Eye Stem Cell Literatres

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Abstract: The definition of stem cell is "an unspecialized cell that gives rise to a specific specialized cell, such as a blood cell". Stem Cell is the original of life. All cells come from stem cells. Serving as a repair system for the living body, the stem cells can divide without limit to replenish other cells as long as the living body is still alive. When a stem cell divides, each new cell has the potential to either remain a stem cell situation or become another type of cell with a more specialized function, such as a muscle cell, a red blood cell, a bone cell, a nerve cell, or a brain cell. Stem cell research is a typical and important topic of life science. This material collects some literatures on eye stem cell.
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Key words: stem cell; life; gene; DNA; protein; eye

Literatures

Ahmad, S., R. Stewart, et al. (2007). "Differentiation of human embryonic stem cells into corneal epithelial-like cells by in vitro replication of the corneal epithelial stem cell niche." Stem Cells **25**(5): 1145-55.

Human embryonic stem cells (hESCs) are pluripotent cells capable of differentiating into any cell type of the body. It has long been known that the adult stem cell niche is vital for the maintenance of adult stem cells. The cornea at the front of the eye is covered by a stratified epithelium that is renewed by stem cells located at its periphery in a region known as the limbus. These so-called limbal stem cells are maintained by factors within the limbal microenvironment, including collagen IV in basement membrane and limbal fibroblasts in the stroma. Because this niche is very specific to the stem cells (rather than to the more differentiated cells) of the corneal epithelium, it was hypothesized that replication of these factors in vitro would result in hESC differentiation into corneal epithelial-like cells. Indeed, here we show that culturing of hESC on collagen IV using medium conditioned by the limbal fibroblasts results in the loss of pluripotency and differentiation into epithelial-like cells. Further differentiation results in the formation of terminally differentiated epithelial-like cells not only of the cornea but also of skin. Scanning electron microscopy shows that some differences exist between hESC-derived and adult limbal epithelial-like cells, necessitating further investigation using in vivo animal models of limbal stem cell deficiency. Such a model of hESC differentiation is useful for understanding the early events of epithelial lineage specification and to the eventual potential application of epithelium differentiated from hESC for clinical conditions of epithelial stem cell loss. Disclosure of potential conflicts of interest is found at the end of this article.

Akinci, M. A., H. Turner, et al. (2009). "Molecular profiling of conjunctival epithelial side-population stem cells: atypical cell surface markers and sources of a slow-cycling phenotype." Invest Ophthalmol Vis Sci **50**(9): 4162-72.

PURPOSE: Side-population (SP) cells isolated from limbal and conjunctival epithelia derive from cells that are slow cycling in vivo, a known feature of tissue stem cells. The purpose of this study was to define the molecular signature of the conjunctival SP cells and identify markers and signaling pathways associated with the phenotype of these cells. METHODS: Overnight cultures of freshly isolated human conjunctival epithelial cells stained with Hoechst 33342 were sorted by flow cytometry into SP and non-SP cohorts. Isolated RNA was processed for microarray analysis using a commercial oligonucleotide spotted array. Results were validated at the gene and protein levels by quantitative PCR and immunologic methods. Data mining methods were used to identify cellular processes relevant for stem cell function. RESULTS: Comparative analyses of transcripts expression based on present and absent software calls across four replicate experiments identified 16,993 conjunctival epithelial transcripts including 10,266 unique known genes of approximately 24,000 represented in the array. Of those genes, 1254 and 363 were overexpressed (>2-fold) or underexpressed (<0.5-fold), respectively, in the SP. The overexpressed set included genes coding for proteins that have been associated with (1) embryonic development and/or stem cell self renewal (MSX, MEIS, ID, Hes1, and SIX homeodomain genes); (2) cell survival (e.g., CYP1A1 to degrade

aromatic genotoxic compounds); (3) cycling rate (e.g., DUSPs and Pax6 to foster slow cycling); and (4) genes whose expression is not typical in epithelia (e.g., CD62E). CONCLUSIONS: The molecular signature of conjunctival SP cells is consistent with a stem cell phenotype. Their gene expression patterns underpin slow cycling and plasticity, features associated with tissue stem cells. The results provide valuable insights for the preservation and/or expansion of epithelial stem cells.

Akinci, M. A., H. Turner, et al. (2009). "Differential gene expression in the pig limbal side population: implications for stem cell cycling, replication, and survival." Invest Ophthalmol Vis Sci **50**(12): 5630-8.

PURPOSE: To define the molecular signature of limbal SP cells and identify signaling pathways associated with the phenotype of these putative stem cells. METHODS: Primary cultures of pig limbal epithelial cells stained with Hoechst 33342 were sorted by flow cytometry into SP and non-SP cells, and purified RNA was processed for microarray analysis with an oligonucleotide spotted array. Expressed transcripts for which SP and non-SP expressions differed by more that 1.5-fold in each paired set and by twofold overall were considered to be differentially expressed. Differential expression was validated by quantitative PCR and immunostaining. Data-mining methods were used to identify cellular processes that are either salient or depressed in the SP cells. RESULTS: The microarray identified approximately 9000 distinct, expressed, and identifiable genes. Of those, 382 and 296 were either over- or underexpressed in the SP cells, respectively. Overrepresentation analysis indicated that SP cells are in a low metabolic and biosynthetic state. In addition, a pattern of elevated MXD1, MAXI2, DUSP5, p27/KIP1, and p57/KIP2 and decreased Cyclin D and CDK genes can be expected to slow intrinsic and mitogen-induced G(1)-to-S cell cycle transition. SP cells were also rich in genes associated with stem cell phenotype and genes providing protection against oxidative and/or xenobiotic damage. CONCLUSIONS: Microarray analysis of pig limbal SP cells yielded a molecular signature underscoring a phenotype characterized by slow cycling and low metabolic activity. The results provide valuable insights for the preservation and/or replication of epithelial stem cells.

Anderson, D. F., P. Ellies, et al. (2001). "Amniotic membrane transplantation for partial limbal stem cell deficiency." Br J Ophthalmol **85**(5): 567-75.

AIM: To examine the efficacy, safety, and long term outcomes of amniotic membrane transplantation for corneal surface reconstruction in cases of partial limbal stem cell deficiency. METHODS: 17 eyes of 15 patients with partial limbal stem cell deficiency underwent superficial keratectomy of the conjunctivalised corneal surface followed by amniotic membrane transplantation. Cases were followed up for at least a year. RESULTS: All eyes exhibited a stable, intact corneal epithelial surface after a mean follow up period of 25.8 months with no eyes developing recurrent erosion or persistent epithelial defect. The mean time to re-epithelialisation was 22.8 days. Overall improvement in visual acuity was observed in 92.9% of 14 eyes with visual potential. Of those, five eyes gained six or more lines, two eyes gained between four and five lines, six eyes gained between one and three lines, and one eye lost three lines of Snellen acuity. Pain and photophobia were abolished in 86% of cases and substantially reduced in 14%, with all eyes exhibiting decreased vascularisation and inflammation at final follow up. CONCLUSIONS: Amniotic membrane transplantation appears to be a safe and effective method of restoring a stable corneal epithelium for cases of partial limbal stem cell deficiency and can be considered as an alternative to limbal autograft or allograft.

Askmyr, M., J. Holmberg, et al. (2009). "Low-dose busulphan conditioning and neonatal stem cell transplantation preserves vision and restores hematopoiesis in severe murine osteopetrosis." Exp Hematol **37**(2): 302-8.

OBJECTIVE: Infantile malignant osteopetrosis is a fatal disease caused by lack of functional osteoclasts. In most of patients, TCIRG1, encoding a subunit of a proton pump essential for bone resorption, is mutated. Osteopetrosis leads to bone marrow failure and blindness due to optic nerve compression. Oc/oc mice have a deletion in Tcirg1 and die around 3 to 4 weeks, but can be rescued by neonatal stem cell transplantation (SCT) after irradiation conditioning. However, as irradiation of neonatal mice results in retinal degeneration, we wanted to investigate whether conditioning with busulphan prior to SCT can lead to preservation of vision and reversal of osteopetrosis in the oc/oc mouse model. MATERIALS AND METHODS: Pregnant dams were conditioned with busulphan and their litters transplanted with 1 x 10(6) normal lineage-depleted bone marrow cells intravenously or intraperitoneally. Mice were followed in terms of survival and engraftment level, as well as with peripheral blood lineage analysis, bone and eye histopathology and a visual-tracking drum test to assess vision. RESULTS: Busulphan at 15 mg/kg was toxic to oc/oc mice. However, six of seven oc/oc mice conditioned with busulphan 7.5 mg/kg survived past

the normal lifespan with 10% engraftment, correction of the skeletal phenotype, and normalization of peripheral blood lineages. Busulphan, in contrast to irradiation, did not have adverse effects on the retina as determined by histopathology, and 8 weeks after transplantation control and oc/oc mice retained their vision. CONCLUSION: Low-dose busulphan conditioning and neonatal SCT leads to prolonged survival of oc/oc mice, reverses osteopetrosis and prevents blindness even at low engraftment levels.

Auw-Haedrich, C., C. Potsch, et al. (2007). "Histological and immunohistochemical characterisation of conjunctival graft vs host disease following haematopoietic stem cell transplantation." Graefes Arch Clin Exp Ophthalmol **245**(7): 1001-7.

BACKGROUND: Conjunctival graft vs host disease (cnGvHD) is a complication of haematopoietic stem cell transplantation, in most cases as part of systemic GvHD. Diagnostic biopsies are commonly collected from bulbar conjunctiva only. The aims of our study were to evaluate whether additional biopsies from the tarsal conjunctiva increase sensitivity upon histopathologic evaluation and to investigate the staining profile for common immunohistochemical markers in cnGvHD. We additionally propose an adaptive histological classification for cnGvHD analogous to Lerner's GvHD skin classification for predicting patient survival. METHODS: Formalin-fixed and paraffin-embedded conjunctival specimens from 23 post-mortem control eyes and 42 patients after haematopoietic stem cell transplantation (HSCT) were stained with haematoxylin and eosin (HE), periodic acid-Schiff (PAS) stain and with antibodies against CD1a, CD4, CD8, CD25, CD45RO, CD68, Fas ligand, TIA-1, HLA-DRalpha by means of immunohistochemistry. Cell counting took place in ten representative fields at 64.4 microm (length) x 21.2 microm (width). Multifactorial analysis of variance was performed to assess any influence of cnGvHD on the staining pattern for the immunohistochemical markers. Survival times were estimated by the Kaplan-Meier method. RESULTS: All 42 specimens and none of the controls were diagnosed as cnGvHD. The bulbar specimens were staged according to the modified Lerner classification: grade (G) I: 0; G II: 17 (tarsal with G<or=II, 2; G>II, 8); G III: 12 (tarsal with G<or=III: 2; G>III: 1); G IV: 12 (tarsal with G<or=IV: 6); G V: 1. The number of pairs with either the tarsal or bulbar counterpart being more severely affected was almost equal (10 vs 9). A tendency towards shorter survival in advanced bulbar cnGvHD was demonstrated (G III-V vs G I-II, p =0.06). Staining for the immunohistochemical markers in cnGvHD differed significantly from that in controls ($p<0.01$). Proposed markers for cnGvHD (e = epithelium, s = stroma; mean cell counts +/- SD; cnGvHD vs controls) are: CD8 s (15.7 +/- 18.4 vs 6 +/- 5.6), CD25 s (2.6 +/- 2.8 vs 0.7 +/- 1.6), CD68 s (8 +/- 9 vs 3.9 +/- 3.5) at the bulbar site and CD1a e (1.2 +/- 1.6 vs 0.3 +/- 0.6) and TIA-1 e (2.2 +/- 2.2 vs 1.1 +/- 1.3) at the tarsal site. CONCLUSIONS: Additional tarsal biopsy does not seem to add relevant diagnostic sensitivity for cnGvHD when the modified Lerner classification is applied. The modified Lerner classification of the bulbar cnGvHD seems to be of prognostic value.

Balla, M. M., G. K. Vemuganti, et al. (2009). "Phenotypic characterization of retinoblastoma for the presence of putative cancer stem-like cell markers by flow cytometry." Invest Ophthalmol Vis Sci **50**(4): 1506-14.

PURPOSE: Retinoblastoma (Rb) is an intraocular tumor that grows rapidly and poses a threat to sight and life. Similar to other tumors, there is increasing speculation that the Rb tumor also contains cancer stem-like cells that could influence the prognosis and response to therapy. This study was undertaken in an attempt to identify putative stem-like cells by characterizing different subpopulations of cells in retinoblastoma. METHODS: Freshly isolated tumor cells obtained from unfixed eye specimens (n=7) were analyzed for the presence of CD44, ABCG2, CXCR4, CD133, and CD90 using flow cytometry. RT-PCR was performed to analyze the expression of human Syntaxin1A, PROX1, CD133, and NSE in the sorted subpopulation of tumor cells. RESULTS: Two different subpopulations of cells were observed in seven samples. The small cells, assigned FSC(lo)/SSC(lo) (forward scatter low/side scatter low, ranging from 1.7% to 17.7%) were characterized as positive for CD44 and negative for CD133, CXCR4, and CD90. The large cells were designated as FSC(hi)/SSC(lo) (ranging from 2.7% to 35.1%) and characterized as positive for all markers. RT-PCR analysis revealed that sorted cells of FSC(lo)/SSC(lo) subpopulation expressed the retinal progenitor cell markers PROX1 and Syntaxin1A. CONCLUSIONS: Retinoblastoma, on flow cytometric analysis, revealed two distinct subpopulations with variable expression of stem cell and retinal progenitor markers. In these populations, the FSC(lo)/SSC(lo) subpopulation appeared to be more primitive, since they expressed stem cell (CD44) and retinal progenitor markers (PROX1 and Syntaxin 1A) combined with a relatively lower percentage of differentiated markers. Moreover, the FSC(hi)/SSC(lo) subpopulation showed a higher percentage of differentiated markers (CD90 and CD133).

Brandl, C., C. Florian, et al. (2009). "Identification of neural crest-derived stem cell-like cells from the corneal limbus of juvenile mice." Exp Eye Res **89**(2): 209-17.

The neural crest is a transient embryonic tissue alongside the lateral margins of the neural folds. It contains cells involved in the development of anterior eye segments such as the cornea. Previous studies have revealed the presence of neural crest-derived stem cells in the cornea of the adult murine eye. However, less is known about cell populations of the developing eye. In this study, we have identified neural crest-derived murine corneal cells (MCCs) with stem cell-like properties derived from the corneal limbus of mice between postnatal days 1 and 8. RT-PCR analysis and immunofluorescence staining demonstrate that MCCs express a unique profile of markers including typical neural crest-originated stem cell transcripts like Sca1. MCCs show a limited self-renewing capacity but possess multipotency under in vitro conditions after differentiation into cells with features resembling adipocytes, osteoblasts and neuronal cells. Interestingly, MCCs could not be isolated from corneas of adult animals. We conclude that MCCs are stem cell-like cells of an early postnatal period of murine eye growth, probably involved in the early development of the postnatal cornea.

Bull, N. D., G. A. Limb, et al. (2008). "Human Muller stem cell (MIO-M1) transplantation in a rat model of glaucoma: survival, differentiation, and integration." Invest Ophthalmol Vis Sci **49**(8): 3449-56.

PURPOSE: Stem cell transplantation is a potential treatment strategy for neurodegenerative diseases such as glaucoma. The Muller stem cell line MIO-M1 can be differentiated to produce retinal neurons and glia. The survival, migration, differentiation, and integration of MIO-M1 cells were investigated in a rat model of glaucoma. The effect of modulating the retinal environment with either chondroitinase ABC or erythropoietin was also studied. METHODS: Intraocular pressure was chronically increased unilaterally by using a laser glaucoma model in adult rats. EGFP-transduced MIO-M1 cells were transplanted into the vitreous or subretinal space of glaucomatous or untreated eyes. Oral immune suppressants were administered to reduce xenograft rejection. Survival, migration, differentiation, and integration of grafted cells were assessed by immunohistochemistry. RESULTS: Transplanted cells survived for 2 to 3 weeks in vivo, although microglia/macrophage infiltration and a reduction in graft survival were seen by 4 weeks. Grafted cells displayed a migratory phenotype with an elongated bipolar shape often oriented toward the retina. Transplanted cells expressed markers such as PSA-NCAM, GFAP, and beta-III-tubulin. The host retina was resistant to MIO-M1 migration, but modification of the local environment with erythropoietin or chondroitinase ABC facilitated retinal infiltration by MIO-M1 cells. CONCLUSIONS: The results demonstrate that differentiating MIO-M1 cells within the glaucomatous eye produced cells that expressed neuronal and glial cell markers. The retina was relatively resistant to transplant integration, and long-term xenograft survival was limited. However, local modulation of the retinal environment enhanced the integration of MIO-M1 cells into the glaucomatous retina.

Burman, S. and V. Sangwan (2008). "Cultivated limbal stem cell transplantation for ocular surface reconstruction." Clin Ophthalmol **2**(3): 489-502.

Severe damage to cell repair mechanisms of the limbal region can lead to many disorders such as vascularized conjunctivalization, keratinization, corneal scarring, and corneal opacification, collectively described as limbal stem cell deficiency (LSCD). Limbal stem cell deficiency may occur as a result of depletion of stem cells or destruction of their stromal niche. In such cases, apart from conventional corneal transplantation, limbal stem cell transplantation would be needed to restore vision. Limbal stem cells may be replenished by autologous limbal transplants from the healthy fellow eye in unilateral cases, and allografts from living related donors or cadaveric donors in bilateral cases. The induction of iatrogenic LSCD and its sequelae in donor eyes have motivated researchers to cultivate sheets of limbal epithelium ex vivo, from small fragments of donor tissue for the purpose of ocular surface reconstruction.

Carr, A. J., A. Vugler, et al. (2009). "Molecular characterization and functional analysis of phagocytosis by human embryonic stem cell-derived RPE cells using a novel human retinal assay." Mol Vis **15**: 283-95.

PURPOSE: To examine the ability of retinal pigment epithelial (RPE) cells derived from human embryonic stem cells (HESC) to phagocytose photoreceptor outer segments, and to determine whether exposure to human retina induces any morphological changes in these cells. METHODS: HESC-RPE cells were derived from a super-confluent preparation of the Shef1 HESC line. Pigmented colonies were isolated and expanded into pigmented monolayers on Matrigel matrix-coated dishes or filters. Cells were exposed to fluorescently labeled outer segments isolated from the porcine eye and

assessed for phagocytic activity at regular intervals. Expression of molecules associated with RPE phagocytosis was analyzed by RT-PCR, immunocytochemistry, and western blot. The role of Mer Tyrosine Kinase (MERTK) in the phagocytosis of outer segments was investigated using antibodies directed against MERTK to block function. In a novel approach, cells were also exposed to fresh human neural retina tissue then examined by electron microscopy for evidence of phagocytosis and changes in cell morphology. RESULTS: HESC-derived RPE cells are capable of phagocytosing isolated porcine outer segments and express molecules associated with RPE-specific phagocytosis, including MERTK. Pre-incubation with antibodies against MERTK blocked phagocytosis of photoreceptor outer segments, but not polystyrene beads. HESC-RPE cells also phagocytosed outer segments in a novel human retinal explant system. Furthermore co-culture adjacent to human retina tissue in this preparation resulted in the appearance of features in HESC-derived RPE cells normally observed only as the RPE matures. CONCLUSIONS: The ingestion of photoreceptor outer segments from an isolated population and an artificial ex vivo human retina system demonstrates HESC-derived RPE cells are functional. HESC-derived RPE possess the relevant molecules required for phagocytosis, including MERTK, which is essential for the phagocytosis of outer segments but not latex beads. Furthermore, some changes observed in cell morphology after co-culture with human retina may have implications for understanding the full development and differentiation of RPE cells.

Chaudhry, G. R., C. Fecek, et al. (2009). "Fate of embryonic stem cell derivatives implanted into the vitreous of a slow retinal degenerative mouse model." Stem Cells Dev **18**(2): 247-58.

Stem cell therapy may be used potentially to treat retinal degeneration and restore vision. Since embryonic stem cells (ESCs) can differentiate into almost any cell types, including those found in the eye, they can be transplanted to repair or replace damaged or injured retinal tissue resulting from inherited diseases or traumas. In this investigation, we explored the potential of ESCs and ESC-derived neuroprogenitors to proliferate and integrate into the diseased retinal tissue of rd12 mice. These rd12 mice mimic the slow and progressive retinal degeneration seen in humans. Both ESCs and ESC-derived neuroprogenitors from ESCs survived and proliferated as evidenced from an increase in yellow fluorescent protein fluorescence. Quantification analysis of cryosectioned retinal tissue initially revealed that both ESCs and neuroprogenitors differentiated into cells expressing neural markers. However, ESC proliferation was robust and resulted in the disruption of the retinal structure and the eventual formation of teratomas beyond 6 weeks postimplantation. In contrast, the neuroprogenitors proliferated slowly, but differentiated further and integrated into the retinal layers of the eye. The differentiation of neuroprogenitors represented various retinal cell types, as judged from the expression of cell-specific markers including Nestin, Olig1, and glial fibrillary acidic protein. These results suggest that ESC-derived neuroprogenitors can survive, proliferate, and differentiate when implanted into the eyes of experimental mice and may be used potentially as cell therapy for treating degenerated or damaged retinal tissue.

Chen, W., K. Zhao, et al. (2007). "Keratoconjunctivitis sicca modifies epithelial stem cell proliferation kinetics in conjunctiva." Cornea **26**(9): 1101-6.

PURPOSE: The objective of this study was to examine the epithelial stem cell proliferation kinetics in a rat model with keratoconjunctivitis sicca (KCS). METHODS: Wistar rats received a daily injection of 5-bromo-2-deoxyuridine (BrdU) at a dose of 5 mg/100 g of body weight for 2 weeks. Dry eye was induced in 2 groups of rats by subcutaneous injection of scopolamine and placed in a desiccating environment: The first group received dry eye treatment at the beginning of BrdU labeling for 2 weeks; the second group received dry eye treatment after BrdU labeling for 4 weeks. Rats receiving no dry eye treatment were used as controls. Aqueous tear production, tear clearance, and corneal barrier function of dry eye rats were compared with those of control rats. Ocular epithelial morphology and goblet cell density were also evaluated in histologic sections. One month after BrdU injection, epithelial stem cell proliferation kinetics was assessed by BrdU labeling. RESULTS: Significant decreases in tear fluid secretion and tear clearance were noted in rats 5 days after dry eye treatment, with significantly increased corneal carboxy fluorescein uptake. Changes in ocular surface epithelial morphology and significantly reduced density of conjunctival goblet cells were found in dry eye groups. The number of conjunctival BrdU label-retaining cells in the rats with dry eye was significantly decreased compared with control rats ($P < 0.01$ for both groups). Furthermore, BrdU labeling in the before dry eye induction group showed more label-retaining basal cells in the conjunctiva than labeling in the dry eye state group ($P < 0.01$). CONCLUSIONS: Experimentally induced KCS in rats causes significant modification of epithelial stem cell proliferation kinetics in conjunctiva. The modification of epithelial stem cell proliferation

kinetics in conjunctiva may play a crucial role in the development of KCS and may be a therapeutic target for this condition.

Coles, B. L., D. J. Horsford, et al. (2006). "Loss of retinal progenitor cells leads to an increase in the retinal stem cell population in vivo." Eur J Neurosci **23**(1): 75-82.

Retinal stem cells [with the potential to produce either neural retinal progenitors or retinal pigment epithelial (RPE) progenitors] exist in the mammalian eye throughout life, and indeed the greatest absolute increase in the stem population occurs postnatally. The stem cells proliferate embryonically and thus may help to build the retina initially, but in postnatal mammals they clearly do not proliferate to regenerate the retina in response to injury. Using Chx10(orJ/orJ) and Mitf(mi/mi) mice, with small eye phenotypes due to the reduction of the neural retinal progenitor population and the retinal pigmented epithelial progenitor population, respectively, we now report that the retinal stem cell population, when assayed from the ciliary margin, increases 3-8-fold in both mutants. These findings suggest that the mammalian retinal stem cell population may be capable of responding to genetically induced signals from the progenitor populations.

Collinson, J. M., S. A. Chanas, et al. (2004). "Corneal development, limbal stem cell function, and corneal epithelial cell migration in the Pax6(+/-) mouse." Invest Ophthalmol Vis Sci **45**(4): 1101-8.

