Corneal neovascularization (CNV) was induced in 20 corneas of ten 6- to 8-week-old C57BL6/N mice by alkali burn (n = 10) and sutures (n = 10) assays. All corneal flatmounts were stained with CD31 and LVEY1. Three independent operators assessed blood and lymphatic CNV with both a quantitative manual (mCNV) and an automatic (aCNV) method. In the manual method the inner tips of the vessels were connected together. The resulting area was then normalized for the corneal surface and the result was mCNV. Automatic analysis was performed using a novel open-source plugin for ImageJ software, called VesselJ. VesselJ algorithm relies on a corneal background-adjusted threshold, based on the red channel for blood vessels and on the green channel for lymphatic vessels.

Results: Both methods showed a strong reliability (ICC > 0.90) in quantifying hemangiogenesis for the suture and alkali burn models. However, reliability of lymphatic mCNV varied from moderate in the alkali burn (ICC: 0.700) to poor in the suture model (ICC: 0.415), whereas it remained high in aCNV. In the sutured group, a significant correlation between mCNV and aCNV was found among all three operators for blood vessels (P < 0.001) and just for one operator for lymphatic vessels (P < 0.001). Regarding the alkali burn model, correlation between blood mCNV and aCNV was significant for all operators (P < 0.001), whereas no significant correlation was appreciated for lymphatic vessels. Time spent to analyze images with VesselJ was significantly inferior to manual system (P < 0.001), being 6 and 21 times faster than manual method for blood and lymphatic quantification, respectively.

Conclusions: The mouse cornea is a generally accepted model to study blood angiogenesis and lymphangiogenesis and to investigate their role even in non-ocular disorders (e.g. cancer). The majority of the techniques used to quantify CNV relies on manual methods, thus being operator-dependent, time consuming and not always reproducible. VesselJ is a reliable and fast method to quantify corneal hem- and lymph-angiogenesis in corneal flat-mounts.

Commercial Relationships: Alessandro Rabiolo, None; Fabio Bignami, None; Chiara Giacomini, None; Anna Lorusso, None; Giulio Ferrari, None; Paolo Rama, None

Program Number: 4353
Presentation Time: 11:15 AM–11:30 AM

Live Imaging of the Dynamics of Corneal Lymphangiogenesis
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Purpose: The cornea provides an ideal tissue for lymphatic research due to its accessible location, transparent nature, and lymphatic-free but inducible features. Using this tissue, we recently reported that the lymphatic pathway mediates corneal transplant rejection and luminal valves are formed inside corneal lymphatics as lymphangiogenesis proceeds. The purpose of this study is to assess the dynamic processes of lymphangiogenesis within live cornea.

Methods: Some recently developed live imaging system and fluorescent protein labeled Prox-1 mice were used to study lymphatic processes in several pathologic model systems, including suture placement, growth factor implantation, and orthotopic transplantation. Intravitral images were collected for longitudinal data analysis.

Results: Lymphangiogenesis is a dynamic and complex process from the initiation to regression phases. This process includes multiple steps involving vessel sprouting, pruning, maturation, and recession, which occur progressively and within certain time windows.

Conclusions: Our study offers new insights into in vivo processes of corneal lymphangiogenesis. Further investigation using the intravitral technology promises to reveal new mechanisms and therapeutic strategies for lymphatic diseases which occur in many parts of the body.

Commercial Relationships: Gyeong Jin Kang, None; Tan N. Truong, None; Valerie Su, None; Lu Chen, None

Program Number: 4354
Presentation Time: 11:30 AM–11:45 AM

Anti-VEGF-B therapy in a rat model of corneal neovascularization
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Purpose: Anti-vascular endothelial growth factor-A (VEGF-A) therapy has shown promise for treating newly-formed corneal vessels, but has little effect on established vessels. VEGF-B is a potent survival factor for vascular endothelial cells. VEGF-B deficiency reduced survival of blood vessels in a murine model of corneal neovascularization (CoNV). We developed an anti-VEGF-B antibody fragment (scFv format) and tested its activity on growing and established vessels in a rat model of CoNV.

