	<.0001	.
	Isograft - Tacrolimus Only	-14.116(-17.036, -11.196)	<.0001	.
	Isograft - tacrolimus end csatcr	-0.215(-2.842, 2.413)	0.8697	.
	Isograft - tacrolimus start rejection	-11.167(-13.794, -8.539)	<.0001	.
	Tacrolimus Only - tacrolimus end csatcr	13.901(10.981, 16.821)	<.0001	.
	Tacrolimus Only - tacrolimus start rejection	2.949(0.029, 5.869)	0.0479	.
	tacrolimus end csatcr - tacrolimus start rejection	-10.952(-13.580, -8.324)	<.0001	.

Results from time to rejection data analysis

Table 46- Provides the hazard ratios between groups from a Cox Proportional Hazard model. Isograft, protopic end csa/tcr, protopic start rejection csa/tcr, and allograft were not included in the analysis. The overall p value of <0.0001 indicated that there were significant difference in risk of rejection among the 5 groups. Specifically, the Clobetasol start rejection csa/tcr group had a lower risk of rejection than the clobetasol only group (HR= 0.025, P<0.001). The clobetasol end csa/tcr had a lower risk of rejection than the clobetasol only group (HR= 0.019, P<0.001). The clobetasol end csa/tcr had a lower risk of rejection than the only csa/tcr group(HR= 0.142, P=0.002). The clobetasol only group had a higher risk of rejection than the protopic only group (HR= 27.73, P<0.001)

Table 46. Comparisons on hazard of rejection among groups. A Bonferroni adjusted significance level was 0.005=0.05/10. P values were from Cox proportional hazard model.

<i>Group</i>	<i>Hazard Ratio(95% CI)</i>	<i>P value</i>	<i>Overall P value</i>
			<.0001
Clobetasol start rejection csa/tcr VS clobetasol end csa/tcr	1.295(0.433,3.872)	0.643	
Clobetasol start rejection csa/tcr VS clobetasol only	0.025(0.005,0.133)	<.001	
Clobetasol start rejection csa/tcr VS only csa/tcr	0.184(0.056,0.605)	0.005	
Clobetasol start rejection csa/tcr VS protopic only	0.694(0.231,2.088)	0.516	
clobetasol end csa/tcr VS clobetasol only	0.019(0.004,0.107)	<.001	
clobetasol end csa/tcr VS only csa/tcr	0.142(0.041,0.492)	0.002	
clobetasol end csa/tcr VS protopic only	0.536(0.172,1.670)	0.282	
clobetasol only VS only csa/tcr	7.338(1.718,31.334)	0.007	
clobetasol only VS protopic only	27.73(5.12,150.16)	<.001	
only csa/tcr VS protopic only	3.780(1.101,12.970)	0.035	