PURPOSE: To investigate the etiology of corneal dysfunction in the Pax6(+/-) mouse model of aniridia-related keratopathy. METHODS: Mosaic patterns of X-gal staining were compared in the corneal and limbal epithelia of female Pax6(+/-) and Pax6(+/+) littermates, age 3 to 28 weeks, hemizygous for an X-linked LacZ transgene, and Pax6(+/+), LacZ(-)<-->Pax6(+/+), LacZ(+) and Pax6(+/+), LacZ(-)<-->Pax6(+/-), LacZ(+) chimeras. Histologic examination of chimeric corneas was performed. RESULTS: Disrupted patterns of X-gal staining showed that heterozygosity for Pax6 perturbed clonal patterns of growth and development in the corneal and limbal epithelium. Centripetal migration of Pax6(+/-) corneal epithelial cells was diverted. Normal patterns of centripetal Pax6(+/-) cell migration and epithelial morphology were restored in Pax6(+/+)<-->Pax6(+/-) chimeras. Fewer, larger clones of limbal stem cells were present in Pax6(+/-) eyes, compared with wild-type. In the chimeras, Pax6(+/-) limbal stem cells were cell-autonomously depleted or less efficient than wild-type cells at producing progeny to populate the corneal epithelium. CONCLUSIONS: The correct Pax6 dosage is necessary for normal clonal growth during corneal development, normal limbal stem cell activity, and correct corneal epithelial cell migration. Disruption of normal cell movement in heterozygotes may be the consequence of failure of nonautonomous guidance cues. Degeneration of the corneal surface in aniridia-related keratopathy relates to both a deficiency within the limbal stem cell niche and nonautonomous diversion of corneal epithelial cell migration.

Coster, D. J., R. K. Aggarwal, et al. (1995). "Surgical management of ocular surface disorders using conjunctival and stem cell allografts." Br J Ophthalmol **79**(11): 977-82.

AIMS: The aim of this work was to investigate different surgical options for the repair of the ocular surface, using various extensions of the procedure of limbal stem cell allotransplantation. METHODS/RESULTS: Straightforward lamellar limbal transplantation was performed in one patient with contact lens induced limbal stem cell failure. A second patient with a neoplastic corneal lesion underwent limbal allotransplantation, followed later by a second procedure in which 360 degrees of limbus and the entire ocular surface was transplanted. A third patient who had suffered extensive chemical burns was treated by penetrating keratoplasty to restore central corneal clarity, followed later by a lamellar allograft comprising a 360 degrees annulus of peripheral cornea to repair the ocular surface. A fourth patient with long standing, chronic trachomatous eye disease underwent allotransplantation of the upper lid tarsal plate and conjunctiva, with reconstruction of the fornix. Finally, a child with Goldenhar's syndrome underwent reconstruction of the medial fornix with autologous buccal mucosa, followed by a lamellar corneal and conjunctival allograft. A stable ocular surface has been achieved in each case and there have been no obvious rejection episodes. CONCLUSION: Limbal allotransplantation can be extended to engraftment of the entire superficial cornea, limbus, conjunctiva, and tarsal plate in patients with a range of pathologies. We have described the surgical management of five cases which demonstrate the potential of the technique, but which raise questions which still need to be explored.

Das, A. V., K. B. Mallya, et al. (2006). "Neural stem cell properties of Muller glia in the mammalian retina: regulation by Notch and Wnt signaling." Dev Biol **299**(1): 283-302.

The retina in adult mammals, unlike those in lower vertebrates such as fish and amphibians, is not known to support neurogenesis. However, when injured, the adult mammalian retina displays

neurogenic changes, raising the possibility that neurogenic potential may be evolutionarily conserved and could be exploited for regenerative therapy. Here, we show that Muller cells, when retrospectively enriched from the normal retina, like their radial glial counterparts in the central nervous system (CNS), display cardinal features of neural stem cells (NSCs), i.e., they self-renew and generate all three basic cell types of the CNS. In addition, they possess the potential to generate retinal neurons, both in vitro and in vivo. We also provide direct evidence, by transplanting prospectively enriched injury-activated Muller cells into normal eye, that Muller cells have neurogenic potential and can generate retinal neurons, confirming a hypothesis, first proposed in lower vertebrates. This potential is likely due to the NSC nature of Muller cells that remains dormant under the constraint of non-neurogenic environment of the adult normal retina. Additionally, we demonstrate that the mechanism of activating the dormant stem cell properties in Muller cells involves Wnt and Notch pathways. Together, these results identify Muller cells as latent NSCs in the mammalian retina and hence, may serve as a potential target for cellular manipulation for treating retinal degeneration.

Daya, S. M. and F. A. Ilari (2001). "Living related conjunctival limbal allograft for the treatment of stem cell deficiency." Ophthalmology **108**(1): 126-33; discussion 133-4.

PURPOSE: To evaluate the outcomes of living related conjunctival limbal allograft transplantation for the treatment of stem cell deficiency. DESIGN: Retrospective, noncomparative case series. PARTICIPANTS: Nine living related donors, eight recipients (10 eyes) with Stevens-Johnson syndrome (3 eyes), ectodermal dysplasia (3 eyes), chemical injury (2 eyes), ocular cicatricial pemphigoid (1 eye), and atopic keratoconjunctivitis (n = 1). INTERVENTION: Four clock hours of limbal conjunctival tissue from the best matched human leukocyte antigen (HLA) relative donor were transplanted to the recipient eye superiorly and inferiorly after conjunctival peritomy and removal of conjunctival pannus. Systemic cyclosporine was administered to all recipients. MAIN OUTCOME MEASURES: Restoration of corneal epithelium, reduction of vascularity and conjunctivalization, improved comfort, improved corneal clarity, and visual improvement. RESULTS: Mean follow-up period was 26.2 months. Two highly inflamed eyes failed to initially epithelialize. The remainder all survived with restoration of corneal epithelium and reduction of vascularization. Corneal opacification was reduced (four of eight eyes) and visual improvement was achieved in seven eyes. All five eyes with pain had an improvement in symptoms. Allograft rejection occurred in two eyes (25%), and both were treated successfully. Both eyes had two class I HLA mismatches, and both had an underlying diagnosis of Stevens-Johnson syndrome. One eye developed a recurrent epithelial defect and perforated, requiring a penetrating keratoplasty that remained clear with an intact epithelial surface. The two initial failures also perforated and required penetrating keratoplasties that failed. None of the donor eyes had any complications. CONCLUSIONS: Restoration of the ocular surface by HLA-matched conjunctival limbal allograft transplantation can be accomplished in selected recipients. Systemic cyclosporine, even at low doses, is useful in ensuring long-term survival.

Daya, S. M., A. Watson, et al. (2005). "Outcomes and DNA analysis of ex vivo expanded stem cell allograft for ocular surface reconstruction." Ophthalmology **112**(3): 470-7.

PURPOSE: To investigate the outcome of a new technique of ex vivo expanded stem cell allograft for limbal stem cell deficiency (LSCD), and to characterize the ocular surface genotype after surgery. DESIGN: Retrospective noncomparative case series. PARTICIPANTS: Ten eyes of 10 patients with profound LSCD arising from ectodermal dysplasia (3 eyes), Stevens-Johnson syndrome (3 eyes), chemical injury (2 eyes), thermal injury (1 eye), and rosacea blepharoconjunctivitis (1 eye). INTERVENTION: Allogeneic corneal limbal stem cells were cultured on plastic and transplanted to the recipient eye after removal of conjunctival pannus. Amniotic membrane was applied in a bandage capacity. The procedure was combined with other reconstructive surgery in 2 cases. Nine patients received systemic cyclosporin A immunosuppression, and the DNA genotype was investigated with surface impression cytology. MAIN OUTCOME MEASURES: Parameters of LSCD, including vascularization, conjunctivalization, inflammation, epithelial defect, photophobia, and pain. RESULTS: The mean follow-up period was 28 months (range, 12-50). Seven of 10 eyes (70%) had improved parameters of LSCD at final follow-up and were considered successes. Four (40%) had improved visual acuity, including 3 having had further procedures for visual rehabilitation. Three patients failed to improve-1 with a thermal burn and lid deformity, 1 with Stevens-Johnson syndrome and severe dry eye, and 1 with ectodermal dysplasia who developed an epithelial defect at 26 months. DNA analysis of the first 7 cases showed no ex vivo donor stem cell DNA present beyond 9 months. CONCLUSIONS: Ex vivo expanded stem cell allograft is a useful technique for restoring the ocular surface in profound LSCD. The absence of donor

DNA beyond 9 months suggests that ongoing immunosuppression may be unnecessary and raises questions regarding the origin of the host corneal epithelium.

DeSousa, J. L., S. Daya, et al. (2009). "Adnexal surgery in patients undergoing ocular surface stem cell transplantation." Ophthalmology **116**(2): 235-42.

PURPOSE: To examine the role of adnexal disease and surgery in the outcome of ocular surface stem cell transplantation. DESIGN: Retrospective, noncomparative case series. PARTICIPANTS: Twenty-two patients (23 eyes) with severe corneal stem cell deficiency undergoing ocular surface stem cell transplantation. METHODS: Consecutive cases of stem cell grafting for ocular surface disease over a 6-year period at a single institution were studied. MAIN OUTCOME MEASURES: Main outcome measures were 2-fold: (1) nature of eyelid, fornix, and lacrimal abnormalities encountered; indications for treatment; methods; and outcome of adnexal surgery; (2) stem cell allograft success with respect to underlying disease, indication for stem cell grafting, preoperative adnexal involvement, and adnexal surgery after stem cell grafting. RESULTS: Twenty-nine limbal stem cell grafts on 23 eyes of 22 patients were identified during the study. Seventeen ex vivo expanded stem cell allografts, 11 keratolimbal allografts, and 1 living-related donor limbal allograft were performed, with combined stem cell techniques used in 5 patients. Median follow-up was 26.5 months (standard deviation, 18.6 months). Overall, stem cell graft success at final review was 69%. Thirty-four percent (10/29) of cases had eyelid involvement and 41% (12/29) had fornix involvement at the time of stem cell grafting. Trichiasis and symblepharon were the most common abnormalities. Fornix involvement was associated with a 50% chance of stem cell graft failure. After stem cell grafting, 66% (19/29) of cases required adnexal surgery. This was for lagophthalmos in 11 cases (46%), eyelid malposition in 7 cases (23%), fornix shortening in 5 cases (19%), and punctal patency in 8 cases (31%). Punctal patency and lagophthalmos frequently required repeated procedures for successful correction. Epithelial healing promptly followed correction of the adnexal abnormality with surgery; however, the need for adnexal surgery was associated with a 50% chance of stem cell graft failure. CONCLUSIONS: Concurrent adnexal abnormalities are associated with worse graft outcomes after stem cell transplantation and can compromise epithelial healing if uncorrected. Surgery for eyelid malposition and closure is essential before and after transplantation for surface epithelial integrity and often requires multiple procedures. FINANCIAL DISCLOSURE(S): The author(s) have no proprietary or commercial interest in any materials discussed in this article.

Di Rosa, P., J. C. Villaescusa, et al. (2007). "The homeodomain transcription factor Prep1 (pKnox1) is required for hematopoietic stem and progenitor cell activity." Dev Biol **311**(2): 324-34.

Most of the hypomorphic Prep1(i/i) embryos (expressing 3-10% of the Prep1 protein), die between E17.5 and P0, with profound anemia, eye malformations and angiogenic anomalies [Ferretti, E., Villaescusa, J.C., Di Rosa, P., Fernandez-Diaz, L.-C., Longobardi, E., Mazzieri, R., Miccio, A., Micali, N., Selleri, L., Ferrari G., Blasi, F. (2006). Hypomorphic mutation of the TALE gene Prep1 (pKnox1) causes a major reduction of Pbx and Meis proteins and a pleiotropic embryonic phenotype. Mol. Cell. Biol. 26, 5650-5662]. We now report on the hematopoietic phenotype of these embryos. Prep1(i/i) fetal livers (FL) are hypoplastic, produce less common myeloid progenitors colonies (CFU-GEMM) in cytokine-supplemented methylcellulose and have an increased number of B-cells precursors that differentiate poorly. Prep1(i/i) FL is able to protect lethally irradiated mice only at high cell doses but the few protected mice show major anomalies in all hematopoietic lineages in both bone marrow (BM) and peripheral organs. Prep1(i/i) FL cells compete inefficiently with wild type bone marrow in competitive repopulation experiments, suggesting that the major defect lies in long-term repopulating hematopoietic stem cells (LTR-HSC). Indeed, wt embryonic expression of Prep1 in the aorta-gonad-mesonephros (AGM) region, fetal liver (FL), cKit(+)Sca1(+)Lin(-)AA4.1(+) (KSLA) cells and B-lymphocytes precursors agrees with the observed phenotype. We therefore conclude that Prep1 is required for a correct and complete hematopoiesis.

Donahue, L. M., P. W. Coates, et al. (1996). "Characterization of developmental stage and neuronal potential of the rat PNS-derived stem cell line, RT4-AC." Brain Res Dev Brain Res **94**(1): 67-80.

RT4 is a family of cell lines derived from a rat peripheral neurotumor and consists of a multipotential stem cell line that spontaneously gives rise to three derivative cell types: one glial-like and two neuronal-like. Previous studies have established that the RT4 glial derivative expresses many properties of Schwann cells; however, the neuronal designation of the other RT4 derivatives is less well substantiated. To further characterize the developmental stage and lineages represented by the RT4 stem cell and its derivatives we examined the expression of 16 marker genes whose expression is

either specific to neurons or in some cases, neural tissue. Taken together our results indicate that (i) the RT4 neuronal-like derivatives express only immature neuronal properties, (ii) the RT4 cell lines most closely resemble neural crest derivatives from embryonic day 10 to 12 in the rat, (iii) treatment with cAMP and steroids, although capable of promoting process extension by the RT4 neuronal-like derivatives, did not affect the expression of any of the 16 marker genes examined, and (iv) when compared to other neural stem cell systems, RT4-AC generates the most immature neuronal derivatives.

Donisi, P. M., P. Rama, et al. (2003). "Analysis of limbal stem cell deficiency by corneal impression cytology." Cornea **22**(6): 533-8.

PURPOSE: The impaired function of corneal epithelial stem cells, located in the limbus, is responsible for corneal surface damage and is clinically characterized by recurrent epithelial defects, conjunctivalization, neovascularization, and corneal opacity. The aim of this study was to investigate corneal limbal stem cell deficiency (LSCD) by means of the impression cytology (IC) technique, using antibodies against cytokeratin 19 (CK19) and cytokeratin 3 (CK3), and to evaluate the diagnostic potential of this approach. METHODS: Over a 3-year period (October 1998-June 2001), we collected 113 pairs of IC samples from the eyes of 85 patients with a range of ocular surface diseases and performed an immunocytochemical analysis of CK19 and CK3. Samples with more than 50% cellularity were considered suitable for diagnostic purposes, while samples with less than 50% cellularity were considered with caution. CK19-positive cells in corneal IC were considered an expression of LSCD. We arbitrarily scored LSCD as mild (<25% of CK19-positive cells), moderate (25-50%), and severe (>50%). RESULTS: One hundred thirteen pairs of IC specimens were obtained from 85 patients; 32 patients (37.6%) had alkaline burns, 18 (21.2%) had other chemical or physical corneal injuries, 13 (15.3%) had complications from wearing contact lenses, 8 (9.4%) had severe microbial keratitis, and 14 (16.5%) had suspicious limbal deficit due to other causes. Nine patients underwent bilateral sampling and 12 had to be resampled. Thirteen pairs of IC specimens were obtained during the follow-up of 8 patients who had undergone limbal stem cell transplantation. In 3 of these patients, IC confirmed reversion to corneal immunophenotype (CK3+/CK19-), whereas in 4, residual limbal damage was still evident; 1 patient relapsed. In the remaining 100 pairs of impressions, we found 77 cases of LSCD, whereas in 16 pairs, we did not find LSCD. Seven pairs were defined as "not valuable" because of the poor quality of both CK samples. Diffuse LSCD, moderate or severe in degree, was found in 26 of 32 patients (81.2%) with alkali burns, whereas mild diffuse LSCD or sectoral LSCD was found in 13 of 18 patients (72.2%) with other chemical-physical injuries, in 10 of 13 patients (76.9%) wearing contact lenses, in 7 of 8 patients (87.5%) with severe microbial keratitis, and in 12 of 14 patients (85.7%) with other corneal pathologies. The quality of impressions was assessed in 77 cases and found to be good or discrete for both CKs in 32 cases (41.5%) and poor in 45 (58.5%): in 46.7% of these cases, the IC was poor only for CK19 and in 45.4% only for CK3. CONCLUSIONS: Immunocytochemistry for seeking out CK19- and CK3-positive cells on corneal IC is a simple and practical method to investigate LSCD. We believe that this technique could have an important role in evaluating patients undergoing therapeutic penetrating keratoplasty to select those who would benefit from limbal stem cell transplantation. Since sampling has been shown to be a critical point, we believe that any improvement in this area will also help to improve the methodology and will contribute to its wider utilization.

Dua, H. S. and A. Azuara-Blanco (1999). "Allo-limbal transplantation in patients with limbal stem cell deficiency." Br J Ophthalmol **83**(4): 414-9.

AIM: To report the outcome of a series of patients with stem cell deficiency who underwent allo-limbal transplantation and to describe a technique for this procedure. METHODS: Six consecutive patients underwent allo-limbal stem cell transplantation. The primary diagnosis included alkali burn (n = 2), trachoma (n = 1), chronic rosacea blepharitis and kerato-conjunctivitis (n = 1), aniridia (n = 1), and Stevens-Johnson syndrome (n = 1). The limbal rim consisted of peripheral cornea and perilimbal sclera. FK-506 was used postoperatively for immunosuppression. RESULTS: The length of follow up ranged from 3 to 24 months (mean follow up 11.8 (SD 9.3) months). The outcome was considered satisfactory in five of six cases. The corneal surface was completely epithelialised within 2 weeks, and there was a substantial improvement in vision and symptoms. One patient had recurrent epithelial defects related to eyelid abnormalities. No side effects associated with systemic immunosuppression were noted. CONCLUSION: Allo-limbal transplantation, with systemic immunosuppression with FK-506 is useful in reconstruction of the ocular surface with improvement in vision in patients with severe stem cell deficiency.

Dua, H. S. and A. Azuara-Blanco (2000). "Autologous limbal transplantation in patients with

unilateral corneal stem cell deficiency." Br J Ophthalmol **84**(3): 273-8.

AIM: To describe a surgical technique for autologous limbal stem cell transplantation and the outcome of a series of patients with unilateral stem cell deficiency. METHODS: A report of six consecutive patients who underwent autologous limbal stem cell transplantation is presented. The primary diagnosis included alkali burn (n=3), conjunctival intraepithelial neoplasia (CIN) (n=1), recurrent pterygium (n=1), and contact lens induced keratopathy (n=1). The autologous transplanted tissue consisted of peripheral cornea, limbus, and conjunctiva obtained from the contralateral eye. Three of the above patients underwent penetrating keratoplasty in association with auto-limbal transplantation. A significant modification to established techniques was the close monitoring of conjunctival epithelial migration in the immediate postoperative period. If conjunctival epithelium threatened to migrate on to the corneal surface, it was mechanically removed at the slit lamp and prevented from crossing the limbus. This was required in three patients. RESULTS: The mean follow up was 18.8 months. The outcome was satisfactory in all cases: a stable corneal surface was restored and there was a substantial improvement in vision and symptoms. One patient had a primary failure of the corneal allograft associated with glaucoma, and 6 months later developed a retinal detachment. No complications were noted in the donor eye with the exception of one patient who developed filamentary keratitis along the edge of the donor site. CONCLUSION: Autologous limbal transplantation with corneal, limbal, and conjunctival carriers was found to be useful for ocular surface reconstruction, over a mid-term follow up, in patients with unilateral stem cell deficiency. Close monitoring of the migration of conjunctival epithelium in the immediate postoperative period, and preventing it from crossing the limbus, ensured that the corneal surface was re-epithelialised exclusively from epithelial cells derived from the transplanted limbal tissue. This approach should improve the success of this procedure.

Dudney, B. W. and M. A. Malecha (2004). "Limbal stem cell deficiency following topical mitomycin C treatment of conjunctival-corneal intraepithelial neoplasia." Am J Ophthalmol **137**(5): 950-1.

PURPOSE: To report a case of conjunctival-corneal intraepithelial neoplasia (CCIN) in an elderly African American patient treated with topical mitomycin C and the subsequent complication of limbal stem cell deficiency. DESIGN: Interventional case report. METHODS: A 92-year-old African American woman was diagnosed with CCIN in the right eye. Following incisional biopsy, the patient received five 1-week courses of 0.04% mitomycin C and was followed over a period of 10 months. RESULTS: The CCIN regressed completely following mitomycin C therapy. Three months later, the patient developed recurrent nonhealing epithelial defects in the right cornea. CONCLUSIONS: Conjunctival-corneal intraepithelial neoplasia may occur in the African American population. Although MMC is effective in eradicating CCIN, a limbal stem cell deficiency may complicate the treatment.

Edman, L., J. Larsen, et al. (2001). "Health-related quality of life, symptom distress and sense of coherence in adult survivors of allogeneic stem-cell transplantation." Eur J Cancer Care (Engl) **10**(2): 124-30.

This is the first Swedish study to evaluate the health-related quality of life and sense of coherence in adult survivors of allogeneic, haematopoietic stem cell transplantation (HSCT). Twenty-five recipients completed three questionnaires 2-4 years after the transplantation. The questionnaires used were the Sickness Impact Profile (SIP), the Symptom Frequency Intensity and Distress (SFID-BMT) scale and the Sense of Coherence (SOC) scale measuring subjective functional status, symptom distress and coping ability. Impairments in functional status were found, as compared with a population norm. The most common impairments were found in the areas of social interaction and sleep and rest. Eye problems, dry mouth, cough, sexual problems, tiredness, anxiety and changes of taste were symptoms reported by more than half of the patients. Despite impaired functioning and a high incidence of symptoms, the general health was described as quite good or excellent by 80% (n = 20) of the patients. The majority (20/22) had also been able to return to work or to attend school. No difference in the sense of coherence was seen, as compared with the population norm. Functional impairments were significantly correlated to a lower degree of sense of coherence.

Espana, E. M., M. Grueterich, et al. (2003). "Phenotypic study of a case receiving a keratolimbal allograft and amniotic membrane for total limbal stem cell deficiency." Ophthalmology **110**(3): 481-6.

PURPOSE: To report the expression pattern of key molecules by the reconstructed corneal epithelium after a keratolimbal allograft (KLAL) and amniotic membrane transplantation (AMT) for total limbal stem cell deficiency. DESIGN: Interventional case report. METHOD: A 50-year-old woman with severe chemical burns in both eyes received an AMT as a temporary patch at the acute stage, and a KLAL with AMT as a graft at the chronic stage for total

limbal stem cell deficiency. The corneal button removed during subsequent corneal transplantation was submitted for immunofluorescence staining with monoclonal antibodies against keratin K3, MUC5AC, connexin 43, integrins alpha3beta1 and alpha6beta4, and laminin 5 for comparison with a normal cornea. RESULTS: Histologically, a normal stratified corneal epithelium has five to six cell layers that lay on the thick amniotic membrane basement membrane. The phenotype was of a corneal origin, based on expression of positive keratin K3, negative MUC5AC, and positive connexin 43. Furthermore, intact basement membrane complexes were present, evidenced by positive staining to integrins alpha3beta1 and alpha6beta4 and to laminin 5. CONCLUSIONS: A normal corneal epithelial phenotype with normal basement membrane complexes was restored after a KLAL and AMT in a case with total limbal stem cell deficiency.

Fagerholm, P. and G. Lisha (1999). "Corneal stem cell grafting after chemical injury." Acta Ophthalmol Scand **77**(2): 165-9.

PURPOSE: To evaluate the clinical and histological outcome after corneal stem cell grafting in unilateral chemical burns in eight consecutive patients. METHODS: The visual performance and degree of irritation were evaluated following autologous corneal stem cell grafting. Scar tissue overlying the injured corneas as well as two corneal buttons obtained at penetrating graft performed a year or more after the stem cell graft were evaluated histologically. RESULTS: Seven of the 8 grafted eyes obtained useful vision postoperatively. Two of these eyes had undergone a penetrating graft following initial surgery. The chronic irritation before surgery was significantly reduced. In one eye a penetrating graft was opacified due to a late developing scar entropion. CONCLUSIONS: Autologous corneal stem cell grafting proved successful in restoring vision and reduce irritation in unilateral chemical burns. Histological examination indicates that the conjunctival overgrowth is replaced by regular corneal epithelium.

Fahnehjelm, K. T., A. L. Tornquist, et al. (2006). "Ocular findings in four children with mucopolysaccharidosis I-Hurler (MPS I-H) treated early with haematopoietic stem cell transplantation." Acta Ophthalmol Scand **84**(6): 781-5.