Methods: CoNV was induced in male and female SD rats (12 week – 1 year old) by superficial cautery with silver nitrate. Topical anti-VEGF-B scFv therapy (5 x 5 μg eye drops per day for 14 days) was commenced one day after cautery to determine the effect on growing vessels, or 14 days after cautery to determine the effect on established vessels. In a subset of animals, an additional subconjunctival injection (SCINJ) of 50 μg scFv on days 1 and 8 of treatment was applied. Vessels were perfused with haematoxylin and the cornea was flatmounted. The percentage of the cornea covered by vessels was determined with NIH ImageJ. Data were analysed by Kruskal-Wallis test and Mann-Whitney U with correction for multiple testing.

Results: After topical therapy the corneal neovascular area was not significantly different between untreated, control scFv treated and anti-VEGF-B scFv treated animals (growing vessels p=0.46, established vessels p=0.86). Topical anti-VEGF-B scFv therapy supplemented with SCINJ in the established vessel group significantly reduced the area of corneal vessels (12.9%, SD=±3.6, n=21) when compared with untreated (20.5%, SD=±7, n=19) or control scFv treated (22.5%, SD=±3.2, n=9) animals (p<0.001).

Conclusions: Anti-VEGF-B scFv therapy caused regression of established vessels in a rat model of CoNV. Combining anti-VEGF-B treatment with anti-VEGF-A therapy could be useful in the treatment of human CoNV.

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Plasmacytoid Dendritic Cells Demonstrate Vital Neuro-protective Properties in the Cornea and Induce Corneal Nerve Regeneration

Resident corneal pDCs harbor neurotrophic properties through secretion of NGF and are crucial for corneal nerve regeneration after corneal nerve loss.

**Purpose:** Plasmacytoid dendritic cells (pDCs), a recently identified resident bone marrow-derived cell population in the cornea, are potent orchestrators of innate and adaptive immunity. Despite the traditional perspective that immune cells are deleterious for nerve regeneration, more recent evidence suggests that immune cells may have beneficial effects in neuronal regeneration. This study aims to characterize the role of pDCs in corneal nerve maintenance, function, and regeneration.

**Methods:** Corneal pDCs were locally depleted by constitutive subconjunctival injection of 30ng Diphtheria toxin (DT) in BDCA2-DTR mice. Wild-type C57BL/6 mice treated with DT and BDCA2-DTR mice receiving PBS served as controls. Corneal sensation was evaluated by an 8.0 thread; corneas were stained for βIII-tubulin (pan-neuronal marker) and underwent confocal microscopy. Corneal nerve density was measured on stacked confocal micrographs via NeuronJ. Relative corneal NGF mRNA levels were assessed via real-time PCR. Naïve and sutured corneas underwent flow cytometry for CD45 (pan-leukocyte marker), Siglec-H, PDCA-1, B220 (pDC markers), and NGF. Chi square, T-test and ANOVA were used to assess statistical significance.

**Results:** Upon local depletion of resident corneal pDCs, central corneal nerve density was reduced to 120±14.9 mm/mm² on day1, 21.9±14.8 on day 3, and 1.1±0.7 on day 7 compared to 143±8.1 in control corneas (p<0.001). In the peripheral cornea, pDC depletion resulted in nerve diminishment to 102±16.9, 20.9±8.6, 5.3±3.9, on day 1, 3, and 7, respectively compared to 112±9.3 in controls (p<0.001). Corneal sensation was diminished in all pDC-depleted mice by day 3 (p<0.01). Relative NGF mRNA levels were decreased to 22.5% and 17.9% of controls after pDC depletion on day 7 and 14, respectively (p<0.01). Flow cytometry showed that NGF co-stains with pDCs in both naïve and suture-induced inflamed corneal single cell suspensions. After 7-day pDC depletion, mice were kept for 14 days to allow pDC repopulation. Upon pDC repopulation, corneal nerves regenerated to 81.5±0.2 in periphery and to 48.4±5.0 in central cornea, and corneal sensation was recovered (p<0.001).