PURPOSE: To present visual functions and ocular findings in four children with mucopolysaccharidosis I-Hurler (MPS I-H) treated early with stem cell transplantation (SCT). METHODS: Clinical ophthalmological evaluations including visual evoked potentials (VEPs) were carried out. RESULTS: Stem cell transplantation was performed before 20 months of age. Ocular follow-up lasted 1.3-5.6 years (median 4.1 years). Reductions in corneal opacities were observed in all four children post-SCT, but a slight cloudiness persisted. Decreased visual acuity and high hyperopia (median + 6.25 dioptres, range + 4.0 D to + 7.5 D spherical equivalents) were noted in all children. Hyperopia was initially undetected due to dull retinal reflexes and photophobia. Two children developed esotropia, one with amblyopia. Keratometry, performed in two children, demonstrated subnormal values with a mean of 39.33 D (range 37.62-41.00 D). Visual evoked potentials and intraocular pressures were normal. Neither cataract nor dry eye were detected during follow-up. CONCLUSIONS: Early SCT appears to be beneficial in reducing, but not eliminating, corneal opacities in children with MPS I-H. Subjects are at risk of developing high hyperopia and esotropia. Hyperopia might be caused by the storage of glucosaminoglucans that increase corneal rigidity, thereby straightening the curvature of the cornea and reducing refractive power. As early diagnosis and treatment are very important, paediatric ophthalmologists should remember to rule out MPS I-H in children with corneal opacities.

Fahnehjelm, K. T., A. L. Tornquist, et al. (2008). "Dry-eye syndrome after allogeneic stem-cell transplantation in children." Acta Ophthalmol **86**(3): 253-8.

PURPOSE: To report the prevalence of dry-eye syndrome (DES) in children and young adults treated with allogeneic stem-cell transplantation (SCT) during childhood; to relate DES to conditioning regimes, including total body irradiation (TBI) and chemotherapy, and to immunosuppressive drugs and graft-versus-host disease (GVHD). METHODS: This cross-sectional study included 60 children/young adults transplanted because of leukaemia, various haematological disorders and inborn errors of metabolism between 1986 and 2004, with a follow-up time of 7.0 years (median, range 2-18). Clinical assessments, performed at a median age of 15.6 years (range 5.5-23.5), included an inquiry form on dry-eye symptoms, corneal status including fluorescein staining, 'break-up time' (BUT) and Schirmer test. RESULTS: A total of 37 of 60 patients had DES defined as presence of corneal epithelial lesions with a pathological BUT and/or Schirmer test. Twenty-nine had had staining <1-10% of the corneal surface while eight patients had staining > or =10-25% of the corneal surface. All 37 patients with objective signs of DES, graded and not graded, had significant associations to subjective symptoms of dry eyes including dry eyes, red eyes, ocular irritation,

secretion and sensitivity to light. Frequent occasions (above median; n = 7) of high cyclosporine A trough levels above 250 ng/ml were associated significantly with DES (P = 0.002). However, there was no association between DES and conditioning with single-dose (s-TBI) or fractionated TBI (f-TBI), busulfan or other chemotherapy. There were no associations between prolonged corticosteroid treatment or chronic GVHD and DES in the present study. DES was more common in patients with malignant diseases (P = 0.02). Malignant disease increased the risk of DES in girls but not in boys. Increased age at SCT increased the risk for DES in boys but not in girls (P = 0.02). Although severe keratitis occurred in three patients, nobody suffered corneal perforation. CONCLUSION: DES with epithelial punctata keratopathy was common in children/young adults treated with SCT and more common if the patients were exposed to repeated high trough levels of cyclosporine A; however, DES was not associated with irradiation, corticosteroids or GVHD in the present study. Patients with objective DES also had subjective symptoms of dry eyes, which facilitate diagnosis. Girls with malignant diseases and boys who underwent SCT at later ages seem to demand higher attention and more frequent check-ups regarding DES. Patients with diagnosed severe DES needed frequent and continuous ophthalmological care to maintain treatment motivation.

Fan, Z., T. Yamaza, et al. (2009). "BCOR regulates mesenchymal stem cell function by epigenetic mechanisms." Nat Cell Biol **11**(8): 1002-9.

The BCL-6 co-repressor (BCOR) represses gene transcription by interacting with BCL-6 (Refs 1, 2). BCOR mutation is responsible for oculo-facio-cardio-dental (OFCD) syndrome, which is characterized by canine teeth with extremely long roots, congenital cataracts, craniofacial defects and congenital heart disease. Here we show that BCOR mutation increased the osteo-dentinogenic potential of mesenchymal stem cells (MSCs) isolated from a patient with OFCD, providing a molecular explanation for abnormal root growth. AP-2alpha was identified as a repressive target of BCOR, and BCOR mutation resulted in abnormal activation of AP-2alpha. Gain- and loss-of-function assays suggest that AP-2alpha is a key factor that mediates the increased osteo-dentinogenic capacity of MSCs. Moreover, we found that BCOR maintained tissue homeostasis and gene silencing through epigenetic mechanisms. BCOR mutation increased histone H3K4 and H3K36 methylation in MSCs, thereby reactivating transcription of silenced target genes. By studying a rare human genetic disease, we have unravelled an epigenetic mechanism for control of human adult stem cell function.

Fernandes, M., V. S. Sangwan, et al. (2004). "Limbal stem cell transplantation." Indian J Ophthalmol **52**(1): 5-22.

The past two decades have witnessed remarkable progress in limbal stem cell transplantation. In addition to harvesting stem cells from a cadaver or a live related donor, it is now possible to cultivate limbal stem cells in vitro and then transplant them onto the recipient bed. A clear understanding of the basic disease pathology and a correct assessment of the extent of stem cell deficiency are essential. A holistic approach towards management of limbal stem cell deficiency is needed. This also includes management of the underlying systemic disease, ocular adnexal pathology and dry eye. Conjunctival limbal autografts from the healthy contralateral eye are performed for unilateral cases. In bilateral cases, tissue may be harvested from a cadaver or a living related donor; prolonged immunosuppression is needed to avoid allograft rejection in such cases. This review describes the surgical techniques, postoperative treatment regimes (including immunosuppression for allografts), the complications and their management. The short and long-term outcomes of the various modalities reported in the literature are also described.

Fisher, V. L. (1999). "Long-term follow-up in hematopoietic stem-cell transplant patients." Pediatr Transplant **3 Suppl 1**: 122-9.

Hematopoietic stem-cell transplantation (HSCT) has increasingly become an accepted treatment for many childhood diseases and disorders. Potential HSCT recipients can be children with hematological malignancies or solid tumors, as well as congenital and acquired disorders. In the past decade, the use of HSCT in the treatment of pediatric disorders has grown exponentially while advances in supportive care have improved survival rate, contributing to a rapidly growing population of transplant survivors. Although numerous similarities can be found between pediatric and adult long-term HSCT survivors, this article provides a brief overview of the pediatric patients, emphasizing the aspects of surveillance and late effects. Understanding the long-term complications that can occur after HSCT is important in determining the appropriate evaluations and medical treatment for the patient involved. The goal of this article is to assist caregivers in providing optimal care for long-term survivors of HSCT. The initial section of this work comprises the three major causes of late effects in HSCT. It will then encompass

a system review of the different potential complications that are seen with HSCT.

Fogla, R. and P. Padmanabhan (2005). "Deep anterior lamellar keratoplasty combined with autologous limbal stem cell transplantation in unilateral severe chemical injury." Cornea **24**(4): 421-5.

PURPOSE: To evaluate the efficacy of deep anterior lamellar keratoplasty combined with autologous limbal stem cell transplantation for ocular surface reconstruction and visual rehabilitation in eyes with unilateral, late-stage, severe chemical injury. METHODS: This was a retrospective, noncomparative, interventional case series that included 7 eyes of 7 patients, with severe unilateral late stage chemical injury, exhibiting corneal vascularization, conjunctivalization, and extensive corneal scarring were treated at the C. J. Shah Cornea Service, Sankara Nethralaya, a tertiary care center. Surgical procedures included releasing symblepharon adhesions, excising epibulbar fibrous tissue, superficial keratectomy to remove fibrovascular tissues over cornea, deep anterior lamellar dissection, grafting a lamellar corneal button, and transplanting autologous limbal graft, with or without amniotic membrane transplantation. The main outcome measures were relief of patient symptoms, postoperative recovery of the ocular surface, corneal clarity, corneal epithelial stability, and best corrected visual acuity. RESULTS: The mean duration between the injury and surgery was 24.4 +/- 21.8 months. No intraoperative complications were noted. Successful epithelialization was achieved in all eyes. The reconstructed corneal surface remained stable during the entire follow-up period (mean follow-up, 16.57 +/- 5.12 months). All patients had resolution of ocular symptoms. Remarkable improvement in vision was noted in all (85.7%) except 1 eye in which recovery was limited due to amblyopia. The average best corrected visual acuity at last follow-up was 20/50. No complications were noted in the donor fellow eye. CONCLUSIONS: DALK combined with autologous limbal transplantation can restore a healthy, stable ocular surface, besides providing a clear cornea that remarkably improves the visual acuity, in patients with unilateral, late stage, severe chemical injury.

Fujishima, H., J. Shimazaki, et al. (1996). "Temporary corneal stem cell dysfunction after radiation therapy." Br J Ophthalmol **80**(10): 911-4.

BACKGROUND: Radiation therapy can cause corneal and conjunctival abnormalities that sometimes require surgical treatment. Corneal stem cell dysfunction is described, which recovered after the cessation of radiation. METHODS: A 44-year-old man developed a corneal epithelial abnormality associated with conjunctival and corneal inflammation following radiation therapy for maxillary cancer. He experienced ocular pain and loss of vision followed by conjunctival epithelialisation of the upper and lower parts of the cornea. RESULTS: Examination of brush cytology samples showed goblet cells in the upper and lower parts of the cornea, which showed increased fluorescein permeability, and intraepithelial lymphocytes. Impression cytology showed goblet cells in the same part of the cornea. Specular microscopy revealed spindle type epithelial cells. Patient follow up included artificial tears and an antibiotic ophthalmic ointment. The corneal abnormalities resolved after 4 months with improved visual acuity without any surgical intervention, but the disappearance of the palisades of Vogt did not recover at 1 year after radiation. CONCLUSION: Radiation therapy in this patient caused temporary stem cell dysfunction which resulted in conjunctivalisation in a part of the cornea. Although limbal stem cell function did not fully recover, this rare case suggested that medical options should be considered before surgery.

Furst, D. E. (2002). "Stem cell transplantation for autoimmune disease: progress and problems." Curr Opin Rheumatol **14**(3): 220-4.

The current status of stem cell transplantation in rheumatoid arthritis, juvenile chronic arthritis, systemic lupus erythematosus, and systemic sclerosis are reviewed. From a large European bone marrow transplant registry, a birds' eye view of stem cell transplantation for autoimmune disease can be obtained. Among 43 rheumatoid arthritis patients, 35 juvenile chronic arthritis patients, 34 systemic lupus erythematosus patients, and 58 systemic sclerosis patients who underwent stem cell transplantation, initial responses in most patients were good to excellent. Although initial transplant related mortality was low for rheumatoid arthritis, somewhat higher rates for juvenile chronic arthritis, systemic lupus erythematosus, and systemic sclerosis may be falling with modifications in the stem cell transplantation regimens. In rheumatoid arthritis and systemic lupus erythematosus treatment, the criteria for patient selection are still not clear and the therapeutic regimens for stem cell transplantation (and whether follow-up treatment is necessary) are not fully defined. In juvenile chronic arthritis, responses are encouraging although little fully published data beyond that from the European Bone Marrow Transplant Registry exist. In systemic sclerosis, criteria for patient selection and a limited number of stem cell transplantation regimens have been agreed on and controlled trials are underway.

Gomes, J. A., M. S. dos Santos, et al. (2003). "Amniotic membrane transplantation for partial and total limbal stem cell deficiency secondary to chemical burn." Ophthalmology **110**(3): 466-73.

PURPOSE: To evaluate the surgical outcome of preserved amniotic membrane transplantation (AMT) for ocular surface reconstruction in chemical burn with limbal stem cell deficiency. DESIGN: Prospective, noncomparative, interventional case series. PARTICIPANTS: Twenty eyes of 20 consecutive patients with limbal stem cell deficiency secondary to ocular chemical injury. INTERVENTION: AMT with or without adjunctive limbal transplantation using limbal tissue from either the healthy contralateral eye (CLAU) or a living related donor (lr-CLAL). MAIN OUTCOME MEASURES: Reconstruction of corneal epithelium (clear appearance without epithelial defect, normal fluorescein permeability and the absence of conjunctiva-derived goblet cells on impression cytology), decrease in corneal vascularization and improvement in visual acuity. RESULTS: With a mean follow-up time of 19 months (range, 8-27 months), satisfactory ocular surface reconstruction was obtained in 15 eyes (75%), with reduced inflammation and vascularization of the ocular surface and a mean epithelialization time of 3.3 weeks. Success was observed in all cases of partial limbal stem cell deficiency (PLD) and in 68.75% (11 eyes) of cases of total limbal stem cell deficiency (TLD). Surgical failure was observed in five severe cases (31.25%). A significant visual improvement was observed in all cases after surgery, except for 2 eyes that maintained preoperative visual acuity. CONCLUSIONS: AMT seems to be an efficient adjunct for ocular surface reconstruction in chemical burns with PLD. When performed in conjunction with limbal stem cell transplantation, it is also effective in most cases of TLD.

Grueterich, M., E. M. Espana, et al. (2002). "Phenotypic study of a case with successful transplantation of ex vivo expanded human limbal epithelium for unilateral total limbal stem cell deficiency." Ophthalmology **109**(8): 1547-52.

OBJECTIVE: To minimize the risk to the donor eye when a conjunctival limbal autograft is performed for unilateral total limbal stem cell deficiency (LSCD), a new approach has been reported of expanding limbal epithelial progenitor cells from a small limbal biopsy cultured on amniotic membrane (AM). Herein, we present for the first time the morphologic and phenotypic outcome of one such patient. DESIGN: Interventional case report. METHODS: A 31-year-old male with a severe acid burn to his left eye received AM transplantation at the acute stage and a keratolimbal allograft (KLAL) at the chronic stage for total LSCD. As an alternative to combat the failed KLAL, the above-mentioned new surgical procedure was performed. The corneal button, obtained after a penetrating keratoplasty performed 5.5 months later, and a normal corneal button as a control were submitted to hematoxylin-eosin and immunofluorescence staining for keratin K3, connexin 43, goblet-cell mucin MUC 5AC, laminin 5, and integrins alpha3beta1 and alpha6beta4. MAIN OUTCOME MEASURES: Clinical and immunohistologic features. RESULTS: The resultant epithelium was stratified with five to six cell layers and anchored to laminin 5 of the amniotic basement membrane via integrins alpha3beta1 and alpha6beta4 in a manner similar to the normal corneal epithelium. Intriguingly, the epithelial phenotype was limbal and not corneal, based on the negative expression of keratin K3 and connexin 43 of the basal epithelium. CONCLUSIONS: The technique described ensures the preservation of amniotic basement membrane, which allows formation of adhesion complexes and maintains normal corneal architecture. The preservation of a limbal epithelial phenotype on the reconstructed corneal surface indicates that AM provides a unique stromal environment conducive to the preservation and expansion of limbal epithelial progenitor cells.

Harkin, D. G., Z. Barnard, et al. (2004). "Analysis of p63 and cytokeratin expression in a cultivated limbal autograft used in the treatment of limbal stem cell deficiency." Br J Ophthalmol **88**(9): 1154-8.

AIM: To investigate the expression of p63 and cytokeratins throughout the course of producing a cultivated autograft of limbal epithelial cells. METHODS: A 75 year old male with a severe alkali burn to his right eye received two cultivated autografts of limbal epithelial cells on amniotic membrane followed by a corneal allograft. Immunostaining for p63 and cytokeratins was performed during ex vivo expansion with 3T3 fibroblasts, following subcultivation on amniotic membrane, and on the excised corneal button. RESULTS: Cultures grown in the presence of 3T3 fibroblasts or on amniotic membrane displayed positive staining for keratins 14 and 19, and p63, but poor staining for keratin 3 (K3). The excised corneal button possessed a stratified epithelium of K3 positive cells residing on amniotic membrane. CONCLUSIONS: Our results document for the first time the co-expression of cytokeratins 14 and 19 with p63 in a cultivated limbal graft. These data support the conclusion that cultivated grafts of limbal epithelium contain predominantly undifferentiated cells with the potential to regenerate a normal corneal epithelium.

Hatakeyama, J., Y. Bessho, et al. (2004). "Hes genes regulate size, shape and histogenesis of the nervous system by control of the timing of neural stem cell differentiation." Development **131**(22): 5539-50.

Radial glial cells derive from neuroepithelial cells, and both cell types are identified as neural stem cells. Neural stem cells are known to change their competency over time during development: they initially undergo self-renewal only and then give rise to neurons first and glial cells later. Maintenance of neural stem cells until late stages is thus believed to be essential for generation of cells in correct numbers and diverse types, but little is known about how the timing of cell differentiation is regulated and how its deregulation influences brain organogenesis. Here, we report that inactivation of Hes1 and Hes5, known Notch effectors, and additional inactivation of Hes3 extensively accelerate cell differentiation and cause a wide range of defects in brain formation. In Hes-deficient embryos, initially formed neuroepithelial cells are not properly maintained, and radial glial cells are prematurely differentiated into neurons and depleted without generation of late-born cells. Furthermore, loss of radial glia disrupts the inner and outer barriers of the neural tube, disorganizing the histogenesis. In addition, the forebrain lacks the optic vesicles and the ganglionic eminences. Thus, Hes genes are essential for generation of brain structures of appropriate size, shape and cell arrangement by controlling the timing of cell differentiation. Our data also indicate that embryonic neural stem cells change their characters over time in the following order: Hes-independent neuroepithelial cells, transitory Hes-dependent neuroepithelial cells and Hes-dependent radial glial cells.

Haynes, T., C. Gutierrez, et al. (2007). "BMP signaling mediates stem/progenitor cell-induced retina regeneration." Proc Natl Acad Sci U S A **104**(51): 20380-5.

We identified a mechanism whereby retina regeneration in the embryonic chick can be induced by the contribution of stem/progenitor cells. We show that bone morphogenetic protein (BMP) signaling is sufficient and necessary to induce retina regeneration and that its action can be divided into two phases. By 3 days after postretinectomy (d PR), the BMP pathway directs proliferation and regeneration through the activation of Smad (canonical BMP pathway) and the up-regulation of FGF signaling by the MAPK pathway. By 7d PR, it induces apoptosis by activating p38 (a noncanonical BMP pathway) and down-regulating FGF signaling (by both MAPK and AKT pathways). Apoptosis at this later stage can be prevented, and BMP-induced regeneration can be further induced by inhibition of p38. These results unravel a mechanism for stem/progenitor cell-mediated retina regeneration, where BMP activation establishes a cross-talk with the FGF pathway and selectively activates the canonical and noncanonical BMP pathways. Retina stem/progenitor cells exist in other species, including humans. Thus, our findings provide insights on how retinal stem cells can be activated for possible regenerative therapies.

Heath, J. A., E. H. Broxson, Jr., et al. (2002). "Epstein-Barr virus-associated lymphoma in a child undergoing an autologous stem cell rescue." J Pediatr Hematol Oncol **24**(2): 160-3.

Epstein-Barr virus-associated lymphoproliferative disease (EBV-LPD) is a serious disorder seen in various states of immunodeficiency, often with a fatal outcome. In this article, a patient with EBV-lymphoma after autologous stem cell rescue for treatment of a nonhematologic solid tumor is described. The child, a 4-year-old boy, had unilateral retinoblastoma with metastatic spread to the central nervous system. He had previously received both local tumor bed and craniospinal radiation therapy together with intensive myeloablative alkylator chemotherapy before autologous stem cell rescue. Histologically confirmed lymphoma with evidence of active EBV proliferation developed within cervical lymph nodes 3 weeks after his first autologous stem cell rescue. A complete clinical remission of the lymphadenopathy was obtained after infusions of rituximab (an anti-CD20 monoclonal antibody), acyclovir, and high-titer anticytomegalovirus immunoglobulin. The patient died approximately 6 months later of persistent and progressive retinoblastoma without any clinical evidence of lymphoma. It is concluded that EBV-LPD should be included in the differential diagnosis in patients in whom lymphadenopathy develops after autologous stem cell rescue.

Huck, K., H. J. Laws, et al. (2006). "Three cases of renal relapse after allogeneic hematopoietic stem cell transplantation for childhood acute lymphoblastic leukemia." Haematologica **91**(5 Suppl): ECR07.

Isolated renal relapse after allogeneic hematopoietic stem cell transplantation (alloHSCT) in children with acute lymphoblastic leukemia (ALL) is a rare condition. Generally, in ALL, the sites most frequently affected by extramedullary relapse are the central nervous system (CNS) and the testicles. Here we report on three young boys with relapsed B-precursor ALL, who underwent alloHSCT from HLA-identical siblings and suffered a histopathologically proven isolated unilateral renal relapse (two patients) or a combined renal and testicular relapse (one

patient) 6, 10 and 12 months post alloHSCT. In all patients at the time of relapse bone marrow showed complete remission with complete donor hematopoiesis. They all received total body irradiation with partial shielding of the kidneys as part of their conditioning therapy, such that renal shielding could be an explanation for the observed accumulation of renal relapses. Moreover, during the past few years so called immune privilege has been postulated for frequent relapse sites such as the CNS, the testicles and the anterior chamber of the eye. Impaired accessability of these organs by cytotoxic T-cells (CTLs) with a reduced graft-versus-leukemia (GvL) effect after alloHSCT is based on a number of different molecular and cellular mechanisms. Similar mechanisms have been shown to be effective in the tubulointerstitial space of the kidney, rendering the kidney a potentially immune privileged site. Due to these observations we advocate sufficient treatment of the kidneys during conditioning therapy.

Insua, M. F., M. V. Simon, et al. (2008). "Trophic factors and neuronal interactions regulate the cell cycle and Pax6 expression in Muller stem cells." J Neurosci Res **86**(7): 1459-71.

The finding that Muller cells have stem cell properties in the retina has led to the hypothesis that they might be a source for replacing neurons lost in neurodegenerative diseases. However, utilization of Muller cells for regenerative purposes in the mammalian eye still requires identifying those factors that regulate their multipotentiality and proliferation. In addition, because Pax6 expression is indispensable for eye development, its regulation would be required during regeneration. In the present study we investigated the regulation of cell-cycle progression and Pax6 expression in pure Muller glial cell cultures and neuroglial cocultures from rat retinas. At early times in vitro, glial cells showed high expression of Pax6 and of nestin, a stem cell marker, and of markers of cell-cycle progression; expression of these markers decreased during development in parallel with increased glial differentiation. The addition of glial-derived neurotrophic factor, basic fibroblast growth factor, and insulin restored proliferation and also Pax6 and nestin expression in glial cells. Noteworthy, in neuroglial cocultures Muller cells retained Pax6 expression for longer periods, and, in turn, neuronal progenitors preserved their proliferative potential for several days in vitro. This suggests that neuroglial interactions mutually regulate their mitogenic capacity. In addition, in glial secondary cultures incubated with insulin, many neuroblast-like cells expressed the neuronal marker NeuN. Our results suggest that the proliferative capacity and the features of eye stem cells of Muller glial cells are regulated by molecular and cellular factors, which might then provide potential tools for manipulating retinal regeneration.

Kal, H. B., M. Loes van Kempen-Harteveld, et al. (2006). "Biologically effective dose in total-body irradiation and hematopoietic stem cell transplantation." Strahlenther Onkol **182**(11): 672-9.

BACKGROUND AND PURPOSE: Total-body irradiation (TBI) is an important part of the conditioning regimen for hematopoietic stem cell transplantation (HSCT) in patients with hematologic malignancies. The results after treatment with various TBI regimens were compared, and dose-effect relationships for the endpoints relapse incidence, disease-free survival, treatment-related mortality, and overall survival were derived. The aim was to define requirements for an optimal treatment schedule with respect to leukemic cell kill and late normal-tissue morbidity. MATERIAL AND METHODS: A literature search was performed. Three randomized studies, four studies comparing results of two or three TBI regimens, and nine reports with results of one specific TBI regimen were identified. Biologically effective doses (BEDs) were calculated. The results of the randomized studies and the studies comparing results of two or three TBI regimens were pooled, and the pooled relative risk (RR) was calculated for the treatments with high BED values versus treatments with a low BED. BED-effect relationships were obtained. RESULTS: RRs for the high BED treatments were significantly lower for relapse incidence, not significantly different for disease- free survival and treatment-related mortality, and significantly higher for overall survival. BED-effect relationships indicate a decrease in relapse incidence and treatment-related mortality and an increase in disease-free and overall survival with higher BED values. CONCLUSION: "More dose is better", provided that a TBI setting is used limiting the BEDs of lungs, kidneys, and eye lenses.

Kim, J. T., Y. S. Chun, et al. (2008). "The effect of in vivo grown corneal epithelium transplantation on persistent epithelial defects with limbal stem cell deficiency." J Korean Med Sci **23**(3): 502-8.

We report our experience with corneal epithelium, grown in vivo, transplantation in three patients with persistent epithelial defect (PED). The three patients had ocular surface disease unresponsive to standard treatments and were therefore chosen for transplantation. They underwent transplantation of epithelial sheets, grown in vivo, to the most affected eye. In vivo cultivation was carried out in the cornea of a living related donor. After epithelialization was completed, the epithelium grown on an amniotic

membrane was harvested gently; it was then transplanted into the patient's eye after debridement of fibrovascular tissue. The cultivated epithelium was completely epithelialized by 2 weeks; it was well-differentiated with well-formed hemidesmosome. On immunohistochemical staining, p63, connexin 43, and Integrin beta4 were expressed in the cells on the epithelial sheet. The PED was covered completely and maintained for 4 weeks in all cases. However, corneal erosion recurred after 5 weeks in two cases. This novel technique demonstrates the corneal epithelial cells can be expanded in vivo successfully on denuded amniotic membrane of a healthy cornea and harvested safely. A corneal epithelial sheet, grown in vivo, can be transplanted to treat eye with a severe ocular surface disease, such as total limbal deficiency.

Kitadate, Y., S. Shigenobu, et al. (2007). "Boss/Sev signaling from germline to soma restricts germline-stem-cell-niche formation in the anterior region of Drosophila male gonads." Dev Cell **13**(1): 151-9.

Drosophila germline stem cells are regulated by the somatic microenvironment, or "niche," which ensures that the stem cells can both self-renew and produce functional gametes throughout adult life. However, despite its prime importance, little is known about how niche formation is regulated during gonadal development. Here, we demonstrate that a receptor tyrosine kinase, Sevenless (Sev), is required to ensure that the niche develops in the anterior region of the male embryonic gonads. Sev is expressed in somatic cells within the posterior region of the gonads. Sev is activated by a ligand, Bride of sevenless (Boss), which is expressed by the germline, to prevent ectopic niche differentiation in the posterior gonadal somatic cells. Thus, we propose that signal transduction from germline to soma restricts expansion of the germline-stem-cell niche in the gonads.

Koch, T. G., L. C. Berg, et al. (2009). "Current and future regenerative medicine - principles, concepts, and therapeutic use of stem cell therapy and tissue engineering in equine medicine." Can Vet J **50**(2): 155-65.

This paper provides a bird's-eye perspective of the general principles of stem-cell therapy and tissue engineering; it relates comparative knowledge in this area to the current and future status of equine regenerative medicine.The understanding of equine stem cell biology, biofactors, and scaffolds, and their potential therapeutic use in horses are rudimentary at present. Mesenchymal stem cell isolation has been proclaimed from several equine tissues in the past few years. Based on the criteria of the International Society for Cellular Therapy, most of these cells are more correctly referred to as multipotent mesenchymal stromal cells, unless there is proof that they exhibit the fundamental in vivo characteristics of pluripotency and the ability to self-renew. That said, these cells from various tissues hold great promise for therapeutic use in horses. The 3 components of tissue engineering - cells, biological factors, and biomaterials - are increasingly being applied in equine medicine, fuelled by better scaffolds and increased understanding of individual biofactors and cell sources.The effectiveness of stem cell-based therapies and most tissue engineering concepts has not been demonstrated sufficiently in controlled clinical trials in equine patients to be regarded as evidence-based medicine. In the meantime, the medical mantra "do no harm" should prevail, and the application of stem cell-based therapies in the horse should be done critically and cautiously, and treatment outcomes (good and bad) should be recorded and reported.Stem cell and tissue engineering research in the horse has exciting comparative and equine specific perspectives that most likely will benefit the health of horses and humans. Controlled, well-designed studies are needed to move this new equine research field forward.

Kosinski, C., V. S. Li, et al. (2007). "Gene expression patterns of human colon tops and basal crypts and BMP antagonists as intestinal stem cell niche factors." Proc Natl Acad Sci U S A **104**(39): 15418-23.

Human colonic epithelial cell renewal, proliferation, and differentiation are stringently controlled by numerous regulatory pathways. To identify genetic programs of human colonic epithelial cell differentiation in vivo as well as candidate marker genes that define colonic epithelial stem/progenitor cells and the stem cell niche, we applied gene expression analysis of normal human colon tops and basal crypts by using expression microarrays with 30,000 genes. Nine hundred and sixty-nine cDNA clones were found to be differentially expressed between human colon crypts and tops. Pathway analysis revealed the differential expression of genes involved in cell cycle maintenance and apoptosis, as well as genes in bone morphogenetic protein (BMP), Notch, Wnt, EPH, and MYC signaling pathways. BMP antagonists gremlin 1, gremlin 2, and chordin-like 1 were found to be expressed by colon crypts. In situ hybridization and RT-PCR confirmed that these BMP antagonists are expressed by intestinal cryptal myofibroblasts and smooth muscle cells at the colon crypt. In vitro analysis demonstrated that gremlin 1 partially inhibits Caco-2 cell differentiation upon confluence and activates Wnt signaling in normal rat intestinal epithelial cells. Collectively, the expression data set provides a comprehensive picture of human colonic epithelial cell differentiation. Our study also

suggests that BMP antagonists are candidate signaling components that make up the intestinal epithelial stem cell niche.

Kremens, B., R. Wieland, et al. (2003). "High-dose chemotherapy with autologous stem cell rescue in children with retinoblastoma." Bone Marrow Transplant **31**(4): 281-4.

Children with metastatic retinoblastoma are considered to have a poor prognosis after conventional chemotherapy. We used high-dose chemotherapy (HDC) with peripheral hematopoietic stem cell transplantation in such patients in an attempt to improve their survival. Four patients with bone marrow metastases and one child with extraorbital disease were treated with HDC after achieving complete remission by enucleation and conventional chemotherapy. The child with extraorbital tumor was the only one to receive local irradiation. The conditioning regimen included thiotepa (900 mg/m(2)), etoposide (40 mg/kg) and carboplatin (1.5 g/m(2)) in four patients, and BCNU (300 mg/m(2)), cyclophosphamide (6.8 g/m(2)) and etoposide (1.6 g/m(2)) in one child. Hematologic recovery occurred without delay in all patients. The main toxicities were diarrhea, mucositis and infectious complications. No toxic deaths or any major late toxicities were observed. The child treated with the BCNU regimen developed a meningeal relapse 10 months after HDC, which was partially resected and treated with conventional chemotherapy, but not with radiotherapy. He is in complete remission (CR) 105 months off treatment. The other patients are in CCR for 107, 57, 9 and 8 months after HDC. HDC with thiotepa, etoposide and carboplatin may represent a curative option for children with extrabulbar or disseminated retinoblastoma responsive to chemotherapy. It may control occult CNS disease. The necessity to irradiate these children and the curative potential of this strategy for patients with bulky CNS disease remain to be determined.

Kruse, F. E. and C. Cursiefen (2008). "Surgery of the cornea: corneal, limbal stem cell and amniotic membrane transplantation." Dev Ophthalmol **41**: 159-70.

PURPOSE: To demonstrate surgical treatment options for complications of severe forms of dry eye at the cornea, limbus and conjunctiva. METHODS: Corneal, limbal and conjunctival surgical treatment strategies are outlined. Results: Amniotic membrane transplantation, different forms of corneal transplantation and limbal stem cell surgery all are treatment options for complications of dry eye disease. CONCLUSIONS: Nowadays a broad spectrum of surgical treatment options exists to treat corneal complications of severe forms of dry eye at the ocular surface. Currently available conservative therapy for patients with 'dry eye' is primarily focused on augmenting or stabilizing the tear film and reducing primary or secondary causative factors such as inflammation of the ocular surface. While most patients with 'mild' and 'moderate' forms of dry eye (accounting for more than 95% of all patients with dry eye) can be treated sufficiently with drug treatment as well as environmental measures, some patients with very severe forms of dry eye need surgical intervention. Corneal surgery in the context of dry eye has primarily the objective to correct surface pathologies of the cornea caused by severe dysfunctions of the precorneal tear film. This primarily means persistent epithelial defects of the ocular surface, corneal ulcerations and consecutive corneal scarring. Besides conservative approaches, the first can be treated by amniotic membrane transplantation. Lamellar or perforating corneal transplantations are used to treat stromal scarring or perforated ulcerations as a sequel of persistent epithelial defects and associated apoptotic degeneration of stromal keratocytes. Finally, limbal stem cell transplantation can correct limbal stem cell deficiency states associated with or caused by diseases leading to severe forms of dry eye (e.g. chemical burns leading to destruction of conjunctival mucus-producing cells). All three surgical approaches will be discussed below.

Kuo, T. K., J. H. Ho, et al. (2009). "Mesenchymal stem cell therapy for nonmusculoskeletal diseases: emerging applications." Cell Transplant **18**(9): 1013-28.

Mesenchymal stem cells are stem/progenitor cells originated from the mesoderm and can different into multiple cell types of the musculoskeletal system. The vast differentiation potential and the relative ease for culture expansion have established mesenchymal stem cells as the building blocks in cell therapy and tissue engineering applications for a variety of musculoskeletal diseases, including repair of fractures and bone defects, cartilage regeneration, treatment of osteonecrosis of the femoral head, and correction of genetic diseases such as osteogenesis imperfect. However, research in the past decade has revealed differentiation potentials of mesenchymal stem cells beyond lineages of the mesoderm, suggesting broader applications than originally perceived. In this article, we review the recent developments in mesenchymal stem cell research with respect to their emerging properties and applications in nonmusculoskeletal diseases.

Lawrence, J. M., S. Singhal, et al. (2007). "MIO-M1 cells and similar muller glial cell lines derived from adult human retina exhibit neural stem cell characteristics." Stem Cells **25**(8): 2033-43.

Growing evidence suggests that glial cells may have a role as neural precursors in the adult central nervous system. Although it has been shown that Muller cells exhibit progenitor characteristics in the postnatal chick and rat retinae, their progenitor-like role in developed human retina is unknown. We first reported the Muller glial characteristics of the spontaneously immortalized human cell line MIO-M1, but recently we have derived similar cell lines from the neural retina of several adult eye donors. Since immortalization is one of the main properties of stem cells, we investigated whether these cells expressed stem cell markers. Cells were grown as adherent monolayers, responded to epidermal growth factor, and could be expanded indefinitely without growth factors under normal culture conditions. They could be frozen and thawed without losing their characteristics. In the presence of extracellular matrix and fibroblast growth factor-2 or retinoic acid, they acquired neural morphology, formed neurospheres, and expressed neural stem cell markers including betaIII tubulin, Sox2, Pax6, Chx10, and Notch 1. They also expressed markers of postmitotic retinal neurons, including peripherin, recoverin, calretinin, S-opsin, and Brn3. When grafted into the subretinal space of dystrophic Royal College of Surgeons rats or neonatal Lister hooded rats, immortalized cells migrated into the retina, where they expressed various markers of retinal neurons. These observations indicate that adult human neural retina harbors a population of cells that express both Muller glial and stem cell markers and suggest that these cells may have potential use for cell-based therapies to restore retinal function. Disclosure of potential conflicts of interest is found at the end of this article.

Lee, S. H., K. H. Yoo, et al. (2008). "Tandem high-dose chemotherapy and autologous stem cell rescue in children with bilateral advanced retinoblastoma." Bone Marrow Transplant **42**(6): 385-91.

Although external-beam radiation therapy (EBRT) has been an effective treatment modality in patients with bilateral advanced retinoblastoma, it significantly increases the risk of second malignancies and facial deformities. This study aimed to evaluate the efficacy of tandem high-dose chemotherapy and autologous stem cell rescue (HDCT/ASCR) for treatment, instead of EBRT, in children with bilateral advanced retinoblastoma. Fourteen patients with bilateral retinoblastoma received chemotherapy, and local therapy was provided whenever possible. When at least one functional eye could not be saved by chemoreduction and local therapy, tandem HDCT/ASCR was provided to avoid EBRT. As a result, nine patients received tandem HDCT/ASCR. The toxicities were tolerable and there was no TRM. All nine patients who received tandem HDCT/ASCR had at least one functional eye without EBRT, and in two patients, both eyes were saved. No second malignancy has developed to date. HDCT/ASCR might be an effective treatment for bilateral advanced retinoblastoma, especially in cases in which at least one functional eye could not be preserved with chemoreduction and local therapy alone, and where EBRT was unavoidable. Long-term follow-up and further studies are needed to evaluate the efficacy and toxicity of HDCT/ASCR as an alternative treatment to EBRT.

Liang, L., H. Sheha, et al. (2009). "Long-term outcomes of keratolimbal allograft for total limbal stem cell deficiency using combined immunosuppressive agents and correction of ocular surface deficits." Arch Ophthalmol **127**(11): 1428-34.

OBJECTIVE: To determine the long-term outcomes of keratolimbal allograft (KLAL). METHODS: Scores of such risks as infrequent blinking, blink-related microtrauma, conjunctival inflammation, elevated intraocular pressure, dry eye, symblepharon, lagophthalmos, and previous KLAL or penetrating keratoplasty (PKP) failure were calculated and recorded before, during, and after KLAL. Prolonged oral mycophenolate mofetil and tacrolimus and short-term prednisone and acyclovir were administered in 12 eyes (10 consecutive patients) with total limbal stem cell deficiency after KLAL. Ten eyes underwent subsequent PKP. RESULTS: More corrective measures were required in eyes with higher risk scores. During a follow-up of 61.2 months (standard deviation [SD], 18.2; range, 36-91 months) after KLAL, postoperative epithelial breakdown due to exposure occurred late in the period after PKP and remained a primary risk. Mean daily doses of 1.4 g of mycophenolate mofetil and 1.6 mg of tacrolimus were administered for 52.7 months (SD, 22.5; range, 23-91 months) with few adverse effects and reached trough levels of 1.6 microg/mL (SD, 0.6 microg/mL) and 4.5 ng/mL (SD, 2 ng/mL), respectively. Keratolimbal allograft and PKP rejection was noted in 2 and 3 eyes, respectively, though there was a reversal in 1 eye in each group, yielding final KLAL and PKP survivals in 10 and 8 eyes, respectively, and ambulatory visual acuity of up to 20/20 in 10 eyes for 67.2% of the entire follow-up period. CONCLUSION: Correction of ocular surface deficits combined with an immunosuppressive regimen further improves the long-term outcome of KLAL in eyes with total limbal stem cell deficiency.

Lim, K. H., S. Kim, et al. (2008). "Central pontine myelinolysis in a patient with acute lymphoblastic leukemia after hematopoietic stem cell transplantation: a case report." J Korean Med Sci **23**(2): 324-7.

We describe a 37-yr-old man who developed central pontine myelinolysis (CPM) after allogeneic hematopoietic stem cell transplantation (HSCT) for acute lymphoblastic leukemia. After HSCT, desquamation developed on the whole body accompanied by hyperbilirubinemia. The liver biopsy of the patient indicated graft-versus-host disease-related liver disease, and the dose of methylprednisolone was increased. Then, the patient developed altered mentality with eye ball deviation to the left, for which electroencephalogram and magnetic resonance imaging (MRI) scans were done. Brain MRI scan demonstrated the imaging findings consistent with central pontine myelinolysis and extrapontine myelinolysis. He did not have any hyponatremia episode during hospitalization prior to the MRI scan. To the best of our knowledge, presentation of CPM after allogeneic HSCT is extremely rare in cases where patients have not exhibited any episodes of significant hyponatremia. We report a rare case in which hepatic dysfunction due to graft-versus-host disease has a strong association with CPM after HSCT.

Limb, G. A., J. T. Daniels, et al. (2006). "Current prospects for adult stem cell-based therapies in ocular repair and regeneration." Curr Eye Res **31**(5): 381-90.

Recent advances in stem cell biology have led to the exploration of stem cell-based therapies to treat a wide range of human diseases. In the ophthalmic field, much hope has been placed on the potential use of these cells to restore sight, particularly in those conditions in which other established treatments have failed and in which visual function has been irreversibly damaged by disease or injury. At present, there are many limitations for the immediate use of embryonic stem cells to treat ocular disease, and as more evidence emerges that adult stem cells are present in the adult human eye, it is clear that these cells may have advantages to develop into feasible therapeutic treatments without the problems associated with embryonic research and immune rejection. Here we discuss the current prospects for the application of various adult ocular stem cells to human therapies for restoration of vision.

Lord-Grignon, J., M. Abdouh, et al. (2006). "Identification of genes expressed in retinal progenitor/stem cell colonies isolated from the ocular ciliary body of adult mice." Gene Expr Patterns **6**(8): 992-9.

Rare pigmented cells showing retinal stem cell characteristics have been identified in the ocular ciliary body (CB) of adult mammals. In vitro, these cells were reported to clonally proliferate and generate pigmented sphere colonies (PSC) containing multipotent retinal progenitor-like cells. Because these cells may have important clinical applications and because their embryonic origin is unclear, we have analyzed their local environment and gene expression profile. We found that transcription factors Pax6, Six3, and Rx, all involved in early eye morphogenesis, were expressed in the CB of adult mice. By sequencing a PSC cDNA library, we found that PSC expressed at high levels transcripts involved in the control of redox metabolism and cellular proliferation. PSC also expressed the retinal transcription factor Six6, which expression was not detected in the CB epithelium. By in situ hybridization screen, we found that Palmdelphin (Palm), Hmga2, and a novel transcript were expressed in the central nervous system of early embryos. Palm expression delineated the pigmented epithelium of the future CB and the developing myotome. Hmga2 was expressed in the ventricular zone of the telencephalon, the developing retinal ciliary margin and lens. Several genes expressed in PSC were also expressed in the nasal anlagen. Taken together, our study reveals that PSC isolated from the ocular CB express genes involved in the control of embryonic development, retinal identity, redox metabolism, and cellular proliferation.

Luengo Gimeno, F., V. Lavigne, et al. (2007). "Advances in corneal stem-cell transplantation in rabbits with severe ocular alkali burns." J Cataract Refract Surg **33**(11): 1958-65.

PURPOSE: To evaluate the efficacy of autologous corneal epithelial sheet implantation in restoring transparency of rabbit corneas severely injured by alkaline and the effect of photocoagulation in arresting corneal neovessel ingrowth. SETTING: Ophthalmology Department, School of Biomedical Sciences, Universidad Austral, Buenos Aires, Argentina. METHODS: Limbal stem-cell deficiency (LSCD) was induced in 14 rabbits by alkali burns. A limbal cell biopsy was done in the contralateral eye, and the cells were cultured on a fibroblast feeder layer grown on autologous clotted platelet-poor plasma or commercial fibrin for 21 days. Anterior keratectomy was followed by suturing corneal cell sheets over the stroma. If regrowth of vessels occurred, argon laser photocoagulation was applied to them. Rabbits were killed at 30, 60, 90, 180, and 360 days and the corneas processed for histopathology and

inmunohistochemistry. RESULTS: A small (2.5 mm(2)) limbal biopsy achieved stem-cell replication in vitro. Corneal clarity and epithelial defects evolved with a trend toward improvement. There was a significant reduction in corneal neovascularization. Histology showed a multilayered stratified epithelium including several epithelial-like cells with clear cytoplasm in the deepest part. There were no signs of intraepithelial mucin cells on the implanted corneas. Immunohistochemical results showed expression of cytokeratins 3 and 12 in the central corneal epithelium and an absence of cytokeratin 19. CONCLUSIONS: Autologous limbal epithelial cell transplantation improved the corneal surface in eyes with LSCD. Photocoagulation of neovessel ingrowth was effective over the 1-year follow-up. Results may facilitate the application of this technique in patients.

Lund, R. D., S. Wang, et al. (2006). "Human embryonic stem cell-derived cells rescue visual function in dystrophic RCS rats." Cloning Stem Cells **8**(3): 189-99.

Embryonic stem cells promise to provide a well-characterized and reproducible source of replacement tissue for human clinical studies. An early potential application of this technology is the use of retinal pigment epithelium (RPE) for the treatment of retinal degenerative diseases such as macular degeneration. Here we show the reproducible generation of RPE (67 passageable cultures established from 18 different hES cell lines); batches of RPE derived from NIH-approved hES cells (H9) were tested and shown capable of extensive photoreceptor rescue in an animal model of retinal disease, the Royal College of Surgeons (RCS) rat, in which photoreceptor loss is caused by a defect in the adjacent retinal pigment epithelium. Improvement in visual performance was 100% over untreated controls (spatial acuity was approximately 70% that of normal nondystrophic rats) without evidence of untoward pathology. The use of somatic cell nuclear transfer (SCNT) and/or the creation of banks of reduced complexity human leucocyte antigen (HLA) hES-RPE lines could minimize or eliminate the need for immunosuppressive drugs and/or immunomodulatory protocols.

Lyall, D. A., S. Srinivasan, et al. (2009). "Limbal stem cell failure secondary to advanced conjunctival squamous cell carcinoma: a clinicopathological case report." BMJ Case Rep **2009**.

A 67-year-old man with a history of multiple myeloma (treated with chemotherapy) was referred with a left hyperaemic conjunctival lesion covering almost 360 degrees of the limbus and extending onto the corneal surface. Conjunctival biopsy revealed conjunctival intraepithelial neoplasia. Initial treatment consisted of topical and intralesional injections of interferon alpha-2b. The patient subsequently developed limbal stem cell deficiency resulting in a persistent non-healing corneal epithelial defect. This was successfully managed with total excisional biopsy of the lesion, combined with limbal stem cell autograft (from the fellow eye) and amniotic membrane transplantation. Histopathology revealed a conjunctival squamous cell carcinoma. The corneal epithelium completely healed postoperatively and there is no evidence of tumour recurrence at 1 year follow-up. This case highlights a rare case of advanced ocular surface neoplasia causing secondary limbal stem cell deficiency. Medical and surgical management of ocular surface neoplasia with limbal stem cell transplantation is effective in treating such cases.

Lyngholm, M., H. Vorum, et al. (2008). "Differences in the protein expression in limbal versus central human corneal epithelium--a search for stem cell markers." Exp Eye Res **87**(2): 96-105.

In the search for potential limbal stem cell protein markers, the purpose of this study was to characterize differences in protein expression between human central and limbal corneal epithelium by a proteomic approach using two-dimensional polyacrylamide gel electrophoresis (2D PAGE) combined with mass spectrometry (LC-MS/MS). The results were subsequently confirmed by Western blotting and immunohistochemistry. We detected more than 1000 protein spots in each gel. Thirty-two spots were significantly over-expressed in the central part and 70 spots were significantly over-expressed in the limbal part. We identified 25 different proteins. Among these 11 proteins representing different cellular locations and functions were selected for further investigations. Most interestingly, superoxide dismutase 2 (SOD2), was expressed in clusters of cells in the basal limbal epithelium. Heat shock protein 70 protein 1 (HSP70.1) and annexin I were highly abundant in limbal epithelium, although they were also present in the central epithelium to a minor extent. Among the proteins primarily expressed in the limbal fraction we further identified cytokeratin (CK) 15, CK19 and alpha enolase, which have been reported previously to be related to the limbal basal epithelium. The basal limbal epithelium consists of clusters of slow cycling limbal stem cells and rapid cycling transient amplifying cells. Ideally, proteins exclusively expressed in the limbal part of the epithelium may serve as markers for the basal limbal cells. SOD2 and CK15 identify clusters of limbal basal cells and therefore they may serve as markers

for limbal stem cells in conjunction with the earliest transient amplifying cells.

Marena, C., M. Zecca, et al. (2001). "Incidence of, and risk factors for, nosocomial infections among hematopoietic stem cell transplantation recipients, with impact on procedure-related mortality." Infect Control Hosp Epidemiol **22**(8): 510-7.

OBJECTIVES: To determine the incidence of, and risk factors for, nosocomial infections (NIs) occurring among hematopoietic stem cell transplantation (HSCT) recipients during hospitalization and to evaluate the impact of these NIs on patient outcome. DESIGN: A two-year prospective observational study in two HSCT units. PATIENTS: All patients admitted to the HSCT units between February 1997 and March 1999. SETTING: A teaching hospital. METHODS: After admission to the HSCT units, the patients were followed prospectively on a daily basis to collect all pertinent variables for the development of NIs. RESULTS: 49 NIs were identified in 34 of the 143 patients screened. The incidence of NIs and infected patients was 34.2% and 23.7%, respectively. The incidence density of NI was 8.96 per 1,000 patient-days. The most frequent NIs were bloodstream infections ([BSIs], 42.8%) and respiratory tract infections (28.6%). Other sites involved were as follows: eye (8.2%), urinary tract (6.1%), gastrointestinal tract (6.1%), skin (4.1%), ear (2%), and central venous catheter ([CVC], 2%). Because of the predominance and clinical relevance of BSIs, we examined both intrinsic and extrinsic risk factors associated with these infections. Independent risk factors for BSIs were allograft from matched unrelated or partially matched family donor, graft-versus-host disease (GVHD) prophylaxis without methotrexate (MTX), type of CVC, and duration of total parenteral nutrition. Four variables were independently associated with mortality occurring during hospitalization: culture-proven BSIs, advanced disease phase at transplant, type of transplant, and absence of MTX for GVHD prophylaxis. CONCLUSIONS: The study identified several factors associated with increased risk of BSIs among HSCT patients. Because BSIs are life-threatening complications for HSCT recipients, preventive measures aimed at reducing the incidence of these infections among patients given HSCT should be adopted.