**Conclusions:** Resident corneal pDCs harbor neurotrophic properties through secretion of NGF and are crucial for corneal nerve maintenance and function. Moreover, corneal pDCs induce corneal nerve regeneration after corneal nerve loss.

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Conclusions: Altogether these results demonstrate the potential of meganuclease to protect against endothelitis.

Commercial Relationships: Eric Gabison, None; Marc Labetoulle, None; Jose A. Sahel, None; Roman Galetto, Cellectis Therapeutics SAS (E); isabelle cochereau, None; Benoit Chapelier, None

Support: OSEO Active Grant

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Presentation Time: 12:30 PM–12:45 PM
Corneal Transplant Follow-up Study II (CTFS II) - HLA class II matching does not reduce risk of allograft rejection in high-risk penetrating keratoplasty

John Armitage1, Mark Jones1, Helen Winton1, Chris Rogers1, Derek Tole1, Andrew D. Dick1. 1Clinical Sciences, University of Bristol, Bristol, United Kingdom; 2Bristol Eye Hospital, Bristol, United Kingdom; 3NHS Blood and Transplant, Bristol, United Kingdom. 40%.

Purpose: The role of HLA matching in corneal transplantation remains controversial. We designed an observational, prospective, longitudinal clinical trial (ISRCTN25094892) to determine the influence of HLA class II matching on the risk of allograft rejection in high-risk, HLA class I matched penetrating keratoplasties (PK).

Methods: Patients at increased risk of rejecting corneal allografts were registered for the study after giving informed consent and placed on a waiting list held by NHS Blood & Transplant (NHSBT). Tissue typing used DNA methodology to avoid the errors inherent in serological typing. All corneas were stored by organ culture for up to 4 weeks and had a minimum endothelial cell density of 2200 cells/mm². When corneas from a tissue-typed donor became available, the HLA type was compared with those of patients on the waiting list. Patients matched at HLA class I (≤2 mismatches) were identified and then the corneas allocated by cohort minimization to patients in this group with 0, 1 or 2 HLA class II mismatches. Patients were followed for 5 years. The primary outcome measure was time to first rejection episode. Data were analyzed both by univariate and multiple regression methods (Cox proportional hazards). The level of significance was set at p<0.05. Survival estimates and relative risks (RR) are quoted with 95% confidence intervals (95% CI).

Results: Recruitment closed with 1137 transplants. The overall rejection-free survival at 5 years was 60% (95% CI 56, 63; n=1072). Univariate Kaplan-Meier rejection-free survivals for 0, 1 and 2 HLA class II mismatches were, respectively, 60% (95% CI 51, 67; n=180), 63% (95% CI 57, 67; n=480), and 57% (95% CI 51, 62; n=412) (p=0.4). This lack of influence of HLA class II was confirmed in the Cox regression model (p=0.2). Recipient age had a major influence on rejection with recipients ≥40 years having a 3-fold greater risk of rejection than those over 60 years (RR 2.9, 95% CI 1.8, 4.7; p<0.0001). The next most influential factor was the number of preoperative risk factors. Recipients with ≥3 preoperative risk factors were at ≥2-fold higher risk of rejection than those with no risk factors (RR 2.3, 95% CI 1.5, 3.7; p=0.0004).

Conclusions: Matching for HLA class II did not reduce the risk of rejection in high-risk PK matched for HLA class I.

Commercial Relationships: John Armitage, None; Mark Jones, None; Helen Winton, None; Chris Rogers, None; Derek Tole, None; Andrew D. Dick, None

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Clinical Trial: ISRCTN25094892

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