Meallet, M. A., E. M. Espana, et al. (2003). "Amniotic membrane transplantation with conjunctival limbal autograft for total limbal stem cell deficiency." Ophthalmology **110**(8): 1585-92.

PURPOSE: To evaluate the outcomes of corneal surface reconstruction with conjunctival limbal autograft when combined with amniotic membrane transplantation on both the donor and recipient eyes. DESIGN: Retrospective, noncomparative, interventional small case series. PARTICIPANTS: Five eyes of five patients with total limbal stem cell deficiency (LSCD) resulting from pseudopemphigoid (n = 1), chemical burns (n = 3), and extensive removal of conjunctival intraepithelial neoplasia (n = 1) were operated on by one surgeon (SCGT). INTERVENTION: After the removal of fibrovascular pannus from the corneal surface, two conjunctival limbal free grafts were harvested from the fellow eyes in all five patients with unilateral LSCD. Amniotic membrane, with the basement membrane side up, was grafted onto the defect created at the donor site and onto the recipient corneal and limbal sclera before placement of conjunctival limbal grafts. MAIN OUTCOME MEASURES: Symptomatic relief, improvement in visual acuity, fornix deepening, and rapid healing and restoration of normal cornea and limbus in the recipient and donor eyes were assessed. RESULTS: During the mean follow-up of 22 months (range, 11-48 months), all eyes experienced symptomatic relief. All recipient eyes had a mean improvement in visual acuity of nine lines (range, 7-12). The three eyes with stromal vascularization showed regression, and all recipient eyes had marked improvement in corneal clarity. Three eyes receiving simultaneous symblepharon lysis and fornix reconstruction successfully regained deep, stable fornices. The donor eyes showed rapid healing and restoration of the normal limbal landmark, even in one eye where nearly the entire limbus was removed. CONCLUSIONS: Limbal conjunctival transplantation is an effective procedure for restoring the corneal surface integrity in eyes with total LSCD. The additional use of amniotic membrane may contribute to a higher rate of success in the recipient eye and a lower rate of complications in the donor eye, as well as allow the simultaneous correction of concomitant cicatricial abnormalities.

Meisler, D. M., V. L. Perez, et al. (2005). "A device to facilitate limbal stem cell procurement from eye bank donor tissue for keratolimbal allograft procedures." Am J Ophthalmol **139**(1): 212-4.

PURPOSE: To develop a device that facilitates the procurement of corneal limbal stem cell grafts for keratolimbal allograft procedures used in the treatment of ocular surface disease associated with stem cell deficiency. DESIGN: Description of device design and technique for use. METHODS: The device is composed of a pedestal with a convex surface mounted to a flat platform. A corneoscleral button placed endothelial side down and centrally upon the convexity is secured by suction conveyed through a

hollowed core in the pedestal that connects to fenestrated openings on the convex surface. A donut-shaped stainless steel ring placed on tension by springs braces the peripheral tissue. A circular corneal incision is created of a desired thickness by a suction trephine, and a crescent blade is utilized to peripherally dissect a donut-shaped keratolimbal allograft. RESULTS: This device facilitated the harvesting of the keratolimbal allograft tissue from four eye bank donor practice corneoscleral buttons and was then used to successfully procure grafts from six corneoscleral buttons used in three keratolimbal allograft procedures in three patients, one each with aniridia, alkali burn, and drug-induced limbal stem cell deficiency. CONCLUSIONS: The described device effectively facilitates procurement of corneoscleral buttons for keratolimbal allograft procedures. It appears to offer advantages over freehanded techniques and previously described devices used for the same purpose.

Meller, D., T. Fuchsluger, et al. (2009). "Ocular surface reconstruction in graft-versus-host disease with HLA-identical living-related allogeneic cultivated limbal epithelium after hematopoietic stem cell transplantation from the same donor." Cornea **28**(2): 233-6.

PURPOSE: To report a case of HLA-identical allogeneic living-related ex vivo expanded limbal epithelium in ocular surface reconstruction for chronic graft-versus-host disease (cGVHD). METHODS: Review the clinical findings in a 58-year-old woman with bilateral limbal stem cell deficiency caused by cGVHD who underwent ocular surface reconstruction on the left eye with cultivated limbal epithelial cells (LECs) on intact human amniotic membrane combined with extracapsular cataract extraction and intraocular lens implantation. LECs were harvested from a small biopsy of the same HLA-identical living-related donor who already donated peripheral blood cells for hematopoietic stem cell transplantation. RESULTS: At the present state, after a follow-up of 31 months, the patient shows a successful ocular surface reconstruction with a clear, smooth, and stable corneal ocular surface without recurrence of limbal stem cell deficiency. Nine months postoperatively, patients' corneal thinning progressed to a small perforation, which was successfully treated with a tectonic perforating keratoplasty combined with the removal of irritating lid lashes. CONCLUSION: The technique of HLA-typed allogeneic ex vivo expansion of LECs harvested from the same living-related donors who already donated hematopoietic stem cells offers a possibility to reconstruct ocular surface in cGVHD.

Mendicute, J., A. Bidaguren, et al. (2008). "Automatized large diameter lamellar keratoplasty and stem cell transplantation for the treatment of ocular surface diseases with limbal insufficiency." Eur J Ophthalmol **18**(4): 641-4.

PURPOSE: To report a new surgical procedure for the treatment of ocular surface diseases associated with severe limbal insufficiency. METHODS: A retrospective review of four patients with severe ocular surface disease who required stem cell transplantation and keratoplasty for the correction of limbal insufficiencies. They underwent large diameter lamellar keratoplasty with microkeratome. When limbal dysfunction was associated with limited alteration of the ocular surface and transparent deep corneal stroma only the anterior corneal stroma was transplanted. When the entire corneal thickness was compromised, both anterior and deep donor buttons were transplanted. RESULTS: Patients remained stable and improved their visual acuity after surgery. Best-corrected visual acuity ranged from 20/200 to 20/30. No corneal graft rejections were found. The main complication found in one of our patients was a central stromal opacity which required a secondary penetrating keratoplasty. CONCLUSIONS: Automatized large diameter lamellar keratoplasty provides a safe and successful alternative to limbal transplantation for limbal insufficiency associated with corneal opacity. This technique enables a single-stage surgical procedure and the use of a single donor which reduces the risk of rejection. In addition, better refractive results are achieved due to the quality of the interface and the absence of corneal sutures.

Mimeault, M. and S. K. Batra (2008). "Recent progress on tissue-resident adult stem cell biology and their therapeutic implications." Stem Cell Rev **4**(1): 27-49.

Recent progress in the field of the stem cell research has given new hopes to treat and even cure diverse degenerative disorders and incurable diseases in human. Particularly, the identification of a rare population of adult stem cells in the most tissues/organs in human has emerged as an attractive source of multipotent stem/progenitor cells for cell replacement-based therapies and tissue engineering in regenerative medicine. The tissue-resident adult stem/progenitor cells offer the possibility to stimulate their in vivo differentiation or to use their ex vivo expanded progenies for cell replacement-based therapies with multiple applications in human. Among the human diseases that could be treated by the stem cell-based therapies, there are hematopoietic and immune disorders, multiple degenerative disorders, such as Parkinson's and Alzheimer's diseases, type 1 or 2 diabetes mellitus as well as eye, liver, lung, skin

and cardiovascular disorders and aggressive and metastatic cancers. In addition, the genetically-modified adult stem/progenitor cells could also be used as delivery system for expressing the therapeutic molecules in specific damaged areas of different tissues. Recent advances in cancer stem/progenitor cell research also offer the possibility to targeting these undifferentiated and malignant cells that provide critical functions in cancer initiation and progression and disease relapse for treating the patients diagnosed with the advanced and metastatic cancers which remain incurable in the clinics with the current therapies.

Mimeault, M., R. Hauke, et al. (2007). "Stem cells: a revolution in therapeutics-recent advances in stem cell biology and their therapeutic applications in regenerative medicine and cancer therapies." Clin Pharmacol Ther **82**(3): 252-64.

Basic and clinical research accomplished during the last few years on embryonic, fetal, amniotic, umbilical cord blood, and adult stem cells has constituted a revolution in regenerative medicine and cancer therapies by providing the possibility of generating multiple therapeutically useful cell types. These new cells could be used for treating numerous genetic and degenerative disorders. Among them, age-related functional defects, hematopoietic and immune system disorders, heart failures, chronic liver injuries, diabetes, Parkinson's and Alzheimer's diseases, arthritis, and muscular, skin, lung, eye, and digestive disorders as well as aggressive and recurrent cancers could be successfully treated by stem cell-based therapies. This review focuses on the recent advancements in adult stem cell biology in normal and pathological conditions. We describe how these results have improved our understanding on critical and unique functions of these rare sub-populations of multipotent and undifferentiated cells with an unlimited self-renewal capacity and high plasticity. Finally, we discuss some major advances to translate the experimental models on ex vivo and in vivo expanded and/or differentiated stem cells into clinical applications for the development of novel cellular therapies aimed at repairing genetically altered or damaged tissues/organs in humans. A particular emphasis is made on the therapeutic potential of different tissue-resident adult stem cell types and their in vivo modulation for treating and curing specific pathological disorders.

Miyamura, F., S. Kako, et al. (2009). "Successful treatment of young-onset adult T cell leukemia/lymphoma and preceding chronic refractory eczema and corneal injury by allogeneic hematopoietic stem cell transplantation." Int J Hematol **90**(3): 397-401.

Only some carriers of human T cell lymphotropic virus type I (HTLV-1) develop adult T cell leukemia/lymphoma (ATLL) after a long latency period, and an association has been reported between chronic refractory eczema, known as infective dermatitis, and young-onset ATLL. A 25-year-old female developed ATLL and underwent allogeneic hematopoietic stem cell transplantation (HSCT) in non-remission. She had chronic refractory eczema and corneal injury at the onset of ATLL. Remission of ATLL was achieved, and the HTLV-1 proviral load decreased after HSCT. In addition, her pre-existing eczema and corneal injuries almost disappeared. More than a year has passed since the transplantation was performed, and she has had no recurrence of either ATLL or lesions in the skin and eye. Her clinical course suggests a possible association between skin and eye lesions and HTLV-1 infection. Changes in the immunological condition after HSCT might play a key role. Special attention is needed when HTLV-1 carriers develop eye or skin lesions.

Mohammadpour, M., M. A. Javadi, et al. (2006). "Limbal stem cell deficiency in the context of autoimmune polyendocrinopathy." Eur J Ophthalmol **16**(6): 870-2.

PURPOSE: To report two sisters with bilateral progressive visual loss and photophobia secondary to stem cell deficiency due to multiple endocrine deficiency. METHODS: Case reports and review of medical literature. RESULTS: The younger sister had severe photophobia and decreased visual acuity since May 2000. Despite multiple outpatient visits no definite cause was found and conservative treatments failed. On slit lamp examination severe meibomian gland dysfunction, loss of eyelashes, decreased tear meniscus, diffuse corneal vascularization, and delayed punctate fluorescein staining of corneal epithelium were detected. She also had episodes of hypotension, oral candidiasis, and seizures. Her systemic workup revealed multiple endocrine deficiency (Addison's disease and hypoparathyroidism). Hormone replacement therapy with fludrocortisone and oral calcium accompanied by punctual occlusion led to significant clinical recovery . The older sister showed a similar pattern but interestingly the onset was later and the signs and symptoms were milder. CONCLUSIONS: In the pediatric age group with diffuse corneal vascularization and no definite cause, systemic workup should be done to rule out multiple endocrine deficiencies. The therapy consists of hormone replacement therapy and management of dry eye.

Mohty, M., M. Kuentz, et al. (2002). "Chronic graft-versus-host disease after allogeneic blood stem cell transplantation: long-term results of a randomized study." Blood **100**(9): 3128-34.

The use of peripheral blood stem cells (PBSCs) is rapidly growing in the allogeneic transplantation setting as an alternative to bone marrow (BM). We previously reported a higher incidence of chronic graft-versus-host disease (cGVHD) associated with allogeneic PBSC transplantation in a randomized trial. In this follow-up report, we analyzed the evolution of cGVHD in the patients (n = 101) enrolled on this study. At a median follow-up of 45 months (range, 31-57 months), we found that the 3-year cumulative incidence of cGVHD was 65% (95% confidence interval [CI] 51%-78%) in the PBSC group and 36% (95% CI 23%-49%) in the BM group (P =.004). We also found that extensive cGVHD was more frequent in the PBSC group (44% [95% CI 30%-58%] vs 17% [95% CI 7%-27%]; P =.004). The prevalence of cGVHD was always higher in the PBSC arm. Ocular involvement was more frequent in PBSC recipients (P =.02). Cutaneous and liver involvement was similar among BM and PBSC recipients. Chronic GVHD required multiple courses of immunosuppressive therapy in addition to cyclosporine and corticosteroids during longer periods (P =.03). Altogether, this translated into longer periods of hospitalization after transplantation in the PBSC group (P =.04). Finally, we also confirm that cGVHD after PBSC transplantation is associated with an antileukemic effect that is at least as potent as after BM. However, to date, this has not translated into a survival difference, possibly due to the early-stage leukemic status of these patients or to the relatively small size of the study population.

Mori, T., M. Watanabe, et al. (2008). "Reduced efficacy of topical corticosteroid in preventing cytarabine-induced kerato-conjunctivitis in patients receiving high-dose cytarabine and total body irradiation for allogeneic hematopoietic stem cell transplantation." Bone Marrow Transplant **42**(3): 197-9.

This study aimed to retrospectively evaluate the incidence of kerato-conjunctivitis in patients receiving TBI followed by high-dose cytarabine, and to clarify how effectively topical corticosteroid eye drops prevent kerato-conjunctivitis in these patients. Fifty-three patients who received cytarabine at a dose of 3 g/m2 every 12 h for 4 days after receiving TBI (12 Gy) as a conditioning for allogeneic hematopoietic stem cell transplantation (HSCT) were evaluated. For the prophylaxis of kerato-conjunctivitis, all patients received betamethasone sodium phosphate eye drops every 6 h, starting 1 day before the first dose of cytarabine and continuing until 1 day after the last dose of cytarabine or the complete resolution of ocular symptoms. For grading of kerato-conjuncitivitis, the National Cancer Institute-Common Toxicity Criteria were used. Among the 53 patients, the grades of kerato-conjunctivitis were grade 0 in 13 patients, grade 1 in 6 patients (11.3%), grade 2 in 10 patients (18.9%) and grade 3 in 25 patients (47.2%). These results strongly suggest that topical corticosteroid eye drops could not effectively prevent the development of cytarabine-induced kerato-conjunctivitis in HSCT recipients who receive high-dose cytarabine following TBI. Further investigation into a more effective prophylaxis for cytarabine-induced kerato-conjunctivitis in this setting is required.

Mouriaux, F., F. Chahud, et al. (2001). "Implication of stem cell factor in the proliferation of choroidal melanocytes." Exp Eye Res **73**(2): 151-7.

The tyrosine kinase receptor c-kit and its ligand stem cell factor exert a broad range of biological activities during organogenesis. It also improves normal cell development including complex biological responses involved in the differentiation and proliferation of the melanocytes. Diffuse uveal melanocytic proliferation is a rare paraneoplasic syndrome, resulting in rapid bilateral visual loss due to proliferation of melanocytes within the choroid. We have therefore investigated whether the c-kit/stem cell factor pathway regulates the proliferation of choroidal melanocytes and also if such pathway plays a role in bilateral uveal melanocytic proliferation. Normal cultured melanocytes of the choroid and paraffin-embedded sections of melanocytic proliferation were studied. C-kit expression and effects of stem cell factor were measured. Western blot assays of cell extracts demonstrated that c-kit was expressed in choroidal melanocytes. Immunocytochemical analysis on cultured melanocytes showed a cytoplasmic distribution. Immunohistochemical analysis on melanocytic proliferation showed a strong cytoplasmic distribution in the pigmented spindle-shaped melanocytes localized in the multiple focal areas of choroidal thickening. The addition of stem cell factor did not change melanocyte morphologies and was mitogenic in the presence of bFGF, isobutyl-1-methylxanthine and cholera toxin. In contrast, stem cell factor was not able to produce any significant melanin. Activation of c-kit by its ligand may contribute to the proliferation of choroidal melanocytes.

Nguyen, D. Q., S. Srinivasan, et al. (2007). "Thimerosal-induced limbal stem cell failure: report

of a case and review of the literature." Eye Contact Lens **33**(4): 196-8.

PURPOSE: To report a case of unilateral total limbal stem cell (LSC) failure and corneal opacification secondary to thimerosal- and contact lens-induced ocular surface toxicity. METHODS: Interventional case report and review of the literature on thimerosal-induced ocular surface changes. RESULTS: A 49-year-old woman with a 2-year history of long-term soft contact lens wear developed unilateral total LSC failure and corneal opacification secondary to presumed thimerosal-induced toxicity and contact lens wear. At presentation, best-corrected visual acuities were 20/120 in the right eye and 20/15 in the left eye. The patient underwent a keratolimbal allograft and amniotic membrane graft followed by a penetrating keratoplasty. At the last follow-up, the right eye showed a clear corneal graft with a best-corrected visual acuity of 20/30. CONCLUSIONS: Thimerosal toxicity can lead to total LSC failure with secondary corneal vascularization and opacification. Keratolimbal allograft followed by penetrating keratoplasty can be successful in reconstructing the ocular surface in such cases.

Nishida, K., M. Yamato, et al. (2004). "Functional bioengineered corneal epithelial sheet grafts from corneal stem cells expanded ex vivo on a temperature-responsive cell culture surface." Transplantation **77**(3): 379-85.

BACKGROUND: Limbal stem-cell deficiency by ocular trauma or diseases causes corneal opacification and visual loss. Recent attempts have been made to fabricate corneal epithelial graft constructs, but the technology is still evolving. We have developed a novel cell-sheet manipulation technology using temperature-responsive culture surfaces to generate functional, cultivated corneal epithelial cell sheet grafts. METHODS: Human or rabbit limbal stem cells were cocultured with mitomycin C-treated 3T3 feeder layers on temperature-responsive culture dishes at 37 degrees C. Cell sheets were harvested from the dishes after 2 weeks by reducing temperature to 20 degrees C. Histologic analyses, immunoblotting, and colony-forming assay were performed to characterize the cell sheets. Autologous transplantation was undertaken to reconstruct the corneal surfaces of rabbits with experimentally induced limbal stem cell deficiencies. RESULTS: Multilayered corneal epithelial sheets were harvested intact simply by reducing the temperature, without the use of proteases. Cell-cell junctions and extracellular matrix on the basal side of the sheet, critical to sheet integrity and function, remained intact. A viable population of corneal progenitor cells, close in number to that originally seeded, was found in the sheets. Harvested sheets were easily manipulated, transplantable without any carriers, and readily adhesive to corneal stroma so that suturing was not required. Corneal surface reconstruction in rabbits was highly successful. CONCLUSIONS: Cell sheet engineering technology allows us to create intact, transplantable corneal epithelial cell sheets that retain stem cells from limbal stem cells expanded ex vivo. Our research indicates highly promising clinical capabilities for our bioengineered corneal epithelial sheet.

Ogawa, Y. and M. Kuwana (2003). "Dry eye as a major complication associated with chronic graft-versus-host disease after hematopoietic stem cell transplantation." Cornea **22**(7 Suppl): S19-27.

PURPOSE: To review the condition of dry eye associated with chronic graft-versus-host disease (GVHD). METHODS: The immunopathogenic processes and therapeutic options for lacrimal gland chronic GVHD are discussed. RESULTS: Dry eye is the most frequent ocular complication after hematopoietic stem cell transplantation. The condition typically occurs around 6 months post-operation and is recognized as a complication of chronic GVHD. Lacrimal gland specimens from patients with dry eye show prominent fibrosis and an increase in CD34+ stromal fibroblasts in the glandular interstitium in addition to infiltration of T cells into the periductal areas. In periductal areas, CD4+ and CD8+ T cells colocalize with stromal fibroblasts that express the full component of surface molecules necessary for antigen presentation. These findings strongly suggest that periductal fibroblasts are involved in fibrogenic and immune processes by interacting with T cells in the lacrimal gland of patients with chronic GVHD, resulting in rapidly progressive dry eye. Current therapies for dry eye related to chronic GVHD include tear supplements and nonspecific immunosuppressants. CONCLUSION: We report a significant role for stromal fibroblasts in the pathogenic processes of dry eye related to chronic GVHD. Although several supportive therapies can reduce the symptoms, specific therapies that suppress fibrotic and immune processes in the lacrimal glands are necessary to control dry eye associated with chronic GVHD.

Ogawa, Y., S. Okamoto, et al. (1999). "Dry eye after haematopoietic stem cell transplantation." Br J Ophthalmol **83**(10): 1125-30.

AIMS: To determine the incidence, natural course, and severity of dry eye occurring or worsening after haematopoietic stem cell transplantation (SCT). METHODS: At a tertiary care hospital, 53 patients undergoing allogeneic or autologous SCT followed by

at least 180 days of follow up were studied prospectively. Examination included grading of symptoms of dry eye, evaluation of ocular surface, tear break up time, and Schirmer tests with and without nasal stimulation. Meibomian gland secretion was also examined using a slit lamp while applying steady digital pressure. RESULTS: Of the 53 patients, 44 received allografts. Half of these patients (22) developed dry eye or their pre-existing dry eye worsened after SCT, while none of nine autograft recipients did. Onset of dry eye was 171 (SD 59) days after SCT. Two types of dry eye occurred. One (n=10) was severe with ocular surface findings resembling Sjogren's syndrome and reduction of reflex tearing soon after onset. A mild type (n=12) had unimpaired reflex tearing. Meibomian gland dysfunction (MGD) was more frequent and severe in patients with dry eye and chronic graft versus host disease (GVHD), and overall severity of dry eye was greater in patients with MGD and chronic GVHD. CONCLUSIONS: Dry eye after SCT occurred only in allograft recipients, and was not evident in autograft recipients. The severe form of dry eye had a tendency to develop rapidly. Further study on the prediction and treatment of severe dry eye after SCT is necessary.

Oji, Y., Y. Oka, et al. (1999). "Successful treatment of relapsed T-cell non-Hodgkin's lymphoma with allogeneic peripheral blood stem cell transplantation with double conditioning." Int J Hematol **69**(4): 263-7.

We report a patient with T-cell non-Hodgkin's lymphoma (NHL) who relapsed after treatment with relatively intensive third-generation chemotherapy, VACOP-B, and who was safely and effectively treated with allogeneic peripheral blood stem cell transplantation (allo PBSCT) with double conditioning. The first conditioning consisted of carboplatin and etoposide. Twenty-one days later, the second conditioning was performed with cytosine arabinoside, cyclophosphamide, and total body irradiation (AraC/Cy/TBI). Between the periods of the first and second conditioning, autologous (auto) PBSCT (4.4 x 10(5) colony-forming units granulocyte/macrophage (CFU-GM)/kg, 3.8 x 10(6) CD34+ cells/kg) was performed to rescue marrow aplasia after the first conditioning. After the second conditioning, allo PBSCT (2.1 x 10(5) CFU-GM/kg, 8.2 x 10(6) CD34+ cells/kg) was performed from a human leukocyte antigen-identical sibling. Marrow reconstitution after allo PBSCT was rapid. Grade I acute graft-vs.-host disease (GVHD) involving skin and chronic GVHD on the eye was observed. No severe transplantation-related complications occurred. With a follow-up of 22 months after allogeneic PBSCT, the patient is alive without evidence of the disease. This case shows that allo PBSCT with intensive double conditioning may become a new treatment strategy to achieve long-term disease-free survival for young NHL patients of resistant relapse with a great deal of tumor burden and invasion of lymphoma cells in bone marrow.

Oyama, Y., R. K. Burt, et al. (2009). "A case of autoimmune-related retinopathy and optic neuropathy syndrome treated by autologous nonmyeloablative hematopoietic stem cell transplantation." J Neuroophthalmol **29**(1): 43-9.

Autoimmune-related retinopathy and optic neuropathy (ARRON) syndrome is characterized by visual loss and often the presence of antibodies against retinal or optic nerve antigens in the absence of cancer. Limited success has been reported in treatment of ARRON syndrome with medications that suppress the immune system. In many patients, current strategies are insufficient to control the disease. A 47-year-old woman with progressive visual and hearing loss attributed to ARRON syndrome that was resistant to conventional therapies underwent autologous hematopoietic stem cell transplantation (HSCT). Clinical manifestations appeared to stabilize. This report suggests that autologous HSCT may have a therapeutic role in ARRON syndrome.

Rama, P., S. Bonini, et al. (2001). "Autologous fibrin-cultured limbal stem cells permanently restore the corneal surface of patients with total limbal stem cell deficiency." Transplantation **72**(9): 1478-85.

BACKGROUND: Ocular burns cause depletion of limbal stem cells, which leads to corneal opacification and visual loss. Autologous cultured epithelial cells can restore damaged corneas, but this technology is still developing. We sought to establish a culture system that allows preservation of limbal stem cells and preparation of manageable epithelial sheets and to investigate whether such cultures can permanently restore total limbal stem cell deficiency. METHODS: We selected a homogeneous group of patients whose limbal cell deficiency was evaluated by scoring the gravity of the clinical picture and the keratin expression pattern. Stem cells, obtained from the limbus of the contralateral eye, were cultivated onto a fibrin substrate and their preservation was evaluated by clonal analysis. Fibrin cultures were grafted onto damaged corneas. RESULTS: Fibrin-cultured limbal stem cells were successful in 14 of 18 patients. Re-epithelialization occurred within the first week. Inflammation and vascularization regressed within the first 3-4 weeks. By the first month, the corneal surface was covered by a transparent, normal-looking epithelium. At 12-27 months follow-up, corneal surfaces were clinically and cytologically

stable. Three patients had a penetrating keratoplasty approximately 1 year after restoration of their corneal surface. Their visual acuity improved from light perception or counting fingers to 0.8-1.0. CONCLUSIONS: Preservation of limbal stem cells in culture gives new perspectives on the treatment of ocular disorders characterized by complete limbal stem cell deficiency. The multicenter nature of this study and the handiness and ease of long-distance transportation of the fibrin-cultured epithelial sheets suggest that this technology can now be widely applied.

Samson, C. M., C. Nduaguba, et al. (2002). "Limbal stem cell transplantation in chronic inflammatory eye disease." Ophthalmology **109**(5): 862-8.

OBJECTIVE: The goal of this study was to describe the outcome of limbal stem cell transplantation (LSCT) in patients with severe ocular surface disease caused by underlying chronic inflammatory eye disease. DESIGN: Retrospective noncomparative case series. PARTICIPANTS: Nine patients with limbal stem cell deficiency caused by an underlying ocular inflammatory disease who underwent LSCT. METHODS: The authors reviewed the records of 11 eyes of 9 patients with immunologically mediated ocular surface disease that underwent LSCT. MAIN OUTCOME MEASURES: The main outcome measures were reepithelialization of the corneal surface, restoration of corneal surface, and improvement in visual acuity. RESULTS: A total of 11 eyes underwent either autologous (n = 1) or HLA-matched living related donor (n = 10) LSCT for ocular surface disease secondary to inflammatory disease. Reepithelialization of the corneal surface in the immediate postoperative period occurred in 10 eyes (91%) within an average of 10 days (range, 3-21 days). Long-term restoration of the corneal surface was achieved in six (55%) eyes. Visual acuity improved in six eyes (55%). Reasons for poor outcomes included microbial infection, limbal stem cell graft rejection, and corneal ulceration. No donor eyes had complications. CONCLUSIONS: Patients with underlying immunologically mediated diseases, such as Stevens-Johnson syndrome, toxic epidermal necrolysis, or ocular cicatricial pemphigoid, who undergo LSCT have lower success rates than do those patients with noninflammatory ocular surface diseases.

Sanders, J. E. (2002). "Chronic graft-versus-host disease and late effects after hematopoietic stem cell transplantation." Int J Hematol **76 Suppl 2**: 15-28.

Late effects following HSCT are related to either the transplant process or to the transplant preparative regimen. Problems related to the transplant process include delayed recovery of the immune system and chronic GVHD. Chronic GVHD presents between 3-14 months post-HSCT in approximately 20% of matched sibling transplants and 40% of matched unrelated donor recipients. Most commonly involved sites are skin, mouth, liver, gastrointestinal tract, and eye. Patients with platelet count < 100,000/ml and receiving cortocosteroid therapy at day 80 with any clinical manifestations of chronic GVHD require prolonged immune suppressive therapy with prednisone, cyclosporine +/- other agents. Treatment should be administered until all clinical and pathological signs and symptoms of chronic GVHD have resolved which may take one to several years. Problems related to the transplant preparative regimen include those involving the endocrine system, eyes, lungs, bone, and development of secondary malignancies. Endocrine deficiencies include growth failure with growth hormone (GH) deficiency, overt hypothyroidism, primary gonadal failure, Type 1 or Type 2 diabetes, and exocrine pancreatic insufficiency. These problems develop at any time post-HSCT, but usually occur within the first few years and should be treated with appropriate hormone supplementation. Eye problems are primarily related to development of cateracts secondary to total body irradiation (TBI) or prolonged corticosteroid use. Cateracts developing after fractionated frequently do not require removal. Pulmonary problems may be due to bronchiolitis obliterans (BO) or to restrictive lung disease. BO may be associated with chronic GVHD and may respond to chronic GVHD therapy. Restrictive lung disease does not occur for many years after HSCT. There is not therapy for this problem. Development of decreased bone mineral density (BMD) is related to GH deficiency and/or corticosteroid therapy. Treatment includes withdrawal of corticosteroids, administration of GH and calcium, Vitamin D and antiresorptive agents. All malignant disease survivors are at risk for development of secondary malignancies, including survivors of HSCT. Recipients of TBI are at highest risk as are children. All pediatric and adult survivors of HSCT should be followed for their life-time for development of delayed effects of transplantation.

Sangwan, V. S., M. Fernandes, et al. (2005). "Early results of penetrating keratoplasty following limbal stem cell transplantation." Indian J Ophthalmol **53**(1): 31-5.

PURPOSE: To describe the early results of penetrating keratoplasty (PKP) in patients who had earlier received limbal transplantation (LT). METHODS: Prospective, non-comparative interventional case series comprising of four patients with limbal stem cell deficiency (LSCD) due to

chemical injury (Cases 1, 2, 4) and xeroderma pigmentosum (Case 3). Cadaveric kerato-limbal allografts or living-related conjunctival-limbal allografts were done in four eyes followed by PKP for visual rehabilitation 3-4.5 months later. The following details were noted: demographics, primary aetiology, type of limbal transplant (cadaveric or living-related), immunosuppression, vision and ocular surface stability before and after LT and PKP, surgical complications and outcome of PKP. RESULTS: Three eyes received living-related conjunctival-limbal allotransplantation and one received cadaveric kerato-limbal allograft. Duration of follow up after PKP ranged from 4 to 11 months. Visual acuity improved in the early postoperative period in all patients but reduced in 2 due to endothelial rejection and after trans-scleral cyclophotocoagulation for medically uncontrolled glaucoma. The ocular surface remained stable in all patients. All patients were started on immunosuppression on the first postoperative day. This was continued till the last follow-up visit. Post-PKP complications were punctate epithelial keratopathy, corneal allograft rejection and secondary glaucoma (one patient each). CONCLUSION: Satisfactory visual rehabilitation is possible after PKP following LT without compromising ocular surface stability. However, a prolonged and close follow-up is warranted to avert complications.

Sansom, S. N., D. S. Griffiths, et al. (2009). "The level of the transcription factor Pax6 is essential for controlling the balance between neural stem cell self-renewal and neurogenesis." PLoS Genet **5**(6): e1000511.

Neural stem cell self-renewal, neurogenesis, and cell fate determination are processes that control the generation of specific classes of neurons at the correct place and time. The transcription factor Pax6 is essential for neural stem cell proliferation, multipotency, and neurogenesis in many regions of the central nervous system, including the cerebral cortex. We used Pax6 as an entry point to define the cellular networks controlling neural stem cell self-renewal and neurogenesis in stem cells of the developing mouse cerebral cortex. We identified the genomic binding locations of Pax6 in neocortical stem cells during normal development and ascertained the functional significance of genes that we found to be regulated by Pax6, finding that Pax6 positively and directly regulates cohorts of genes that promote neural stem cell self-renewal, basal progenitor cell genesis, and neurogenesis. Notably, we defined a core network regulating neocortical stem cell decision-making in which Pax6 interacts with three other regulators of neurogenesis, Neurog2, Ascl1, and Hes1. Analyses of the biological function of Pax6 in neural stem cells through phenotypic analyses of Pax6 gain- and loss-of-function mutant cortices demonstrated that the Pax6-regulated networks operating in neural stem cells are highly dosage sensitive. Increasing Pax6 levels drives the system towards neurogenesis and basal progenitor cell genesis by increasing expression of a cohort of basal progenitor cell determinants, including the key transcription factor Eomes/Tbr2, and thus towards neurogenesis at the expense of self-renewal. Removing Pax6 reduces cortical stem cell self-renewal by decreasing expression of key cell cycle regulators, resulting in excess early neurogenesis. We find that the relative levels of Pax6, Hes1, and Neurog2 are key determinants of a dynamic network that controls whether neural stem cells self-renew, generate cortical neurons, or generate basal progenitor cells, a mechanism that has marked parallels with the transcriptional control of embryonic stem cell self-renewal.

Santos, M. S., J. A. Gomes, et al. (2005). "Survival analysis of conjunctival limbal grafts and amniotic membrane transplantation in eyes with total limbal stem cell deficiency." Am J Ophthalmol **140**(2): 223-30.

PURPOSE: To evaluate the survival of conjunctival limbal grafts and amniotic membrane transplantation (AMT) for total limbal stem cell deficiency (LSCD) and the influence of several parameters as cause of LSCD, dry eye, keratinization, eyelid abnormalities, HLA compatibility, systemic immunosuppression, and keratoplasty (PKP) on surgical outcome. DESIGN: Prospective, noncomparative, interventional case series. METHODS: Thirty-three eyes of 31 patients with total LSCD that underwent conjunctival limbal grafts and AMT at the Department of Ophthalmology, Federal University of Sao Paulo were included in this study. Cumulative graft survival as well as the influence of several variables on surgical outcome was analyzed. RESULTS: Ten eyes (30%) underwent conjunctival limbal autograft and 23 (70%) underwent conjunctival limbal allograft from living HLA-matched donor. Graft survival was seen in 13 eyes (40%) at 1 year and in 11 eyes (33.3%) at 2 years, with a cumulative survival of 33% after a mean follow-up time of 33 months. Increase in postoperative visual acuity was observed in 20 eyes (60.6%) during this period. Marked impact on graft survival was observed for patients with Stevens-Johnson syndrome, dry eye, keratinization, eyelid abnormalities, and allogeneic conjunctival limbal transplantation (independently of HLA compatibility) ($P < .05$). Preoperative dry eye was the most important prognostic parameter for surgical outcome ($P < .001$). CONCLUSIONS: Conjunctival limbal

grafts associated with AMT are useful for restoring corneal epithelium phenotype in eyes with total LSCD. However, the cumulative survival declines substantially over a 2-year period. Considering all investigated variables, dry eye was the most important prognostic parameter.

Sarojini, H., R. Estrada, et al. (2008). "PEDF from mouse mesenchymal stem cell secretome attracts fibroblasts." J Cell Biochem **104**(5): 1793-802.

Conditioned medium (secretome) derived from an enriched stem cell culture stimulates chemotaxis of human fibroblasts. These cells are classified as multipotent murine mesenchymal stromal cells (mMSC) by immunochemical analysis of marker proteins. Proteomic analysis of mMSC secretome identifies nineteen secreted proteins, including extracellular matrix structural proteins, collagen processing enzymes, pigment epithelium-derived factor (PEDF) and cystatin C. Immunodepletion and reconstitution experiments show that PEDF is the predominant fibroblast chemoattractant in the conditioned medium, and immunofluorescence microscopy shows strong staining for PEDF in the cytoplasm, at the cell surface, and in intercellular space between mMSCs. This stimulatory effect of PEDF on fibroblast chemotaxis is in contrast to the PEDF-mediated inhibition of endothelial cell migration, reported previously. These differential functional effects of PEDF toward fibroblasts and endothelial cells may serve to program an ordered temporal sequence of scaffold building followed by angiogenesis during wound healing.

Schlotzer-Schrehardt, U., T. Dietrich, et al. (2007). "Characterization of extracellular matrix components in the limbal epithelial stem cell compartment." Exp Eye Res **85**(6): 845-60.

A specialized microenvironment or niche, which regulates maintenance, self-renewal, activation, and proliferation of stem cells by external signals, is one of the key prerequisites for stem cell function. However, the parameters determining the limbal stem cell niche are not yet defined. In order to characterize the role of basement membrane (BM) and extracellular matrix components in the generation of a microenvironmental niche for limbal stem and progenitor cells, we extensively analyzed the topographical variations of the BM zone of human ocular surface epithelia using immunohistochemistry and a large panel of antibodies to most of the presently described intrinsic and associated BM components. Apart from BM components uniformly expressed throughout all ocular surface epithelia (e.g. type IV collagen alpha5 and alpha6 chains, collagen types VII, XV, XVII, and XVIII, laminin-111, laminin-332, laminin chains alpha3, beta3,and gamma2, fibronectin, matrilin-2 and -4, and perlecan), the BM of the limbal epithelium shared many similarities with that of the conjunctival epithelium, including positive labelling for type IV collagen alpha1 and alpha2 chains, laminin alpha5, beta2, and gamma1 chains, nidogen-1 and -2, and thrombospondin-4, whereas type IV collagen alpha3, type V collagen, fibrillin-1 and -2, thrombospondin-1, and endostatin were present in the corneal BM, but lacking or more weakly expressed in the limbal and conjunctival BMs. As compared to both the corneal and conjunctival BMs, the limbal BM showed a markedly increased immunoreactivity for laminin alpha1, alpha2, beta1 chains, and agrin, and a specific but patchy immunoreactivity for laminin gamma3 chain, BM40/SPARC, and tenascin-C, which co-localized with ABCG2/p63/K19-positive and K3/Cx43/desmoglein/integrin-alpha2-negative cell clusters comprising putative stem and early progenitor cells in the basal epithelium of the limbal palisades. Components that were particularly expressed in the corneal-limbal transition zone included type XVI collagen, fibulin-2, tenascin-C/R, vitronectin, bamacan, chondroitin sulfate, and versican, all of which co-localized with vimentin-positive cell clusters comprising putative late progenitor cells in the basal epithelium. This pronounced heterogeneity of the BM in the limbal area, both in the region of limbal palisades and the corneal-limbal transition zone, appears to be involved in providing unique microenvironments for corneal epithelial stem and late progenitor cells. Identification of specific niche parameters might not only help to understand limbal stem cell regulation, but also to improve their selective enrichment and in vitro expansion for therapeutic strategies.

Sheedlo, H. J., T. J. Bartosh, et al. (2007). "RPE-derived factors modulate photoreceptor differentiation: a possible role in the retinal stem cell niche." In Vitro Cell Dev Biol Anim **43**(10): 361-70.

A photoreceptor cell line, designated 661W, was tested for its response to growth factors secreted by retinal pigment epithelial cells including basic fibroblast growth factor, epidermal growth factor, and nerve growth factor. Early passaged 661W cells expressed high levels of retinal progenitor markers such as nestin and Pax6, but not opsin or glial fibrillary acidic protein. 661W cells grown in FGF-2 or EGF exhibited a multiple-process morphology with small phase-bright nuclei similar to neurons, whereas cells cultured in nerve growth factor (NGF) or retinal pigment epithelium (RPE)-conditioned medium (RPE-CM) displayed rounded profiles lacking processes. 661W cells grown in FGF-2 were slightly elevated,

but not significantly above, control cultures; but cells treated with RPE-CM or NGF were fewer, approximately 63% and 49% of control, respectively. NGF immunodepletion of RPE-CM strongly suppressed the inhibitory activity of RPE-CM on cell proliferation. Cells treated with FGF-2, but not NGF, upregulated their expression of opsin. All treatment conditions resulted in almost 100% viability based on calcium AM staining. Cells grown on extracellular matrix proteins laminin, fibronectin, and/or collagen resembled those grown on untreated dishes. This study showed that early passaged 661W cells displayed characteristics of retinal progenitor cells. The 661W cells proliferated and appeared to mature morphologically expressing rod photoreceptor phenotype in response to FGF-2. In contrast, NGF and RPE-CM inhibited proliferation and morphological differentiation of 661W cells, possibly inducing cell cycle arrest. These findings are consistent with reports that the RPE modulates photoreceptor differentiation and retinal progenitor cells via secreted factors and may play a role in the regulation of the retinal stem cell niche.

Song, W. K., Y. H. Min, et al. (2008). "Cytomegalovirus retinitis after hematopoietic stem cell transplantation with alemtuzumab." Ophthalmology **115**(10): 1766-70.

OBJECTIVE: To report on the clinical characteristics and treatment outcomes of cytomegalovirus (CMV) retinitis cases that occurred after allogeneic hematopoietic stem cell transplantation (HSCT) using an alemtuzumab-based (Campath-1H, Genzyme, Cambridge, MA) conditioning regimen. DESIGN: A retrospective noncomparative interventional case series. PARTICIPANTS: Seven eyes of 4 patients in whom CMV retinitis developed after allogeneic HSCT using alemtuzumab. METHODS: A retrospective chart review was performed. CMV retinitis was diagnosed by the presence of characteristic ophthalmoscopic findings and confirmed by polymerase chain reaction-based detection of CMV in vitreal biopsy specimens. The affected eyes received intravitreal injections of 2 mg/0.1 mL of ganciclovir twice weekly during induction therapy until the lesions were inactive, followed by weekly injections as maintenance therapy. Maintenance intravitreal therapy continued until the lesions consisted of an atrophic retina with pigment epithelium mottling and attenuated vessels. MAIN OUTCOME MEASURES: Visual acuity, response of retinitis lesions, and postoperative complications. RESULTS: From 1999 to 2007, 294 patients received allogeneic HSCTs at our institution. Among the HSCTs, 65 were unrelated transplants, and of these, 17 were performed using alemtuzumab-based conditioning regimens. Only 4 patients went on to develop CMV retinitis. These 4 patients had several features in common. All patients received transplants from unrelated donors after an alemtuzumab-conditioning regimen for acute leukemia. One patient died before initiation of treatment. Three patients exhibited a bilateral disease, and 3 patients had neutropenia. Patients underwent a mean of 8.3 intravitreal ganciclovir injections in each eye. All 3 treated patients showed a good response. The treatment was well tolerated without serious adverse events during the mean follow-up period of 8.5 months (range, 4.5-16 months). CONCLUSIONS: An increased incidence of CMV retinitis was noted in unrelated patients undergoing HSCT using a nonmyeloablative alemtuzumab-based conditioning regimen. Intravitreal ganciclovir therapy seems to be an acceptable therapeutic option in these patients given the nature of their systemic illness, which prohibits the use of typical systemic anti-CMV drugs.

Soussain, C., K. Hoang-Xuan, et al. (2008). "Intensive chemotherapy followed by hematopoietic stem-cell rescue for refractory and recurrent primary CNS and intraocular lymphoma: Societe Francaise de Greffe de Moelle Osseuse-Therapie Cellulaire." J Clin Oncol **26**(15): 2512-8.

PURPOSE: The prognosis of relapsing primary CNS lymphoma (PCNSL) is poor. We report the results of a prospective multicenter trial of intensive chemotherapy followed by autologous hematopoietic stem-cell rescue (IC + HCR) in immunocompetent adult patients with PCNSL or intraocular lymphoma (IOL) after failure of high-dose methotrexate-based treatment. PATIENTS AND METHODS: Salvage treatment consisted of two cycles of high-dose cytarabine and etoposide (CYVE). Intensive chemotherapy combined thiotepa, busulfan, and cyclophosphamide. Forty-three patients (median age, 52 years; range, 23 to 65 years) were included, with relapse (n = 22), refractory disease (n = 17), or a partial response to first-line treatment (n = 4). The response to CYVE was not assessable in three cases because of treatment-related death. Twenty patients (47%) were chemosensitive to CYVE: 15 of them proceeded to IC + HCR. IC + HCR was also administered to 12 patients who did not respond to CYVE. All but one of the 27 patients who underwent IC + HCR entered complete remission. RESULTS: With a median follow-up of 36 months, the median overall survival was 18.3 months in the overall population, and 58.6 months among patients who completed IC + HCR. The respective median progression-free survival (PFS) times after IC + HCR were 11.6 and 41.1 months. The 2-year overall survival probability was 45% in the whole population

and 69% among the 27 patients who received IC + HCR. The 2-year PFS probability was 43% among all the patients and 58% in the IC + HCR subpopulation. CONCLUSION: IC + HCR is an effective treatment for refractory and recurrent PCNSL.

Soussain, C., F. Suzan, et al. (2001). "Results of intensive chemotherapy followed by hematopoietic stem-cell rescue in 22 patients with refractory or recurrent primary CNS lymphoma or intraocular lymphoma." J Clin Oncol **19**(3): 742-9.

PURPOSE: To assess the feasibility and efficacy of intensive chemotherapy with hematopoietic stem-cell rescue (IC + HCR) in patients with refractory or recurrent primary CNS lymphoma (PCNSL) or intraocular lymphoma (IOL). PATIENTS AND METHODS: IC consisted of thiotepa 250 mg/m(2)/d days -9 through -7, busulfan 10 mg/kg (total dose) days -6 through -4, and cyclophosphamide 60 mg/kg/d days -3 and -2. Intravenous clonazepam 2 mg/d was given prophylactically from the day before initiation of busulfan therapy to the day after completion of busulfan therapy. Patients with refractory or recurrent PCNSL underwent IC + HCR only if they were chemosensitive to two cycles of salvage treatment with cytarabine (2 g/m(2)/d days 2 through 5 and 50 mg/m(2)/d days 1 through 5 in a 12-hour infusion) and etoposide (VP-16; 200 mg/m(2)/d days 2 through 5) (CYVE). Patients with IOL refractory to high-dose methotrexate (MTX) and cytarabine entered the IC + HCR program directly. RESULTS: Twenty-two patients (10 with relapses, 12 with refractory disease) were enrolled. Twenty patients entered the IC + HCR program: twelve entered after CYVE treatment, seven entered directly, and one had previously been retreated with high-dose MTX. Before IC, eight patients were in complete remission (CR), four were in partial remission (PR), one had stable disease, and seven had refractory disease. After IC + HCR, 16 patients entered CR, two remained in PR, one had stable disease, and one had disease progression. Fourteen patients remained alive (median follow-up time, 41.5 months). The overall probability of survival at 3 years was 63.7%. After IC, that probability was 60% and the 3-year probability of event-free survival was 53%. Seven patients had neurologic adverse events during the entire procedure. CONCLUSION: IC + HCR proved feasible and effective in patients with refractory or recurrent PCNSL or IOL. The entire procedure seemed to be most toxic in patients > or = 60 years. A prospective multicenter study is ongoing.

Sridhar, M. S., G. K. Vemuganti, et al. (2001). "Impression cytology-proven corneal stem cell deficiency in patients after surgeries involving the limbus." Cornea **20**(2): 145-8.

PURPOSE: To report three cases of limbal stem cell deficiency (confirmed by impression cytology) that followed multiple pterygium surgeries and therapeutic penetrating keratoplasty. METHODS: The first case, after multiple pterygium surgeries, presented with corneal scarring and thickened epithelium with vascularization sparing the central cornea in the right eye and involving the entire cornea in the left eye. The second case presented with superficial scarring and extensive vascularization after failed therapeutic graft performed for a large perforated corneal ulcer. The third case was a clear graft performed for a progressing fungal ulcer with signs of conjunctivalization inferotemporally. Limbal stem cell deficiency was clinically suspected in all of these cases. RESULTS: Goblet cells with mucin globules were found on the corneal surface by impression cytology in all three cases. CONCLUSIONS: We report three cases of limbal stem cell deficiency (proven by impression cytology) that followed multiple pterygium surgeries and therapeutic penetrating keratoplasty. Surgical insult to the limbus is the predisposing factor for stem cell damage in these cases. Involvement of the limbus by infection and use of intensive medications are probable contributing factors for stem cell damage in cases of therapeutic penetrating keratoplasty.

Stabile, B. (2009). "What's the matter with Kansas? Legislative debates over stem cell research in Kansas and Massachusetts." Politics Life Sci **28**(1): 17-30.

This paper examines the contextual factors shaping legislative debates affecting stem cell research in two states, Kansas and Massachusetts, which both permit therapeutic cloning for stem cell research but markedly vary in their legislative approach to the issue. In Kansas, restrictive legislation was proposed but effectively blocked by research proponents, while in Massachusetts permissive legislation was successfully implemented under the auspices of an act to promote stem cell research. The importance of university and industry involvement is highlighted in each case, as are the roles of enterprising and persistent policy entrepreneurs. Providing a close examination of the policy process attending the cloning debate in these states is intended to contribute to an enhanced understanding of the cloning-policy process as it has played out at the state level, with an eye toward informing legislative debates over related biotechnical advances in the future.

Stoiber, J., W. H. Muss, et al. (2002). "Histopathology of human corneas after amniotic membrane and

limbal stem cell transplantation for severe chemical burn." Cornea **21**(5): 482-9.

PURPOSE: To describe the histopathologic changes in the cornea following amniotic membrane transplantation (AMT) combined with limbal transplantation. METHODS: Four eyes with complete limbal stem cell deficiency after severe chemical burn underwent AMT with either a living-related conjunctival limbal allograft (lr-CLAL) (three eyes) or a conjunctival limbal autograft (CLAU) (one eye) for ocular surface reconstruction. Penetrating keratoplasty was performed several months after the initial procedure for further visual rehabilitation. Mean follow up time was 20 months. Light and transmission electron microscopy (TEM) and indirect immunofluorescence microscopy of the excised corneal buttons were performed. RESULTS: All specimens displayed a multilayered epithelium without conjunctival goblet cells over the entire corneal surface. Basal epithelial cells demonstrated a firm connection to the remnants of the transplanted amniotic membrane (AM), which at some places appeared to be in a state of "modification" or "remodeling" in the collagen layers. The basement membrane zone displayed a positive staining when using antibodies against collagen IV and VII, integrin alpha6 and beta4, laminin 5, and bullous pemphigoid antigen 2. Remnants of the AM in the specimen showed staining of collagen IV, which was found also in cross-sections of cryopreserved AM. The recipients Bowman's membranes that were only partially present after the initial trauma were significantly disturbed. CONCLUSION: Within the time frame studied, the transplanted AM apparently survives and integrates into the host tissue being modified or remodeled by recipient cells. AMT in combination with a CLAU or lr-CLAL is a useful technique in promoting a rapid and stable reepithelialization of a corneal surface following severe chemical or thermal damage.

Sundmacher, R. and T. Reinhard (1996). "Central corneolimbal transplantation under systemic ciclosporin A cover for severe limbal stem cell insufficiency." Graefes Arch Clin Exp Ophthalmol **234 Suppl 1**: S122-5.

BACKGROUND: Severe stem cell deficiencies uniformly lead to superficial conjunctivalization of corneal grafts with subsequent functional failure. We sought better long-term results by transplanting central corneolimbal grafts and simultaneously protecting the graft and its stem cells from immunological destruction by means of systemic administration of ciclosporin A. PATIENTS AND METHODS: In an ongoing pilot study, up to April 1995 20 eyes with stem cell dysfunctions of various etiology (e.g. chemical burn, ocular pseudopemphigoid, congenital aniridia) received eccentrically trephined fresh corneal grafts of 7.7-10.0 mm diameter. About one third of the circumference of the grafts contained limbal area. The mean age of the patients was 46.2 years (range 9-84 years). All patients received systemic ciclosporin A for at least 12 months. At present, the mean follow-up period is 9.6 months (mean 1-20.6 months). RESULTS: Fourteen of 20 grafts (70%) have remained clear so far. Reasons for six graft failures were surface disorders in four eyes, immune reactions in one eye and surface disorders in combination with immune reactions in another eye. Ten of 20 grafts (50%) experienced severe surface disorders. In six eyes surface disorders were coincident with endothelial immune reactions, in four eyes they were not. In four of 20 grafts (20%) conjunctivalization was observed in front of the transplanted limbal area; in seven of 20 grafts (35%) conjunctivalization occurred only distant from the transplanted limbal stem cells. CONCLUSIONS: Our method of central corneolimbal transplantation with simultaneous protection of the transplanted stem cell population from immunological destruction by means of systemic ciclosporin A has been successful for 14 eyes with severe stem cell deficiencies up to 20.6 months postoperatively. This new treatment principle promises - for the first time - long-term rehabilitation for a majority of eyes with severe limbal stem cell deficiencies.

Swift, G. J., R. K. Aggarwal, et al. (1996). "Survival of rabbit limbal stem cell allografts." Transplantation **62**(5): 568-74.

Failure of a specialized population of corneal epithelial stem cells found in the peripheral cornea and limbus results in ocular surface disease, which may be amenable to treatment by transplantation of limbal tissue. This study was designed to investigate donor limbal stem cell allograft survival in rabbits with ocular surface disease. Rabbits underwent corneal epithelial debridement and limbal ablation to induce ocular surface disease and were then treated by limbal stem cell allotransplantation, by allotransplantation plus topical steroid, or by topical steroid only (n = 7 for each group). Donors and recipients were sex mismatched. Recipients were followed for up to 5 months. Outcome was assessed by daily slit-lamp examination, weekly impression cytology and photographic record, end-point sex chromatin and fluorescent cell tracer analyses, histology, and immunohistochemistry. In no case was a completely normal ocular surface regained, but some animals that received grafts plus corticosteroids fared best by all criteria used. In the absence of immunosuppression, graft hemorrhagia (believed to be a manifestation of graft rejection) occurred within

the first month, the cornea became resurfaced with conjunctiva-derived cells, and no donor cells survived centrally in the long term. Topical corticosteroids reduced the number and severity of these episodes significantly, and were associated with survival of some donor-derived cells in the central cornea of some grafted animals. Thus, rabbit limbal stem cell allografts appeared to undergo rejection, which could be modified by immunosuppression, but useful regeneration of the ocular surface occurred only where rejection was circumvented.

Tabbara, K. F., A. Al-Ghamdi, et al. (2009). "Ocular findings after allogeneic hematopoietic stem cell transplantation." Ophthalmology **116**(9): 1624-9.

OBJECTIVE: To study the incidence, causes, and outcome of major ocular complications in patients undergoing allogeneic hematopoietic stem cell transplantation (HSCT). DESIGN: Retrospective, noncomparative, observational clinical study. PARTICIPANTS: The study included a total of 620 patients who underwent allogeneic HSCT in the period from 1997 to 2007 at King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia. INTERVENTION: Allogeneic HSCT. MAIN OUTCOME MEASURES: Patients with ocular complications were referred to the ophthalmology division for complete ophthalmologic examination, including visual acuity, tonometry, Schirmer test, biomicroscopy, and dilated ophthalmoscopy. Laboratory investigations were performed whenever indicated. The incidence and causes of major ocular complications after allogeneic HSCT were determined. Visual acuity at 1 year after allogeneic HSCT was recorded. RESULTS: Major ocular complications occurred in 80 (13%) of 620 patients who underwent allogeneic HSCT. There were 36 male patients (45%) and 44 female patients (55%) with a mean age of 29 years and an age range of 9 to 65 years. Prophylaxis for graft-versus-host disease (GVHD) consisted of cyclosporine and methotrexate in 69 patients, and cyclosporine, methotrexate and corticosteroids, or mycophenolate mofetil in 11 patients. The most frequently encountered ocular complications were chronic GVHD, dry eye syndrome without GVHD, corneal ulcers, cataract, glaucoma, cytomegalovirus retinitis, fungal endophthalmitis, and acquisition of allergic conjunctivitis from atopic donors. There was no correlation between the pattern of ocular complications and the transplanted stem cell source. Best-corrected visual acuity (BCVA) at 1 year after transplantation was less than 20/200 in 13 patients (16%), less than 20/50 in 17 patients (21%), and better than 20/50 in 50 patients (63%). CONCLUSIONS: Ocular complications are common in patients undergoing allogeneic HSCT. Early recognition and prompt treatment are important. FINANCIAL DISCLOSURE(S): The author(s) have no proprietary or commercial interest in any materials discussed in this article.

Takeda, H., K. Nishimura, et al. (2009). "Planarians maintain a constant ratio of different cell types during changes in body size by using the stem cell system." Zoolog Sci **26**(12): 805-13.

Planarians change in body size depending upon whether they are in feeding or starving conditions. To investigate how planarians regulate this flexible system, the numbers of total cells and specific cell types were counted and compared among worms 2 mm to 9 mm in body length. The total cell number increased linearly with increasing body length, but the ratio of cell numbers between the head and the trunk portion was constant (1:3). Interestingly, counting the numbers of specific neurons in the eye and brain after immunostaining using cell type-specific antibodies revealed that the ratio between different neuron types was constant regardless of the brain and body size. These results suggest that planarians can maintain proportionality while changing their body size by maintaining a constant ratio of different cell types. To understand this system and reveal how planarians restore the original ratio during eye and brain regeneration, the numbers of specialized cells were Investigated during regeneration. The results further substantiate the existence of some form of "counting mechanism" that has the ability to regulate both the absolute and relative numbers of different cell types in complex organs such as the brain during cell turnover, starvation, and regeneration.

Toba, K., J. Tsuchiyama, et al. (2004). "Sensitive measurement of fragmented red cell population using flow cytometry, and its application for estimating thrombotic microangiopathy after stem cell transplantation." Cytometry B Clin Cytom **58**(1): 39-46.

BACKGROUND: Thrombotic microangiopathy (TMA) is one of the lethal complications after hematopoietic stem cell transplantation (SCT). The levels of fragmented red cells (FRCs), thrombomodulin (TM), and factor VIII-related antigen in the blood are the most important markers for estimating TMA. However, the FRC level has been measured by using microscopy and the naked eye; therefore, an improvement in technology to objectively count FRC is necessary. METHODS: We established a novel technique to sensitively measure FRC as glycophorin A dull-positive small particles using a flow cytometer and estimated its reliability in patients treated with SCT. The blood level of FRC was compared with other clinical data in

257 blood samples in 16 clinical courses after SCT of 15 patients. RESULTS: Sorted glycophorin A dull-positive small particles morphologically showed FRC. Measured FRC percentage had a weak correlation with serum levels of lactate dehydrogenase (LDH) and total bilirubin but not with TM level, whereas TM showed a weak correlation with the levels of aspartate aminotransferase and LDH. In a patient with fulminant TMA, decrement of the FRC level led to improvement in liver parameters after treatment, presumably due to the rapid clearance of FRC, and increased simultaneously with the levels of LDH and bilirubin by the TMA recurrence. CONCLUSIONS: Levels of FRC percentage and TM were independent parameters of TMA. This novel technique may be used as a standard methodology in diagnosing TMA.

Tseng, S. C. and D. Q. Li (1996). "Comparison of protein kinase C subtype expression between normal and aniridic human ocular surfaces: implications for limbal stem cell dysfunction in aniridia." Cornea **15**(2): 168-78.

Frozen sections of corneoscleral buttons from normal and aniridic donors were stained with hematoxylin and periodic acid-Schiff, monoclonal antibodies AE-5 and AK-2 (to cornea-specific K3 and K12 keratins, respectively), and AM-3 (to conjunctival goblet cells) as well as with subtype-specific antibodies to seven different protein kinase C (PKC) subtypes, the signal transduction isoenzymes increasingly implicated in the regulation of cell growth and differentiation. Compared with the normal cornea, the aniridic cornea showed decreased AE-5 and AK-2 stainings, increased AM-3 staining, attenuated Bowman's membrane, invasion of new blood vessels, and limbal epithelial hyperplasia. In the normal tissue, the corneal epithelium expressed PKC alpha, lambda, and zeta; the limbal and conjunctival epithelia expressed additional PKC gamma. Conjunctival goblet cells expressed only PKC lambda. Within a given epithelium, different PKC subtypes had different cell-layer distributions. In the aniridic tissue, some of the four normally expressed subtypes were expressed in different cell layers, especially at the limbal region. PKC beta and PKC delta, which were normally weakly expressed, were markedly up-regulated. These results support the conclusion that the aniridic cornea does indeed manifest features of limbal stem cell deficiency with decreased corneal epithelial phenotype and increased conjunctival epithelial phenotype. Different capacities of proliferation and differentiation may be affected by the differential expression of PKC subtypes by different cell layers of normal ocular surface epithelia. The aberrant expression of PKC subtypes in aniridia may thus result in abnormal proliferation and differentiation noted in its ocular surface epithelia. Because limbal stem cells are the ultimate source of corneal proliferation and differentiation, we postulate that limbal deficiency in aniridia is a result of abnormal limbal stem cells.

Tsubota, K., Y. Satake, et al. (1999). "Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation." N Engl J Med **340**(22): 1697-703.

BACKGROUND: Conditions that destroy the limbal area of the peripheral cornea, such as the Stevens-Johnson syndrome, ocular pemphigoid, and chemical and thermal injuries, can deplete stem cells of the corneal epithelium. The result is scarring and opacification of the normally clear cornea. Standard corneal transplantation cannot treat this form of functional blindness. METHODS: We performed and evaluated 70 transplantations of corneal epithelial stem cells from cadaveric eyes into 43 eyes of 39 patients with severe ocular-surface disorders and limbal dysfunction. Medical treatment had failed in all patients. The patients had a mean preoperative visual acuity of 0.004 (only being able to count the number of fingers presented by the examiner) in the affected eyes, which satisfies the criteria for legal blindness in most countries. In 28 eyes, we also performed standard corneal transplantation. Stem-cell transplantations were performed as many as four times on 1 eye if the initial results were not satisfactory; 19 eyes had multiple transplantations. Patients were followed for at least one year after transplantation. RESULTS: A mean of 1163 days after stem-cell transplantation, 22 of the 43 eyes (51 percent) had corneal epithelialization; of the 22 eyes, 7 eyes had corneal stromal edema and 15 eyes had clear corneas. Mean visual acuity improved from 0.004 to 0.02 (vision sufficient to distinguish the largest symbol on the visual-acuity chart from a distance of 1 m) ($P<0.001$). The 15 eyes in which the cornea remained clear had a final mean visual acuity of 0.11 (the ability to distinguish the largest symbol from a distance of 5 m). Complications of the first transplantation included persistent defects in the corneal epithelium in 26 eyes, ocular hypertension in 16 eyes, and rejection of the corneal graft in 13 of 28 eyes. The epithelial defects eventually healed in all but two of the eyes. CONCLUSIONS: Transplantation of corneal epithelial stem cells can restore useful vision in some patients with severe ocular-surface disorders.

Tunici, P., S. Pellegatta, et al. (2003). "The potential of stem cells for the treatment of brain tumors and globoid cell leukodystrophy." Cytotechnology **41**(2-3): 93-101.

Stem cells of different origin are under careful scrutiny as potential new tools for the treatment of several neurological diseases. The major focus of these reaserches have been neurodegenerative disorders, such as Huntington Chorea or Parkinson Disease (Shihabuddin et al., 1999). More recently attention has been devoted to their use for brain repair after stroke (Savitz et al., 2002). In this review we will focus on the potential of stem cell treatments for glioblastoma multiforme (Holland, 2000), the most aggressive primary brain tumor, and globoid cell leukodystrophy (Krabbe disease), a metabolic disorder of the white matter (Berger et al., 2001). These two diseases may offer a paradigm of what the stem cell approach may offer in term of treatment, alone or in combination with other therapeutic approaches. Two kinds of stem cells will be consideredhere: neural stem cells and hematopoietic stem cells, both obtained after birth. The review will focus on experimental models, with an eye on clinical perspectives.

Wehman, A. M., W. Staub, et al. (2005). "Genetic dissection of the zebrafish retinal stem-cell compartment." Dev Biol **281**(1): 53-65.

In a large-scale forward-genetic screen, we discovered that a limited number of genes are required for the regulation of retinal stem cells after embryogenesis in zebrafish. In 18 mutants out of almost 2000 F2 families screened, the eye undergoes normal embryonic development, but fails to continue growth from the ciliary marginal zone (CMZ), the post-embryonic stem-cell niche. Class I-A mutants (5 loci) display lower amounts of proliferation in the CMZ, while nearly all cells in the retina appear differentiated. Class I-B mutants (2 loci) have a reduced CMZ with a concomitant expansion in the retinal pigmented epithelium (RPE), suggesting a common post-embryonic stem cell is the source for these neighboring cell types. Class II encompasses three distinct types of mutants (11 loci) with expanded CMZ, in which the progenitor population is arrested in the cell cycle. We also show that in at least one combination, the reduced CMZ phenotype is genetically epistatic to the expanded CMZ phenotype, suggesting that Class I genes are more likely to affect the stem cells and Class II the progenitor cells. Finally, a comparative mapping analysis demonstrates that the new genes isolated do not correspond to genes previously implicated in stem-cell regulation. Our study suggests that embryonic and post-embryonic stem cells utilize separable genetic programs in the zebrafish retina.

Wiles, M. V. and B. M. Johansson (1997). "Analysis of factors controlling primary germ layer formation and early hematopoiesis using embryonic stem cell in vitro differentiation." Leukemia **11 Suppl 3**: 454-6.

Differentiation and subsequent development are intricately interwoven processes operating as an integrated whole to form the organism. As an approach to examine these interactions in early mammalian development, we used embryonic stem (ES) cell in vitro differentiation. ES cells can, depending upon the environment differentiated to neuroectoderm, mesoderm and hematopoietic cells. We developed a serum-free, chemically defined medium (CDM) in which ES cells survive and differentiate. In CDM, in the absence of exogenous factors, ES cells form neuroectoderm, upregulating the early neural marker Pax-6. This is consistent with the view that neuroectoderm development can represent a default state, where the absence or sequestration of mesoderm inducing factors permits neuroectoderm formation. In contrast, if CDM is supplemented with bone morphogenetic protein (BMP) 2 or 4 a process resembling primitive streak formation, least at the molecular level occurs, with the formation of mesoderm and subsequently endothelial and hematopoietic cells. If used with care, ES cell in vitro differentiation can act as a guide in understanding the environment which controls early differentiation events in mammals.

Wiles, M. V. and B. M. Johansson (1999). "Embryonic stem cell development in a chemically defined medium." Exp Cell Res **247**(1): 241-8.

Vertebrate germ layer development is an intricately interwoven process with the organism operating as an integrated whole. To examine these processes we have used embryonic stem (ES) cell in vitro differentiation in a serum-free, chemically defined medium (CDM). In CDM, ES cells differentiate as embryoid bodies to neuroectoderm with upregulation of pax-6, without commensurate expression of Brachyury. In the presence of Activin A, pax-6 and Brachyury mRNAs are readily detectable, suggestive of both neuroectoderm and mesoderm formation, while in the presence of BMP-4 a process resembling primitive streak formation at the molecular level occurs. Neuroectoderm development in CDM alone is consistent with the view that this process can occur by default, as reported in Xenopus, due to the absence or sequestration of mesoderm-inducing factors. Additionally, these data show that BMP-4 alone is capable of instigating a process resembling primitive streak formation in ES cells and possibly in vivo.

Williams, K. A., H. M. Brereton, et al. (1995). "Use of DNA polymorphisms and the polymerase chain

reaction to examine the survival of a human limbal stem cell allograft." Am J Ophthalmol **120**(3): 342-50.

PURPOSE: The extent to which limbal epithelial stem cell allografts will repopulate the human corneal ocular surface, and the time frame over which such cells survive, are uncertain. We investigated the survival of donor-derived epithelial cells after limbal stem cell allotransplantation in a patient with bilateral limbal stem cell failure by using short tandem-repeat DNA polymorphisms to distinguish donor and recipient cells. METHODS: Epithelial cells were harvested by impression cytology from the grafted eye before and at various times after transplantation. DNA was extracted and amplified by the polymerase chain reaction at an informative locus, D8S264. RESULTS: Cells of donor genotype were present over the grafted areas at the time of surgery but were not detected in the central cornea until 12 weeks postoperatively, indicating that repopulation of the epithelial surface from transplanted limbal stem cells took considerable time. However, by the 20th postoperative week, only recipient-type cells were detected in the grafted eye, despite systemic immunosuppression of the recipient with azathioprine and cyclosporine. CONCLUSIONS: Discrimination between donor and recipient cells on the ocular surface after limbal allotransplantation was possible using genotypic variation at DNA polymorphic sites (microsatellites). Long-term survival of donor cells after limbal transplantation did not occur in this patient. Detection of DNA polymorphisms amplified by the polymerase chain reaction is a simple, rapid, and noninvasive method of following the course of transplanted cells at the ocular surface.

Williams, K. A. and D. J. Coster (1997). "Rethinking immunological privilege: implications for corneal and limbal stem cell transplantation." Mol Med Today **3**(11): 495-501.

Immunological privilege operates within the normal eye by multiple passive and active mechanisms, including antigen sequestration, maintenance of an immunosuppressive local environment and induction of apoptotic death in infiltrating cells of the immune system. Ocular privilege might have developed to protect the eye from the collateral damage associated with an inflammatory response to invading pathogens. Nevertheless, corneal grafts do undergo irreversible immunological rejection and, furthermore, corneal graft rejection is very similar at a histological level to the rejection processes that operate in vascularized organ grafts. Ocular privilege is thus relative. The question arises as to how corneal grafts are rejected in the face of so many mechanisms designed to prevent immune responses from operating inside the eye--a question that is still essentially unanswered.

Wolosin, J. M., M. T. Budak, et al. (2004). "Ocular surface epithelial and stem cell development." Int J Dev Biol **48**(8-9): 981-91.

Phenotypic features and developmental events involved in the genesis of the limbo-corneal and conjunctival epithelia are described. Together, these two epithelia define the ocular surface. They derive from a small cohort of optic vesicle-induced PAX6+ head ectodermal cells that remain on the surface following lens vesicle formation by the main PAX6+ cell cohort. Both epithelia are stratified, and display wet, non-keratinizing phenotypes. The most significant spatial feature of the limbo-corneal epithelium is the segregation of its supporting stem and early precursor cells to the limbus, the outer vascularized rim separating the cornea from the conjunctiva. These stem cells express ABCG2, a xenobiotic transporter present in stem cells from other organs. ABCG2 transport activity excludes the DNA dye Hoechst 33342, allowing the isolation of the ocular stem cells by flow cytometry, as a unique cohort known as a side 'side population'. Limbal stem cells do not form gap junctions and exist as metabolically isolated entities. Tracking of expression changes in Cx43, the main gap junction protein expressed in both the pre-epithelial ectoderm and in the mature central corneal epithelium, indicates that a limbal stem cell phenotype starts developing very soon after lens vesicle invagination, in advance of the appearance of any recognizable anatomical sub-epithelial limbal feature. Differences in Cx43 expression also reveal the very early nature of the divergence in limbo-corneal and conjunctival lineages. The putative involvement of several early genes, including gradients of PAX6 and differences in expression patterns for members of the Id or msh gene expression regulators are reviewed.

Ye, J., K. Yao, et al. (2006). "Mesenchymal stem cell transplantation in a rabbit corneal alkali burn model: engraftment and involvement in wound healing." Eye (Lond) **20**(4): 482-90.

PURPOSE: To investigate whether systemically transplanted mesenchymal stem cells (MSCs) can home and engraft in tissue to promote cornea wound healing after alkali burn, as a new source for treatment. METHODS: Corneal alkali burn was created in four group rabbits: Group I, normal bone marrow function, without MSCs transplantation; Group II, normal bone marrow function, with MSCs transplantation; Group III, bone marrow suppressed by cyclophosphamide, without MSCs; Group IV, bone marrow suppressed by cyclophosphamide, with

MSCs. Clinical outcome was evaluated by cornea re-epithelization, cornea opacity, and neovascularization. Cell engraftment into bone marrow, circulation, and cornea was monitored. Immunohistochemistry, using proliferating cell nuclear antigen (PCNA), P63, vimentin, and alpha-smooth muscle actin (alpha-SMA) was carried out to assess the cell proliferative and differentiative ability. RESULTS: At the time of 1-month follow-up, Group II rabbits showed the best clinical results with a clearer healed cornea compared with other groups. Well-formed neovascularization appeared on day 14 after alkali burn in Group II, that coincided with the maximum engraftment of MSCs. PCNA, P63, vimentin were more strongly expressed in Group II at multiple time points. DiI-labelled MSCs were differentiated into myofibroblast by the expression of alpha-SMA. Delayed and insufficient cell engraftment, with malformed neovascularization and retarded corneal wound healing was found in Groups III and IV. CONCLUSIONS: Systemically transplanted MSCs can engraft to injured cornea to promote wound healing, by differentiation, proliferation, and synergizing with haemotopoietic stem cells.

Zaghloul, N. A. and S. A. Moody (2007). "Alterations of rx1 and pax6 expression levels at neural plate stages differentially affect the production of retinal cell types and maintenance of retinal stem cell qualities." Dev Biol **306**(1): 222-40.

rx1 and pax6 are necessary for the establishment of the vertebrate eye field and for the maintenance of the retinal stem cells that give rise to multiple retinal cell types. They also are differentially expressed in cellular layers in the retina when cell fates are being specified, and their expression levels differentially affect the production of amacrine cell subtypes. To determine whether rx1 and pax6 expression after the eye field is established simply maintains stem cell-like qualities or affects cell type differentiation, we used hormone-inducible constructs to increase or decrease levels/activity of each protein at two different neural plate stages. Our results indicate that rx1 regulates the size of the retinal stem cell pool because it broadly affected all cell types, whereas pax6 regulates more restricted retinal progenitor cells because it selectively affected different cell types in a time-dependent manner. Analysis of rx1 and pax6 effects on proliferation, and expression of stem cell or differentiation markers demonstrates that rx1 maintains cells in a stem cell state by promoting proliferation and delaying expression of neural identity and differentiation markers. Although pax6 also promotes proliferation, it differentially regulates neural identity and differentiation genes. Thus, these two genes work in parallel to regulate different, but overlapping aspects of retinal cell fate determination.

References

1. Ahmad, S., R. Stewart, et al. (2007). "Differentiation of human embryonic stem cells into corneal epithelial-like cells by in vitro replication of the corneal epithelial stem cell niche." Stem Cells **25**(5): 1145-55.
2. Akinci, M. A., H. Turner, et al. (2009). "Differential gene expression in the pig limbal side population: implications for stem cell cycling, replication, and survival." Invest Ophthalmol Vis Sci **50**(12): 5630-8.
3. Akinci, M. A., H. Turner, et al. (2009). "Molecular profiling of conjunctival epithelial side-population stem cells: atypical cell surface markers and sources of a slow-cycling phenotype." Invest Ophthalmol Vis Sci **50**(9): 4162-72.
4. Anderson, D. F., P. Ellies, et al. (2001). "Amniotic membrane transplantation for partial limbal stem cell deficiency." Br J Ophthalmol **85**(5): 567-75.
5. Askmyr, M., J. Holmberg, et al. (2009). "Low-dose busulphan conditioning and neonatal stem cell transplantation preserves vision and restores hematopoiesis in severe murine osteopetrosis." Exp Hematol **37**(2): 302-8.
6. Auw-Haedrich, C., C. Potsch, et al. (2007). "Histological and immunohistochemical characterisation of conjunctival graft vs host disease following haematopoietic stem cell transplantation." Graefes Arch Clin Exp Ophthalmol **245**(7): 1001-7.
7. Balla, M. M., G. K. Vemuganti, et al. (2009). "Phenotypic characterization of retinoblastoma for the presence of putative cancer stem-like cell markers by flow cytometry." Invest Ophthalmol Vis Sci **50**(4): 1506-14.
8. Brandl, C., C. Florian, et al. (2009). "Identification of neural crest-derived stem cell-like cells from the corneal limbus of juvenile mice." Exp Eye Res **89**(2): 209-17.
9. Bull, N. D., G. A. Limb, et al. (2008). "Human Muller stem cell (MIO-M1) transplantation in a rat model of glaucoma: survival, differentiation, and integration." Invest Ophthalmol Vis Sci **49**(8): 3449-56.
10. Burman, S. and V. Sangwan (2008). "Cultivated limbal stem cell transplantation for ocular surface reconstruction." Clin Ophthalmol **2**(3): 489-502.
11. Carr, A. J., A. Vugler, et al. (2009). "Molecular characterization and functional analysis of phagocytosis by human embryonic stem cell-derived RPE cells using a novel human retinal assay." Mol Vis **15**: 283-95.
12. Chaudhry, G. R., C. Fecek, et al. (2009). "Fate of embryonic stem cell derivatives implanted into the vitreous of a slow retinal degenerative mouse model." Stem Cells Dev **18**(2): 247-58.
13. Chen, W., K. Zhao, et al. (2007). "Keratoconjunctivitis sicca modifies epithelial stem cell proliferation kinetics in conjunctiva." Cornea **26**(9): 1101-6.
14. Coles, B. L., D. J. Horsford, et al. (2006). "Loss of retinal progenitor cells leads to an increase in the retinal stem cell population in vivo." Eur J Neurosci **23**(1): 75-82.
15. Collinson, J. M., S. A. Chanas, et al. (2004). "Corneal development, limbal stem cell function, and corneal epithelial cell migration in the Pax6(+/-) mouse." Invest Ophthalmol Vis Sci **45**(4): 1101-8.
16. Coster, D. J., R. K. Aggarwal, et al. (1995). "Surgical management of ocular surface disorders using conjunctival and stem cell allografts." Br J Ophthalmol **79**(11): 977-82.
17. Das, A. V., K. B. Mallya, et al. (2006). "Neural stem cell properties of Muller glia in the mammalian retina: regulation by Notch and Wnt signaling." Dev Biol **299**(1): 283-302.
18. Daya, S. M. and F. A. Ilari (2001). "Living related conjunctival limbal allograft for the treatment of stem cell

deficiency." Ophthalmology **108**(1): 126-33; discussion 133-4.
19. Daya, S. M., A. Watson, et al. (2005). "Outcomes and DNA analysis of ex vivo expanded stem cell allograft for ocular surface reconstruction." Ophthalmology **112**(3): 470-7.
20. DeSousa, J. L., S. Daya, et al. (2009). "Adnexal surgery in patients undergoing ocular surface stem cell transplantation." Ophthalmology **116**(2): 235-42.
21. Di Rosa, P., J. C. Villaescusa, et al. (2007). "The homeodomain transcription factor Prep1 (pKnox1) is required for hematopoietic stem and progenitor cell activity." Dev Biol **311**(2): 324-34.
22. Donahue, L. M., P. W. Coates, et al. (1996). "Characterization of developmental stage and neuronal potential of the rat PNS-derived stem cell line, RT4-AC." Brain Res Dev Brain Res **94**(1): 67-80.
23. Donisi, P. M., P. Rama, et al. (2003). "Analysis of limbal stem cell deficiency by corneal impression cytology." Cornea **22**(6): 533-8.
24. Dua, H. S. and A. Azuara-Blanco (1999). "Allo-limbal transplantation in patients with limbal stem cell deficiency." Br J Ophthalmol **83**(4): 414-9.
25. Dua, H. S. and A. Azuara-Blanco (2000). "Autologous limbal transplantation in patients with unilateral corneal stem cell deficiency." Br J Ophthalmol **84**(3): 273-8.
26. Dudney, B. W. and M. A. Malecha (2004). "Limbal stem cell deficiency following topical mitomycin C treatment of conjunctival-corneal intraepithelial neoplasia." Am J Ophthalmol **137**(5): 950-1.
27. Edman, L., J. Larsen, et al. (2001). "Health-related quality of life, symptom distress and sense of coherence in adult survivors of allogeneic stem-cell transplantation." Eur J Cancer Care (Engl) **10**(2): 124-30.
28. Espana, E. M., M. Grueterich, et al. (2003). "Phenotypic study of a case receiving a keratolimbal allograft and amniotic membrane for total limbal stem cell deficiency." Ophthalmology **110**(3): 481-6.
29. Fagerholm, P. and G. Lisha (1999). "Corneal stem cell grafting after chemical injury." Acta Ophthalmol Scand **77**(2): 165-9.
30. Fahnehjelm, K. T., A. L. Tornquist, et al. (2006). "Ocular findings in four children with mucopolysaccharidosis I-Hurler (MPS I-H) treated early with haematopoietic stem cell transplantation." Acta Ophthalmol Scand **84**(6): 781-5.
31. Fahnehjelm, K. T., A. L. Tornquist, et al. (2008). "Dry-eye syndrome after allogeneic stem-cell transplantation in children." Acta Ophthalmol **86**(3): 253-8.
32. Fan, Z., T. Yamaza, et al. (2009). "BCOR regulates mesenchymal stem cell function by epigenetic mechanisms." Nat Cell Biol **11**(8): 1002-9.
33. Fernandes, M., V. S. Sangwan, et al. (2004). "Limbal stem cell transplantation." Indian J Ophthalmol **52**(1): 5-22.
34. Fisher, V. L. (1999). "Long-term follow-up in hematopoietic stem-cell transplant patients." Pediatr Transplant **3 Suppl 1**: 122-9.
35. Fogla, R. and P. Padmanabhan (2005). "Deep anterior lamellar keratoplasty combined with autologous limbal stem cell transplantation in unilateral severe chemical injury." Cornea **24**(4): 421-5.
36. Fujishima, H., J. Shimazaki, et al. (1996). "Temporary corneal stem cell dysfunction after radiation therapy." Br J Ophthalmol **80**(10): 911-4.
37. Furst, D. E. (2002). "Stem cell transplantation for autoimmune disease: progress and problems." Curr Opin Rheumatol **14**(3): 220-4.
38. Gomes, J. A., M. S. dos Santos, et al. (2003). "Amniotic membrane transplantation for partial and total limbal stem cell deficiency secondary to chemical burn." Ophthalmology **110**(3): 466-73.
39. Grueterich, M., E. M. Espana, et al. (2002). "Phenotypic study of a case with successful transplantation of ex vivo expanded human limbal epithelium for unilateral total limbal stem cell deficiency." Ophthalmology **109**(8): 1547-52.
40. Harkin, D. G., Z. Barnard, et al. (2004). "Analysis of p63 and cytokeratin expression in a cultivated limbal autograft used in the treatment of limbal stem cell deficiency." Br J Ophthalmol **88**(9): 1154-8.
41. Hatakeyama, J., Y. Bessho, et al. (2004). "Hes genes regulate size, shape and histogenesis of the nervous system by control of the timing of neural stem cell differentiation." Development **131**(22): 5539-50.
42. Haynes, T., C. Gutierrez, et al. (2007). "BMP signaling mediates stem/progenitor cell-induced retina regeneration." Proc Natl Acad Sci U S A **104**(51): 20380-5.
43. Heath, J. A., E. H. Broxson, Jr., et al. (2002). "Epstein-Barr virus-associated lymphoma in a child undergoing an autologous stem cell rescue." J Pediatr Hematol Oncol **24**(2): 160-3.
44. Huck, K., H. J. Laws, et al. (2006). "Three cases of renal relapse after allogeneic hematopoietic stem cell transplantation for childhood acute lymphoblastic leukemia." Haematologica **91**(5 Suppl): ECR07.
45. Insua, M. F., M. V. Simon, et al. (2008). "Trophic factors and neuronal interactions regulate the cell cycle and Pax6 expression in Muller stem cells." J Neurosci Res **86**(7): 1459-71.
46. Kal, H. B., M. Loes van Kempen-Harteveld, et al. (2006). "Biologically effective dose in total-body irradiation and hematopoietic stem cell transplantation." Strahlenther Onkol **182**(11): 672-9.
47. Kim, J. T., Y. S. Chun, et al. (2008). "The effect of in vivo grown corneal epithelium transplantation on persistent epithelial defects with limbal stem cell deficiency." J Korean Med Sci **23**(3): 502-8.
48. Kitadate, Y., S. Shigenobu, et al. (2007). "Boss/Sev signaling from germline to soma restricts germline-stem-cell-niche formation in the anterior region of Drosophila male gonads." Dev Cell **13**(1): 151-9.
49. Koch, T. G., L. C. Berg, et al. (2009). "Current and future regenerative medicine - principles, concepts, and therapeutic use of stem cell therapy and tissue engineering in equine medicine." Can Vet J **50**(2): 155-65.
50. Kosinski, C., V. S. Li, et al. (2007). "Gene expression patterns of human colon tops and basal crypts and BMP antagonists as intestinal stem cell niche factors." Proc Natl Acad Sci U S A **104**(39): 15418-23.
51. Kremens, B., R. Wieland, et al. (2003). "High-dose chemotherapy with autologous stem cell rescue in children with retinoblastoma." Bone Marrow Transplant **31**(4): 281-4.
52. Kruse, F. E. and C. Cursiefen (2008). "Surgery of the cornea: corneal, limbal stem cell and amniotic membrane transplantation." Dev Ophthalmol **41**: 159-70.
53. Kuo, T. K., J. H. Ho, et al. (2009). "Mesenchymal stem cell therapy for nonmusculoskeletal diseases: emerging applications." Cell Transplant **18**(9): 1013-28.
54. Lawrence, J. M., S. Singhal, et al. (2007). "MIO-M1 cells and similar muller glial cell lines derived from adult human retina exhibit neural stem cell characteristics." Stem Cells **25**(8): 2033-43.
55. Lee, S. H., K. H. Yoo, et al. (2008). "Tandem high-dose chemotherapy and autologous stem cell rescue in children with bilateral advanced retinoblastoma." Bone Marrow Transplant **42**(6): 385-91.
56. Liang, L., H. Sheha, et al. (2009). "Long-term outcomes of keratolimbal allograft for total limbal stem cell deficiency using combined immunosuppressive agents and correction of ocular surface deficits." Arch Ophthalmol **127**(11): 1428-34.
57. Lim, K. H., S. Kim, et al. (2008). "Central pontine myelinolysis in a patient with acute lymphoblastic leukemia

after hematopoietic stem cell transplantation: a case report." J Korean Med Sci **23**(2): 324-7.
58. Limb, G. A., J. T. Daniels, et al. (2006). "Current prospects for adult stem cell-based therapies in ocular repair and regeneration." Curr Eye Res **31**(5): 381-90.
59. Lord-Grignon, J., M. Abdouh, et al. (2006). "Identification of genes expressed in retinal progenitor/stem cell colonies isolated from the ocular ciliary body of adult mice." Gene Expr Patterns **6**(8): 992-9.
60. Luengo Gimeno, F., V. Lavigne, et al. (2007). "Advances in corneal stem-cell transplantation in rabbits with severe ocular alkali burns." J Cataract Refract Surg **33**(11): 1958-65.
61. Lund, R. D., S. Wang, et al. (2006). "Human embryonic stem cell-derived cells rescue visual function in dystrophic RCS rats." Cloning Stem Cells **8**(3): 189-99.
62. Lyall, D. A., S. Srinivasan, et al. (2009). "Limbal stem cell failure secondary to advanced conjunctival squamous cell carcinoma: a clinicopathological case report." BMJ Case Rep **2009**.
63. Lyngholm, M., H. Vorum, et al. (2008). "Differences in the protein expression in limbal versus central human corneal epithelium--a search for stem cell markers." Exp Eye Res **87**(2): 96-105.
64. Marena, C., M. Zecca, et al. (2001). "Incidence of, and risk factors for, nosocomial infections among hematopoietic stem cell transplantation recipients, with impact on procedure-related mortality." Infect Control Hosp Epidemiol **22**(8): 510-7.
65. Meallet, M. A., E. M. Espana, et al. (2003). "Amniotic membrane transplantation with conjunctival limbal autograft for total limbal stem cell deficiency." Ophthalmology **110**(8): 1585-92.
66. Meisler, D. M., V. L. Perez, et al. (2005). "A device to facilitate limbal stem cell procurement from eye bank donor tissue for keratolimbal allograft procedures." Am J Ophthalmol **139**(1): 212-4.
67. Meller, D., T. Fuchsluger, et al. (2009). "Ocular surface reconstruction in graft-versus-host disease with HLA-identical living-related allogeneic cultivated limbal epithelium after hematopoietic stem cell transplantation from the same donor." Cornea **28**(2): 233-6.
68. Mendicute, J., A. Bidaguren, et al. (2008). "Automatized large diameter lamellar keratoplasty and stem cell transplantation for the treatment of ocular surface diseases with limbal insufficiency." Eur J Ophthalmol **18**(4): 641-4.
69. Mimeault, M. and S. K. Batra (2008). "Recent progress on tissue-resident adult stem cell biology and their therapeutic implications." Stem Cell Rev **4**(1): 27-49.
70. Mimeault, M., R. Hauke, et al. (2007). "Stem cells: a revolution in therapeutics-recent advances in stem cell biology and their therapeutic applications in regenerative medicine and cancer therapies." Clin Pharmacol Ther **82**(3): 252-64.
71. Miyamura, F., S. Kako, et al. (2009). "Successful treatment of young-onset adult T cell leukemia/lymphoma and preceding chronic refractory eczema and corneal injury by allogeneic hematopoietic stem cell transplantation." Int J Hematol **90**(3): 397-401.
72. Mohammadpour, M., M. A. Javadi, et al. (2006). "Limbal stem cell deficiency in the context of autoimmune polyendocrinopathy." Eur J Ophthalmol **16**(6): 870-2.
73. Mohty, M., M. Kuentz, et al. (2002). "Chronic graft-versus-host disease after allogeneic blood stem cell transplantation: long-term results of a randomized study." Blood **100**(9): 3128-34.
74. Mori, T., M. Watanabe, et al. (2008). "Reduced efficacy of topical corticosteroid in preventing cytarabine-induced kerato-conjunctivitis in patients receiving high-dose cytarabine and total body irradiation for allogeneic hematopoietic stem cell transplantation." Bone Marrow Transplant **42**(3): 197-9.
75. Mouriaux, F., F. Chahud, et al. (2001). "Implication of stem cell factor in the proliferation of choroidal melanocytes." Exp Eye Res **73**(2): 151-7.
76. Nguyen, D. Q., S. Srinivasan, et al. (2007). "Thimerosal-induced limbal stem cell failure: report of a case and review of the literature." Eye Contact Lens **33**(4): 196-8.
77. Nishida, K., M. Yamato, et al. (2004). "Functional bioengineered corneal epithelial sheet grafts from corneal stem cells expanded ex vivo on a temperature-responsive cell culture surface." Transplantation **77**(3): 379-85.
78. Ogawa, Y. and M. Kuwana (2003). "Dry eye as a major complication associated with chronic graft-versus-host disease after hematopoietic stem cell transplantation." Cornea **22**(7 Suppl): S19-27.
79. Ogawa, Y., S. Okamoto, et al. (1999). "Dry eye after haematopoietic stem cell transplantation." Br J Ophthalmol **83**(10): 1125-30.
80. Oji, Y., Y. Oka, et al. (1999). "Successful treatment of relapsed T-cell non-Hodgkin's lymphoma with allogeneic peripheral blood stem cell transplantation with double conditioning." Int J Hematol **69**(4): 263-7.
81. Oyama, Y., R. K. Burt, et al. (2009). "A case of autoimmune-related retinopathy and optic neuropathy syndrome treated by autologous nonmyeloablative hematopoietic stem cell transplantation." J Neuroophthalmol **29**(1): 43-9.
82. Rama, P., S. Bonini, et al. (2001). "Autologous fibrin-cultured limbal stem cells permanently restore the corneal surface of patients with total limbal stem cell deficiency." Transplantation **72**(9): 1478-85.
83. Samson, C. M., C. Nduaguba, et al. (2002). "Limbal stem cell transplantation in chronic inflammatory eye disease." Ophthalmology **109**(5): 862-8.
84. Sanders, J. E. (2002). "Chronic graft-versus-host disease and late effects after hematopoietic stem cell transplantation." Int J Hematol **76 Suppl 2**: 15-28.
85. Sangwan, V. S., M. Fernandes, et al. (2005). "Early results of penetrating keratoplasty following limbal stem cell transplantation." Indian J Ophthalmol **53**(1): 31-5.
86. Sansom, S. N., D. S. Griffiths, et al. (2009). "The level of the transcription factor Pax6 is essential for controlling the balance between neural stem cell self-renewal and neurogenesis." PLoS Genet **5**(6): e1000511.
87. Santos, M. S., J. A. Gomes, et al. (2005). "Survival analysis of conjunctival limbal grafts and amniotic membrane transplantation in eyes with total limbal stem cell deficiency." Am J Ophthalmol **140**(2): 223-30.
88. Sarojini, H., R. Estrada, et al. (2008). "PEDF from mouse mesenchymal stem cell secretome attracts fibroblasts." J Cell Biochem **104**(5): 1793-802.
89. Schlotzer-Schrehardt, U., T. Dietrich, et al. (2007). "Characterization of extracellular matrix components in the limbal epithelial stem cell compartment." Exp Eye Res **85**(6): 845-60.
90. Sheedlo, H. J., T. J. Bartosh, et al. (2007). "RPE-derived factors modulate photoreceptor differentiation: a possible role in the retinal stem cell niche." In Vitro Cell Dev Biol Anim **43**(10): 361-70.
91. Song, W. K., Y. H. Min, et al. (2008). "Cytomegalovirus retinitis after hematopoietic stem cell transplantation with alemtuzumab." Ophthalmology **115**(10): 1766-70.
92. Soussain, C., F. Suzan, et al. (2001). "Results of intensive chemotherapy followed by hematopoietic stem-cell rescue in 22 patients with refractory or recurrent primary CNS lymphoma or intraocular lymphoma." J Clin Oncol **19**(3): 742-9.
93. Soussain, C., K. Hoang-Xuan, et al. (2008). "Intensive chemotherapy followed by hematopoietic stem-cell rescue for refractory and recurrent primary CNS and intraocular

lymphoma: Societe Francaise de Greffe de Moelle Osseuse-Therapie Cellulaire." J Clin Oncol **26**(15): 2512-8.
94. Sridhar, M. S., G. K. Vemuganti, et al. (2001). "Impression cytology-proven corneal stem cell deficiency in patients after surgeries involving the limbus." Cornea **20**(2): 145-8.
95. Stabile, B. (2009). "What's the matter with Kansas? Legislative debates over stem cell research in Kansas and Massachusetts." Politics Life Sci **28**(1): 17-30.
96. Stoiber, J., W. H. Muss, et al. (2002). "Histopathology of human corneas after amniotic membrane and limbal stem cell transplantation for severe chemical burn." Cornea **21**(5): 482-9.
97. Sundmacher, R. and T. Reinhard (1996). "Central corneolimbal transplantation under systemic ciclosporin A cover for severe limbal stem cell insufficiency." Graefes Arch Clin Exp Ophthalmol **234 Suppl 1**: S122-5.
98. Swift, G. J., R. K. Aggarwal, et al. (1996). "Survival of rabbit limbal stem cell allografts." Transplantation **62**(5): 568-74.
99. Tabbara, K. F., A. Al-Ghamdi, et al. (2009). "Ocular findings after allogeneic hematopoietic stem cell transplantation." Ophthalmology **116**(9): 1624-9.
100. Takeda, H., K. Nishimura, et al. (2009). "Planarians maintain a constant ratio of different cell types during changes in body size by using the stem cell system." Zoolog Sci **26**(12): 805-13.
101. Toba, K., J. Tsuchiyama, et al. (2004). "Sensitive measurement of fragmented red cell population using flow cytometry, and its application for estimating thrombotic microangiopathy after stem cell transplantation." Cytometry B Clin Cytom **58**(1): 39-46.
102. Tseng, S. C. and D. Q. Li (1996). "Comparison of protein kinase C subtype expression between normal and aniridic human ocular surfaces: implications for limbal stem cell dysfunction in aniridia." Cornea **15**(2): 168-78.
103. Tsubota, K., Y. Satake, et al. (1999). "Treatment of severe ocular-surface disorders with corneal epithelial stem-cell transplantation." N Engl J Med **340**(22): 1697-703.
104. Tunici, P., S. Pellegatta, et al. (2003). "The potential of stem cells for the treatment of brain tumors and globoid cell leukodystrophy." Cytotechnology **41**(2-3): 93-101.
105. Wehman, A. M., W. Staub, et al. (2005). "Genetic dissection of the zebrafish retinal stem-cell compartment." Dev Biol **281**(1): 53-65.
106. Wiles, M. V. and B. M. Johansson (1997). "Analysis of factors controlling primary germ layer formation and early hematopoiesis using embryonic stem cell in vitro differentiation." Leukemia **11 Suppl 3**: 454-6.
107. Wiles, M. V. and B. M. Johansson (1999). "Embryonic stem cell development in a chemically defined medium." Exp Cell Res **247**(1): 241-8.
108. Williams, K. A. and D. J. Coster (1997). "Rethinking immunological privilege: implications for corneal and limbal stem cell transplantation." Mol Med Today **3**(11): 495-501.
109. Williams, K. A., H. M. Brereton, et al. (1995). "Use of DNA polymorphisms and the polymerase chain reaction to examine the survival of a human limbal stem cell allograft." Am J Ophthalmol **120**(3): 342-50.
110. Wolosin, J. M., M. T. Budak, et al. (2004). "Ocular surface epithelial and stem cell development." Int J Dev Biol **48**(8-9): 981-91.
111. Ye, J., K. Yao, et al. (2006). "Mesenchymal stem cell transplantation in a rabbit corneal alkali burn model: engraftment and involvement in wound healing." Eye (Lond) **20**(4): 482-90.
112. Zaghloul, N. A. and S. A. Moody (2007). "Alterations of rx1 and pax6 expression levels at neural plate stages differentially affect the production of retinal cell types and maintenance of retinal stem cell qualities." Dev Biol **306**(1): 222-40.
113. Ma H, Chen G (2005). Stem Cell. J Am Sci. 1(2):90-92. http://www.sciencepub.net/american/0102/14-mahongbao.pdf.
114. Ma H, Chenrg S (2007). Eternal Life and Stem Cell. Nat Sci. 5(1):81-96. http://www.sciencepub.net/nature/0501/10-0247-mahongbao-eternal-ns.pdf.
115. Ma H, Chenrg S (2007). Review of Stem Cell Studies. Nat Sci. 5(2):45-65. http://www.sciencepub.net/nature/0502/09-0247-mahongbao-stem-ns.pdf.
116. Yang Y, Ma H (2010). Germ Stem Cell. Stem Cell. 1(2):38-60]. http://www.sciencepub.net/stem/stem0102/07_1348stem0102_38_60.pdf.
117. Pubmed. Stem Cell. http://www.ncbi.nlm.nih.gov/pubmed/?term=stem+cell.
118. Wikipedia. Stem Cell. http://en.wikipedia.org/wiki/Stem_cell.

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